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Plant Growth Substances

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# PREFACE

SINCE the dawn of agriculture one of man's principal aims has been the promotion and control of plant growth. These two aspects of his work with plants in the struggle to increase crops are by no means synonymous. He was soon to realize that the promotion of lush and leafy growth does not always produce the best results in terms of fruit and seed, and hence he has evolved such well-known cultural methods as pruning, balanced manuring, etc., in order to regulate the nature as well as the luxuriance of his plant growth. This control of the pattern of the development of the plant, this adjustment of the balance between shoot and root, between leaf and blossom, has, until recent years, been mediated by an appropriate and very largely empirical combination of what may be called dietary and surgery. Within the last twenty years, however, we have seen the advent of chemical growth control, which has brought vast new possibilities of the delicate adjustment of development pattern in the plant and many new techniques. These newcomers in the agricultural and horticultural fields have come to be known, in both scientific and popular literature alike, as plant growth regulating substances, or plant growth substances for short. Starting, as do so many great scientific advances, in a few unobtrusive and purely academic observations, the volume of research upon these substances has swelled to tremendous proportions. The study of plant physiology has never before in its history enjoyed such concentrated interest from agriculturalists and chemists alike.

As has no doubt been widely realized by both the expert agriculturalist and the amateur gardener, the use of these amazing new chemicals involves the considerable risk of doing more harm than good. The action of these materials, derived as they are from naturally occurring substances essential for the normal growth and development of the plant, have a deep-seated effect on fundamental growth processes. Since, in addition, they are usually active in considerable dilution,

extreme care must be exercised in their use if detrimental instead of beneficial effects are not to be obtained. A knowledge, therefore, of the basic physiological principles underlying the action of these new agricultural "tools" would seem to be eminently desirable for all who intend to make use of them.

Bearing this in mind therefore, this book on the plant growth substances has been written against a background of their physiological actions as far as they are known at present. Although an attempt has been made to cover every practical aspect of growth substance application as comprehensively as possible, yet the book is in no way a practical manual, since only the briefest of references will be made to ultimate details of technique, rates of application, etc. Now that there are available for use so many proprietary preparations, different naturally in their detailed and very largely unpublished formulations, such information is better acquired from the instruction sheets issued by the manufacturers or the various agricultural advisory bodies whose prime concern is the working out of such details. The slightly differing actions of such preparations, and the fact that their constitutions and availabilities will change from time to time, makes the compilation of general instructions unwise in a book of this sort.

The book, however, is not devoted entirely to those aspects of plant growth substance activity that have been of proven practical value. Phenomena which show distinct promise of future application in plant cultivation have also been discussed, and many subjects, *e.g.* sexual hormones in the lower plants (Chapter 12), natural plant growth inhibitors (Chapter 14), etc., which have at present only a theoretical interest, have been described in fair detail. In this way the writer has tried to present to the non-botanist the broadest possible picture of growth control in plants by the action of these organic growth-controlling chemicals. It is felt that, in these days, when scientific information is accumulating at such a terrifying rate and the scientist himself is being driven more and more into isolation in his narrow specialist valley, every attempt should be made to preserve a view of the broadest possible horizon.

Since the book is planned to cater for all classes of reader, for the non-scientific layman who spends his week-ends in his

garden as well as the chemist and plant physiologist who are more directly concerned with the production and use of these chemicals, the treatment will be as simple as possible consistent with accuracy and adequate explanation of the phenomena. The author therefore apologizes in advance to any of those exacting specialists to whom such treatment might seem unnecessarily elementary. It is, however, essentially concerned with chemicals, and so the intrusion of what, to the non-chemist, must be "cabbalistic" formulæ and unpronounceable names is unavoidable. Even the most conservative of gardeners must realize, however, that to move with the times in agriculture means ultimate and inevitable contact with organic chemicals, and he must not therefore be too abashed at the names he will meet in this book. Fortunately for him, shortened symbols have been adopted for those compounds in frequent use (*e.g.* IAA for β-indolylacetic acid and 2, 4-D for 2, 4-dichlorophenoxyacetic acid), and they should soon become as familiar as any of the proprietary names so common nowadays. Although the writer likes to think himself somewhat of a purist in these matters, he has adopted, for a number of reasons, this convention of shortened symbols, and a key to them is included in the glossary at the end of the book. After all, the medical man does not need to know the chemical constitution of his drug before he can prescribe it; all he needs is a knowledge of its name and its mode of action. That, then, is very largely the basic principle on which this book is written.

There has, however, been included a separate chapter on the chemistry of the most important group of growth substances, the auxins, in which the relationship between chemical structure and physiological action is dealt with in detail. This is intended solely for the chemist who is not familiar with this particular group of organic compounds and is naturally to be avoided by the non-chemists, together with the few brief chemical sections of the chapters dealing with other aspects of growth substances. Failure to read these portions should in no way detract from a proper understanding of the rest of the book.

I am very happy here to express my very sincere thanks to a number of fellow-scientists who have given generously of their

time to read the manuscript and offered much valuable and constructive criticism of the chapters dealing with those aspects of growth substance phenomena in which they are expert. Friends who have helped me in this way are Professor R. L. Wain of Wye College, University of London (Chapter 3), Dr. E. S. J. Hatcher of East Malling Research Station (Chapter 5), Dr. L. C. Luckwill of Long Ashton Research Station (Chapter 7), Drs. M. C. Vyvyan and H. W. B. Barlow of East Malling Research Station (Chapter 10), Professor F. G. Gregory, F.R.S., of Imperial College, London University (Chapter 12), and Dr. P. W. Brian of Imperial Chemical Industries (Chapters 14 and 15). My thanks are also due to Dr. B. C. Sharman of Bedford College, University of London, for his candid comments on the presentation of the two introductory chapters, always the most difficult part of a book to write.

I also wish to acknowledge with grateful thanks the generosity of those workers in the field of plant growth substances, and the institutions in which they are employed, for the many clear photographic records of experimental observations which they have made available to me and which constitute a considerable proportion of the illustrations in this book. They include Dr. E. S. J. Hatcher and East Malling Research Station, Professor R. L. Wain, Dr. L. C. Luckwill and Long Ashton Research Station, Dr. P. F. Wareing, Plant Protection Ltd., Professor G. E. Blackman, Dr. O. N. Purvis, Dr. W. W. Schwabe, Professor W. T. Williams, Drs. H. E. Street and W. C. Boll, and Dr. P. W. Brian.

Lastly, but by no means least, I must record my deep gratitude to my wife, who has not stinted her active help and criticism during the whole of the preparation of the book and who has thereby contributed so much to its final form.

L. J. AUDUS.

STANMORE, MIDDLESEX.
*April* 1952.

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CHAPTER I

# THE NATURE OF PLANT GROWTH

THE vast majority of living organisms are built up of complex aggregates of small microscopic units of structure known as cells, just as a house is built of bricks. In any one organism the form and detailed constitution of these units will vary from place to place in the body of which they form a part, but all living cells have certain properties and attributes which are fundamental, irrespective of their variations in form or position. In the first place, each cell is mainly composed of a viscous and extremely heterogeneous fluid known as protoplasm, which is the living stuff of the organism. It is in this protoplasm that occur unimaginably complex sequences of chemical and physical events which integrate into what we know as life. The most important of these processes are those by which the protoplasm increases itself. It is this capacity to build itself up from simple food materials that characterizes living from dead protoplasm; it is this which is the basis of all growth.

Thus, in the cell which is actively growing in the presence of a plentiful food supply the protoplasm is rapidly increasing in quantity by these building-up processes and is thus accompanied by a steady increase in the size of the cell. Such a steady increase of the cell volume does not continue indefinitely during growth. Eventually a time comes when the cell reaches a maximum size and then splits into two daughter cells, each half the size of the parent. This process of cell multiplication can best be illustrated by taking an example from the simplest of known organisms, the bacteria. In the bacteria a complete organism consists of one simple microscopic cell with very little visible heterogeneity of structure, or differentiation as biologists call it. In the presence of a plentiful food supply, the bacterial cell will increase in size at a steady rate until a certain critical level is reached; it then splits into two offspring of equal

size which themselves continue to enlarge at the same rate as their parent did. After a definite interval of time, these, too, will have reached the critical size and will divide to form four granddaughters. After further successive and equal time-intervals, 8, 16, 32, etc. cells would be formed. Although at the start the food supply may be plentiful, yet the progressive doubling of the size of this bacterial population cannot go on indefinitely, since the dwindling supply of food material must eventually retard the building-up processes of the cell protoplasm and slow down all growth and the accompanying cell multiplication. When this food is exhausted, growth will cease.

The same fundamental principles underlie the growth of the more highly organized multicellular plant with its wide variety of different types of cell involving an immense range of function. Although, as we shall see later, only certain specific cells of each complex organism retain the power to grow after reaching a certain stage of development, yet those cells which are capable of growth do so first by building up their protoplasm and other associated structural materials, and then, at a certain cell size, by splitting into two daughter cells, which then repeat the process. In contrast, however, to the growth of the simple bacterial cell, all such daughter cells in the organized plant do not continue indefinitely to grow and multiply in this way. The majority of daughter cells thus produced soon develop considerable changes in internal structure and organization and lose their power to grow and divide. These "differentiated" cells then take on other functions consistent with their modified structure and the positions in the plant in which they find themselves.

It would be as well at this point to turn to a consideration of the detailed structure of an actively growing cell of a highly organized green plant, since the reactions of these cells to growth substances are to be the primary concern of this book. A microscopic examination of such a cell shows the protoplasm to be optically heterogeneous, containing a multitude of granules of various sizes and densities. The largest, densest, and most important of these is the nucleus, a spherical or oval structure characterized by its affinity for certain dyes. This

nucleus, which is the most complex of any known cell structure, is the fundamental unit controlling the activities of the cell. Inside it are contained the highly complex systems of protoplasmic "jigs" on which new units of protoplasm are formed, each "jig" producing, as the cell grows, a continuous succession of products peculiar to it. These "jigs" are thus the factors which govern the form and structure of the organism by maintaining a properly balanced production of the varied protoplasmic units which make up its living stuff. If this proper balance is to be maintained, then the constitution of all nuclei in any one species of organism must be constant. In the multiplication of cells it is therefore of vital importance, for continued normal development, that this constant nuclear constitution should be maintained. During cell division the nucleus of a parent cell divides into two exactly equal parts, one going to each of the two daughter cells. A special and complicated process, into which we need not go, is necessary to ensure the exact duplication of all these factors in the two daughter nuclei.

The plant cell differs from the animal cell in that it is encased in a solid box, called the cell-wall; this wall is composed mostly of a substance called cellulose (a compound of the elements carbon, hydrogen and oxygen) which we meet in almost pure form in cotton-wool. In the very young and embryonic plant cell which is actively growing, this cell-wall most probably contains protoplasm and is therefore living. Later on, when the cell matures, this living protoplasmic content disappears, except perhaps from an extremely thin layer in contact with the protoplasm lining its inside surface.

We are now in a position to consider the various stages in the growth cycle of a cell of a green plant. Firstly, there is a stage of intense synthesis of protoplasm culminating at a certain size-limit in the division of the nucleus into the two identical daughter nuclei. The division of the cell itself is completed by the laying down by the cytoplasm * of a new cellulose wall between the two nuclei, thus cutting the cytoplasm into two portions and forming two complete daughter cells. As in the

* The protoplasm of the cell is classified into two major parts, the nucleoplasm making up the nucleus and the rest called the cytoplasm.

case of bacteria, this cell multiplication in all embryonic plant parts may continue for some time, these parts consequently increasing in size as cell numbers increase. Sooner or later, however, some daughter cells lose the power to divide and enter instead into the second stage of the growth process. In this phase, as a result of subtle and as yet unknown biochemical changes, there is a rapid and considerable increase in cell size, accompanied by the appearance in the cytoplasm of large vesicles filled with a dilute watery solution of various substances. This is the cell-sap. When this second phase is complete and the cell has reached its maximum size, which is usually many times that of the original embryonic cell from which it came, the appearance of the cell has completely altered. The watery vesicles have merged to form one large central vesicle or vacuole, the cytoplasm now being confined to a thin layer in contact with the cell-wall and containing the nucleus embedded in it. This phase of the growth of the cell is known as "extension growth" and accounts for most of the volume increase in plants. Following this comes a third phase in which the structure of the cell becomes modified in relation to the function it will ultimately perform, these modifications usually taking place with little change in cell, and therefore tissue, volume. For example, cells which are to function in maintaining the strength and rigidity of plant organs develop very considerable thickening of the cell-wall, which becomes at the same time chemically modified and thereby toughened by impregnation with a substance called *lignin.* These particular cells die soon after this modification is complete, and the protoplasm disintegrates leaving only the dead shell of the cell-wall. Other cells become modified for special vital functions and thus remain alive and active throughout the life of the organ containing them. The cells of the green spongy tissue in the interior of a leaf come into this category. The small green bodies (chloroplasts) which develop in these cells are responsible for the manufacture of food which takes place therein in the light. The line of demarcation between these two latter growth-phases is not always clear-cut, and in some tissues cell differentiation may start before cell extension is completed. Successive stages in the growth and development

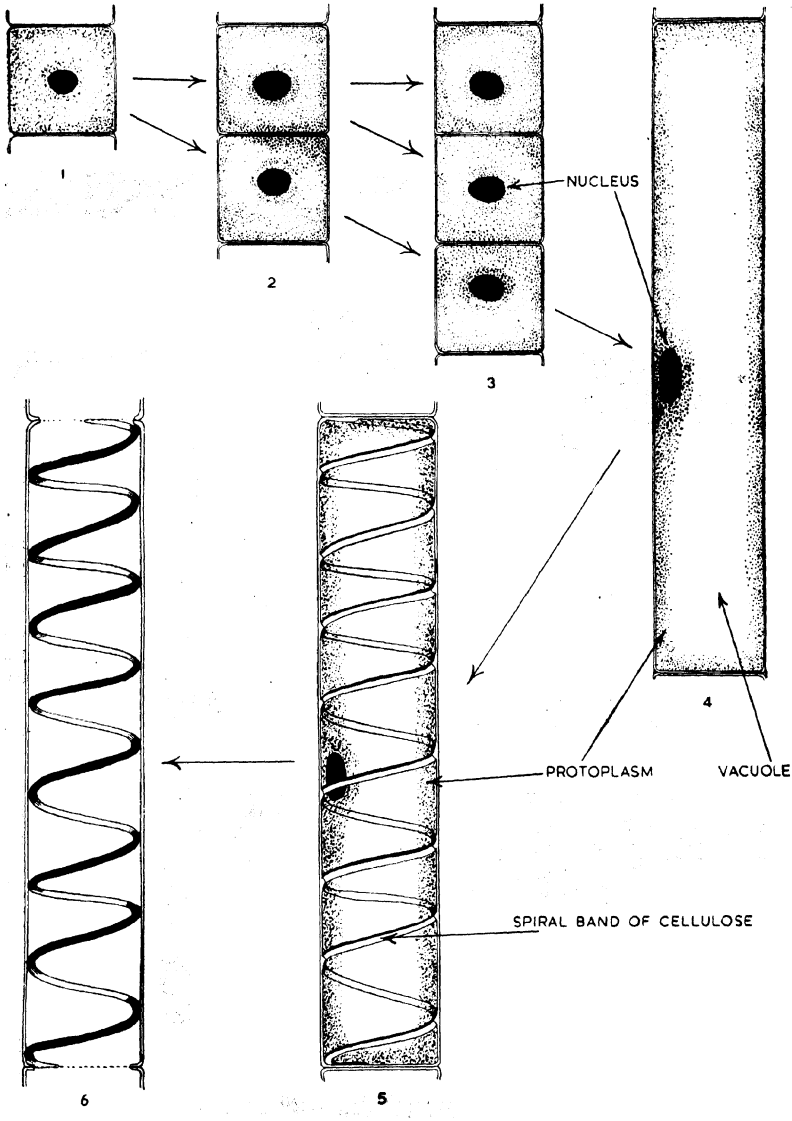



FIG. 1.—Semi-diagrammatic representation of the successive stages in the growth and development of a water-conducting cell of a flowering plant. The arrows mark the progress of the cell through these stages. 1–3 shows the first stage of growth, which is predominantly by the manufacture of living protoplasm and is characterized by active cell multiplication. 4 illustrates the second stage (stretching growth), marked by a great elongation of the cell and the appearance of a large central water-containing vesicle, the vacuole. In the third stage (5) a special spiral strengthening band of cellulose is deposited on the interior of the cell wall and shortly afterwards the cell dies, the end walls break down and the living protoplasm disappears (6). In this last state the cell conducts water. (All cells × 500.)

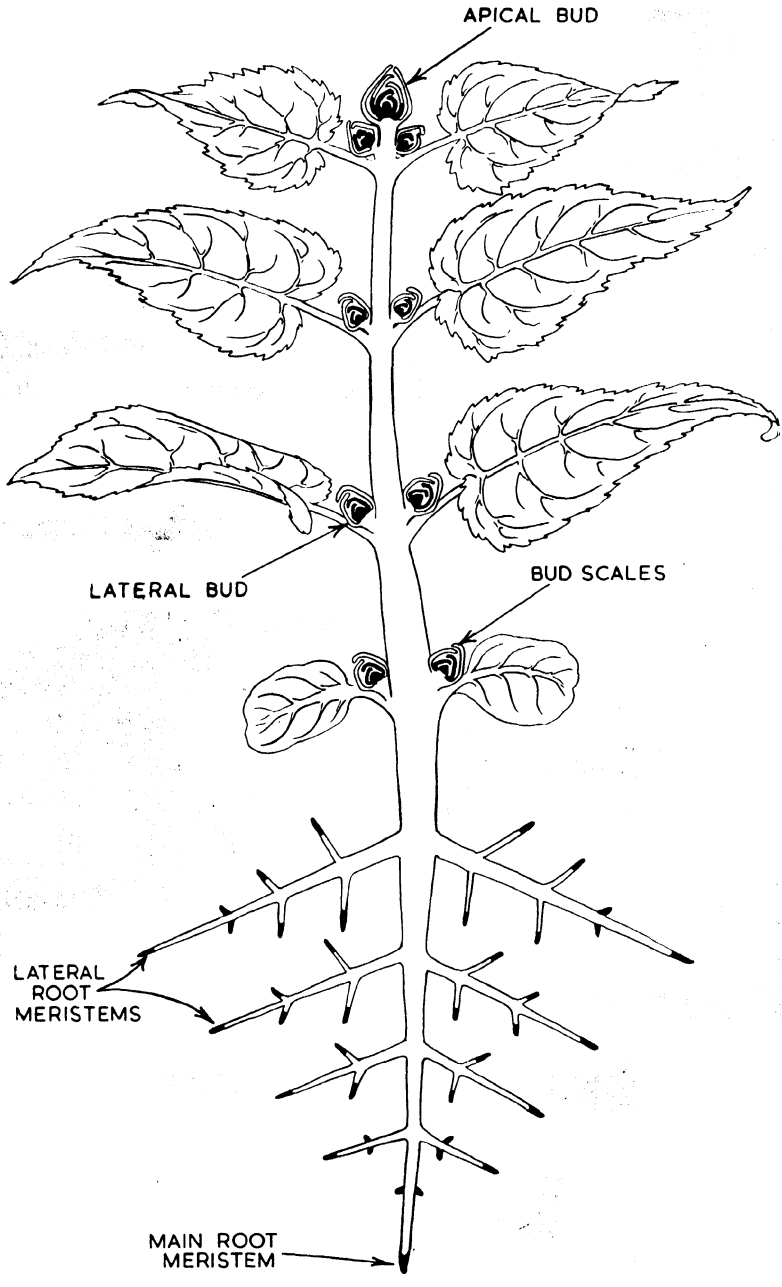



FIG. 2.—Diagram showing the distribution of meristems in a broad-leaved flowering plant. The meristems are shown in black.

of a water-conducting cell of a green flowering plant are illustrated in Fig. 1.

In the very young embryonic plant emerging during germination from the seed-coat, nearly all of its constituent cells are in the first stage of growth and therefore actively dividing. Very soon, however, as distinct organs begin to take shape, the necessary cell modifications (differentiation) follow and, as the plant grows, there is obviously a progressive loss by maturing tissues of the power to divide. Certain parts of the plant, however, retain this power indefinitely and maintain, during active plant growth, a steady production of daughter cells which develop and differentiate into the constituent units of the various plant organs as they form and mature. These regions of permanently embryonic cells, that are either active or potential dividers, depending on conditions, are known as *meristems* and are to be found in certain localized regions in mature plants; thus they are characteristic of stem and root-tips. It is in the region just behind the tips that the most active growth is visible, due to the extension growth of the products of these meristems. They are also found as a thin delicate layer between bark and wood of many plants, and this meristematic layer, called the *cambium*, is responsible for the growth in girth of these plants. Buds are small islands of meristematic tissue left behind in mature tissue as the plant grows and are protected by mature non-meristematic structures, the bud scales. These buds subsequently grow into new branches and when they do their meristems become the apical meristems of the new branch, leaving behind in their turn in regular succession new meristematic islands, the lateral buds of the branch. A diagram illustrating the distribution of these meristem "islands" in a broad-leaved plant is shown in Fig. 2. It is very largely the behaviour of such buds which determines the shapes (or morphology) of plants. It is by exerting an influence on these meristems and those of the stem apex that we can effect the most fundamental control of plant growth. We shall see later how the new growth substances enable us to do this. These do not by any means exhaust the types of meristem found in plants, but they are undoubtedly the most important and all that need be mentioned at the moment.

## THE FACTORS CONTROLLING PLANT GROWTH

*Introduction.*—Since growth is such a complex affair, it is not surprising to find that the factors which contribute to its control are no less complex. We can distinguish three groups of such factors: the first and most obvious of these are broadly categorized as "food factors". Under such a heading can be included all those influences which contribute directly to the *accumulation* of "plant capital", *e.g.* the supply of the raw materials for the manufacture of protoplasm and the cell-wall: these are most conveniently designated *nutritional* factors. The second group are those concerned, not with the gross augmentation of that capital, but with its *distribution* between the various plant organs. Such factors, as we shall see later, show a very close resemblance, in their general mode of action, to the animal hormones, those chemical messengers which are secreted into the blood-stream by various internal glands and which control the nature and intensity of growth in the several organs of the body; these I shall call *hormonal* factors. Thirdly, there are those influences which spring from the plant's own constitution. These are the hereditary factors which have come down to it from its parents and which the biologist calls *genetic.* It should not be necessary to point out that there can be no sharp line of demarcation between these three categories; there is a very considerable overlapping and dovetailing. Thus it is quite possible for any one individual factor, *e.g.* light intensity, to have deep-seated influences on both nutritional and hormonal control of growth. Again, the plant's genetic constitution will determine the nature and extent of its behaviour in relation to both nutritional and hormonal factors.

*Nutritional Aspects.*—Nutritional factors in growth are those which contribute directly to the manufacture of the various organic materials of which the plant body is composed. These complex organic compounds are derived ultimately by an intricate series of chemical events from very simple inorganic materials such as the gas carbon dioxide absorbed from the atmosphere, water, simple salts containing the elements nitrogen phosphorus, sulphur, etc. All these elaborate organic compounds are characterized by possessing in their molecules a

greater store of chemical energy than the simple substances from which they are built up. That this is true is quite apparent from the fact that the breaking up of these organic compounds into their constituent units, *e.g.* in the burning of sugar to form carbon dioxide and water, releases that extra energy in the form of heat. So, in order to make its own body matter, the organism must obtain a supply of these simple components as raw material and also sufficient energy, either chemical or in some other form, to allow the synthesis to take place: indeed this energy is the driving force of the manufacturing processes. All animals and such plants as moulds and toadstools obtain both raw materials and chemical energy from other complex organic substances, which they must first break up into smaller units. During this splitting-up process chemical energy is made available as well as simpler molecular units for the new synthesis. But large amounts of the energy are unavoidably lost as heat, together with many simple products of the breakdown. Although these simple molecular units are always available for subsequent use as building bricks in future growth, since they are retained in the environment of the organism, yet the energy once lost as heat can never be recaptured. It is obvious, therefore, that for life to be maintained on our planet there must be available an independent source of energy for these syntheses: this energy is supplied as light from the sun.

The conversion of this light energy into chemical energy in the living world can be carried out only by the green colouring matter of plants, chlorophyll. The chemical energy generated in the green cell in sunlight is used in the manufacture, from the carbon dioxide and water absorbed from its surroundings, of simple molecules of high chemical energy content. These form the hub from which radiates all the spokes of the great wheel of plant growth syntheses.* There is no need to stress the fact that the gross accumulation of plant capital as dry matter in green plants will be very largely determined by the speed and efficiency of this sunlight-using process, known to the scientist as photosynthesis. It may be controlled by many factors of which the duration and intensity of the light,

* An excellent sketch, for the non-specialist, of the present state of our knowledge of the chemistry of this vital process has been written by Fogg (1951).

the concentration and availability of carbon dioxide, and temperature will be the most important.

However, there are many elements other than carbon, oxygen and hydrogen which go to make up the plant body, which consists of two main components, the living protoplasm and the non-living cell-wall. The latter, consisting almost entirely of complex compounds of the elements carbon, hydrogen and oxygen can be manufactured completely from the raw materials carbon dioxide and water. Other compounds of the same elements occur in cells and act as food reserves, *e.g.* starch, oils, etc. Protoplasm, formed predominantly of complicated substances known as proteins, if we disregard the high water content, contains the additional element nitrogen in high proportion and the elements phosphorus and sulphur in smaller, but still appreciable, amounts. For synthesis of proteins to take place in the green plant, an adequate supply of raw materials in the form of simple salts of these elements must be available in dilute solution in the soil water surrounding the roots. In addition to these elements entering directly into the constitution of the protoplasm and therefore exercising a direct effect on the rate of protoplasm synthesis, certain others must be available in considerable amounts if plant growth is to be normal. Their presence in the right proportions in the cell are in some way, at present not fully understood, essential for the maintenance of vital activities. Finally, there is a long list of other elements which, though essential, are necessary only in very small amounts, and have therefore been termed micronutrients. The more important of these are iron, manganese, magnesium, copper, zinc, boron, silicon and chlorine. Magnesium is an essential constituent of the green molecule chlorophyll. Iron and copper enter into the make-up of some special protoplasmic units which biologists call enzymes and whose function we shall be describing later. Lack or low availability of these latter elements will cause various serious deficiency diseases characterized by marked derangements of the growth and developmental processes, with correlated distortions and abnormalities of the form and structure of the plant. Botanists are still trying to discover the exact rôle of many of these elements. The reader interested in the subject should consult

the recent reviews by Stiles (1945, 1946). It is possible that the supply of some of these micro-nutrients may be exerting an influence on growth by affecting the production of hormones, which we are considering in the next section. This is suspected to be the function of zinc in the plant (see p. 54).

*Hormonal Aspects.*—So far we have considered in outline only that aspect of the control of plant growth which determines primarily the rate of increase of "plant capital". The second aspect, which we have called hormonal, must now be similarly outlined.

In the very simple plants such as yeasts and bacteria there is very little differentiation of structure. As far as one can tell, growth is a simple process of synthesis of cell substance, and multiplication a necessary outcome of reaching a critical size. When, however, we come to consider multicellular organisms, with distinct organs, performing their own special functions (*e.g.* roots, stems, leaves, flowers, etc., in the higher green plant), then the need for control in the development of these different organs becomes obvious if a well-balanced individual is to result.

Thus, the development, both in space and time, of the several parts of organisms, following as it does a regular and, within the limits of variation of the species, an unchanging pattern, demands the existence of some precise mechanism for the co-ordination of the relative growth intensities of these organs. In other words, we must have a system to deal with the *distribution* of "plant capital". An organization of the appropriate type has long been known in the higher animals: this is the endocrine system. In the animal body, in certain organs known as the endocrine glands, special organic chemical substances are produced. These substances, which are called *hormones*, are released into the blood stream in small quantities and pass to various parts of the animal body where they control the activity, and hence growth and development of the organ affected. These hormones are therefore "chemical messengers", enabling a central co-ordinating gland to exert a control on other organs of the body. This "action at a distance" is the essential feature of hormone action. In the normal individual a delicate balance is maintained in the secretion of these several

hormones by their parent glands. Any disturbance of this equilibrium, resulting from over- or under-activity of one or more of these glands, gives rise to unbalanced growth and malformations. Giants and dwarfs are examples of what may result from such disturbances.

In the green land plant we do not find such a rigidly constant form as in the higher animal. Although, for example, the flower of a primrose or a tiger lily has always the same structure, yet the number of flowers produced on any one individual plant may show wide variations. Even so, the plant should also have some similar co-ordinating system for maintaining the proper balance of growth rates between the several organs. Actually, the existence of such a system has been known since the early days of plant physiological study in the late seventeenth century, and the presence in some plants of a plant hormone was suggested as early as 1907. It was not until 1930, however, that a definite chemical substance was isolated and identified. The isolation of this hormone, and more particularly its synthesis in relatively large quantities in the laboratory, gave such a fillip to research on the subject, that within the space of a few years a number of far-reaching discoveries had been made as to its functions in the plant. It is now known that this hormone, and other chemical substances of closely related constitution and properties, will exert far-reaching effects on both cell-extension growth and cell division, the precise action depending on the concentration of the substances present and the sensitivity of the organ concerned. Their main rôle, therefore, is the control of the *growth rate* of the various plant organs. Even more attention has been given to the practical implications of these chemical regulators. They have been given a variety of names which will be discussed in greater detail later. For the moment we shall refer to them as *plant growth hormones.*

The existence of other types of plant hormone has also been postulated; for example, a "flowering hormone", the particular rôle of which is to control flower and fruit production. Although this has not yet been isolated and identified chemically, the phenomena associated with it are of sufficient interest to warrant discussion later in the book.

In the last year or so another group of naturally occurring chemical compounds has come under the scrutiny of the biologist as playing a possible part in growth regulation. They can be called the growth inhibitors since their action on growth, in contrast to that of the hormones, is never a stimulation but always an inhibition. As we shall see later, these may to some extent co-operate with the growth hormones in growth control. This subject is still in its infancy but is already showing promise of application to agriculture.

Before we leave the subject of hormone control we must return for a moment to consider another aspect of nutrition in animals and in many of the colourless plants (moulds, etc.) which has not yet been mentioned. In these organisms, in addition to the supply of raw materials for growth and the chemical energy necessary to bring it about, the food must also contain small quantities of certain complex organic compounds which are called *vitamins*. With insufficient supplies of these vitamins growth will be seriously deranged and death will ultimately result. Diseases such as beriberi and pellagra, which are so rife among the under-nourished nations of the Eastern world, are due to deficiencies of particular vitamins in their diet. This requirement comes about because the organisms concerned lack the ability to make these essential growth factors for themselves. For example, many moulds will grow only when a supply of certain vitamins (*e.g.* thiamin = vitamin $B_1$) is supplied to them. Many organisms which cause plant and animal diseases grow in their hosts' tissues only because of the presence there of such essential growth factors. Most of the known vitamins are synthesized in green plants, which are, as far as we can see, the primary source of these compounds in any quantity. This is yet another way in which the animal is ultimately dependent on the green plant for its existence. In spite of this the rôle of vitamins in plants remained a mystery until recent years, when it has become apparent that their function is essentially the same as in the animals. Thus, until the discovery and isolation of the vitamins in pure form, no one had succeeded in growing *isolated* plant parts by supplying them with simple raw materials for growth. Then it was discovered that isolated roots of plants could be grown

indefinitely in the dark in solutions containing sugar and inorganic salts of the necessary elements, provided traces of certain vitamins (notably thiamin) were added. It was therefore clear that these vitamins were as essential for root growth as they were for animal growth and are normally built up in the green parts of the plant, from whence an adequate supply for growth is obtained by the attached root. This is obviously "action at a distance" and should therefore place these vitamins in the category of plant hormones. Nevertheless, there seems little doubt that the vitamins perform the same vital biochemical functions in plant cells as they do in animal cells. A real problem of nomenclature arises, of course, when we consider what we are to call the same vitamin when it is functioning in the same cell in which it has been synthesized. We shall return to this later.

*Genetic Aspects.*—The effect of hereditary make-up on the development of size and form of living organisms is so well known as to need very little comment. The hereditary factors are carried in the cell nucleus, which is therefore in ultimate control of character expression in growth and development. The exact mechanism whereby the nucleus exerts this fine control is still wrapped in almost complete mystery. There seems little doubt that it may be exerted by the intermediary action of nutritional and hormonal factors. As one example we may quote the observation (Went and Thimann, 1937) that in a number of varieties of the genus *Epilobium* (willow-herb) and their hybrids, size is closely correlated with, and presumably controlled by, specific hormone content. Here the hormone is apparently acting as an intermediary agent of the nucleus in the control of plant size. Work along these lines is still in its infancy.

## THE PLANT HORMONES

Speculations on the nature of the factors underlying the development of plants have occupied the thoughts of many of the early natural historians, and even as far back as the end of the seventeenth century there were emerging tentative suggestions of the existence of a moving "sap", responsible for

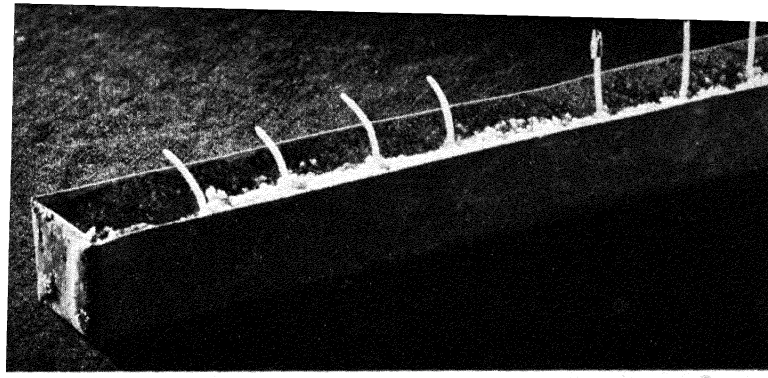
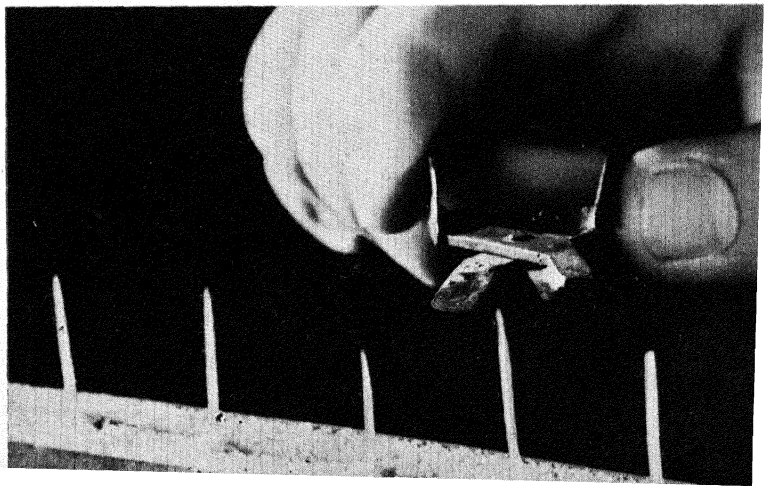


FIG. 3.—Experiments with oat seedlings showing the effect of covering the tips of the coleoptiles with black paper caps on the subsequent reaction to lighting from one side. The four uncapped seedlings on the left show normal bending towards the light, while the four on the right have been capped with black paper and have remained upright.

*Photographs by permission of Dr. E. S. J. Hatcher and East Malling Research Station.*

FIG. 4.—First decapitation of a batch of oat (*Avena*) seedlings in preparation for auxin assay (Went's *Avena* test). The extreme tip of the coleoptile is removed by means of a special double-bladed cutter. This removes the main internal source of auxin.



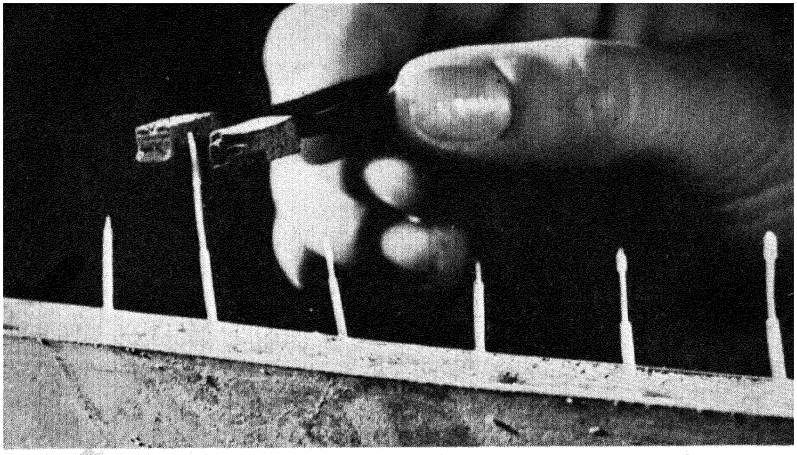
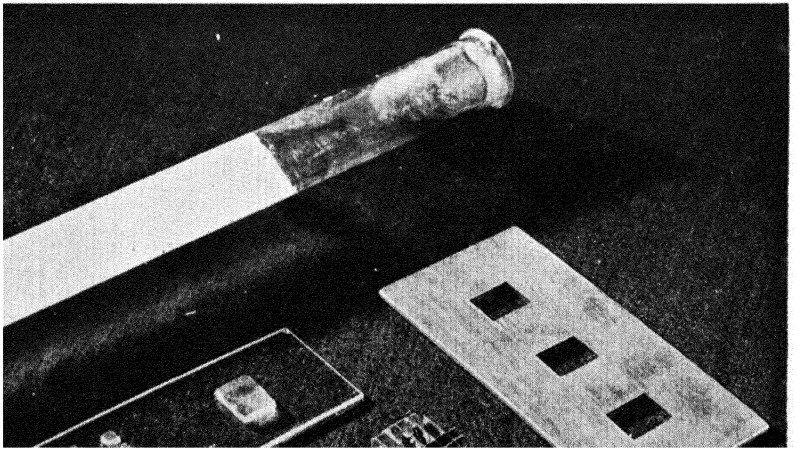


FIG. 5.—Second decapitation of the oat seedlings. A nick is made in the side of the coleoptile by means of a sharp blade just below the cut surface of the stump. Cork-tipped forceps are now used to remove the partially severed segment and at the same time the first leaf inside the coleoptile is pulled out to act as a support to the agar block (see Figs. 6 and 7).

*Photographs by permission of Dr. E. S. J. Hatcher and East Malling Research Station.*

FIG. 6.—Preparation of the agar blocks to act as carriers of auxin for the Went's *Avena* test. The agar, seen in the tube at the top, is melted and a small block cast by pouring it into the brass mould on the right. When set, this block (seen on the right-hand end of the glass slide) is cut into twelve uniform blocks, each of 0·01 ml. volume (seen on left-hand end of slide) by means of the small cutter below.



correlating the growth activities of the separate plant organs. It was not, however, until the latter half of the nineteenth century, when the modern science of plant physiology was really born, that the famous German botanist, Sachs, the father of that science, started a detailed study of the phenomena. As a result of his investigations he proposed a generalized theory involving "organ-forming substances", whose specific actions and localized distributions, which could be affected by external forces (*e.g.* gravity), were made to explain simply the facts of plant growth and organ development. Sachs's views found little favour with his contemporaries and it was the best part of half a century before the existence of his specific organ-forming substances was finally and convincingly proved. During this considerable interval of time, botanists were very concerned with the gross nutritional aspects of plant growth, *e.g.* the supply of its raw food materials, and, as a result, these aspects of physiology tended to dominate theories of growth correlations. Such ideas have lingered on well into the present century, for example, in the carbon-nitrogen ratio theory of flower induction (see Chapter 12). According to this theory, put forward in 1918 in America, the ratio of the level of the carbon to the nitrogen nutrition of the plant was supposed to control its reproductive activity, *i.e.* the prolixity or otherwise of its flower, and therefore fruit, production. As we shall see later, this has now been superseded by a hormonal concept of flowering control.

The original observations that led directly to the first isolation of a plant hormone were published over seventy years ago in a book called *The Power of Movement in Plants*, by the British naturalist Charles Darwin, so famous for his theories of organic evolution. The story of the way in which these ideas of Darwin set in train a series of purely academic enquiries which culminated in the development of techniques now revolutionizing agriculture, is another proof, if such is needed, of the vital importance of fundamental research. The story is interesting and well worth outlining.

All of us who have had any dealings with growing plants know that they are sensitive to external stimuli such as light and the force of gravity. The young parts of plants brought

indoors will bend over towards the light coming from a window, and the leaves will arrange themselves, so that their blades are perpendicular to the light falling on them. Similarly, young plants that have been laid flat by rain or wind will, if the stems have not been broken, eventually bend upwards at the growing tips and the stem grow vertically once more. These movements of the plant in response to external stimuli—unilateral light in the first instance, the force of gravity in the second—are called by the botanist *tropisms*, and are brought about by opposite sides of the organ or organs concerned growing at different rates in response to the stimuli. It was the elucidation of the mechanism of such responses that was Darwin's main concern. His experimental plant was the seedling of the ornamental canary grass (*Phalaris canariensis*). Such seedlings, in common with all members of the same family, produce on germination a tubular first leaf, which remains short in the light, but in the dark grows very much longer. This first leaf, through which a typical flattened leaf-blade eventually bursts, is called a *coleoptile* and is particularly sensitive to light stimuli. Darwin observed that when these organs were illuminated from one side they bent over towards the source of the illumination. The region in which the bending took place was quite a long way below the coleoptile tip. If, however, the extreme tip of the coleoptile were removed or covered by a small cap of light-proof material, such as tin-foil, no bending took place in the lower region on exposure to light. This and similar experiments led Darwin to postulate that the light was perceived by the tip. These phenomena are very clearly demonstrated in Fig. 3, which shows the results of such experiments on the coleoptiles of oat seedlings. Since the bending region was situated lower down the organ, some "influence" must have been transmitted from the stimulated tip to the reacting zone. Experiments on roots and other organs indicated strongly that transmission of similar influences might underlie reactions to gravitational pull. Research at the turn of the century was not marked by the chase after immediate practical results that taints so much of our present-day activities, but, over a period of thirty years following the publication of Darwin's book, an occasional report verified and amplified his

main findings. One of the most important was that the transmission of the "influence" down the coleoptile was not affected by a transverse cut between the tip and the reacting zone. Such evidence showed that the continuity of living protoplasm was not essential for the transmission of the "influence", and led the Danish botanist, Boysen-Jensen, who had chosen to work on the light reactions of the oat coleoptile, to suggest, just before the beginning of the First World War, that a purely chemical mechanism underlay this transfer of stimulus (Boysen-Jensen, 1911). This was proved beyond all doubt when it was shown that the "influence" would pass through non-living tissue such as gelatine, for if the tip were removed and fastened back on to the stump with such material, then the response of the coleoptile to unilateral light was unimpaired. The exact nature of this chemical influence remained obscure until it was shown that the tip of the coleoptile could influence the growth of the stump quite independently of any light stimulus. Thus in 1919 it was demonstrated (Páal, 1919) that if the coleoptile tip were removed and replaced asymmetrically on the stump, then the stump bent over in such a way that the longest side was under the tip. This demonstrated the existence in the tip of a chemical substance which passed into and down the side of the stump in contact with the tip, stimulating the extension growth of the zones below and giving rise to the curvatures noted. The reaction of the coleoptile to light was then presumably caused by an appropriate redistribution of this substance in the tip, greater amounts going into the darkened side and therefore making the coleoptile grow longer on that side. It was not, however, until 1926 that this chemical messenger was finally isolated by the now classical work of the Dutchman F. W. Went. It was this isolation and the technique that sprang from it that mark the beginning of the modern era of plant growth substances.

Went, knowing from the work of Páal that the substance would move out of the isolated coleoptile tip into the stump, hit upon the idea of allowing it to diffuse instead into a non-living material, thus isolating it from the living cells in which it acted. The material he chose was agar-agar, a carbohydrate prepared from sea-weed; this

dissolves in water to form an inert transparent jelly. Small rectangular blocks of this jelly were prepared under sterile conditions and on these blocks were placed a number of severed coleoptile tips with their cut surfaces in contact with the agar-agar. These were left to stand in a humid atmosphere to allow the chemical to diffuse outwards into the jelly. Its presence in the agar was demonstrated when the larger block was cut up into smaller cubes and one of these cubes placed unilaterally on a stump of a coleoptile kept in the dark. After a short lag, the coleoptile was found to bend away from the side on which the block had been placed, demonstrating conclusively the presence in the block of a growth-stimulating substance. All this, however, was nothing more than a combination and refinement of the techniques of other workers and final confirmation of their findings. Went's greatest and original contribution to these studies was when he made quantitative measurements of the curvatures produced by the small agar cubes containing the hormone. By allowing the hormone in one small block to diffuse into larger blocks, known dilutions could be obtained, and, by using small cubes from these latter blocks to produce curvatures, it was shown that, within the limits of experimental error, the curvature produced was directly proportional to the concentration of the hormone in the agar. The importance of these observations will be appreciated when it is realized that these hormones are active in extremely low concentrations, far, far below the sensitivity of any chemical tests. No known chemical test could possibly have revealed the presence of the hormone in the agar, and yet here was a reasonably accurate method of measuring—in arbitrary units, of course—the quantity of hormone coming from any tip. It was the development and refinement of this biological assay technique, now known among plant physiologists as the Went *Avena* test,* that were chiefly responsible for the final isolation of the hormone in a pure crystalline form and its subsequent chemical identification.

But these investigations into the hormones responsible for growth curvatures in plants were not the only activities of the early botanists in this field. The response of plants to wound-

* *Avena sativa* is the botanical name of oat.

ing was also a field for their speculations. When plants are wounded, the otherwise mature cells around the wound often react by reassuming activity and proliferating to form a bulky scar tissue well known to botanists and gardeners as *wound-callus*. As long ago as fifty years the famous German botanist, Weisner, had suggested that wounded cells might produce a chemical substance which could diffuse into neighbouring tissues and evoke this growth response. It was his compatriot Haberlandt, over ten years later, who demonstrated such a substance in crushed plant tissue. Even now, in spite of claims to have identified this hormone chemically, we cannot yet be certain that we have isolated it in pure crystalline form.

In the majority of phenomena, however, in which theoretical chemical substances have been suggested as controlling factors, *e.g.* the "flower-forming" and "root-forming" substances of Sachs, it was not until after the growth hormone of the *Avena* coleoptile ($\beta$-indolylacetic acid) (see Chapter 2) had been isolated that the opportunity arose to test these possibilities directly. We shall see as we progress through this book that such tests have shown that this single hormone can evoke very different growth responses in different organs and indeed in the same organ according to the concentrations employed, and it is becoming more and more likely as data accumulate that it may be the key substance in the control of most of the growth phenomena in the plant.

It is convenient at this point to turn to a consideration of nomenclature, since a wide range of terms have been used to characterize the plant growth regulating substances, and perusal of the very extensive literature will reveal considerable overlapping and ambiguities in terminology. One of the most recent definitions of a plant hormone is given by Thimann (Pincus and Thimann, 1948) as: "*An organic substance produced naturally in higher plants, controlling growth or other physiological functions at a site remote from its place of production and active in minute amounts.*" Thimann favours the term "phytohormone" (Gr. *phyton* = a plant) for these substances to distinguish them from the animal hormones. This definition will therefore include a number of compounds, other than those responsible for growth curvatures in organs

such as the *Avena* coleoptiles. It includes the hormones which induce flowering, wound-healing, etc., and also some vitamins, whose action as growth factors are quite well established but whose precise functions are not so clear cut. However, a distinction is usually made between these general phytohormones and those specifically concerned with the promotion of extension growth of cells, *e.g.* in the *Avena* coleoptile. Phytohormones in this category are given the names of "auxins". We could do worse than to quote Thimann's recent definition of an auxin (Pincus and Thimann, 1948): "*An organic substance which promotes growth* (i.e. *irreversible increase in volume*) *along the longitudinal axis when applied in low concentrations to shoots of plants freed as far as practicable from their own inherent growth-promoting substances. Auxins may, and generally do, have other properties, but this one is critical.*" These two definitions are widely accepted among plant physiologists to-day.

There are, however, difficulties which are arising as a result of our increasing knowledge of the subject. The most important concerns the names we should apply to the already vast and still rapidly expanding list of purely synthetic * compounds whose action, as far as we can see, are identical with those of the naturally occurring hormones. In the past a rigid distinction has been made between such "synthetic growth-regulating substances" and "natural phytohormones". For example, Nicol, in an earlier book on this subject, wrote: "Unless it can be proved that the 'synthetic' growth substances occur naturally in plants and regulate growth within them, it would to-day be as absurd to give the name 'hormone' to the exterior substances which affect plant growth as it would be to confuse vitamins and hormones in speaking of animal physiology." However, the two cases are not strictly comparable, as the boundary between vitamin and hormone in the plant is

* The real meaning of synthesis is "placing together" (Greek: *syn* = together, and *thesis* = a placing) and refers to a building up of a chemical compound, whether performed in the living organism or in the laboratory or factory. Popular usage of the adjective *synthetic* restricts the term to those compounds, made artificially, which are often substitutes for the naturally synthesized materials. Here the term synthetic is, of course, used in its restricted sense of "made artificially".

by no means as clear cut as it is in the animal. In the latter, hormones are produced in the organism itself and are then carried to the site of their action. Vitamins are manufactured by other organisms, mainly green plants, and must be taken by the animal in its food since it cannot manufacture them itself. In the green plant both hormones and vitamins are manufactured, and, since they are both regulators of vital processes, the distinction between them now becomes a matter of the distance between the centres of production and those of action or utilization. That this distinction is highly arbitrary has recently been pointed out by van Overbeek (1950), who asks what the limit of distance should be, that between organs, tissues, cells or even molecules? If molecules, then all naturally occurring organic regulators of plant-physiological processes could be called phytohormones, since the distance is always finite, however small it may be. A case more nearly approaching that of the animal is seen in some colourless plants such as moulds. Moulds all require for their growth a range of accessory growth substances (vitamins), but different species differ in their ability to build them from simple inorganic food materials. Thus the common mould *Aspergillus niger* will grow normally on a medium containing mineral salts and simple sources of carbon and nitrogen: it can synthesize all its own growth substances. Others, such as *Phycomyces Blakesleeanus*, require an external supply of a whole complex of such growth substances. One of these is thiamin (Vitamin $B_1$), which apparently functions in these organisms in precisely the same way as it does in the animal. If we adhere to the nomenclature of the animal physiologist, these obligate food factors are vitamins from the mould's point of view. What, therefore, must we call them when they are manufactured by the organism, whether it be fungus or higher green plant? Are they still vitamins or have they become hormones, or must we coin yet another name for them?

Obviously, the ideal system of nomenclature must eventually be based on their biochemical and physiological functions, irrespective of their mode and site of production and the processes involved in transporting them to the places where they act. Such an ideal nomenclature must therefore

embrace not only naturally occurring compounds, but also all artificially made substances having the same action in the cell. Indeed, a recent treatise (van Overbeek, 1950) has given a very broad definition of plant hormones as "organic compounds which regulate plant-physiological processes . . . regardless of whether these compounds are naturally occurring and/or synthetic, stimulating and/or inhibitory, local activators or substances which act at a distance from the place where they are formed". Similarly, the term auxin is made to include all synthetic compounds having the specific *auxin* action. There is no *a priori* reason why the term hormone should not be used in this very broad sense, since there is no suggestion in its derivation from the Greek word "hormaein" (to step up) of an action necessarily at a distance from the centre of production. A term which is coming into general use on the continent and which is intended to meet this difficulty of nomenclature is *ergon* (van Euler, 1946). An ergon can be defined as any organic compound acting, in low concentrations, as an activator or regulator of a physiological process. An ergon in the plant would then be virtually synonymous with van Overbeek's definition of plant hormone.

In this book, therefore, a definition of phytohormone will be adopted which is similar in its generality to that of van Overbeek but not quite so all-embracing. Thus we can define it as *an organic compound which, in extremely low concentrations, regulates the growth of the plant in one or more of its several aspects.* Thus we see that, on this definition, Phytohormone is synonymous with Plant Growth Regulating Substance, embracing therefore the whole scope of this book. We shall talk of "natural hormones" for all those growth regulators that are synthesized by plants and shall further divide them into "natural auxins", "natural flowering hormones", etc., depending on their observed or suspected actions in the plant. On the other hand, we shall give the term "synthetic hormone" to all those substances having, as far as we can tell, precisely the same action on the cell as the natural hormones, but which, so far, have not been shown to occur naturally in plants. Obviously, this is not entirely without ambiguity. β-indolyl-

acetic acid itself is now manufactured in relatively large quantities for experimental purposes, but is, of course, a "natural hormone". Eventually, when we know enough about the exact mode of action of these hormones and have identified the chemical systems in which they are working, we may be in a position to revise our nomenclature so that the above difficulties may be avoided.

The greater part of this book, however, will be concerned with those growth hormones that are active in Went's *Avena* test, *i.e.* those that have the physiological properties of auxins. We shall refer to these compounds as auxins (natural or synthetic), even though we may be discussing aspects of their action other than the stimulation of extension growth (see Thimann's definition, p. 18). Those other growth-regulating substances which have not the advantage of such definitive and easy categorization will be catalogued under the names by which they are best known. Thus the vitamins will be so called whatever their function in the plant, *e.g.* as accessory rooting hormones, general growth hormones, etc. The auxins have proved to be of the greatest practical importance in agriculture and horticulture and have received the attention of research workers in this field almost to the exclusion of other substances. The next ten chapters of the book are concerned with these hormones. The great success of these compounds naturally makes us aware of the possible potentialities of other plant hormones in practical growth regulation, but so far the field is new and almost unexplored. For the sake of completion and as an indication of future prospects, the last four chapters review in general terms all other aspects of the growth control of plants by natural hormones (*e.g.* flowering hormones, germination inhibitors, etc.).

CHAPTER 2

# THE NATURAL AUXINS

## INTRODUCTION

WE saw in the last chapter that curiosity on the part of Darwin concerning the bending of plants in response to unilateral light gave rise to a series of researches culminating, in 1928, in the isolation of a chemical substance which was active, in very small concentrations, in causing a great acceleration in the rate of extension growth of oat coleoptiles. It now seems likely that the young shoots of all higher plants are equally so affected. After this isolation it was soon shown that the action of light in causing curvatures of these organs was indeed carried out by this active substance. Light from one side induced an accumulation of the hormone on the darker side of the tip, and this excess, diffusing vertically down the coleoptile, made it grow more rapidly on the darker side and hence bend towards the light. Thus, after about fifty years, Darwin's academic questions were answered, but this was only the beginning of the subject of plant hormones as we know it to-day. The really great advances that led to their development for use in practical agriculture and horticulture began when they were identified chemically by the classic work of the chemist Kögl and his colleagues in Utrecht. Milestone as this is in the history of the subject, it would not have been possible but for the botanist Went, who provided, in the *Avena* test, a quantitative assay * on which their whole purification technique depended. Since such quantitative assay has played a fundamental part in both the isolation and identification of natural plant growth hormones (auxins) and, as we shall see later, in the preparation of the majority of similarly active synthetic compounds, it would be as well, before we go on to describe the work of Kögl, to deal in rather more detail with the various

* Assay is a term originally employed for the determination of the proportion of a metal in an ore or an alloy. In recent years it has been applied by biologists to the determination of specific activity of such biological products as hormones, vitamins, antibiotics (see Chapters 14 and 15), etc.

assay methods available. We should, however, first consider the three main functions that such assays have fulfilled in advancing our knowledge of the auxins. Firstly, they enabled chemists, in their efforts to obtain pure crystalline hormones in fair yield from natural sources, to test the activity of their various samples during the separate steps of purification. Since the methods used in these researches were purely empirical, an assay such as the *Avena* test was an indispensable tool in determining their efficiencies. Secondly, when the constitution and activity of the hormones became known, the assay became important as a method of determining the distribution and relative concentrations of hormones in various parts of the plant, since, as we have already noted, direct chemical methods are much too insensitive for the extremely small quantities involved. Thirdly, when it became apparent that a wide range of synthetic chemical compounds had properties similar to those of the natural growth hormones, it became a point of great practical importance to have an accurate method whereby their relative activities might be assessed. Assays of this type play a vital part in the manufacture and standardization of antibiotic drugs such as penicillin and streptomycin, which are the natural products of micro-organisms. The main assay methods which have been employed in growth hormone determinations will now be briefly considered. Only the principles of the methods will be given, the details being of interest only to workers actively engaged in their use.

## ASSAY METHODS

The test which has been most used by plant physiologists, and which still continues to be of the greatest importance, is Went's *Avena* test (Went, 1928). It has undergone slight modifications in detail from time to time but still remains in essentials the same as that originally described. Oat grains are dehusked and then soaked in water for two to three hours, after which time they are sown in a suitable moist medium, usually damp absorbent paper (the chemists "filter paper") in shallow dishes in the dark at a temperature of about 25° C. (77° F.). These environmental conditions, which are held

constant during the whole period of the test, have been found to give maximum sensitivity of the coleoptile to the applied hormone. Germination is allowed to continue for about thirty-six hours and then the seedlings are transferred to a special apparatus where they can grow with their roots in a dilute solution of mineral salts of carefully controlled constitution and their coleoptiles in a strictly vertical position. When the coleoptiles have attained a length of about one inch, the seedlings are ready for the test. Some dozen or so such seedlings constitute a batch to be used in one test. The extreme tip of the coleoptile is then cut off (see Fig. 4), thus removing the main source of its natural auxin and thereby stopping its growth. After a period of some hours the top few millimetres of the stump are similarly removed (see Fig. 5), since it has been found that this apical tissue may gradually acquire the power to produce auxin during the subsequent test, an undesirable state of affairs. The tip of the young foliage leaf is thus disclosed; this is then pulled with a pair of cork-tipped forceps so that it breaks at the bottom of the coleoptile tube where it is attached; it is then withdrawn so that it projects slightly above the cut end of the coleoptile and acts as a support for the agar block containing the auxin when it is finally applied (see Fig. 7). The principle behind this elaborate preparation is to produce a plant organ with a hormone content reduced to vanishingly small proportions so that the reaction to added hormone is clear cut and disturbances due to wide variations in normal hormone content are at a minimum. One refinement of Went's original method is to remove the "seed" from each seedling some hours before carrying out the test. This simple procedure removes the store of material (precursor) from which the growth hormone is made in the seedling, thereby further increasing the sensitivity of the coleoptile by preventing the further production of auxin in the tip of the coleoptile stump.

Since in these tests one is dealing with plants which are extremely sensitive to external influences, very special precautions have to be taken to ensure optimum response and minimum interference from environmental factors. In addition, a collection of plants, even of the same variety, unlike crystals

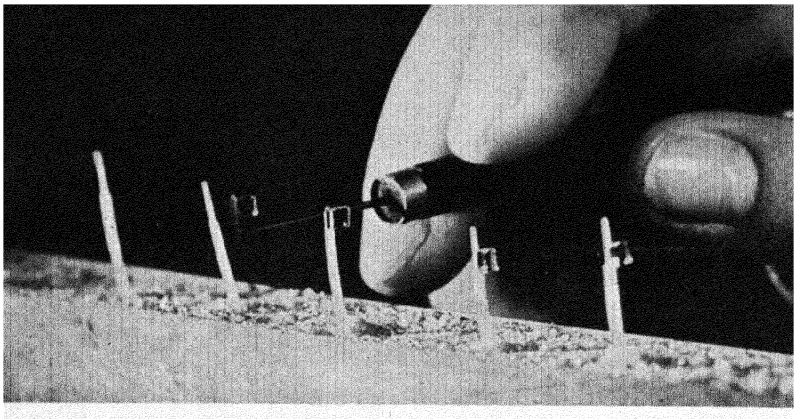
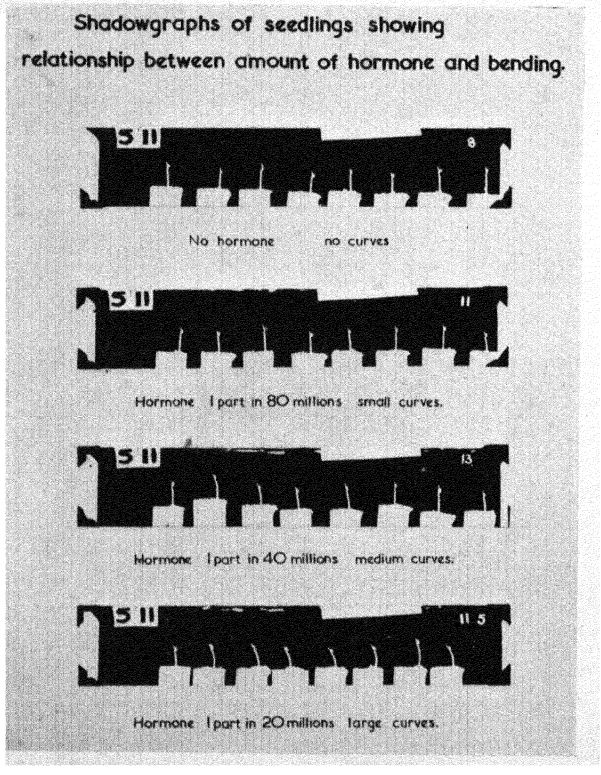


FIG. 7.—Application of the agar blocks containing auxin to the prepared coleoptile stumps.

*Photograph by permission of Dr. E. S. J. Hatcher and East Malling Research Station.*

FIG. 8.—Shadowgraph showing quantitative relationship between curvature of coleoptile and auxin dosage.



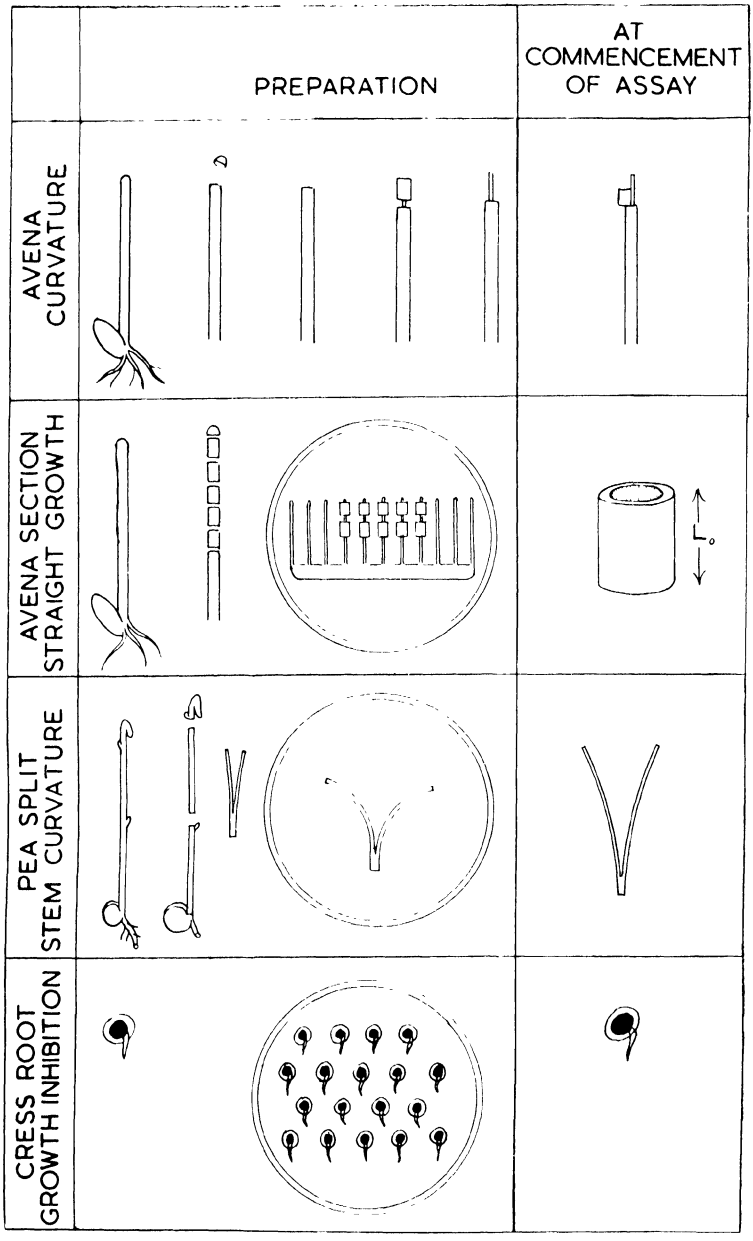
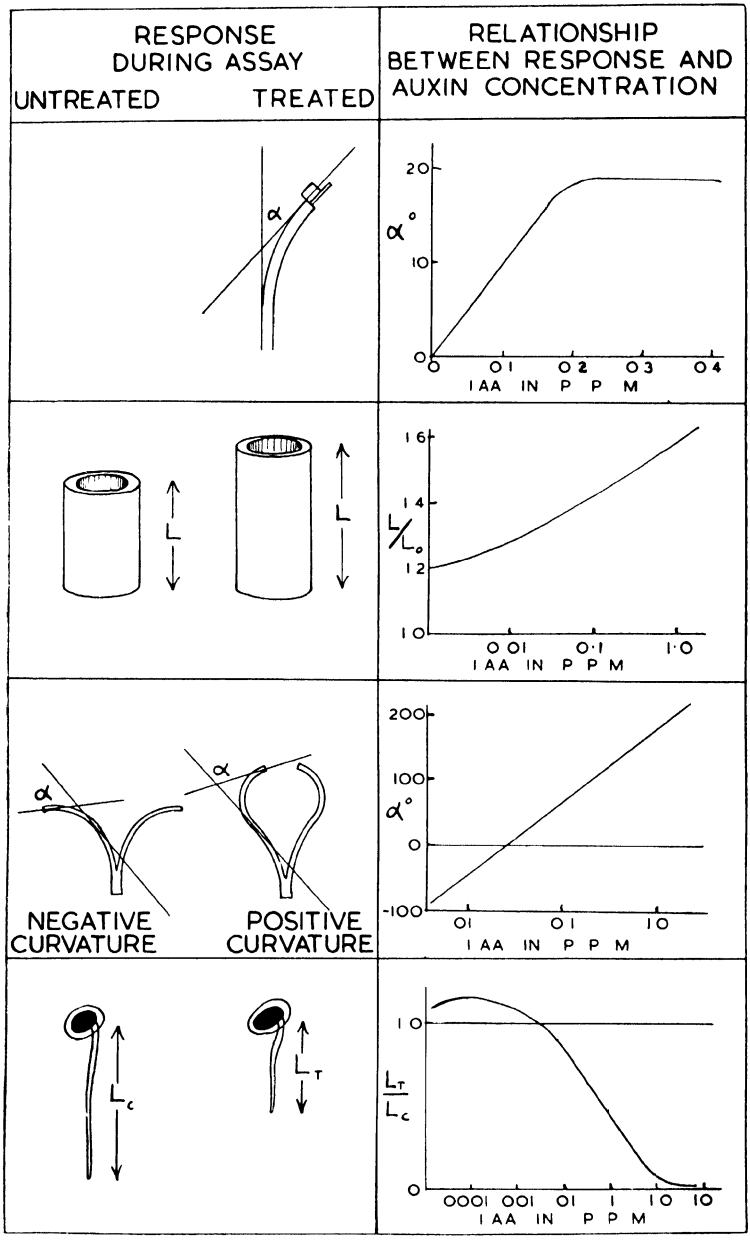



FIG. 9.—Diagram illustrating the salient features of the four main auxin assay techniques (Went's *Avena* curvature test, *Avena* coleoptile cylinder straight growth test, Went's split pea stem curvature test and Moewus' cress root growth test). In the first column are illustrated the major steps in the preparation of the material.




The second column shows the test material ready for the assay. In the third column are drawn the materials as they appear after the assay period together with the controls in water where relevant. The last column shows the quantitative relationships between response and the applied concentration of auxin.

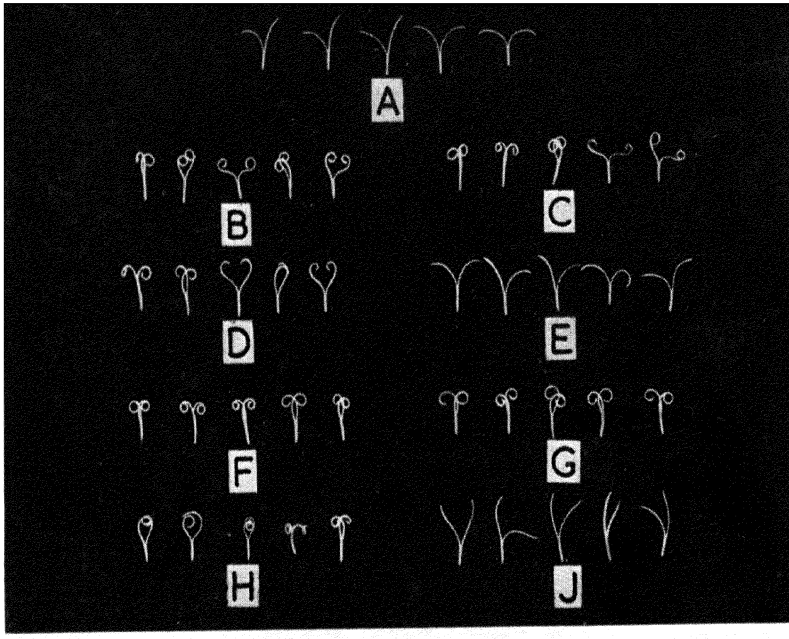



FIG. 10.—The Went's split pea stem curvature test in the comparison of auxin activities of synthetic chemicals. The substances tested here, all at the same concentrations, are:

B. 4-chlorophenoxyacetic acid (active).
C. α-(4-chlorophenoxy)propionic acid (active).
D. γ-(4-chlorophenoxy)-*n*-butyric acid (active).
E. α-(4-chlorophenoxy)-*iso*-butyric acid (inactive).
F. 2, 4-dichlorophenoxyacetic acid (active).
G. α-(2, 4-dichlorophenoxy)propionic acid (active).
H. γ-(2, 4-dichlorophenoxy)-*n*-butyric acid (active).
J. α-(2, 4-dichlorophenoxy)-*iso*-butyric acid (very slightly active).

Compared with A, which is the control in water.

*Photograph by permission of Professor R. L. Wain, Wye College, University of London.*

of a pure chemical substance, is not completely uniform, and this necessitates the use of special pure strains of selected plants to minimize this intrinsic variability and increase the accuracy of the assay.

In addition to preparing the oat seedling, the small agar blocks for carrying the auxin have also to be made; these are cut in cubes of 5–10 cubic millimetres volume from thin sheets of 3 per cent. agar (see Fig. 6). The hormone can be added to them either when the agar is in a molten condition before the gel is allowed to set, and therefore before the blocks are cut, or the blocks can be soaked in a solution of the hormone for an hour or so before the test: either technique is equally satisfactory. The blocks, which must be uniform size and therefore need a special cutter for their preparation (see Fig. 6), are then placed unilaterally one on each coleoptile and supported in the angle made by the cut surface and the slightly projecting first leaf (see Fig. 7). All these operations so far described must be carried out in weak red light to which the coleoptile does not respond. White light cannot be used since it reduces sensitivity and, more important still, can produce growth curvatures which completely invalidate the test (see Fig. 3). The plant then remains in darkness for one and a half to two hours, during which time a maximum curvature is obtained. One important test condition is that the humidity must remain constant at a value somewhat below 90 per cent.; if it is allowed to rise above this point, water may be exuded in drops at the cut surface and may even wash off the agar block. When the period for maximum response has elapsed, the curvatures of all the seedlings in the batch are recorded photographically by projecting their shadows on to a long strip of bromide paper (shadowgraph technique) (see Fig. 8). The response is then determined from the print either by measuring the total angle of curvature between tip and base by means of a protractor, or by estimating from the radius of curvature the difference in length between the two sides of the curved coleoptile.

The responses obtained from plants in any one batch receiving identical treatments will vary considerably from plant to plant, a feature which we have already noted is characteristic of

biological assay. Such variations of response arise mainly from slight differences in age and length of individual coleoptiles. Each assay consequently requires that a considerable number of seedlings be used so that by taking an average these intrinsic variations of response are very largely cancelled out. It is then found that the average response of a batch of test coleoptiles is directly proportional to the concentration of applied hormone up to a certain maximum. This maximum is equivalent to about one part of the naturally occurring hormone $\beta$-indolylacetic acid in five million parts of water (see later, Chapter 3) and the corresponding angle of curvature varies from 15° to 35°, depending on such unexpected factors as the time of day. This type of variation in response between batches of apparently identical seedlings is a further complication of the technique, and so far the causes remain unknown. It does mean, however, that for any one test or set of tests on any one day a calibration run has to be made with known concentrations of hormone so that the relevant response curve can be found (see Fig. 9).

It should be obvious, even from this much simplified description of the *Avena* test, that it requires a very delicate and skilled technique, quite apart from the provision of special dark growth chambers with humidity and temperature control and other devices and precautions to eliminate the complicating influence of varying external conditions. Such a test can therefore be carried out, as a rule, only in research institutions with laboratories devoted to the study of plant physiology. It is therefore not surprising that a number of simpler assay methods, less susceptible to environmental influences, have been devised from time to time. The one much favoured to-day by plant physiologists also uses the coleoptile of the oat, but instead of measuring curvatures obtained by unilateral application of the hormone, it measures the total increase in growth rate (stretching growth) of small cylinders cut from the coleoptile and immersed in aerated solutions of the hormone under investigation. This is known as the "straight growth" test to distinguish it from the previous "curvature" test. It must be pointed out that such cylinders when immersed in water will show growth due to their own hormone content, and that therefore two ob-

servations are necessary for each test, firstly the average growth rate of a batch of cylinders growing in water and secondly the growth rate of a similar set growing in the test solution. The sections, which are usually 3–5 mm. long, are cut from identical regions of similar uniform coleoptiles, and are conveniently measured to the nearest 0·02 mm. by means of a travelling microscope. Growth must be allowed to take place in darkness and at a constant temperature, but no special precautions are needed to maintain a constant humidity, an important practical consideration. Cane sugar is usually added to all solutions, since lack of such a food supply would limit the response to the hormone. In addition, an adequate aeration (supply of oxygen) must be ensured, and this is done by using shallow dishes (Petri dishes) for the tests with the cylinders just breaking the surface of the solution. Gentle continuous agitation with some mechanical rocking device is often used to advantage. For ease of handling and measurement, the cylinders are often slipped on to the teeth of very fine plastic combs before being immersed in the solution. The growth response of these cylinders (increase in growth above those in water) is found to be directly proportional, not to the concentration, but to the logarithm of the concentration of the applied hormone. This means that it can be used for estimations over a very wide range of concentrations but that its accuracy over a restricted range is much smaller than in the *Avena* test of Went (see Fig. 9).

A third test, much favoured by chemists in estimations of the activity of synthetic compounds, is also due to Went (1934): it is the so-called "pea test". The material used consists of portions of the young stem of the garden pea (*Pisum sativum*) which have been growing in the dark for about a week. The peas should be grown in a room where there is no risk of contamination from coal gas, as the compound ethylene, present in small traces in that gas, can cause considerable contortions of the young stem. Stems grown in this way in the dark are long and colourless and have small leaves, a condition known to the botanist as *etiolated.* The apical five millimetres of such a stem are removed and the stem split lengthwise for a distance of 3 cm. by one strictly median cut

made with a razor blade. This split portion is then removed from the main stem by severing it a few millimetres below the slit and it is then washed for a period of an hour or so in glass-distilled water.* After this treatment the split stems have the appearance seen in Fig. 9, the split portions bending outwards in response to a release of tensions present in normal plant tissues. If a section is now placed in a dilute solution of a growth hormone, the cut portions of the stem will respond by bending inwards at its tips (see Figs. 9 and 10) due to the differential stimulation of the growth of the inside and outside of the stem, the cut inside surface growing slower than the intact outside. The cause of this difference in response is partly inherent in the two tissues and partly due to an inhibition of the growth response of the inner tissue by wounding. Maximum response is attained in about six hours. This curvature differs from that in the *Avena* test in being due to a different response of two tissues to the same hormone concentration, not of the same tissue to different concentrations as in the coleoptile test. The simplest procedure to use in this test is to set up in shallow dishes a range of dilutions of the growth substances to be tested and to place a number of identical stem sections in each solution. From the greatest dilution giving an observable response an estimate of the activity of the substance under test can be made with an accuracy of about 50 per cent. However, with careful handling, the method is capable of somewhat more accurate results for assay of material of known specific activity, and as a measure of the absolute response the angle of inward curvature can be determined from shadowgraphs. This is measured, as illustrated in Fig. 9, between the tangent at the extreme tips of the split section and the tangent at the point of inflection between outward and inward curvatures. Calibration experiments show that within certain concentration limits the curvature is directly proportional to the logarithm of the concentration. It has recently been shown that the sensitivity of this stem material to applied growth hormones can be greatly increased

* Water distilled in an all-glass still must be used throughout the test as it has been shown that this material is very sensitive to traces of heavy metals present in ordinary distilled water and may completely fail to respond in this latter medium, which always contains traces of copper.

(about ten times the curvature for the same applied concentration) by exposing the seedling for a short period (4 hours) to very weak red light (10 foot candles) about 1½ days before harvesting for an assay (Kent and Gortner, 1951). This illustrates the extreme care necessary to ensure uniform growth conditions if reproducible assay material is to be obtained. In quantitative assays, therefore, calibration curves of response against concentration must first be constructed using the pure substances. Variation in response is much greater than in the methods previously described and causes a much smaller overall accuracy. The method involves, like the straight growth test with coleoptiles, a large bulk of solution and therefore is not suitable for work with small quantities of extracts of natural hormones. The fact, however, that it is insensitive to temperature and diffuse light over the ranges normally encountered in a laboratory has made it a popular test where rapid routine qualitative methods are sufficient. Other plant material such as split dandelion flower stalks have been used with some success. A modification of this method, convenient when comparing the activities of different auxins, is to determine the concentrations which will reduce the normal outward curvature of the split stems by a convenient angle (usually 100°). The molecular activities of the auxins are then inversely proportional to these standard activity concentrations (Thimann, 1952).

Another assay method which is receiving considerable attention at the moment makes use of the effect of auxins on elongation growth in roots. As we shall see later, concentrations of auxins which stimulate the growth of coleoptiles greatly inhibit that of roots, but at very low concentrations (about one part in $10^{11}$) a slight stimulation of growth is detectable. Cress seedlings are a very suitable material for this assay, and by growing selected seedlings for short periods in a range of dilutions of $\beta$-indolylacetic acid solutions, a curve can be constructed relating growth to the concentration of the hormone. This curve covers a very wide range of concentrations from optimum growth at one part in $10^{11}$ to complete inhibition in about one part in $10^{6}$ (see Fig. 9). In the assay itself similar dilutions of the solution under test are set up and root

growth followed in them. A curve of growth rate against dilution is then constructed and by comparing this with the standard curve for β-indolylacetic acid the auxin content of the test solution (in terms of β-indolylacetic acid equivalents) can be calculated. The exact technique employed varies with the quantities of materials available and the accuracy required (Moewus, 1948; Audus, 1949, 1951).

A convenient and popular test for growth hormone activity depends on the movement of leaves of certain test plants in response to the application of the growth hormone to the surface of the stem or leaf-blade. The suspected hormone is usually dissolved in lanolin * and the paste then applied in small drops to an appropriate part of the plant. The compound, if active, will be absorbed into the plant, will pass to the leaf-stalks and will induce a growth response causing the leaves to bend downwards. This phenomenon is known to the botanists as *epinasty*. The test is of little value from a quantitative point of view.

These tests have all been concerned with the determination of activity in controlling the longitudinal growth of a plant organ, an activity which is confined to the phase of stretching growth of a cell-wall. In subsequent chapters, however, we shall see that these hormones exert other and varied effects on the growth processes of the plant, *e.g.* the initiation of roots, the development of fruit, etc. It has therefore been found necessary in investigations of these phenomena to devise special tests and assays relating to these properties and these will be described in the relevant chapters of the book.

Earlier on in this chapter the extreme sensitiveness of these tests has been pointed out. The *Avena* test can detect quantities of auxins of the order of 1/1000γ †, *i.e.* about 35 billionths of an ounce ($35 \times 10^{-12}$ oz.). The most sensitive chemical tests for these compounds (viz. for β-indolylacetic acid) will detect quantities of the order of 2γ (Mitchell and Brunstetter, 1939), that is about 2000 times less sensitive than the *Avena* test.

* Lanolin is a natural fat obtained from wool and has itself no action when smeared on plant organs.

† γ (Gamma) is the symbol for one microgramme, which is equal to the millionth part of a gramme.

There are, however, occasions when quantitative chemical tests of this sort are to be preferred to biological assay methods on account of their relative simplicity and greater accuracy; for example, when high concentrations of pure substances are being studied. Brief details of such tests will be given in the chapter on the chemistry of the auxins and therefore will not be further considered here.

## THE ISOLATION AND CHEMICAL IDENTIFICATION OF THE AUXINS

The first attempt to isolate the growth hormones, in sufficient quantity to allow a chemical analysis of their structure, was not, as might have been expected, carried out on oat coleoptiles or even plant tissue, since they occur here in far too small quantities. Fortunately, biochemists in their researches found other materials which were far richer in substances active in the *Avena* test, and attention was therefore concentrated on these. Firstly, in 1930, Nielsen, working in Boysen-Jensen's laboratory in Copenhagen, isolated a highly active syrup from the culture medium in which certain moulds (*Rhizopus suinus* was the most important) had been growing. No further attempts were made to purify this syrup, but the active principle was shown later to be a weak acid of approximately the same strength as acetic acid (Dolk and Thimann, 1932). At about the same time, in the laboratories of the University of Utrecht, Kögl and Haagen-Smit (1931) discovered that human urine was a particularly active source and therefore chose it as their experimental material. Starting with 150 litres (about 35 gallons) of urine, they concentrated it down by various processes until about 12½ lb. of a dark brown syrup had been obtained; this syrup was then subjected to a series of empirical purification processes resulting in a progressive removal of impurities. The products of each purification step were tested for activity by the *Avena* test. The final stages of purification consisted of distillation in high vacuum, giving a final yield of 40 milligrammes (equal to about 1/700th of an ounce) of crystalline product. The *Avena* test showed that this corresponded to a 5,000 times increase in concentration of the active substance. From

crystals of this kind * the chemical constitution of the active compound was established and the substance was given the name of *Auxin A* or, chemically speaking, *auxentriolic acid.* Its proposed formula will be discussed in a later chapter.

Understandably, after this success, these workers turned their attentions to plant sources and found that certain cereal products, in particular germ oil (from maize) and malt (from barley), had very high auxin activities. A similar series of purifications of these products yielded auxin A and a new compound of similar structure and almost identical activity which was called *Auxin B* or *auxenolonic acid.* These latter isolations required a degree of concentration of 100,000 times for malt and 300,000 times for germ oil. Both these substances were completely new to chemical science. It was not, however, always possible to extract auxin A from the mixed urine of the hospital supplying this raw material. This type of snag, not unfamiliar to biological workers, proved to be of great importance to the subsequent development of the subject, for it caused these chemists to devote their attentions to one particularly prolific source, the urine of an eighteen-year-old youth. This had been shown by the *Avena* test to possess up to five times the activity of the normal mixed urines. From this source, in addition to auxins A and B, a third active substance was isolated and christened *heteroauxin* ("other auxin") (Kögl, Haagen-Smit and Erxleben, 1934). On subsequent analysis this substance was shown to be the compound $\beta$-indolylacetic acid, which had long been known to the chemists but had never before been suspected of having any biological functions. It had first been synthesized in Germany in 1904 (Ellinger, 1905). The Dutch workers showed that this compound could also be obtained from yeast, and in America it was demonstrated conclusively (Thimann, 1935) that it was also identical with the active substance that Nielsen had obtained from the fungus *Rhizopus suinus.*

In these early days auxins A and B were regarded as the only natural plant growth hormones, and heteroauxin, a typical

* In all about 700 milligrammes, *i.e.* about 1/40th oz., were isolated and used n the analyses.

product of the activity of such microorganisms as moulds and yeasts, was thought not to be so. Of course, plant physiologists made many attempts to settle the doubt on this point, but, owing to the small quantities of substance involved, most of the evidence from the hormones extracted from plants rested on the measurement of such properties as their rates of diffusion through agar jelly and their sensitivity to destruction by hot acids and alkalies. From the former an estimate of the size of the hormone molecule was obtained, and these seemed to correspond more closely to the proposed structure of auxins A and B than to that of β-indolylacetic acid. Again, auxins A and B are destroyed by hot alkalies, whereas β-indolylacetic acid is not, and the results of such treatment of plant extracts also supported the above contentions. It is not surprising, therefore, that dogmatic statements to that effect have dominated the literature of the nineteen-thirties and have been made the theme of all the early text-books on the subject. However, all the evidence for these statements has been of an indirect nature, as crystalline auxin A and B have not since been isolated from plant materials. In recent years, as the result of a considerable accumulation of both direct and indirect evidence, the pendulum has swung in the opposite direction and it now seems likely that β-indolylacetic acid is the most important growth hormone in plants. Haagen-Smit himself, working now in America with other colleagues (1942, 1946), isolated it in pure form from corn meal and corn germ. Recent work on the stability in hot acids and alkalies of the hormone extracted from a wide range of higher plants all point to β-indolylacetic acid and not auxins A and B as the natural hormone. As we shall be seeing in a later chapter (Chapter 3), all the data we now possess concerning the origin and breakdown of the growth hormone in plants are in conformity with this suggestion.

Auxin A and B in urine might not therefore come, as originally supposed, from the green plants eaten by the animal and passed without change from the body. Were it not for the claims of their isolation from malt and corn-germ oil, we might suppose that they were peculiar products of the metabolism of the animal itself. The position is still further complicated in that

recent analyses of human urine have failed to isolate any plant growth hormone other than those of the indole group of compounds. Bennet-Clark and his colleagues (1952), using paper chromatography,* have demonstrated that the greater part of the activity of urine is due either to β-indolylacetic acid or to closely related compounds. It is also very suggestive that the chemists, who could do so much to resolve this mystery, have not yet succeeded in synthesizing these two auxins. In any case, the term "heteroauxin" for β-indolylacetic acid is definitely a misnomer and should be dropped completely.

Whatever the true story concerning the occurrence of auxins A and B in urine, there is no doubt in the minds of many plant physiologists to-day that β-indolylacetic acid is the most important natural auxin. This idea, first formulated in America, is rapidly spreading, although continental workers still cling to the old ideas. In certain circles in Germany, for example, β-indolylacetic acid is still regarded merely as an activator of the natural auxins (v. Guttenberg, 1942; Dettweiler, 1942). In America, however, these new ideas have gained a firm hold and, as stated in a recent book on the subject, several research workers have "privately expressed doubt as to the existence of auxins A and B" (van Overbeek, 1950). Be that as it may, there is no doubt that it is to the discovery of β-indolylacetic acid that we owe the vast expansion of the practical aspect of the subject in recent years. It was with this compound as a model that the chemists subsequently synthesized the very wide range of compounds of similar structure which

* The methods of chromatography depend upon the capacity of certain powders (*e.g.* chalk, cane sugar, etc.) to remove from solution the compounds to be separated and to retain them "stuck" to the surface of the powder grains. The tenacity of this retention varies from compound to compound with any one powder or solvent liquid, and this property can be used to separate the compounds. Thus if the mixture is washed slowly down a uniformly packed column of powder with a suitable solvent, the compounds will be held back in the column to an extent depending on the relative attraction of the powder particles for them, those attracted most strongly remaining at the top of the column and the weakly attracted passing down to lower levels. In this way it is possible to separate closely similar chemical compounds that are inseparable by any other known methods. In paper chromatography a sheet of blotting paper is used instead of a powder column.

have proved to be of such immense value in practical agriculture and horticulture and which we now term the synthetic auxins.

## CHARACTERISTIC PROPERTIES OF NATURAL AUXIN. FUNCTIONS IN EXTENSION GROWTH

*Auxins in the Growth of Coleoptiles and Shoots.*—We have seen in the first chapter that, with the exception of those which stay permanently meristematic, all plant cells exhibit three distinct phases in their cycle of growth, *i.e.* phases of division, extension and differentiation. In the coleoptile of *Avena*, which is convenient for illustrating the growth sequence in young seedling shoots, there is little tissue differentiation except for two small veins running up vertically on opposite sides of this almost cylindrical organ. For the first half-inch of its growth, increase in size is partly by cell division and partly by cell extension. When this size limit is reached, all the cells have passed from the dividing into the extension phase and, from there to the final size limit of 2 to 2½ inches, growth in length is entirely by cell elongation (extension growth). An important characteristic of this growth is that the extension phase sets in first at the base of the coleoptile and gradually passes up towards the tip. This upwardly moving wave of extension growth never reaches the extreme tip, and the cells of the top tenth of an inch scarcely elongate at all. There is, however, a maximum size that any cell can attain and the elongation rate falls off to zero as this size is approached. As a result of this we find in the coleoptile as a whole, as indeed in all organs and organisms, that its growth, being the sum of the growth of all the component cells, also takes place as a wave, starting slowly, reaching a maximum at about half size, and then tailing off to zero as the final size is reached. If at any time during all but the earliest stages of this growth wave the tip of the coleoptile is removed, then growth falls almost to zero. That this is due to the removal of the source of auxin, thus robbing the growing cells below of their necessary hormone, can be shown by replacing the tip or by applying β-indolylacetic acid to the cut surface of the stump in some suitable carrier (*e.g.* agar jelly or lanolin-water emulsion). Since this

period of the growth wave is due entirely to extension growth of the cell, it follows that the action of auxin, both here and in the curvatures of the *Avena* test, is primarily concerned with the stimulation of this particular phase of growth.

Although decapitation * of the coleoptile brings about an immediate stoppage of growth, yet after a short time growth may start again although at a much slower rate. If the top few millimetres of the stump are then removed this renewed growth stops at once. This renewal of growth and its suppression by the second decapitation was explained by assuming that the apical portion of the stump had taken over the functions of the normal tip and was producing a supply of auxin for growth, although in much reduced amounts. This phenomenon, known as the *regeneration of the physiological tip*, was of great practical importance, since it reduced the sensitivity of the coleoptile to applied auxin and therefore a double decapitation technique had to be adopted for the Went *Avena* test (see Figs. 4 and 5). Later the discovery was made (Skoog, 1937) that this regeneration could be prevented by the removal of the "seed" of the oat before the initial decapitation. The logical explanation of this phenomenon was that some forerunner (precursor) of auxin was originating in the "seed" and was passing up to the tip of the coleoptile where it was being converted into auxin. The phenomenon of regeneration of the physiological tip was then merely the acquisition by the apex of the stump of this power of turning the precursor into auxin. Skoog proved this by cutting off the coleoptile at its base and applying an agar block to capture any such precursor diffusing up from the "seed". Such agar blocks were then tested for growth hormone in the *Avena* test. Curvatures were not obtained immediately but only after a lapse of two to six hours, showing that during this time the precursor present in the block had diffused into the coleoptile and had there been slowly converted into growth-promoting auxin. This precursor story seems, as we shall see later, to be of general application. The nature of the precursor will be discussed in Chapter 3.

* Decapitation is a term adopted by the plant physiologist and means removal of the apical portion of a plant organ.

All these experiments indicate that it is essential for the cells of the oat coleoptile to contain auxin before they can grow in length. In the words, therefore, of the pioneer Went: "No auxin—no growth". Observations on the growth of isolated sections of the young aerial parts of various plants, *e.g.* oat coleoptiles, pea shoots, sunflower shoots, etc. isolated from the source of both precursor and auxin alike, still showed small growth in sugar solutions. This may continue at a slowly decreasing rate for a considerable time, and is assumed to be due to the slowly dwindling content of auxin present before isolation. As would be expected, additions of an external supply of auxin greatly stimulated the growth of those sections and, furthermore, a definite relationship exists between this stimulated growth and the concentration applied. This is what would be expected from the curvature-concentration relations of the *Avena* test.

Growth rate in such sections increases with increase of added auxin up to a maximum at a concentration of about 1 part per million, as is shown in the portion A–B of the relevant curve in Fig. 11. It is this order of concentration which occurs in the plant, and naturally changing supplies of auxin would be expected to control growth to the extent indicated by this curve. If, however, auxin is applied in strengths above this optimum, the growth rate will fall and will eventually be inhibited completely in sufficiently high concentrations. It is extremely unlikely that such concentrations will arise in nature, but this inhibitory effect of "supra-optimal" concentrations is a phenomenon of general occurrence and of great importance in the practical field.

*Auxin in the Growth of Roots.*—Roots as well as shoots are sensitive to external stimuli, the best known illustration of this being their reaction to gravity. It is common knowledge that, if a seedling is placed with its root horizontal, the tip will always bend over and grow downwards towards the earth's centre. Darwin showed that the perception by the root of the stimulus of gravitational pull was located at the tip, since its removal eliminated the root's reaction to the stimulus. Here too, as in the oat coleoptile, the reacting zone was in the region of extension growth some distance behind the tip. We shall see

later how this influence can now be explained in terms of the distribution of hormone coming from the tip.

Since it would appear then that in these responses a hormone arises from the root-tip, it might be assumed that it is functioning as a normal regulator of growth as is the auxin from the tip of the coleoptile. We would therefore expect decapitation of the root to have an effect on root growth rate. Here however, in contrast to the effects in the coleoptile, no retardation is immediately observed. In many cases, when suitable precautions are taken to supply the root with adequate water, a temporary acceleration of growth has been claimed (Cholodny, 1924). It would seem, therefore, that instead of a growth-stimulating hormone, a growth-retarding hormone is being produced by the tip. This was made more probable when it was claimed that replacing the tip after decapitation caused the growth to fall again to normal. So far the story runs parallel with that for the coleoptile but with the hormone producing opposite results on growth. The most important discovery, however, in connection with this root hormone, was that coleoptile tips, when placed on decapitated roots, similarly retarded the growth; this was the first indication that the growth-retarding hormone of the root might be identical with the growth-stimulating hormone of shoots. It seemed probable that the hormone that diffused from the root-tip was $\beta$-indolylacetic acid and that, in the range of concentration found in the shoot or coleoptile tip, it normally retards root growth. The logical expectation from this would be that maximum growth would take place without auxin and that in contrast to the situation in shoots, auxin is not an essential factor for root extension. This would be a disturbing conclusion since there seems no reason why the mechanism of extension of root cells should differ in any fundamental way from that of shoots. However, in recent years, evidence has accumulated indicating that in all these early observations on root-growth reactions to externally applied auxin the concentrations employed were above optimal, and that at much lower concentrations a stimulation of the root-growth could be obtained. The optimum fell at a concentration of one part in about $10^{11}$ parts, *i.e.* about 1/100,000th the optimum concentration for shoots.

This relationship is shown in Fig. 11. The difference between root and shoot may therefore be merely one of degree and not of fundamental response. This reaction to applied auxin, however, is difficult to reconcile with the early claims of growth acceleration brought about by the removal of the root-tip and therewith the source of natural auxin. Thus we have the enigmatical situation that normally growing roots are inhibited by their own natural auxin supply but, in spite of this, are stimulated by a further small supply from outside. This enigma has yet to be resolved.

*Auxin and the Growth of Buds.*—The various growing zones and meristematic areas of the aerial parts of green plants by no means show the same sensitivity to auxins. A fact which has long been known among gardeners is that the terminal buds of stems or branches exert a definite influence on the growth of lateral buds. Removal of an apical bud almost always results in prompt development of the highest laterals, which would have remained dormant had the terminal bud been left to grow normally. It was originally thought that this phenomenon was due solely to the diversion of the available food supply to the terminal bud, thereby starving the laterals. In more recent times it has been demonstrated conclusively that auxin is responsible. The terminal bud, just like the apex of the coleoptile, produces a high concentration of auxin, which is presumably involved in the growth of the young shoot from the bud itself. This auxin diffuses down to the lateral buds and stops their development. When the source of this auxin is removed with the apical bud, the inhibition is removed with it and the upper laterals grow out, only to exert a similar influence on still lower buds. Needless to say, this explanation has been somewhat over-simplified for the sake of brevity. The true explanation of the differential effect of auxin is still exercising the ingenuity of interested plant physiologists and we will return to consider this subject in more detail in a later chapter. All we need to emphasize here is the fact that bud growth is more sensitive to auxins than stem growth, but less sensitive than roots. Indeed, one leading worker in this field (Thimann, 1937) has proposed that the basic relation of growth to auxin concentration is the same in root, stem and bud,

with stimulations in low and inhibitions in high concentrations. Such relationships can therefore be represented by the three curves of Fig. 11, which makes clear not only the effect of different concentrations of auxin on the three different organs, but also shows the great differences in overall sensitivity, indicated by the positions of the growth optima and points of complete inhibition.

This differential sensitivity of the several organs of the plant is one of the basic phenomena underlying co-ordination and balance of growth in the plant as a whole. The second important factor of control is the regulation of auxin concentration in these several organs; but here the general picture is not so clear cut although a vast amount of data has been accumulated. Before dealing with the factors and possible mechanisms of this regulation, let us first consider what the actual concentrations are in the normal growing plant organ and how they may be related to growth.

## THE DISTRIBUTION OF AUXIN IN THE PLANT AND ITS RELATION TO GROWTH CONTROL

As we have seen, auxins occur in plant organs in extremely low concentrations even in such actively growing organs as the oat coleoptile. Even if we could be certain of the exact chemical nature of these natural auxins, and simple sensitive chemical tests (*e.g.* colour reactions) existed for them, such low concentrations would make them impossible to demonstrate, let alone estimate, *in situ.* All our methods of estimation are therefore based on some kind of extraction procedure followed by a biological assay of the extract (*e.g.* the *Avena* test). The one great disadvantage of such assays at present is that they do not distinguish one auxin from another, and this means that all our observations on extracts can only give us indications of the distribution of auxin "activity", which is usually expressed in terms of an equivalent amount of $\beta$-indolylacetic acid.

Further difficulties arise in that very different "concentrations" of auxin are observed when different extraction methods are employed. In the earliest methods the auxin was allowed

to diffuse into agar from cut surfaces of the organs concerned. By this method alone it appeared that auxins were of very wide distribution, occurring in greatest concentrations in young actively growing organs such as buds, young leaves, stem tips, etc. Nevertheless, early observations failed to obtain it by diffusion from the root-tip, except in the presence of sugars in the agar used. When, however, chemical solvents were employed to extract the auxin, a particularly suitable substance being pure ethyl ether, much higher yields were obtained, and organs previously thought not to contain it (*e.g.* the coleoptile base) showed its presence in detectable quantities. Even in the coleoptile tip the ether-extractable auxin was much more plentiful than the free diffusible auxin. We can account for this discrepancy, at least partially, by postulating a steady destruction of auxin at the cut surface of the tissue during the diffusive extraction. Such a destruction or "inactivation" has been shown to be stimulated by natural organic catalysts * (*enzymes* of the biochemist) present in the cells of the tissues concerned; such catalysts would have little total effect during ether extractions. Lack of any diffusion from coleoptile base is correlated with, and may be due to, destruction by basal tissue: but inactivation alone cannot account for the magnitude of this discrepancy and two types of auxin have now been suggested as being present in actively growing tissues. Firstly, there is the "free" or diffusible auxin, present in slightly larger concentrations than indicated by diffusive extraction, since some will have been destroyed by enzymatic inactivation. Secondly, there is the "bound" or non-diffusible auxin, which can only be extracted by certain organic solvents such as ether. The amounts obtained by such extraction would represent the "free" plus the "bound" auxin. Some workers (see Went and Thimann, 1937) have regarded this "bound" auxin as being fixed to the centres of hormone action in the cell, the "free" auxin presumably being the excess in equilibrium with it. In this case the "bound" auxin would be the component active in the growth process.

This picture is nevertheless considerably complicated by the

* A catalyst is a substance which, in very small amounts, will greatly speed up a chemical process without itself being changed in that process.

fact that that auxin is extractable from such plant tissues over long periods of time, either by the use of wet ether and successive extractions, or by a long-continued series of diffusive collections. This suggests that, in addition to the "free" and "bound" auxins immediately extractable, there is a third component, bound in some way to cell materials and liberated only slowly over such a period. A slow liberation of this kind does not take place in boiled tissues, indicating that it is due to the action of enzymic cellular catalysts.* A considerable bulk of recent work completely substantiates these views. Thus spinach leaves and wheat and maize grains, when heated with very weak alkaline solutions, yield quantities of auxin far greater than can be obtained by direct extraction with ether. Treatment of spinach leaves etc. with enzymes which will split up protein molecules also liberates large amounts of auxin. These and other similar observations suggest that auxin may be combined in the cells of a variety of tissues with some of their constituent proteins and can be slowly liberated from them when required. It would seem that most organs possess high "auxin potentials" in the form of protein-complexes, the exact form and nature of which have still to be determined. Some discussion of the chemical nature of these auxins and auxin precursors will follow in the next chapter. This store seems to constitute the greater part of the total auxin in plant tissues. In the coleoptile of maize and oat, for example, only about 8 per cent. is present as "free" auxin (van Overbeek, 1941); in the frond of duck-weed (*Lemna*) it is about 3 per cent. (Thimann and Skoog, 1940); in the storage tissue of the maize grain it varies from 10 per cent. (Avery, Berger and Shalucha, 1941) to 30 per cent. (Berger and Avery, 1944). It is very likely that there is an equilibrium in the plant between these various forms of bound auxin, the free diffusible auxin and the somewhat hypothetical auxin fraction fixed at the site of its action in the cell. We can conceive of the regulation of growth as largely effected by adjustments of this delicate equilibrium, resulting in a control

* The enzyme catalysts are complex protein systems which are destroyed by heating above a certain maximum temperature, usually about 60° C. Boiling destroys them in a matter of minutes.

of the supply of the active component either to growth centres in the same organ or, after translocation, to those in other organs.

There is, however, as yet little conclusive evidence of the true nature of this active auxin. It has been variously assumed to be the diffusible fraction and the immediately extractable fraction and considerable data have been accumulated for the distribution of these fractions in growing plants. Attempts have been made to correlate these distributions with growth rates in various parts of plants, but as yet no clear picture has emerged of the precise component or components directly responsible for growth control. The picture is made even more complicated by the recent discovery of the occurrence in the same tissue of substances counteracting auxin action. To these the name of anti-auxins has been given. Clearly, then, further advance will be made only when we can separate and make a chemical identification of the several auxin fractions and also the accompanying anti-auxins; some beginning has been made in this field. Thus the active auxin in maize food reserves has been definitely identified as β-indolylacetic acid (Haagen-Smit *et al*, 1942 and 1946; Berger and Avery, 1944). The inactive "bound" form has also been isolated from the same material (Berger and Avery, 1944) and shown to yield β-indolylacetic acid in hot weak alkaline solutions. Other hormones are not, however, ruled out; indeed, relatively large quantities of the closely related β-indolylacetaldehyde have been recently isolated from a variety of plants (Larsen, 1944), while in cabbage leaves almost the sole active material present is another close relative, β-indolylacetonitrile (Jones, Henbest, Smith and Bentley, 1952). But there is still some discussion whether these are active auxins or easily convertible precursors (see next chapter). New and delicate methods have been recently developed whereby very small amounts of complex mixtures of organic compounds can be separated into their components by chromatography and the components identified with fair certainty; already such methods are being applied in some British laboratories and on the continent to the auxin analysis of plant extracts with very promising results (see further discussion in Chapter 3). Nevertheless, it will be a long time

before experimental data are sufficiently complete to allow us to identify with certainty the active auxin components from the various organs of the plant, and to assess the full extent of their participation in the control of growth in those organs.

## THE CONTROL OF AUXIN MOVEMENT AND CONCENTRATION IN THE PLANT

Although there is little critical experimental data on the exact relationship between the concentration of "active" auxin and the intensity of growth in the various extending zones of plants, yet there can be no doubt, from what has been said so far and from a consideration of other physiological actions of auxin, that the regulation of this concentration is one of the major controlling factors of plant growth. We have seen that immediate supplies of auxin for growth probably come from a precursor and the rate of this production is obviously our first factor affecting auxin concentrations. In the oat coleoptile, this precursor is taken from the seed up to the tip where it is transformed into active auxin. This released hormone has then to be transported to the elongating zone of the coleoptile where its growth-stimulating action is exerted. This, then, introduces our second factor controlling auxin concentration, viz. the speed of its transport from the site of production to the site of action.

This transport is of great interest to the plant physiologist, since it possesses certain unusual features. The most interesting of these is that in young, actively growing tissues like the oat coleoptile, movement is strictly polar (in one direction only), viz. from apex to base. This movement is conditioned by some asymmetric property of the organ and not by external forces such as gravity, since the direction of movement is unaffected by the placing of the organ in space. On the other hand, although this polarity is shown by stem tissue, the phenomenon seems to be absent in roots. The underlying causes of this polarity are amongst the most intriguing of the unsolved mysteries of plant physiology. Such a directive influence on auxin movement is obviously an important factor in growth control. When we turn, however, to movement in more mature

tissues, we find a different state of affairs. In fully differentiated stems or leaf-stalks considerable evidence shows that the hormone movement takes place only with the normal movement of food in the special living cells of the bark (phloem) which perform that function; this movement is not polar. By this means auxin, or its precursor manufactured in the leaves (see later), would be transported with the food to the growing meristems of the plant. Recently the use of synthetic auxins has demonstrated that external application of these compounds in unnaturally high concentrations may result in their spread into the non-living woody tissues, which are concerned with the conduction and distribution of water. In this way hormones may show a rapid and uncontrolled spread throughout the plant. We shall realize the importance of these latter two modes of hormone transport when we come to consider their practical applications.

There is yet a third factor which may play an important part in regulating "active" auxin concentrations: this is the presence or absence of an inactivating enzyme system in the tissues concerned. Recent work has shown that we can isolate from young growing plant tissues, such as pea stems and roots, an enzyme system which can bring about a rapid destruction of auxin in the test-tube. Such a system could be a very important concentration regulator. Indeed, the low concentrations observed at the base of the coleoptile may be due to a system like this. It has been demonstrated that this inactivating enzyme is much more powerful in roots than in stems, a fact which may be closely linked with the much greater sensitivity of roots to auxin concentrations. It seems likely that it is by the action of such a system that the necessarily high concentrations in shoots are prevented from causing correspondingly high concentrations in roots, whose growth would thereby be completely inhibited (see Fig. 11). We still do not know why it should be necessary for roots and shoots to possess these basic differences in sensitivity.

That such an enzyme system may determine the ultimate size of the mature plant has been demonstrated in certain dwarf species of maize, which have very low overall auxin concentrations relative to other varieties, and this low content is directly

correlated with a high capacity of the tissue for auxin inactivation. Thus the hereditary factor determining dwarfness is obviously doing so by causing the generation of an over-active, auxin-destroying enzyme system.

Auxin concentrations, however, will certainly not be the only factor determining the final growth reaction of any one cell or system of cells. Even assuming that the raw materials for growth are present in excess, it is most likely that the sensitivity of the cell itself will come into the picture. One of the most distressing features of the *Avena* test is that the sensitivity of batches of otherwise identical oat plants show periodic fluctuations that have so far resisted attempts at correlation with any known external factors. Similar, although not as marked, variations in sensitivity occur in other biological assay techniques, *e.g.*, straight growth tests with coleoptile sections. Little attention has been given so far to this aspect of auxin action.

The occurrence in the plant of organic molecules counteracting the action of auxin, although not actually destroying it, have recently been demonstrated. These anti-auxins might, as a result of variations in their concentrations and/or activities, bring about inverse changes in the effectiveness of the auxins. This would be yet another component in the complex of growth-controlling factors.

## THE ORIGIN OF AUXINS IN THE PLANT

The subject which we have so far avoided in our discussion is the primary synthesis of the auxins in the plant. We have seen that the production of auxins at the growing points of plants is, as far as we know, entirely the result of the chemical transformation of a precursor. Where and when, therefore, is this precursor made? These questions cannot at the moment be answered. In the first place, we can only guess at what the precursor is, although certain particular compounds are strongly indicated. It was once thought that the initial manufacture of auxin or its precursor could take place only in the presence of light, since plants and plant parts exposed to light showed a much higher content of auxin. But, more recently,

isolated roots growing in the dark on solutions of mineral salts and sugars have been shown to make it. Thus it would seem that auxin (or auxin precursor) synthesis does not need light, and the higher content of free auxin in illuminated plants might be brought about by a stimulated liberation from the "bound" form, or a more rapid conversion of precursor. The precursor now suggested as being the most likely is one of the simple chemical units (amino-acids) which go to build up the proteins of the living protoplasm (see Chapter 3), and it seems logical, therefore, to assume that precursor manufacture is part and parcel of the same set of biochemical processes that brings about protein synthesis.

## AUXIN ACTION IN PLANT GROWTH MOVEMENTS IN RELATION TO STIMULI

Now the wheel has come full circle and we return to consider briefly those phenomena whose early study led ultimately to the discovery of the auxins. We can, in this book, do no more than touch on the subject, which is vast and complicated and which will help the general reader but little to understand the main bulk of this book. However, the phenomena are so integral a part of the growth processes of the plant that some mention should be made of them.

The fundamental characteristics of the reaction of the plant to such external stimuli as light and gravity have already been stated briefly and it was then suggested that the growth response was caused by an unequal distribution, in the growing zone, of auxins diffusing from the apical meristems of the organ concerned. This hypothesis has been verified in a number of organs and for a few simple plant responses by direct analysis of the free auxin available in the growing, and therefore reacting, zone. Thus, in seedling shoots bending towards light coming from one side, a greater concentration of auxin is usually found in the darkened (convex) side. In the case of stems and roots placed with their axes in a horizontal direction, there is generally an accumulation of auxin on the lower side at the expense of the upper. This causes a greater growth of the lower half and the stems bend upwards, whereas, in roots, the higher concentra-

tions cause growth inhibition whereupon the root bends downwards. The causes of the growth response and the rôle of auxin in these simple cases is relatively clear, but the real reasons for the redistribution of the auxin still remain obscure. There is some evidence that light brings about a destruction of auxin ($\beta$-indolylacetic acid) under the action of a specific enzyme which it activates. Such an enzyme has been isolated from pea shoots grown in the dark (Galston, 1950). If such a system were present in all organs showing responses, then its action in the living plant could, by lowering auxin concentrations on the illuminated side, offer a logical explanation of curvatures. When we consider the reaction to gravity we have little but pure theory to explain the redistribution.

Plant responses to stimulation are, however, scarcely ever straightforward curvatures, directly and simply related, as the above would suggest, to the direction of the incident stimuli. A perusal of the older text-books of plant physiology will afford ample evidence of the complicated responses which are the rule rather than the exception. Thus, to take but two examples: firstly, the reaction of the oat coleoptile to unilateral light depends in direction as well as degree on the intensity of the incident illumination. In low illumination the curvature is towards the light (positive curvature), but as it becomes more intense the curvature decreases and is eventually reversed and the coleoptile bends away from the light (negative curvature). Further increase gives a second positive curvature. Secondly, a number of plant organs show different responses to the same stimuli at different stages in their development. Thus the flower-stalks of the ivy-leaved toadflax (*Cymbalaria muralis*), a plant which grows on walls, turn outwards towards the light, but when the fruit is forming these same stalks exhibit the reverse growth-response and turn inwards towards the wall. Again certain flower-stalks grow directly up away from the pull of gravity when the flower is coming to maturity (e.g. *Cyclamen* and groundnut). When, however, the flower has been fertilized and the fruit is beginning to mature, the stalk responds in the reverse manner to the same stimulus and bends downwards, pushing the developing fruit into the earth. Nothing is as yet known of the auxin relations of such complex responses,

but it seems unlikely that a simple redistribution story will hold. In the more complex reactions, for example, the growth of underground stems which grow horizontally and maintain themselves at a constant distance below the soil surface, a complex consisting of auxin growth-stimulant and anti-auxin growth-inhibitor have to be postulated, each with its own particular redistribution behaviour in response to the stimulus of gravity (Bennet-Clark and Ball, 1951). The isolation and identification of such hormones and anti-hormones may give us a new range of chemical growth-regulators. There is no need to emphasize the fact that research on such purely academic branches of botany holds rich promise of discoveries of great practical value.

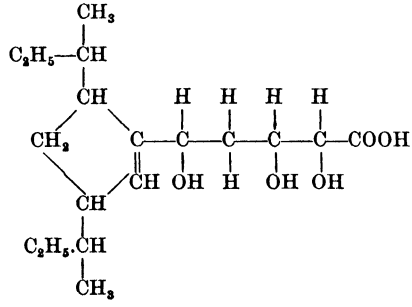
CHAPTER 3

# THE CHEMISTRY OF AUXINS (NATURAL AND SYNTHETIC)

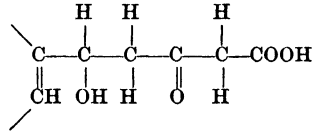
## THE STRUCTURE AND CHEMICAL PROPERTIES OF THE NATURAL AUXINS

THIS chapter is intended only for those readers who have a knowledge of chemistry and can therefore be skipped by all others.

It has been seen in the last chapter that the first attempts at isolating the auxins in sufficient quantity and purity to allow investigations into their chemical structure resulted in the preparation of crystals of three distinct chemical substances, auxin A (auxentriolic acid) (I) from urine, auxin B (auxenolonic acid) (II) from malt and maize germ oil, and heteroauxin ($\beta$-indolylacetic acid) (III) from urine. The two first-mentioned compounds proved to be very similar in chemical structure and the final formulæ proposed for them were as follows:—



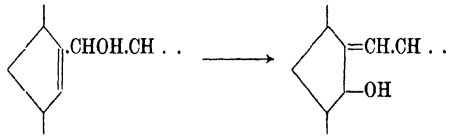
I Auxin A (Auxentriolic acid)



II Auxin B (Auxenolonic acid)

Both these substances were previously unknown to chemical

science and were found to be very unstable *in vitro*. Even *in vacuo* an isomeric change took place resulting in a shift of the double bond to the side chain, giving rise to an inactive pseudo-auxin with the following change of structure:—

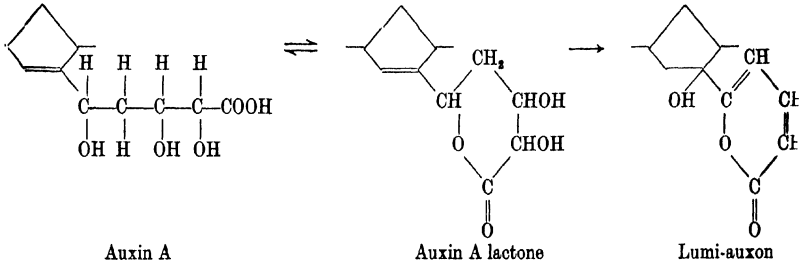



This presumably does not happen in the plant.

During the process of purification of auxin A, the impure concentrate was heated with acid methanol, resulting in the formation of a δ-lactone in the side chain, and this compound was isolated in a pure state. The acid is easily regenerated from the lactone and it has been suggested that they may both be in equilibrium with one another in the plant.*

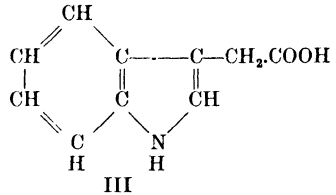
The third compound heteroauxin or β-indolylacetic acid (III), which has now been isolated in crystalline form from a number of plant sources (corn meal, malt, spinach leaves, etc.), has quite a different structure and has long been known to the organic chemist since it had first been synthesized by Ellinger

* It has been claimed that in the light the lactone is converted into a compound called lumi-auxon as follows (Kögl, Koningsberger and Erxleben, 1936):—




Lumi-auxon is supposed to be inactive or may even be a growth inhibitor (Veldstra and Havinga, 1943). This light conversion of auxin A into lumi-auxon has been suggested to underlie the response of plant organs to unilateral illumination (phototropism) although a more recent theory, based on enzymic inactivation of β-indolylacetic acid and better supported by experimental facts, is now replacing it (see Chapter 2, p. 48).

in 1904. Its formula is



III

It was also known to the biologist, since Hopkins and Cole in 1903 had shown that it was produced from the amino-acid, tryptophane, by the activity of bacteria (*Escherichia communis*). Its presence in a higher plant (*Celtis reticulosa*) was even suggested as early as 1909 (Herter, 1909) although at that time there was no clue as to its physiological action.

After the first isolations of auxin A and heteroauxin had been made from urine, much attention was given in the early days to the identity of the naturally occurring hormones of plants. Owing to the extremely small quantities obtainable from the tissues studied (*e.g.* coleoptiles), direct chemical tests could not have been applied even if they had been available, since their sensitivities would have been far too low. Recourse was therefore had to indirect identification methods, involving (*a*) the acid-alkali stability relationships, and (*b*) determination of the diffusion constants in agar. The original work of Kögl had shown that both auxins A and B were destroyed by hot alkali whereas β-indolylacetic acid was stable under the same conditions. On the other hand, hot acid decomposed β-indolylacetic acid and auxin B, but not auxin A. Here, therefore, we have a method of distinguishing the natural plant hormones in extracts, always assuming that they are limited to the above three compounds. Early results of the application of this differentiation technique supported the view that auxin A was the hormone of the oat coleoptile and other seedling shoots (Went and Thimann, 1937). One worker recently, on a basis of such evidence, has refuted the statement that β-indolylacetic acid is a hormone, claiming that it acts merely as an activator of auxins A and B (von Guttenberg, 1942).

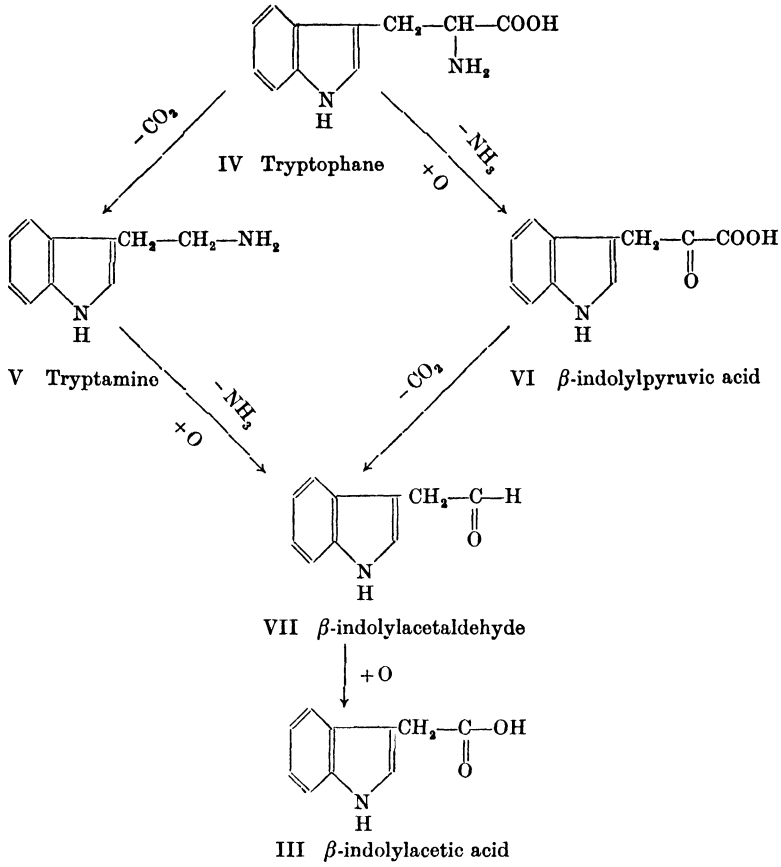
Measurements of diffusion constants have been obtained by allowing diffusion to take place through a series of identical rectangular agar blocks arranged linearly and in close contact.

The distribution of the auxin concentration through the series can be determined by assay of the individual blocks. From such a distribution curve the value of the diffusion constant can be calculated and thereby the molecular weight. Early observations on the auxin from the oat coleoptile (Went, 1928) and on pure auxin A (Kögl, Haagen-Smit and Erxleben, 1934) gave a molecular weight of 376, agreeing reasonably well with that for the proposed formula of auxin A (M = 328) and not with β-indolylacetic acid (M = 175).

In recent years, however, applications of the same technique to potato, tomato, spinach, radish, pineapple and a number of other higher plants have given results characteristic of β-indolylacetic acid rather than auxin A or B. Recent repetition of the early diffusion constant determinations (Wildman and Bonner, 1948) showed that auxin obtained by diffusion from the coleoptile tips into agar gave values corresponding to a molecular weight of 306, closely indicative of auxins A or B. If, however, this auxin were extracted from the agar with ether and then transferred back to fresh agar, a quite different diffusion constant was obtained, quite close to that of β-indolylacetic acid. Apparently the high values obtained in the first estimate and in the earlier determinations of Went were caused by contaminants (viz., growth inhibitors) which seriously interfere with the biological assay tests. The presence of the inhibitor in the assay material would reduce the response of the coleoptile and therefore give too low an estimate of the auxin content. This in its turn would result in too low an estimate of the diffusion constant and therefore too high a value of the molecular weight. Subsequent extractions of 23,000 *Avena* coleoptile tips gave a preparation in which colorimetric chemical tests (Tang and Bonner's test—see later, p. 78) suggested that at least 80 per cent. of the auxin activity could be attributed to β-indolylacetic acid. Nevertheless, such colour reactions still cannot be regarded as conclusive, since it is by no means certain that they are specific for β-indolylacetic acid. In the leaves of cabbage, for instance, there are considerable amounts of a substance extractable with acid ether which gives the Tang and Bonner colour reaction but which is inactive in the *Avena* test (Holley, Boyle, Durfee

and Holley, 1951). The final decision on these questions can only be made when actual crystalline hormones have been isolated. Thus, although auxins A and B have not been isolated from plant materials by workers other than Kögl and his colleagues, yet this does not hold for β-indolylacetic acid. It is not surprising, therefore, that this compound is coming to be regarded as the main natural hormone, and, as we have already noted, a number of plant physiologists are beginning privately to express their doubts as to the existence of auxins A and B.

Very strong supporting evidence for this comes from chemical studies of auxin precursors in the plant. As far back as 1932 it was shown that the mould *Rhizopus suinus*, one of the earliest natural sources of β-indolylacetic acid, developed a high auxin activity by the oxidation of amino-acids from peptone (Bonner, 1932). Later it was shown (Thimann, 1935) that this mould could convert the amino-acid tryptophane to β-indolylacetic acid (*cf.* Hopkins and Cole, 1903). The most probable reaction sequences followed by this biological conversion are shown on page 55. When we turn to the higher plants we find that there are considerable indications that a similar process may be taking place. The evidence for the existence of a precursor easily transformed into auxin by such organs as the coleoptile tip has already been briefly reviewed. By direct experiment with tryptophane it has been shown (Skoog, 1937) that this compound could act as a precursor and was converted to an active auxin by coleoptile tips. Zinc-deficient plants of tomato are very low in auxins (Skoog, 1940; Tsui, 1948) and this is correlated with a low tryptophane content (Tsui, 1948). It seems that zinc is necessary for the synthesis of tryptophane from which the auxin is presumably derived. Spinach leaves have been demonstrated to possess the capacity to produce auxin from solutions of tryptophane injected into them, and an enzyme responsible for this conversion has been isolated (Wildman, Ferri and Bonner, 1946). The auxin produced has all the properties of β-indolylacetic acid although it has not been isolated in the crystalline state. On the other hand, the enzyme does not activate β-indolylpyruvic acid, tryptamine or β-indolylacetaldehyde, which are the supposed intermediates. However, considerable data are now accumulating to show that β-indolyl-



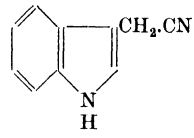

acetaldehyde is a compound occurring widely in plants and that it can be easily converted therein to β-indolylacetic acid. Larsen (1939–44), who made the first demonstration, showed that it can reach a concentration of 4 milligrams per kilogramme in cabbage leaves and that it can be easily oxidized to β-indolylacetic acid by an enzyme system (aldehydrase) present in soil. It was originally thought that the compound itself was a hormone, but the general concensus of opinion now is that it first requires to be oxidized in the plant.* Confirmation of the

* The compound 2, 3, 6-trichlorobenzaldehyde has been shown to have a higher auxin activity than the corresponding acid (Jones, Metcalf and Sexton, 1951), but it is still possible to explain such effects as due to a more rapid absorption of the aldehyde followed by its rapid oxidation in the plant to the effective acid.

aldehyde nature of this neutral "precursor" has come from the formation of a reversible bisulphite addition compound and by inactivation with "dimedon" (Gordon and Sanchez Neiva, 1949). At the same time it was shown that in pineapple tissue there is an enzyme which will convert this aldehyde into the acid and that β-indolylacetic acid production in the leaf is blocked by "dimedon". This is strong evidence for the suggestion that β-indolylacetaldehyde is the immediate precursor of β-indolylacetic acid in the pineapple. In contrast to the results with spinach leaves, both the acid and the aldehyde were shown to be produced from tryptophane and β-indolylpyruvic acid by crude enzyme preparations. This still leaves unsettled the question of whether the path of conversion goes normally through tryptamine or through β-indolylpyruvic acid.

Further evidence that β-indolylacetic acid is the only auxin of pineapple was obtained by use of a specific inactivating enzyme from pea stems (Tang and Bonner, 1947). This attacks β-indolylacetic acid by oxidizing the side chain and seems to be specific for certain indole-alkyl-carboxylic acids, *e.g.* β-indolylacetic, β-indolylpropionic and β-indolylbutyric acids (Wagenknecht and Burris, 1949). The auxin activity of extracts of pineapple leaves was destroyed by the action of this enzyme. All this evidence, however, is very largely indirect, and we must therefore look for more sensitive methods for the purification, concentration and chemical identification of natural auxins in order to resolve this impasse. Much promise is at the present time being given by the application of the various methods of chromatography (Linser, 1951; Bennet-Clark *et al.*, 1952; Jones, Henbest, Smith and Bentley, 1952; Luckwill, 1952 *a*) and by the two solvent counter-current technique of Craig (1944) (Holley, Boyle, Durfee and Holley, 1941). These studies, in which the behaviour of the natural auxin is compared with that of pure crystalline synthetic β-indolylacetic acid, are indicating strongly that this compound, or its derivatives, is the main source of auxin in both urine and green leaves. We have already mentioned above, the occurrence of indole-3-acetaldehyde in cabbages and the possibility of its being an auxin in its own right. Even more recently, a second neutral "auxin" has been isolated from

Brussels sprouts leaves using chromatographic separation on activated gypsum columns, and this has been identified as indole-3-acetonitrile (VIII) (Jones, Henbest, Smith and Bentley, 1952).




VIII

This compound, which is highly active in all the main tests for auxins, is present in amounts greatly in excess of the acid auxins fractions, which are negligible in quantity. This again raises even more urgently the question of whether such neutral "auxins" are true auxins or β-indolylacetic acid precursors: this particular enigma will be more fully discussed later in the chapter. The vital significance of these last isolations lies in the fact that in sprouts, at least, virtually all the auxin "potential" (it is perhaps unwise to talk of auxin activity until we know for certain that the nitrile molecule itself has such activity) resides in this one neutral molecule. The possibility that the insignificant acid fractions may contain the elusive auxins A or B seems very remote.

Direct attempts to solve the mystery of auxins A and B have been made, both in Kögl's laboratory and by E. R. H. Jones at Manchester, by the methods of synthetic chemistry. The problems involved in the synthesis of such complicated molecules with their many asymmetric carbon atoms and the many possible stereo-isomers, is no easy one, and so far no success has been achieved (Kögl and de Bruin, 1950; Brown, Henbest and Jones, 1950). It is interesting to note in passing that of the many synthetic molecules that have been tested, not one with a five-membered ring as nucleus (*cf.* auxins A and B) has been shown to possess any appreciable auxin activity.

In spite of this rather impressive amount of data in favour of β-indolylacetic acid and closely related compounds as the main source of auxin activity in plants, there still remains a small hard core of observation indicating the presence of other auxins. Most of this data concerns the occurrence in plant extracts of residual activity remaining after β-indolylacetic

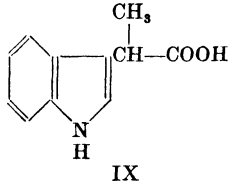
acid has been destroyed by hot acid treatment; such residua activity has been reported from the mould *Phycomyces* an from a variety of green plants (see review by Larsen, 1951) Except for stability to acid there are no other data as yet t identify this residuum with auxins A or B.

Another important outcome of recent chromatographi separations, especially from the point of view of the *Avena* tes and other biological assays on simple extracts, is that the have confirmed the occurrence of highly active growth-inhibitor (Linser, 1951). The bearing of this on the validity of much o the early work has already been discussed. A new and excitin phase in the natural auxin story is now beginning. It i hoped that before long we may be in a position to form a clear picture of the detailed chemistry of the natural auxin and their formation and interconversions.

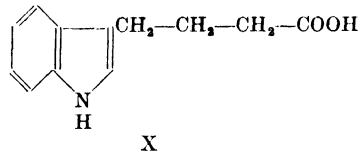
## SYNTHETIC COMPOUNDS

*Structural Requirements.* — Naturally after the isolation of β-indolylacetic acid as an active plant growth hormone, plant physiologists looked around for other compounds of similar chemical constitution and similar physiological activities. In the year 1935, several workers announced the discovery of a number of such compounds, which included other indole carboxylic acids such as α-(indole-3)-propionic acid (IX), γ-(indole-3)-butyric acid (X) (Zimmerman, Hitchcock and Wilcoxon, 1936) and β-indolylpyruvic acid (VI) (Haagen-Smit and Went, 1935), and also α- and β-naphthylacetic acids (XI and XII), phenylacetic acid (XIII) and anthracene acetic acid (XIV) (Zimmerman, Hitchcock and Wilcoxon, 1936). Later on, naphthoxyacetic acid (XVI) was added to the list (Irvine, 1938), and still later the phenoxyacetic acid (XV) series (Zimmerman and Hitchcock, 1942). The relevant formulæ are as shown on page 59.

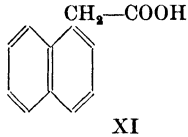
Very early on, however, it became apparent that, although such compounds closely resembled one another in possessing the properties of auxins, they differed widely between themselves as regards specific activity and that estimates of that activity varied with the nature of the assay used in the deter-



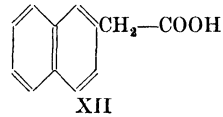

IX



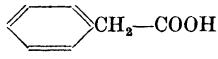

X



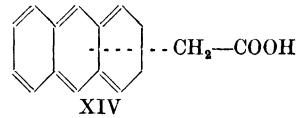

XI



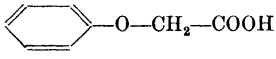

XII



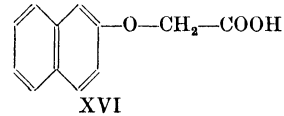

XIII




XIV

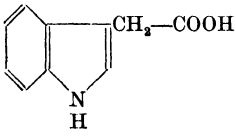



XV

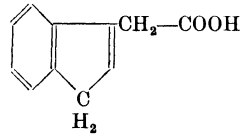



XVI

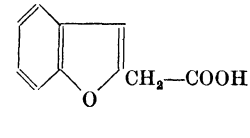
minations. This was shown in the work of Thimann (1935) who investigated the activities of indole-3- (III), indene-3- (XVII) and coumaryl-2-acetic acids (XVIII).




III




XVII




XVIII

Indene-3-acetic acid had a very small and coumaryl-2-acetic acid no activity in the *Avena* curvature test, whereas a considerable activity was shown both in the *Avena* coleoptile straight-growth test and in the pea test. These two latter compounds therefore, although possessing auxin activity, were prevented by some secondary property of the molecule from showing this activity in the *Avena* curvature test. The small curvatures produced were very largely confined to the upper portion of the coleoptile, and suggested that impeded transport of these two latter compounds accounted for their very low activity. Thus it is evident that the overall activity of any one synthetic molecule in any one test may be no indication of its

actual activity in promoting growth. Secondary properties of the molecule affecting its availability at the site of its action, *e.g.* the rate of penetration into cells and tissues, the rate of transport from the site of application and the rate of inactivation or destruction in the tissues of the plant, will all greatly affect the ultimate response. The importance of this last factor has been recently emphasized by the isolation from pea stems of the specific inactivating enzyme for indole carboxylic acids. Thus, whereas transport rates may be the main vitiating factor for accurate activity comparisons in the *Avena* curvature test, the action of this enzyme may introduce the greatest error in the pea test. As we shall see later, the operation of such secondary molecular properties may be of the greatest importance when we come to their application in agriculture and horticulture.

In spite of these difficulties, which are unavoidable in the methods at present available for the estimation of auxin activities, much work has been and is still being done on the interrelationships of chemical structure and activity in a very wide range of synthetic compounds. Before going on to consider the results of such investigations in detail, it will be useful to set down the general structural requirements for auxin activity drawn up from the early work on this subject. These generalizations were first put forward by Koepfli, Thimann and Went (1938) and were based on the pea test (primary activity). They may still be regarded as fundamentally sound, although modifications in the light of some recently discovered exceptions have subsequently been suggested (see later). These generalizations are as follows:—

The active molecule must have

(1) A ring system as a nucleus.
(2) At least one double bond in this ring.
(3) A side chain possessing a —COOH group (or a group easily converted into a —COOH group).
(4) At least one carbon atom between the ring and the —COOH group in the chain.
(5) A particular spatial relationship between the ring system and the —COOH group.

It will be seen that all the compounds so far mentioned, including auxins A and B, conform to these requirements. In addition, secondary properties affecting transport, inactivation rates, etc., are determined by such molecular characteristics as

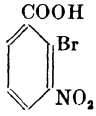
(1) Length of the side chain.
(2) Nature and degree of substitution in the nucleus and the side chain.
(3) The basic structure of the nucleus (Thimann and Went, 1937).

*Effects of Variation in Structure on Auxin Activity.*

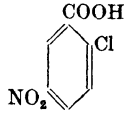
(1) **The nature of the ring.**—It has been seen that the nature of the ring has a great effect on activity. As a rule, five-membered ring compounds are without activity, although auxins A and B would seem to be the exception. Again, the substitution of carbon and of oxygen for nitrogen in the heterocyclic ring of indole greatly reduced the primary growth-activity of the corresponding auxins. On the other hand, naphthalene and anthracene give compounds about as active as $\beta$-indolylacetic acid, although in the former the position of the side chain greatly affects the activity (see later). On the other hand, the phenyl-carboxylic acids have a low relative activity, *i.e.* about 10 per cent. of the three mentioned above. The probable reasons for these differing activities will be considered later, but the number of double bonds apparently play an important part. This has been demonstrated in the case of $\alpha$-naphthylacetic acid and phenylacetic acid in which progressive hydrogenation of the double bonds in the nucleus results in a progressive activity loss which is complete in the saturated ring compounds, cyclohexaneacetic and decahydronaphthaleneacetic acids.

(2) **The nature, length and position of the side chain.**—According to requirements (3) (above) of Koepfli, Thimann and Went, the —COOH group must be separated from the nucleus by at least one carbon atom. It has, however, been suggested by recent work that this minimum requirement is not strictly valid. Thus considerable auxin activity has been reported in the following compounds (Zimmerman and

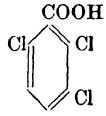
Hitchcock, 1944; Bentley, 1950; Veldstra and van de Westeringh, 1952),



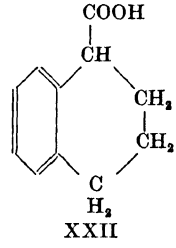

XIX




XX




XXI



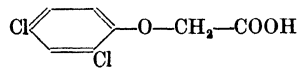

XXII

where the —COOH group is attached directly to the ring. The possible explanation of this activity will be considered later. Nevertheless, there is no doubt that maximum activity is shown by compounds having one bridging carbon atom between nucleus and —COOH group. When the number of these bridging atoms is increased there is generally a fall in activity, but this fall does not run parallel with the increase in the number of carbon atoms. Thus in the higher homologues of $\alpha$-naphthylacetic acid (XI) (Grace, 1939), $\beta$-indolylacetic acid (Thimann and Bonner, 1938) and 2, 4-dichlorophenoxyacetic acid (XXIII) (Synerholm and Zimmerman, 1947), much greater activity is shown by those compounds having an odd number of bridging carbon atoms in the side chain than by those having an even number. The underlying causes of this periodic activity are obscure, although it has been suggested (Synerholm and Zimmerman, 1947) that the low activity of the derivatives with the even number of carbon atoms in the side chain is due to the fact that they are easily oxidized by $\beta$-oxidation to the corresponding inactive phenol, whereas the homologues with the odd number of carbon atoms would yield the corresponding active phenoxy-acetic acid. Direct experimental evidence for this has been obtained by treating flax plants with a range of homologous $\omega$-phenoxy-alkyl-carboxylic acids (general formula $C_6H_5$—O—$(CH_2)_n$COOH (Wain, 1951). After absorption by the roots, considerable quantities of the phenol were shown to be formed only in those plants that had been treated with acids having an even number of carbon atoms between carboxyl groups and ring.

The position of the side chain may also have a very great

effect in asymmetric ring systems. This is best illustrated in the α- and β-naphthylacetic acids: the former has an activity in various auxin assay tests as high as that of β-indolylacetic acid, whereas the activity of the β-compound is very slight. It is possible that this surprising difference may be due to secondary properties of the molecule, although a theory, based on molecular shape, has been advanced to account for it in terms of true auxin activity (see p. 70). If the difference is as fundamental as this, it may well prove a most important lead to the nature of their ultimate action in the growth of the plant cell.

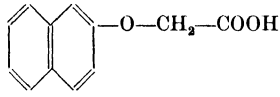
The substitution of other elements to link the side chain to the nucleus can also greatly modify the activity. Oxygen so introduced in phenylacetic acid to form phenoxyacetic acid has little effect on the activity, whereas in the naphthylacetic acids the effect is marked. With α-naphthylacetic acid (XI), which has high activity, its introduction to form α-naphthoxyacetic acid causes a very great reduction in activity, but with the slightly active β-naphthylacetic acid (XII) the reverse is true. Thus the effects of position of the side chain on the resultant activity of the molecule is seen to be closely dependent on the nature of the ring, and one is tempted to look for an explanation of these effects in the stereo-chemistry of the molecule. Such considerations will be dealt with in more detail later when more data have been presented. Although oxygen can bring about this greatly enhanced activity as a linkage element in the side chain, other elements or groups can have the reverse effect. Thus, 2, 4-dichlorophenoxyacetic acid (XXIII) (2, 4-D) is a compound as active as β-indolylacetic acid in certain assay tests (e.g. *Avena* coleoptile cylinder straight-growth test).



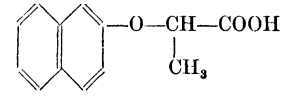

XXIII

Replacement of this oxygen by sulphur lowers the activity considerably (Wain, 1949; Wilske and Burström, 1950). With —NH— groups there still seems to be some doubt, as a high activity has been claimed for the 2, 4-D homologue with that linking group, *i.e.* 2, 4-dichlorophenylglycocoll (Veldstra and Booij, 1949), while the corresponding homologue of β-napthoxy-

acetic acid has negligible activity (Wain, 1949). On the other hand, 2, 4-dibromophenylglycocoll stimulates the proliferation of cells in tissue cultures of Jerusalem artichoke (*Helianthus tuberosus*) in the same way as β-indolylacetic acid and 2, 4-D (Longchamp and Pacheco, 1950). This effect is closely correlated with auxin activity. Nevertheless, in the sulphur homologue the reasonably high activity is reduced to very low values by the oxidation of the —S— group to an —SO— or an —$SO_2$— group (Wilske and Burström, 1950) and it is suggested (Erdtman and Nilsson, 1949) that this is caused by the removal of the two lone electrons of the —S— group, activity being dependent on the presence of these electrons at a suitable distance from the —COOH group. Such considerations are also supposed to determine the activity of the other auxins and of β-indolylacetic acid and 2, 4-dichlorophenoxyacetic acid. On the other hand, Wain (1949) has pointed out the necessity, for auxin activity, of at least one hydrogen atom attached to the α carbon atom of the side chain. Thus



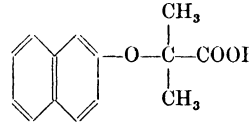

and




XVI
β-naphthoxyacetic acid

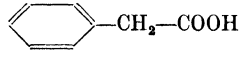
XXIV
α-(2-naphthoxy)propionic acid

are highly active while

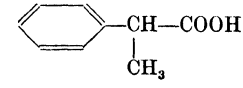



XXV
β-naphthoxy*iso*butyric acid

possesses negligible activity. Similarly in the phenyl-aliphatic acids



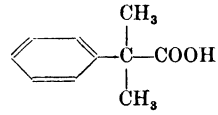

and




XIII
Phenylacetic acid

XXVI
α-phenylpropionic acid

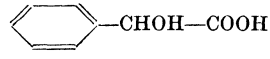
are active whereas



XXVII

phenyl*iso*butyric acid

has negligible activity. The same phenomenon was shown to occur with several series of phenoxy acids (Wain, 1951). Wain suggests that this hydrogen atom plays an essential part in the fundamental biochemical processes in which auxins are involved, including not only the promotion of stretching growth in oat coleoptile curvature and cylinder tests, but also in other growth phenomena (*e.g.* induction of seedless fruits in the tomato, initiation of rooting in cuttings, etc.—see Chapters 5 and 7) (Osborne and Wain, 1951). An effect probably related to this is the disappearance of activity when a hydroxyl group is substituted for hydrogen in the side chain. The best example of this is mandelic acid (XXVIII) which is completely inactive as an auxin, whereas the parent acid, phenylacetic acid (XIII), has a moderate degree of activity (Thimann, 1951).



XXVIII

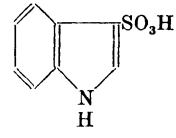
A change in the physical properties of the molecule and a greatly enhanced water solubility may, however, be playing a part in this effect.

(3) **The terminal —COOH group.**—In the majority of instances active compounds have shown a terminal —COOH group or a group easily converted into one. With the esters of $\beta$-indolylacetic acid the activity decreases with the increasing size of the esterifying alcohol, this probably running parallel with the ease of enzymatic hydrolysis of the ester in the plant (Kögl and Kostermans, 1935). From time to time there have been indications in the literature that the activity of sodium salts of the auxins have a greater specific activity than the corresponding acids. It seems most likely that such observed

differences may be due either to secondary properties of the salt (*e.g.* ease of penetration to the site of action) or to a specific stimulation effect of alkaline ions on auxin activity (Thimann and Schneider, 1938). The activities of some esters have been reported to exceed those of the corresponding acids (Zimmerman and Hitchcock, 1936 and 1937), but this again may be merely a secondary property of the molecule bringing about a more rapid tissue penetration. There are, however, a few compounds which do not possess a terminal —COOH group, but which nevertheless have definite activities in some tests. In many instances the activity of these compounds is held to be due to their conversion in the plant to the corresponding acid. Both β-indolylacetaldehyde and β-indolylethylamine (tryptamine) have already been discussed in relation to auxin precursors. Other aldehydes (*i.e.* α-naphthylacetaldehyde and phenylacetaldehyde) have also been shown to be easily converted to the corresponding acid by enzymes present in *Avena* coleoptile tissue and that two molecules of the aldehyde gives one of the acid: the conversion is therefore probably a dismutation (Larsen, 1951*a*). From this one would expect the activities of the aldehydes in the *Avena* test to be exactly half those of the corresponding acids, whereas the actual ratio is of the order of 0·06 to 0·11. This discrepancy may be the reflection of a slow penetration or translocation of the aldehyde or its sluggish conversion to the acid in the coleoptile tissue. Amides may be similarly converted to the corresponding acids, since it has been shown (Jones, Metcalf and Sexton, 1949) that a very close correlation exists between the activities of the amides of a wide range of synthetic auxins (*e.g.* 2, 4-dichlorophenoxyacetic acid, 2-methyl, 4-chlorophenoxyacetic acid, 2, 4, 5-trichlorophenoxyacetic acid and β-naphthoxyacetic acid) and the activity of their corresponding acids. Indole-3-acetonitrile, recently isolated from cabbage leaves (Jones, Henbest, Smith and Bentley, 1952) is another molecule that may be converted to β-indolylacetic acid and this we might expect to account for its high auxin activity. This molecule, however, possesses special properties that put it into a category different from that of indole-3-acetaldehyde. It has, for example, a much higher auxin activity than β-indolylacetic acid itself when tested in

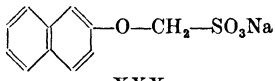
the *Avena* coleoptile section straight-growth test. In Went's *Avena* curvature test their activities are comparable in low concentrations, but β-indolylacetic acid is relatively more active in high concentrations. On the other hand, the nitrile is inactive in the Went's pea-stem curvature test except at high concentrations. Such confusing discrepancies demonstrate the inadequacy of any one assay used by itself in estimating auxin activity and underline the innate difficulties of biological testing. The higher activity of the nitrile in the *Avena* straight-growth test may be explained by its more rapid entry into the tissue and its rapid conversion therein into β-indolylacetic acid. A much more logical explanation arises from the observation that β-indolylacetic acid activity in the pea test is greatly increased by the presence of the relatively inactive nitrile. The nitrile may therefore enter the coleoptile tissues and be partially converted into the acid. The unconverted nitrile could then augment the activity of this acid to an extent sufficient to give the apparently superior activity reported for it in this tissue (Osborne, 1952). The relatively low activity in high concentrations in the *Avena* curvature test is also explainable in terms of its more rapid transverse movement across the coleoptile such that concentration differences and therefore growth-rate differences between the two sides cannot be maintained. This is to some extent supported by indications of a more rapid polar transport of the nitrile in coleoptile tissue. Clearly, at the moment there is no incontrovertible proof that the nitrile is active *per se*.

There are other compounds, however, in which the experimental evidence suggests that activity is not due to partial conversion to compounds with such a terminal —COOH group. One clear exception is indican (XXIX), a constituent of urine, with an activity about 5 per cent. that of β-indolylacetic acid (Veldstra, 1944).



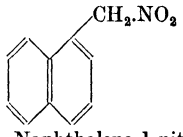

XXIX

On the other hand, compounds of the type

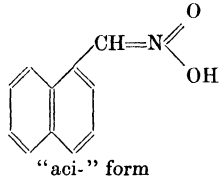


XXX

have been shown to have negligible activity (Wain, 1949). It would seem, therefore, that some activity may be imparted to an otherwise suitable molecule by an acid group other than —COOH. This is one explanation which has been evoked for the small but definite activity of the compound naphthalene-1-nitromethane (XXXI). It has been suggested (Veldstra, 1944) that in solution this compound is present as equilibrium mixtures of two tautomeric forms and that it is the "aci-" form of the molecule which possesses the activity

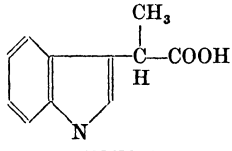


XXXI Naphthalene-1-nitromethane



"aci-" form

(4) **Stereo-considerations.** — We have already noted that a marked difference in activity exists between isomers of naphthylacetic acid and naphthoxyacetic acid, and we have suggested that the explanation for this might be in the difference in the shape of the molecule. This phenomenon was first indicated in 1937 by Kögl, who showed that for the optically active α-(indole-3)-propionic acid (XXXII) the dextro-rotatory isomer was about thirty times more active

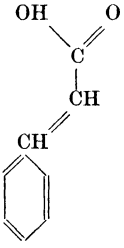


XXXII

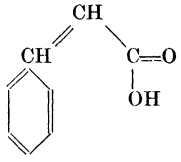
in the *Avena* test than the lævo-rotatory form. It appeared from later work, however (Kögl and Verkaaik, 1944), that both compounds were equally active in the cylinder test. It seems, therefore, that this difference in the *Avena* test is due to a secondary property of the molecule resulting in either

differential basipetal transport rates in the coleoptile or to differential inactivation by enzymes at the surface of the cut stump.

There is, however, strong evidence that the primary action of the auxins may also be determined largely by the configuration of the terminal —COOH group in relation to the ring system. Such evidence comes mainly from observations of the high primary activity in the cylinder test of *cis*-cinnamic acid (XXXIV) and some of its derivatives and the inactivity of the *trans*-isomer (XXXIII).



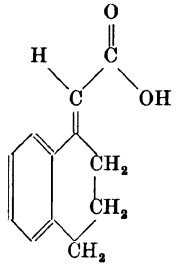

XXXIII *Trans*-cinnamic acid



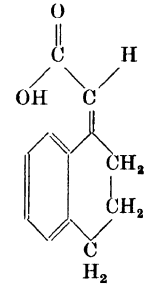

XXXIV *Cis*-cinnamic acid

Such facts have led Veldstra (1944) to postulate that for the molecule to show activity the —COOH group should not lie in the plane of the ring and that the maximum activity will arise when the dipole is perpendicular to that plane. He was led to that conclusion by contemplations of Stuart models of the above compounds. It will be seen that in both compounds the side chain is free to rotate around the —C—C— bond attaching it to the ring. In the *trans*- form the models show that this rotation would take place through 360° and that therefore the average orientation of the —COOH group would be in the plane of the ring. In the *cis*- form, however, complete rotation through 360° was not possible, and movement of the side chain around this bond was restricted to a lateral oscillation on one side. In this case the average orientation of the carboxyl dipole would be at a definite angle to the plane of the ring and have a component perpendicular to it. According to Veldstra this latter component is responsible for the activity of the *cis*-isomer. Similar considerations were shown to apply to the active *cis*- and inactive *trans*- forms of 2-phenyl-cyclopropane-1-carboxylic acid (Veldstra and van de Westeringh, 1951*a*).

This principle was supported by observations on the *cis-* and *trans-* isomers of 1, 2, 3, 4-tetrahydronaphthylidene-1-acetic acid (XXXV, XXXVI).

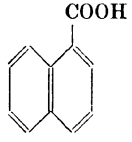


XXXV *cis-*

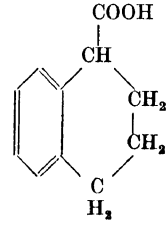


XXXVI *trans-*

Stuart models show that the *trans-* form, which is physiologically inactive, has the carboxyl dipole in the plane of the ring, whereas the *cis-* form cannot be constructed without some strain in the hydrogenated part of the nucleus and the side chain and therefore does not lie in that plane. There is, therefore, a component of the carboxyl dipole perpendicular to the plane of the ring, and this acid is physiologically active (Veldstra, 1944; Havinga and Nivard, 1948). Veldstra has, with some success, applied such theoretical considerations to the explanation of the activity differences of the napthyl- and naphthoxy-acetic acid isomers and also to account for the unexpected activity of the naphthoic acids and the substituted benzoic acids (see p. 62) (Veldstra, 1949). In the former compounds α-naphthoic acid



XXXVII



XXII

(XXXVII) has a very small activity in the pea test. Hydrogenation of the ring to form 1, 2, 3, 4, tetrahydro-1-naphthoic acid (XXII) greatly enhances the activity. This hydrogenation

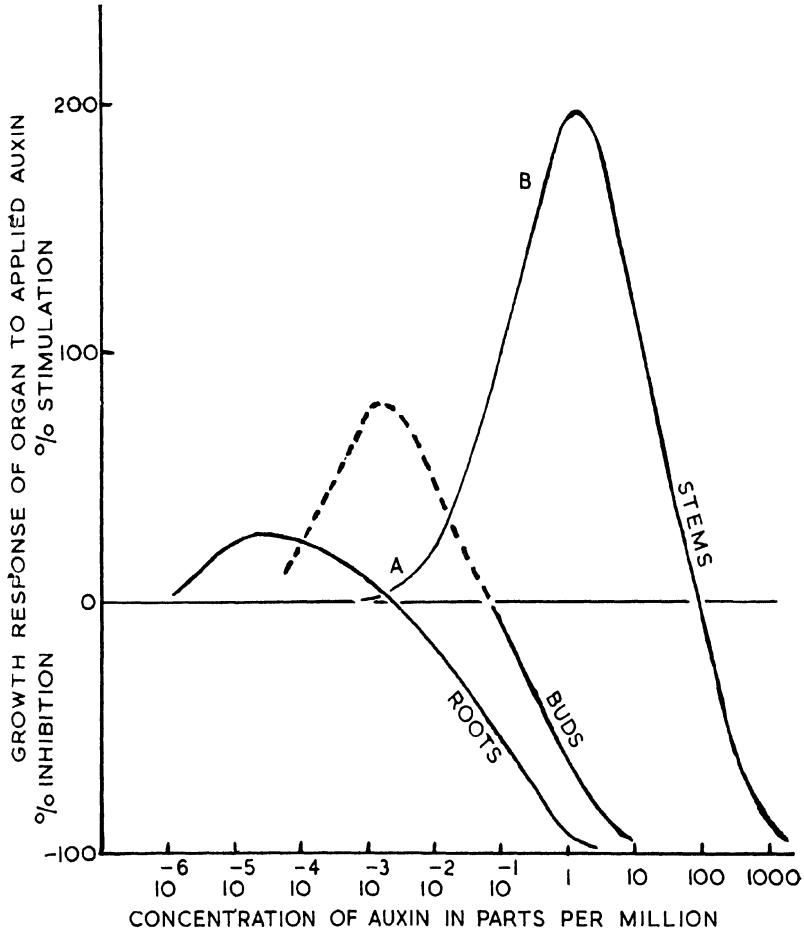



FIG. 11.—Diagram showing the different growth responses of the three main plant organs (stems, roots and buds) to auxins applied from outside. The curves illustrate the responses (stimulation or inhibition of growth rates) in relation to the applied concentrations (expressed as β-indolylacetic acid equivalents). The curves do not necessarily represent the relationships between *internal* auxin concentrations and growth rates.

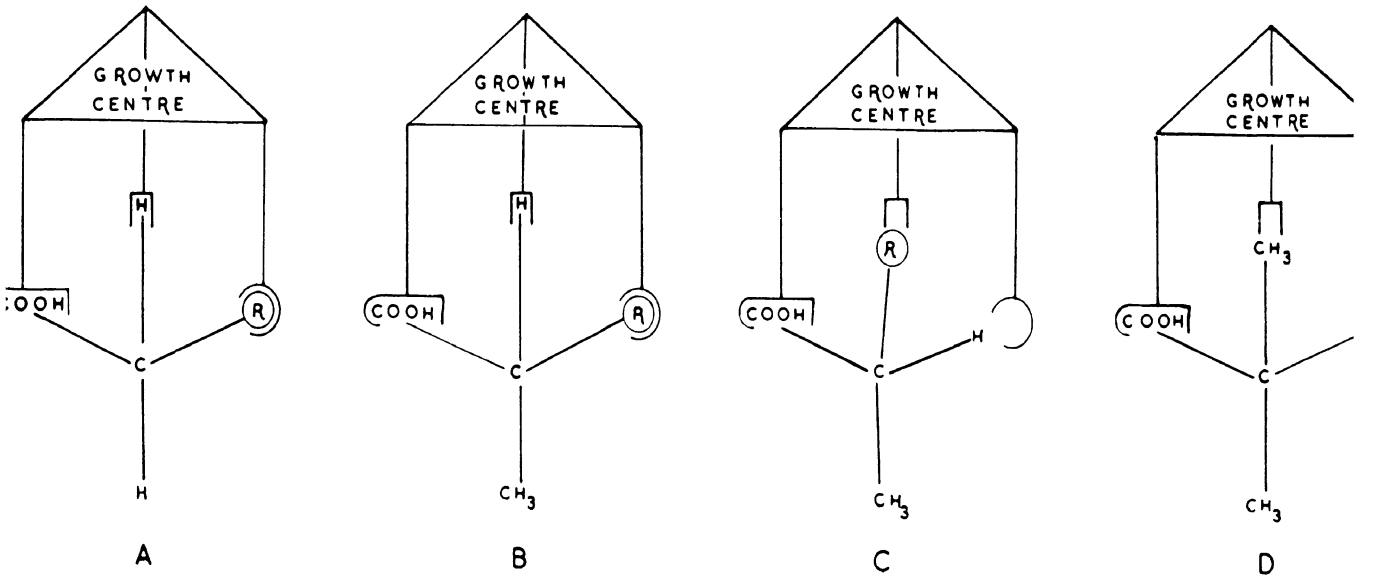



FIG. 12.—Diagrammatic representation of Wain's "three point contact" theory to account for the relationships between structure and activity in the auxins. (R) represents a suitable ring system.

A. Acetic acids (active).

B. (−) α-propionic acids (active).

C. (−) α-propionic acids (inactive).

D. *Iso*-butyric acids (inactive).

has produced an asymmetric carbon atom at the position where the carboxyl is attached, and this necessarily brings the —COOH group out of the plane of the ring and, on Veldstra's theory, increases activity. In certain substituted benzoic acids there is definite experimental evidence to support the view that, owing to interatomic forces, the carboxyl group is directed out of the plane of the ring, thereby giving the compounds auxin activity (Veldstra, 1952) (see p. 69).

Even if this is true it cannot be the whole story since it fails to explain many other equally remarkable phenomena. Thus Veldstra himself has shown that both β-naphthoic acid and 1, 2, 3, 4, tetrahydro-2-naphthoic acid are devoid of activity, whereas, on his theory, it is difficult to see how the latter compound could remain inactive. In addition, he has shown that the 1, 2, 3, 4, tetrahydro-1-naphthoic acid, having an asymmetric carbon atom, exists in two stereo-isomeric forms. These have been resolved from the racemic mixture and he has shown that in that mixture the activity was due entirely to the (−) form, the (+) form being completely inactive (Veldstra and van de Westeringh, 1951). How can we account for this on his theory? A clue to the true explanation comes from the work of Smith and Wain (1952), who have succeeded in resolving a racemic mixture of the compound α-(2-naphthoxy)-propionic acid (XXIV), which also has an asymmetric carbon atom. Here the (+) isomer has high auxin activity, not only in the pea test but also in five other auxin assay tests, whereas the (−) isomer is inactive. They have also shown that the corresponding β-naphthoxy*iso*butyric acid (XXV), which has no α-hydrogen atom in the side chain, has negligible activity. We have already seen that Wain regards the possession of such an α-hydrogen atom as essential for auxin activity (p. 64). As a result of these two findings, Wain suggests that activity depends primarily on three essential units of structure, *i.e.* an unsaturated ring system, a carboxyl group and at least one α-hydrogen atom, all having a definite spatial relationship with each other, so that the auxin molecule can "fit on" at the centre of its action in the growing cell (Smith and Wain, 1952). This "fitting" is visualized as involving a three-point contact of these essential structural units, each with its own specific reaction point in the growth

centre. Lack of any one of these three units or an unsuitable configuration of them will give no activity. This is illustrated in Fig. 12. (Ⓡ=unsaturated ring.) What is more, one would expect inactive compounds, possessing groups that fit at two points only, to compete with active molecules for the growth centres and thereby reduce their activity. That this is indeed the case has been shown by the very strong competitive inhibition of the auxin action of the (+) isomer of α-(2-naphthoxy)-propionic acid by the (−) isomer in the *Avena* cylinder test. This is very strong support indeed for the "three-point contact" theory. Subsequently racemic mixtures of α-(2, 4-dichlorophenoxy)-propionic acid and α-(2, 4, 5-trichlorophenoxy)-propionic acid have been resolved into their respective (+) and (−) isomers, and here too the (+) forms are highly active and the (−) forms inactive. The blocking, by the inactive form, of the expression of the auxin activity of the active form in the *Avena* coleoptile cylinder test has also been confirmed (Smith, Wain and Wightman, 1952).

It is most important that these facts have been established by the use of a number of distinct biological tests, involving the growth responses of whole organs as well as that of excised sections of growing tissue immersed in the solutions. It is, therefore, most unlikely that the differences observed between the two isomeric forms of these propionic acids are due to secondary properties of the molecules, *e.g.* penetrability, lability to inactivating enzymes, etc., but are fundamental differences in auxin activity. This seems to be one of the most profitable lines of research on the chemistry of auxins at present under way.

(5) **Substitution in the nucleus.**—Early work on substitution in the pyrrolene ring of β-indolylacetic acid showed in all cases a reduction in activity (Kögl and Kostermans, 1935). This was least for a $—CH_3$ group, greater for $C_2H_5—$, and activity was completely destroyed by an $—OCH_3$ group in any position. Substitution of the hydrogen atom attached to the nitrogen brought about the greatest reduction in activity. On the other hand, mono-substitution of halogens or a methyl group in the phenylene moiety gives an increased activity (pea test), the

greatest effect being obtained in the 4 or 6 position (Hoffman, Fox and Bullock, 1952). Similar marked effects of substitution are seen in all the synthetic auxins. We may take as the most studied example the series derived from phenoxyacetic acid, which itself has a weak auxin activity. Here substitution of halogen or lower alkyl groups into the ring may greatly increase the activity, which in some compounds (*e.g.* 2, 4-dichloro-phenoxyacetic acid) and in some tests (i.e. *Avena* cylinder test) equals that of $\beta$-indolylacetic acid itself. Owing to their relative ease of synthesis and to their high activity, a very wide range of such substituted phenoxyacetic acids have been prepared and tested. So far it has not been possible to formulate any rigid laws relating activity to nature and degree of substitution. The following broad generalizations can be drawn however. Effectiveness of substitution decreases in the following sequence:

Cl Br $-CH_3$ I

Substitution in the 2 and 4 positions is usually more effective than in the 3 position. Single substitutions usually give less active compounds than double substitutions, but further increase to tri- substituted compounds and over gives no further improvement and may reduce the activity considerably. There are very strong indications that substitution in both ortho-positions in phenoxyacetic acids completely destroys activity (Muir, Hansch and Gallup, 1949; Hansch and Muir, 1950; Seeley and Wain, 1950), suggesting that essential chemical reactions in the plant in which auxins take part involves a combination at this carbon atom. A more generalized form of this requirement has appeared from work on the toxicity in high concentrations (see Chapter 9) of the complete range of —Cl substituted acids (Leaper and Bishop, 1951). These authors state that at least two carbon atoms para to each other in the ring must be left unsubstituted for such activity to be retained. Whether this same relationship holds for true auxin activity still remains to be confirmed. When we consider the substituted benzoic acids, however, the above considerations certainly do not hold, since, of the di- and trichloro- derivatives, 2, 3, 6-trichlorobenzoic acid shows the highest auxin activity

and also the greatest toxicity to seedlings when used in high concentrations (Jones, Metcalf and Sexton, 1951). Two independent hypotheses have been advanced to explain the activity of this compound. One we have already considered is in terms of the —COOH configuration theory (see p. 69) in which the two ortho-substituted chlorine atoms force the —COOH dipole out of the plane of the highly lipophilic ring, giving it thereby a configuration similar to that in the phenoxyacetic and naphthoxyacetic acids. The other hypothesis (Muir and Hansch, 1951; Hansch, Muir and Metzenberg, 1951) attempts to reconcile these results with the above theory of chemical action at the ortho position. On theoretical grounds, that cannot be discussed here, it is held that the ortho chlorine atoms are easily displaced by nucleophilic plant substrate groups in the relevant growth reaction. A production of ionic chlorine from this molecule during response has also been claimed. Weighing strongly against the latter theory is the observation that 3-chloro-2, 6-dimethylbenzoic acid is just as active in the pea test as the 2, 3, 6-trichloro- homologue and in this former compound any displacement of the methyl groups by nucleophilic substrate groups is entirely precluded (Veldstra and van de Westeringh, 1952). The activity is, however, rationally explainable on Veldstra's own theory. There are of course many other puzzling problems. Thus, one of the most active of the substituted phenoxyacetic acids is 2, 4-dichlorophenoxyacetic acid (XXIII). The replacement of chlorine by a methyl group in the 2 position gives a slight increase in activity, whereas, as judged by the relatively higher activities of the monochlor- over the monomethyl-compound, the activity might have been expected to have been lower. A possible explanation of these substitution effects will be considered in the next section.

### *The Physico-Chemical Attributes of Active Molecules*

In recent years, in the search which has been going on for new and active synthetic auxins, it is obvious that an exact knowledge of the mechanism of auxin action in the plant would have been of great service. To this end, therefore, much

attention has been paid to those chemical and physical properties of active molecules which are closely correlated with hormone activity. Thus it has been repeatedly observed by many workers that extension growth of such diverse organs as *Avena* coleoptiles, pea and cress roots, etc., is much greater in acid than in neutral or alkaline media. The optimum pH usually falls between 4 and 5. An explanation, on an auxin basis, was soon forthcoming and turned on the fact that all known auxins were weak acids. Assuming, therefore, that the hormone is active only in the undissociated state, we can account for the optimum of growth on the acid side of neutrality. Further support for this hypothesis came from a number of independent researches, which showed that the activity of synthetic auxins in various tests, *e.g.* the cylinder test, was considerably greater in acid than in alkaline solutions, and that, within experimental error, it varied inversely with the degree of ionic dissociation of the auxin, as calculated from its pK value. It should be noted, notwithstanding, that these facts have not remained entirely undisputed (Lundegårdh, 1949; Rietsema, 1950). Furthermore, in comparing a number of synthetic hormones, it was noted that their activities were inversely proportional to the strengths as acids, but that, if calculated per unit of undissociated acid, activities were of the same order (Bonner, 1938). Thus cinnamic acid, with an activity, calculated on a total concentration basis, of 10 per cent. that of $\beta$-indolylacetic acid, now showed an activity identical with it. It was therefore supposed that the main differences in activity of the synthetic auxins were due to this ionic dissociation in aqueous solution. More recently, however, it has been shown that this correlation is by no means universal. It does not explain the different activities of the naphthaleneacetic acids and their higher homologues (Veldstra, 1944), or of phenoxyacetic acid and its mono-halogen derivatives (Muir, Hansch and Gallup, 1949). The author also has shown that, on this basis, 2, 4-dichlorophenoxyacetic acid is about 500 times as active as $\beta$-indolylacetic acid (Audus, 1948) (see also van Overbeek, Blondeau and Horne, 1951). Although, therefore, ionic dissociation of the acid undoubtedly affects the overall activity of the molecule under any given total concentration,

yet there are other overriding properties that determine its intrinsic activity.

Another line of research was stimulated by theoretical deliberations on the possible function of the obligate double bond in the nucleus. On the analogy of the mode of action of certain known enzyme systems, it was supposed that the auxins might be playing the part of organic catalysts in some metabolic process essential for extension growth and that the catalytic action might depend on a reversible oxidation-reduction of this double bond. As a result of experiments designed to check this possibility, the Dutch worker Veldstra (1944) stumbled upon a property of the auxin molecule which shows a considerable degree of correlation with the growth-promoting activity. This was the tendency of active compounds to be adsorbed at a water-mercury interface. Thus compounds of high auxin activity were strongly adsorbed at the interface and those of low activity were not. This was shown to hold for the naphthaleneacetic acids and the chlorinated phenoxyacetic acids. Exceptions were found, for example, in *cis*- and *trans*- cinnamic acids which showed identical surface activities but markedly different auxin actions (see p. 69). As a result of these findings Veldstra has modified the structural requirements postulated by Koepfli, Thimann and Went. This modification states that the primary requirement of the ring is that it shall have a high surface activity, and, as we have already seen, the —COOH dipole must have a component perpendicular to the plane of the ring. Veldstra visualized the action of the auxins as depending on their adsorption on to the lipoid constituents of the coloidal membranes which are supposed to form the outer boundary of the cell protoplast. Here a powerful "turgescent" action is exerted on the membrane, whereby its permeability to dissolved substances is increased. In this way food supply to the growing cell, and thereby its growth, is regulated. Using the rate of outward diffusion of the red pigment from beetroot slices as a measure of the permeability of the cell membranes to that substance, Veldstra (1949) compared the surface activity and effect on permeability of several members of a homologous series of halogenated phenoxyacetic acids. He found that the two ran closely parallel, but the auxin

activity in the series was not similarly correlated. Indeed, it rose to a maximum in compounds having an intermediate value of surface activity. Further modifications were therefore suggested; namely, that a correct balance should be maintained between the lyophilic activity of the ring and the hydrophilic activity of the carboxyl (or similar) group terminating the side chain. Much evidence has been produced in support of these contentions, but there are still too many exceptional cases for these proposals to be regarded as more than an interesting working hypothesis.

## CHEMICAL TESTS

We have seen that in all physiological work on natural auxins the only tests that could be applied up to the present were the very sensitive but, by chemical standards, inaccurate and extremely non-specific biological assays, such as the *Avena* test, the pea test, the cress root test, etc. Mention has also been made of the promise held out for the future by the application to this assay of the methods of chromatography which will provide both sensitivity and specificity but will still retain the inherent inaccuracies of the biological assay. It will, however, be possible with this method to identify and measure the various active substances from complex biological mixtures, a procedure which has so far been impossible.

It is, however, often desirable to determine the nature, and estimate the quantities, of synthetic hormones present in relatively large concentrations in some experimental material. For some synthetic compounds there are colorimetric chemical tests which are reasonably accurate and much more specific than the biological assay, although in most cases complete specificity is not obtainable. Some of the most important are discussed below.

### *β-indolylacetic Acid*

This is probably the most important auxin that biologists require to detect and estimate. Two of the most sensitive quantitative tests so far described are due to Mitchell and Brunstetter (1939).

(a) *Nitrite test.*—This is suitable for a concentration range of β-indolylacetic acid from 0·01 to 0·15 mg. per ml. To 50 ml. of the acid solution are added 0·5 ml. of 5 per cent. gum arabic solution, 2·0 ml. of a 0·5 per cent. solution of potassium nitrite and 0·4 ml. of concentrated nitric acid in that order. The mixture is shaken and left to stand for 2 hours at room temperature when a red colour will develop, the intensity of which can be estimated in a colorimeter against a standard β-indolylacetic acid solution.

(b) *Ferric chloride test.*—This is suitable for a concentration range of 0·02 to 0·1 mg. per ml. and can usually detect total quantities of the acid of the order of 2 γ. To 10 ml. of syrupy sulphuric acid add 0·5 ml. of M/10 ferric chloride. Then add 14·5 ml. of distilled water, shake and cool to room temperature. Then add 5 ml. of the aqueous β-indolylacetic acid solution. Shake again and cool to room temperature, when a red colour will develop. This can be similarly estimated in a colorimeter.

A slight modification of this method (Tang and Bonner, 1947) is claimed to give a more accurate quantitative measure over the range 5 to 100 γ β-indolylacetic acid. Two ml. of the aqueous β-indolylacetic acid solution are added to 8 ml. of the reagent which has the following constitution:

15 ml. of 0·5 M.$FeCl_3$,
300 ml. of $H_2SO_4$, sp. gr. 1·84,
500 ml. of distilled water.

After mixing, the colour is allowed to develop for a standard time of 30 minutes, since it fades slowly after the maximum has been reached.

A number of qualitative tests for the detection of this acid are also known and have been collected together and described by Nicol (1940). There is still no positive evidence, however, that any of these tests are completely specific for β-indolylacetic acid, although a modification of Tang and Bonner's ferric chloride reagent has been recently described and claimed to give a much better colour development and to be much more specific for β-indolylacetic acid than for other indole compounds (Gordon and Weber, 1951). In the modification perchloric acid is used in place of sulphuric acid. An analytical

scheme for the separation of the principal indole acids and related compounds is overdue. Here again the problem may be solved by the application of chromatographic techniques.

### *The Substituted Phenoxyacetic Acids*

These compounds, particularly the chlorinated derivatives, have, in the last few years, become perhaps the most important of the auxins, in view of their application to agriculture and horticulture. The compounds that are in most frequent use are 2, 4-dichlorophenoxyacetic acid, 2-methyl-4-chlorophenoxyacetic acid and 2, 4, 5-trichlorophenoxyacetic acid. It is obvious, therefore, that a method of detecting and estimating these compounds extracted from such materials as plant organs (estimation of absorption and distribution after external application), soils (to which they have been added as herbicides), etc., would be of great value to the experimenter. Very sensitive biological tests using the reactions of seedlings do exist (see Chapter 9), but, like the other biological assay methods, they are completely non-specific. One of the characteristic properties of these molecules is that they are very stable biochemically and, with very few exceptions, are not easily broken down by living organisms. This stability is probably linked with the fact that no satisfactory specific chemical tests have yet been devised for the detection and estimation of these compounds in low concentrations. It is possible, for example, to demonstrate 2, 4-dichlorophenoxyacetic acid as its uranyl salt (Freed, 1948) which has a very characteristic morphology, but the limits of concentration which are admissible for this test are 200 to 400 $\gamma$ per ml. One very sensitive colorimetric test has been devised by which concentrations of as low as 0·05 $\gamma$ per ml. can be detected. This is based on the reaction with 1, 8-dihydroxynaphthalene-3, 6-disulphonic acid (chromotropic acid) (Freed, 1948). The material under investigation is extracted with benzene and the extract evaporated to dryness in a test-tube. A few crystals of chromotropic acid are then added, followed by 2 ml. of syrupy sulphuric acid, the mixture being heated on a glycerine bath at 150° C. for 1½ to 2 minutes. The temperature must not be allowed to rise above this value

or charring will obscure the deep wine-purple colour which develops. This test, sensitive as it is, is by no means specific, being given by all the chlorinated phenoxyacetic acids that have been tested. In addition, purples, pinks and browns are given by a whole range of other organic acids such as acetic, butyric, oxalic and benzoic. However, it appears that if due care is taken to remove all interfering organic substances that might char in the treatment and if the density of the wine-red colour (after diluting the 5 ml. of heated sulphuric acid mixture to 50 ml. with water and filtering) is estimated from the light transmission at 5650 Å., then accurate determinations of concentration can be made down to 0·2 p.p.m. (Marquardt and Luce, 1951).

The high ultra-violet absorption by dilute aqueous or ether solutions of 2, 4-dichlorophenoxyacetic acid has recently been used (Bandurski, 1947). In both ether and water, absorption maxima were demonstrated at 2840 and 2300 Å. units. Using a spectrophotometer and silica absorption cells of varying length, concentrations of from 0·5 to 300 $\gamma$ per ml. could be estimated. In the original paper no indications are given of the accuracy of the method, although it is pointed out that it depends on the absence in the experimental solution of other compounds having appreciable ultra-violet absorptions. No doubt the method could be extended to other homologous compounds, but in many laboratories it is precluded on account of the expensive spectrophotometric equipment necessary.

Infra-red spectrophotometry also holds great promise for the exact identification and estimation of the various chlorinated phenoxyacetic acids which show very characteristic absorption spectra. It is even possible to use it for precise analysis of a mixture of these homologous acids, as has been done for the measurement of impurities in commercial preparations of the synthetic auxin 2-methyl-4-chlorophenoxyacetic acid (Sjöberg, 1950) (for practical use of this compound see Chapter 9).

CHAPTER 4

# AUXINS AS GENERAL GROWTH STIMULANTS

## GENERAL PRINCIPLES

IT might be thought from what has gone before, that the most obvious use of the auxins, both natural and synthetic, would be, by one or another means, to augment the general hormone concentration level in the various growing-points of the plant and so to increase their rates of growth. In this way an earlier, and perhaps even a greater, final crop might be obtained. A careful consideration, however, of our knowledge of the physiology of auxins in the general growth of the plant shows that the chances of this increase are not great. The picture is also complicated by the probability that the reaction of the plant to treatment will vary markedly, depending on the stage of development which it has reached.

The first stage in the growth of a plant is the germination of the seed, a structure containing inside the thin seed-coat an embryonic plant, complete with leaves, root and shoot, and containing, either in the embryonic plant itself or in a tissue surrounding it, a large reserve of food for the early growth stages. Given the right conditions of moisture supply and temperature, a complex sequence of biochemical processes will start inside the seed. The food reserves will be rapidly mobilized and the young embryonic plant will grow, bursting the seed-coat and sending roots downwards into the soil and shoot and leaves upwards into the light. In addition to the actual structural elements in the food needed for the growing organs, it has been shown that auxins, too, are rapidly produced during these early stages of germination, coming presumably from stored inactive precursors in the dry seed. We have seen that in the oat this conversion is carried out mainly by the tip of the first leaf. The evidence suggests that the supply of this auxin does not limit the rate of growth in the young plant. In these stages the supply of additional active auxin from an

external source would not be expected to accelerate growth-ates above normal. However, it is well known that seeds will ıot always germinate immediately on reaching full development n the seed-case. Many seeds require various maturation treat-nents even under otherwise extremely favourable external onditions. The seeds of hawthorn, for example, need an 'after-ripening" period of up to a year before germination vill start. In addition, many plants need special conditions uring this "after-ripening" period, *e.g.* exposure to low emperatures, before they are ready to germinate. An example f such a plant is the apple. Furthermore, as we shall show in later chapter, such pre-germination treatment may radically ffect the subsequent development of the plant; for example, ıe rapidity with which it reaches the flowering stage. Although irect proof is still needed, such phenomena have come to be onnected in the minds of physiologists with the hormone alance of the seed, and if such is indeed the case, supply of uxin to "immature" seed might easily modify the maturation equirements (see also Chapter 14).

When the plant passes from the stage in which it is dependent n seed reserves for its growth, the growth-rate becomes largely etermined by the net rate of carbohydrate production in the ght (=photosynthesis). Auxin concentrations do not directly ffect this photosynthetic rate, and its supply does not seem ) be growth-limiting except under certain adverse nutrient onditions. Thus there is some evidence to suggest that, in )mato and sunflower (Skoog, 1940), deficiency of zinc leads to ccessive inactivation of the natural auxin supply and that the esulting low auxin concentrations limit the growth of these nc-deficient plants. Nevertheless, although auxin treatments ıight not be expected to augment the growth-rate of a plant as whole, except in so far as this might be promoted by a more ıpid germination and greater seedling vigour, yet applied ıxin has a marked effect on the distribution of growth in the veral organs of the plant. Such reactions, which undoubtedly fect crop yield, will be dealt with briefly in certain of their spects in this chapter. Indirect effects of auxins on crop elds are dealt with in Chapter 9 on the use of auxins as lective weed-killers.

## TREATMENT OF SEEDS

### *Introduction*

Much attention was paid by workers in many countries during the period 1936–42 to the prospect of increasing crop yield by the treatment of seeds with auxins before sowing. In recent years, mainly owing perhaps to lack of positive results, the interest has flagged. The main aims of such treatments were—

(*a*) to improve the percentage germination of the seed,
(*b*) to accelerate the speed of germination, especially in those seeds where this is normally delayed,
(*c*) to increase the general vigour of the young seedling plant, hoping thereby
(*d*) to increase the final crop from the plant, whether root, leaf, seed or fruit.

Two main methods of seed treatment have been tried. The first involves soaking the seed for periods of 24 to 48 hours in very dilute solutions at concentrations ranging from one part in a hundred million to one part in a thousand. The seeds are then sown, either in the field or under a variety of experimental conditions in the laboratory, and subsequent germination and growth compared with similar seed soaked in water only. The compounds most frequently used were $\beta$-indolylacetic acid (IAA) * and $\alpha$-naphthylacetic acid (NAA), either free or as their alkali salts, but $\gamma$-(indole-3)-butyric acid (IBA) has also received attention. It should be noted that in some cases, *e.g.* sugar-beet (Zika, 1941; Bouillenne and Roubaix, 1942), water soaking alone was reported to cause an increased yield, although in both these crops a still higher yield came from auxin-treated samples.

The second method avoids the complication due to soaking and consists of dusting the seeds before sowing with a mixture of the dry auxin crystals suitably diluted with an inert powder such as talc. The same three compounds were investigated mainly at rates varying from two parts per million to the pure compound.

* From this point on, these abbreviated symbols will be used for all the more commonly used synthetic growth substances. For the sake of easy reference, they have also been included in the glossary at the end of the book.

This is not the place to attempt to analyze the welter of much conflicting data that has accumulated from these investigations. Some indication of the range and type of result can be gathered from two reviews by Avery and Johnson (1947) and Pearse (1948), and the interested reader should consult these works. It is notable that the most striking positive results have been obtained by two groups of workers only; Grace and his co-workers (1937–8) in Canada, and Amlong and Naundorf (1937–9) in Germany. Other intensive and extensive individual investigations designed to test these results have mainly failed to do so. The following is a brief summary of the most important of them.

*Results*

**Germination.**—A stimulation of the germination of seeds of oats, wheat, tomato and radish has been severally reported, but in each case an equal number of workers have had completely negative results. In sugar-beet and cabbage, where stimulations have also been recorded, later investigators found a partial suppression of the germination. In these latter plants the concentrations of the auxin had obviously been at toxic levels. Such a deleterious action of auxins in all but the lowest concentrations has been known for some time, and is particularly marked in the case of the highly active and stable 2, 4-dichlorophenoxyacetic acid (2, 4-D). This action is used in the inhibition of germination of weed seeds, a subject to be treated in more detail in a subsequent chapter (Chapter 9).

The above results were for normal quality seeds. Some attempts have been made, however, to increase the germinative capacity of old stored seeds. Some success was claimed for a variety of species (Amlong and Naundorf, 1937) by soaking for 24 hours in solutions of ·02 to 0·2 per cent. IAA. Some experiments have been carried out on the treatment of seeds requiring an "after-ripening" period for their germination (Barton, 1940). Apple seeds, which need a period of low temperature for this "after-ripening", were not affected by soaking in solutions of a number of auxins at concentrations ranging from 3·7 to 320 parts per million. Obviously treatment with auxins could not replace the low temperature "after-

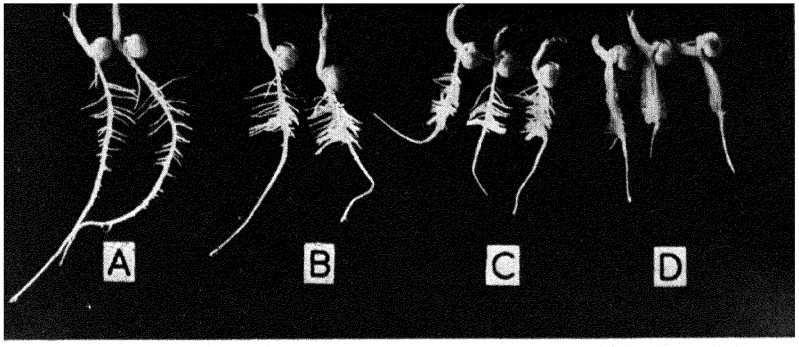



FIG. 13.—Seedlings of garden pea (*Pisum sativum*) showing the effect on root growth of a short exposure to auxins.

A. Ten-day-old plants grown continuously in aerated tap water.
B. Ten-day-old plants that had their main roots immersed for 24 hours on the third day of germination in 1 p.p.m. 2, 4-D, and then grown subsequently in aerated tap water.
C. As B, but treated with 3 p.p.m. 2, 4-D solution.
D. As B and C, but treated with 10 p.p.m. 2, 4-D solution.

Note the much greater lateral root production in all treated seedlings. On the average plants treated as in B produced two to three times as many laterals as the control in tap water, with otherwise little harmful effect on the seedling. In D the lateral root production has been so prolific that the roots have fused into three continuous sheets running the whole length of the treated portion of the main root. Growth, however, is very stunted

FIG. 14.—Hour-glass shaped radishes produced by soaking seed for 20 hours in 200 p.p.m. $\alpha$-naphthylacetic acid before sowing.

*Photograph by permission of Dr. L. C. Luckwill and Long Ashton Research Station.*



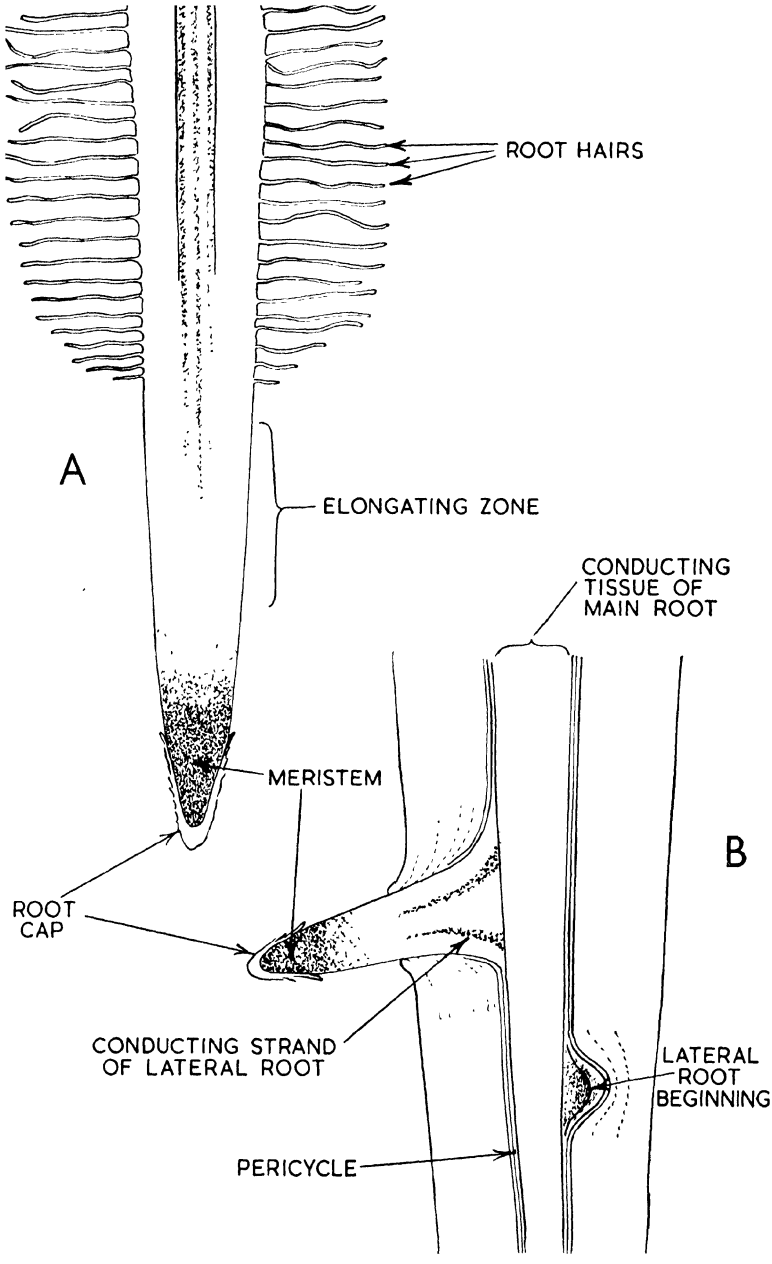



FIG. 15.—Diagram showing the principal features of root structure. A. Vertical section through the tip of a root from root hair region to root cap. B. Similar section from higher up the root showing the origin of lateral roots. (×20.)

ripening" conditions. With the American elm, a tree of similar requirements, a slight stimulation of germination was indicated with low concentrations of potassium naphthalene acetate. This was far below the response effected by the normal "after-ripening" procedure. It would seem that this latter process is not acting simply by increasing the auxin content of the seed (see Chapters 12 and 14).

Hormone treatment of seeds has also been used to try to counteract the deleterious effect of fungicides. On the assumption that fungicide treatment might inactivate the normal auxin complement and thereby impair germination, it was hoped that treatment with auxin preparations might offset this and restore normal germinative capacity. Formalin in dilute solutions has been extensively used in Canada for suppressing the growth of the cereal disease known as *smut*. Infected samples have varying proportions of non-viable seeds, and formalin treatment further reduces the germinative vigour of the viable fraction. Grace (1938) claimed that the inclusion of 0·01 to 1·0 parts per million of IAA or NAA in the formalin solution completely offset this harmful effect, and this was thought to be due to a direct stimulation of the young embryos. A small but significant effect of a similar type was reported as a result of the incorporation of up to 10 parts per million of the same two compounds into mercurial dust fungicides (McRostie *et al.*, 1938), by which the yields of both grain and straw of winter wheat were improved. More recently, a similar neutralization of the harmful effects of copper, mercury and zinc fungicides has been reported from England (Croxall and Ogilvie, 1940) by using 5 to 20 parts per million of NAA and "mixed naphthalene acetic acids" on lettuce and pea. With pea, an increase in crop yield of up to 80 per cent. was claimed, as compared with the controls sterilized by the normal heavy metal dressing alone. On the other hand, other workers (Templeman and Marmoy, 1940) have not been able to confirm such an effect in cereals and sugar-beet using mixtures of the above two hormones with the fungicides Angrosan G and Granosan. We must therefore conclude that these effects cannot be thought sufficiently well established to warrant modification of the routine dressing of infected seeds.

**Growth and Yield.**—Results in this field show that the response of plants varies widely with the species. In the summary of some of the main results which follows, the data will therefore be considered for the various types of crop in turn.

(1) *Cereals*: Most of the work has been done on wheat and oats in which a number of early workers reported considerable increase in both root and shoot growth as a result of seed treatment mainly with the diluted IAA preparations. Increases in total crop yields of up to 55 per cent. were reported by one worker for oats (Cholodny, 1936) and of 40 per cent. for maize (Bouillenne-Walrand, 1943). Smaller increases have been reported for barley and rice. With all these cereals, however, there is an equal amount of data showing no effect whatever on either plant growth or yield, and there are also observations of an inhibition caused obviously by the use of toxic concentration levels.

It is extremely improbable that auxin treatment of seeds will cause any augmentation of the auxin content of the mature plants and thereby bring about increased crop yields. Any such effects on yields would be very indirect ones and there is experimental evidence suggesting that they might be the result of an early direct stimulation of root-growth. Thus it has been reported (Friedrich, 1940) that treatment of a variety of common vegetables caused a great increase in the root/shoot ratios of the plants. Such a stimulation has been supposed (Thimann 1936, Thimann and Lane, 1938) to underlie the considerable augmentation in total dry matter in oat and wheat plants produced from hormone treated seeds. We shall see in a subsequent chapter (Chapter 5) that auxins have the property of initiating the production of roots on stems and leaf-stalks, and suitable concentrations applied for a short time may produce a manifold increase in laterals on a seedling tap-root (see Fig. 13). Auxins may even cause roots to branch even though they never normally do so (*e.g.* onion). Such an action of high hormone concentrations exerted for a short time in the early stages of seedling growth could produce a greatly extended root system. If such an early advantage were maintained during the later growth of the plant, increased yields

might easily be given as a result of increased water and mineral nutrient absorption. Until such points have been checked, however, and consistent reproducible results obtained, no practical routine hormone treatment of cereals is to be contemplated.

(2) "*Root*" *crops*: A stimulation of root-growth might be of particular advantage in the cultivation of "root" crops where the yield depends on the size and quality of a given tap-root, *e.g.* sugar-beet, carrot, parsnip, etc. The plant response noticed above, however (see Fig. 13), is purely one of increased production of side roots and not a promotion of growth in the main tap-root. But caution is needed in speculations on the possible reactions of such storage organs, since, for example, in the sugar-beet the base of the stem enters partly into the structure of the mature beet. In the radish the whole of the organ originates from the stem and not from root tissue. It is of relevant interest, however, that low concentrations of IAA in the medium in which seedlings of cress and mustard are grown can cause a considerable increase in the growth of the stem base of these young seedlings. Direct experimental work with such crops has been mainly concerned with sugar-beet, stimulated presumably by the early claims of Amlong and Naundorf to have obtained marked increases in yield by soaking seed for 24 hours in 0·2 and 0·02 per cent. solutions of potassium indoleacetate and potassium indolebutyrate. Their figures showed 31–40 per cent. increase in roots, giving 23–52 per cent. increase in sugar and 24–29 per cent. increase in tops. Results from a number of subsequent independent investigations, using the soaking technique, have been conflicting. Some have claimed increased root-growth, others no detectable effect, while a third group have noted reductions of yield. Dusting with 10 to 100 parts per million of the same synthetic auxins have no effect on the yield (Stout and Tolman, 1944). In the mangold no positive results have been reported. Interesting results have, however, been reported for carrot (a true root) from Germany (Söding, Bömeke and Funke, 1949). Treatment with a solution of IAA, supplemented by vitamins $B_1$, C and oestrone and also caffeine and nicotinic acid, gave increased crop yields up to 30 per cent. in plants grown in the shade but

no such stimulations in normal sunlight. How much of the stimulation was due to IAA alone is impossible to say from their data. They explain these results by suggesting that, in the shade, the growth substance is not produced at its maximum rate. In the sun the production is sufficient for the plant's requirements and so further additions can give no effect. This reasoning, however, rests on the assumption that, during and after treatment, seeds absorb and retain enough of this growth substance to exert an action for the whole period of its active growth; this seems unlikely. However, if this effect of light can be substantiated it may very well explain the discrepancies observed from the several results of sugar-beet investigations. In the case of radish, a stem storage organ, the claims of Amlong and Naundorf of a 98 per cent. increase in yield have not been borne out by any later work. Fig. 14 shows some results obtained on radish by Luckwill at Long Ashton. The seeds of these French breakfast radishes were soaked for 20 hours in 200 p.p.m. NAA before sowing. This treatment has obviously produced a marked effect, but the strange hour-glass-shaped product is hardly an improvement on a normal fully-formed radish.

(3) *Other crops*: A wide range of other crop plants have been investigated. They include tomato, tobacco, cabbage, lettuce, buckwheat, onion and white mustard, but, except for the claims of Amlong and Naundorf, no consistently positive results have been obtained.

**Hastening of the Onset of Flowering.**—Scattered throughout the relevant literature are a number of claims that seed-treatment significantly hastens the onset of flowering in plants developed from them and thereby brings about an earlier crop. This has been reported for oats, wheat and tomato by different workers. Some support for the plausibility of these claims comes from recent work on flower initiation by applied auxin in the mature pineapple plant. Since this subject belongs properly to another chapter, a further discussion of this aspect of auxin physiology will be left till then (Chapter 12).

**Treatment of "Seed" Potatoes.**—Increased yields of potatoes of up to 100 per cent. have been claimed (Zika, 1939; Malcher and Zika, 1943) as a result of soaking "seed" potatoes for

24 hours in dilute auxin solutions before planting. A net increase in the size of the starch grains was also claimed. Subsequent careful repetition of this work (Whittenberger and Nutting, 1949), using identical treatments (63 p.p.m. IAA), could confirm no effect on starch-grain size and showed that treatment caused delayed development and corresponding decrease in yields. Such a result is in line with the use of auxins to prolong dormancy in tubers (see Chapter 8), and it has been shown (Gandarillas *et al.*, 1949) that tubers, stored in the presence of such auxins and then used as seed, give yields that are as much as 25 per cent. lower than normal untreated tubers.

**General Conclusions.**—In surveying this mass of conflicting data it would be premature to discount the positive results obtained. In the conduct of such investigations, the number of possible experimental variables are very considerable. Thus the degree to which the applied auxins are absorbed by the seed has never been checked, and this may well vary widely with variety within the same species. Large differences in environmental conditions in the field (*cf.* the effect mentioned above of light action in carrot) may easily account for the large discrepancies observed. It is obvious, therefore, that the subject needs long and careful systematic study under accurately controlled experimental conditions before dogmatic statements can be made, but the predominantly negative results that we do possess hold out little hope of any marked economic gain from such treatments.

## TREATMENT OF SEEDLINGS AND GROWING PLANTS

Not so much attention has been paid to the auxin treatment of growing plants as to the treatment of seed before sowing. Furthermore, the wide range of treatment conditions, coupled with the scantier and equally confusing data, make any general conclusions very difficult. Nevertheless, there is no doubt that auxin applied to growing plants in very dilute solutions to roots or shoots is absorbed by the plant, and can, if suitable concentrations are used, provoke characteristic growth responses. These responses are generally in the nature of

modification of the growth of specific parts of the plant so treated, with no significant effects on the total gross accumulation of dry matter. These responses of specific organs belong to another aspect of auxin action and will be fully dealt with later. In recent years, use has been made of the fact that in concentrations above a certain minimum, auxins applied to growing plants are markedly toxic and can be used as weed-killers. This, too, will be considered in another chapter of the book (Chapter 9).

One general conclusion of relevance to this and the previous section is that the application of dilute auxin solutions to the roots of growing plants produces a definite stimulation of side root production. At this same concentration, however, there is a correlated inhibition of root elongation and thus a bushy stunted root system is produced. (See Fig. 13, C and D.) If the concentrations used are not too high or the application too long sustained, then a larger root system could theoretically result with consequent benefit to the plant. In practice these concentrations and exposures are usually very difficult to hit off, and an overall stunting may be the result. It seems that such treatment of plants for the promotion of a general increase in vigour is never likely to become a regular agricultural practice. The interested reader will obtain details of the most important results from the review by Pearse (1948).

## USE IN TRANSPLANTING

One of the main factors contributing to the growth of plants immediately after transplanting is the speed with which an efficient root system can be established. This is particularly so in the transplanting of nursery trees, and, in view of the reactions discussed above, the possibility of the use of auxins in accelerating this process would seem to be quite promising.

In most of the experiments described in the literature, the technique employed has been to soak the root systems of the uprooted plants for periods of up to 48 hours in dilute solutions of auxin ranging from $2\frac{1}{2}$ to 150 p.p.m. The auxin most frequently studied has been IBA and maximum effects have been evoked by the lower concentrations of 24 to 40 p.p.m.;

higher concentrations are definitely toxic. Considerable stimulation of root-growth was obtained in certain shrubby plants such as *Viburnum*, *Cotoneaster* and honeysuckle, and for some hardwood trees, *e.g.*, American elm, hard maple, black oak, pin oak, black walnut and eastern red oak in the United States. Conifers, on the other hand, seem much less responsive, although small positive results are reported for slash pine (Plank, 1939), loblolly pine (Maki and Minshall, 1945) and juniper (Swartley, 1941) with low concentrations. In some cases a correlated stimulation of shoot-growth was also claimed.

An ingenious method of treatment was devised for nursery pecan trees (Romberg and Smith, 1939). Five- to seven-year-old trees were taken and small transverse holes were drilled through the main roots. Into these holes were then inserted the centre 4 centimetre portions of ordinary wooden toothpicks which had been impregnated with various small quantities of IBA (1, 2 and 4 mg.). The growth response obtained was a greatly increased root production, but no effects were observable on the shoots. This has since been repeated by two independent workers using a slightly modified technique and with closely similar results. As far as the writer is aware, this treatment has not been adopted as a routine procedure in transplanting trees.

Results of the soak method on herbaceous plants are rather less consistent, markedly positive results having been claimed for broad bean, tobacco, cabbage and pansy (Amlong and Naundorf, 1941*b*), and for tomato, aster and certain perennial bedding plants (Swartley, 1941). Conversely, other workers could obtain no such beneficial results for tomato (Sayre, 1939) and onion (Sparks, 1945). The prospect here seems less favourable than for woody plants.

CHAPTER 5

# AUXINS AS INITIATORS OF NEW ORGANS: THE ROOTING OF CUTTINGS

## INTRODUCTION

So far we have considered the characteristic property of auxins for which the name was first applied, namely, the action in stimulating growth in length ("stretching growth") of cells in the relevant growth stage (Stage II: see Chapter 1). It has, by virtue of its historical priority, come to be regarded as the basic property. Auxins have, however, a number of other actions not only in this, but in other stages of cell-growth, and from their equally fundamental nature it is becoming increasingly obvious that the true function of auxin in the plant is a multiple one. The nature of the cell reaction depends on the state of its development, the types of tissue in which it is found, the concentration of the active auxin and so on. Since, as far as our information goes, all auxins possess the power to evoke the full range of plant response, of course with certain quantitative differences between various molecules depending presumably on secondary properties, it would seem that the time has come to broaden, in terms of plant response, the connotation of the name auxin, or perhaps substitute a new word.

One of the most important of these additional effects of auxin is its action in Stage I of cell-growth, *i.e.*, cell division. The effect is usually to stimulate this process, *i.e.* to induce meristematic activity in dormant, potentially meristematic cells, or to initiate it in mature cells of other undifferentiated tissues, and to stimulate further this activity when once it has started. Thus we have seen in Chapter 1 that in the higher plant left to its own devices the power to divide in this way is retained by the cells of apical meristems which are the growing-points of stems, buds and root-tips. We have also seen that these meristems are a source of auxin resulting probably from the



FIG. 16.—Range of rooting response of softwood cuttings of apple and plum after treatment with synthetic auxins.

*Top row.* Myrobalan B plum rootstock (moderate rooter). Control (untreated) showing callus formation and traces of roots.

*Second row.* Myrobalan B plum rootstock. Treated with a 24 hours soak in 30 p.p.m. NAA solution. Well rooted.

*Third row.* M.I. Apple rootstock (poor rooter). *Poor source.* Treated 20 p.p.m. IBA. No roots.

*Fourth row.* M.I. Apple rootstock. *Good source.* Control untreated. No roots.

*Bottom row.* M.I. Apple rootstock. *Good source.* Treated 20 p.p.m. IBA. Well rooted.

Ref.: Pearse, H. L. (1939). *Photographs by permission of East Malling Research Station.*

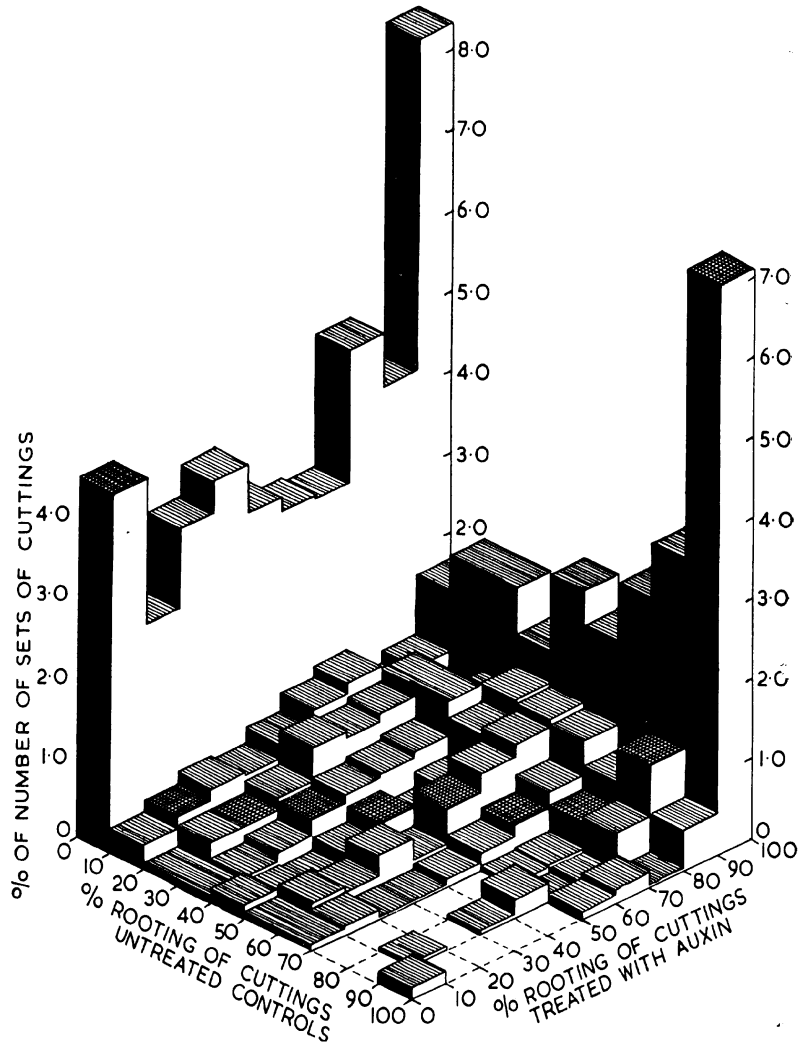



FIG. 17.--Three-dimensional frequency distribution diagram (histogram) showing the results of analysis of the rooting responses of woody cuttings (untreated and treated with auxins) taken from the lists of Thimann and Behnke (1947). For explanation see text (pp. 115–116).

conversion of a stored precursor of some kind. We shall see in a later chapter how this hormone production governs the growth of buds in many plants, such as the deciduous trees. The power to divide is also retained by a tissue known as the cambium, which is situated on the inner surface of the back in both stem and root and which brings about the growth in girth of these organs by the inward (centripetal) production of new wood cells and the outward (centrifugal) production of new bark cells. In this way the conductive capacity of the stem and root for water (carried in the outer layers of the wood) and for organic food made in the green parts (carried in the tissues of the bark) is increased to cater for the greater size of the plant. In all plants showing seasonal growth this activity is a periodic one, heightened in the spring and early summer and tailing away as autumn approaches. Both the quantity and nature of the tissue so produced changes during the period of activity, and this is the origin of the annual rings that characterize the wood of such trees. Recent work has shown that these annual waves of cambial activity are closely correlated with similar waves of auxin production by buds and young developing leaves, and it is not unlikely that a flow of auxin into the cambium from the buds may be the initiating stimulus for its renewed activity in the spring.

A more direct demonstration of this auxin effect is in its action in initiating root meristems in various plant organs. We have already seen that the application of auxins to roots may cause not only an inhibition of longitudinal growth but a great multiplication of the number of lateral roots, making the growth stunted and bushy. This is a direct result of the above action, in that a greatly increased number of lateral root meristems are thereby brought into being. These new side roots do not elongate, however, unless the auxin concentration is later lowered below the inhibiting level (see Fig. 13). The effect is much more striking and of great practical importance in other organs such as stems and leaves. Here application of suitable auxin concentrations can initiate root meristems in a range of different mature non-meristematic tissues, which would normally not show any further appreciable growth changes and would certainly not normally grow roots. The present chapter will

be concerned with this phenomenon and with other similar activities of auxins, but before analyzing it in more detail it will be convenient at this point to indicate some other auxin effects of horticultural significance.

Auxins are not always growth initiators or stimulants. They often exert the opposite effect and inhibit the growth of plant parts. In the growth of any particular organ, inhibition sets in after a certain maximum concentration of auxin has been passed and different plant organs show different values of this maximum (Fig. 11). Further increase in concentration leads to a general toxic action on all plant tissues. These phenomena and their practical importance will be considered in Chapters 8 and 9.

A third phenomenon of great interest, in which auxin plays a leading rôle, is the initiation and control of fruit-growth, or fruit-set as it is popularly called. The type of auxin action involved is still not fully elucidated, but it seems to be concerned principally with the general increase in cell size characterizing the final stages of fruit-growth. Full consideration to this aspect of auxin action will follow in Chapter 7.

In addition to the main reactions outlined above, there are large numbers of other plant responses that have some practical value. We can do no more here than point to their existence and refer to Chapters 10 to 12. But in case the reader should feel somewhat confused at the multiplicity of auxin actions and should begin to wonder however they can remain discrete, let it be reiterated that the secret is probably that each separate response has its own effective auxin concentration range, and furthermore, the behaviour of each organ will be determined by age, state of development and other factors. Nevertheless it is hoped that these underlying general principles will become clear as the separate phenomena are dealt with in turn.

## THE NATURE AND ORIGIN OF ROOTS

As an introduction to the subject it might not be out of place to discuss briefly the nature and function of roots. What then, may we ask, is a root? A root is a cylindrical organ, often branched, growing vertically or obliquely downwards

into the substratum on which the plant is living and serving thereby two important functions:

1. Anchorage and support for the aerial portions of the plant; and

2. The absorption of water and dissolved mineral salts essential for continued plant-growth.

All roots have an actively meristematic tip protected by a sheath known as a root-cap. This root-cap is constantly being worn away during the growth of the tip into the soil but is continually renewed from the underlying meristem. The growth in length of all roots follows the plan outlined in Chapter 1, cell production being carried on in the meristem by the process of cell division (Stage I). Older daughter cells furthest from the tip meristem then pass over into Stage II and elongate to many times their original length, possibly under the action of auxins in very low concentrations. Then follows Stage III in which these fully elongated cells are differentiated and take on specific functions in the mature root. Those in the central region become modified, some for the upward conduction of the water and dissolved salts and others for the downward transport of organic food from the stem to provide the raw materials for root-growth itself. The cells on the outside of the root develop long unicellular strands (root hairs) that grow out between the solid soil particles and therefore come into close contact with the thin films of soil solution that surround them. In this way the total area of contact between living root cells and soil is greatly increased and their absorptive activity correspondingly enhanced (see Fig. 15). Still higher up the root, where further differentiation and growth in thickness has taken place, the outer layer of the root forms an impervious protective layer of corky tissue, and the root hairs in these upper regions, being robbed of their food supply, die and are lost.

From these few considerations of root structure and growth the following important observations can be made:

(*a*) Active growth in length of the root is confined to the apical few millimetres, and so there is no movement of the root ***through*** the soil except in this limited tip region. That all such

relative movement should be over before the root hairs develop is an obvious necessity to prevent damage to these delicate structures.

(*b*) The region of active absorption is confined to the root-hair region, supplemented slightly by the actively growing tip. This absorbing region of the root may be quite limited in its extent, depending largely on the rate at which differentiation and growth in girth is taking place above. The efficiency of a root as an absorbing system is determined therefore by the number and size of its root-hair regions.

Most broad-leaved plants (dicotyledons as the botanist calls them) have a main stout central root (the *tap-root*) from which arise numerous side roots which in turn bear secondary branches to give ultimately a root system of great complexity. The second great group of higher plants, the monocotyledons, usually have unbranched roots which arise in large numbers from specific areas of the stem, to give a dense brush-like nature to the resulting *fibrous root* system. Both the laterals of the tap-root system and the individual fibrous roots arise in the internal tissues of the parent root or stem respectively. Their origin is usually from a particular layer of undifferentiated cells at the edge of the central conducting tissue. This layer is called the *pericycle* (see Fig. 15). Their ultimate origins can be traced to a cell or small group of cells in the parent tissue which, for some reason, changes from undifferentiated non-growing cells into actively dividing cells of the new root-tip. Growth and development, according to the normal pattern, results in the new lateral root bursting its way through the outer tissues of the parent organ and finally establishing itself in the soil. Meanwhile the conducting elements of the new root establishes early connection with the main conducting system. Evidence is now rapidly accumulating that the stimulus which starts the original cell on its career as a root-tip meristem could be auxin, and this opens up a whole field for speculation and further research.

Apart from the normal production of roots by pre-existing roots in tap-root systems and from well-defined portions of the stem in fibrous-root systems, roots may often be produced under certain conditions from parts of the plant not normally

associated with their production. Thus many plant stems, if for some reason they become prostrate, may develop roots from the base where they come into contact with the soil. Typical examples are strawberry runners and carnation layers. Again, it is thought highly probable that one of the main stimuli starting root production is a higher auxin concentration induced on the lower side of the stem by virtue of its horizontal position. We have already seen in Chapter 2 how such an accumulation in horizontal stems and roots is held to give rise to the phenomenon of geotropism.

The fact that such rooted organs may be separated from the parent and grow into new plants is the key to the valuable horticultural practice of increasing plants by vegetative propagation. The importance of this culture method, in contrast to propagation by the sowing of seed, is that a uniform race of plants identical with the parent is obtained. Such uniformity is usually quite impossible to obtain from seed reproduction. The reason for this takes us into the realm of genetics or inheritance and cannot be considered here. The horticulturalist induces the rooting of organs while still attached to the parent by the methods of layering, stooling, and marcotting, in which the appropriate stem regions are covered with soil or other suitable medium. By far the most commonly used method, however, is the rooting of plant organs after separation from the parent plant. Such isolated organs or *cuttings*, as they are called, may, when placed with their basal cut ends in a suitable medium (*e.g.* light soil, moist sand or *Sphagnum* moss) develop roots and establish themselves as new plants. All these practices have been the stock-in-trade of the gardener for centuries and descriptions of them can be found in the writings of the ancient Greeks and Romans.

It is now known that the rooting response of the cutting is initiated by the accumulation, at the basal end of the stem, of auxins arriving from the buds and leaves. The augmentation of this natural hormone supply by synthetic auxins supplied externally results in most cases in a manifold increase in root production. This use of synthetic auxins marked the beginning of their employment in practical agriculture and horticulture. But now we should return to the days that preceded the "auxin

era" and consider for a moment some of the features of normal rooting of the cutting and how the discovery of the participation of auxin was made.

## THE NORMAL ROOTING OF CUTTINGS

*Factors affecting.*—Almost any portion of the plant may serve as a cutting, but the organs most commonly used for this purpose are young vigorous stems. The ease with which such cuttings root, however, varies enormously with the species, as gardeners know only too well. Some species, such as willow and currant, form roots with the greatest of ease, even if placed simply with their cut ends in water. Many others root only with very great difficulty or not at all, though some intractable cuttings respond if given special treatments. In the pine family (Coniferæ), for example, the stems possess many fine canals running vertically in the bark, and these canals are filled with the resins characteristic of this family. These resins flow out over the ends of the cuttings and virtually seal them off, and this prevents adequate absorption of water. Initial soaking of the ends in very hot water will prevent this and result in a higher percentage of successful cuttings.

But these examples represent the two extremes, and the majority of plants show responses of an intermediate character, the number and vigour of the roots produced being greatly influenced by the source of the material (*i.e.* the part of the plant from which it was taken), the culture relations of the parent plant and the external conditions prevailing during the "striking" period. We can draw up no hard and fast rules for determining the most favourable conditions for any selected plant, as these conditions vary considerably from species to species. However, a few points may be made. In the first place there is a very wide range of types of cutting that can be used. The most common is the stem cutting, but root and leaf cuttings may sometimes be very satisfactory. With leaf cuttings the bud at the base, together with a small portion of stem, may or may not be included. Stem cuttings may be "softwood", from young growing stems that have not developed an appreciable amount of wood; they may also be "hardwood"

and are then taken from suitable mature woody twigs at the end of their growth period in the late autumn or early winter. The type of cutting and the time of taking it will depend on the nature of the plant, whether herbaceous, deciduous tree or shrub, evergreen or conifer, and suitable horticultural manuals should be consulted for details. As a general rule, however, leaves should be retained on all but the most basal portions of the cuttings in softwood and evergreen hardwood cuttings and sturdy buds left on deciduous hardwood cuttings, since these are most important sources of auxin and other rooting factors. The very largely empirical procedures of cutting preparation will also vary from species to species and with the type of cutting.

Having taken the cutting, we have to make sure that the conditions to which we subject it shall be optimum for striking. Briefly, such conditions are—

(*a*) Adequate water supply to the whole cutting. This can be ensured by making absorption as easy as possible and impeding the loss by that portion (including leaves) left above the rooting medium.

(*b*) Adequate aeration of the base of the cutting since the development and growth of roots are processes very susceptible to oxygen concentration and are completely stopped if it is too low.

(*c*) Suitable diffuse lighting and maintenance of an optimum temperature.

These conditions can be obtained by the use of a loose spongy rooting medium that will hold a large amount of water and yet be well aerated. A mixture of sand and chopped *Sphagnum* moss is usually ideal. With softwood cuttings a warm, moist frame or greenhouse in diffuse light is necessary. Hardwood cuttings can be struck outdoors with no special precautions of this kind, but for this reason they are correspondingly more difficult to strike.

Needless to say, long before the isolation of auxins, botanists had tried to stimulate cuttings to root by the application of a variety of substances. The idea of applying extra nutrients for growth was an obvious one, and some considerable success

followed the application of solutions of cane sugar. On the other hand, nitrate treatment prior to planting had the reverse effect (Knight, 1926). In addition, various other chemically unrelated substances were tested, some of which gave positive stimulations. One of these treatments was dilute potassium permanganate solution, another dilute acetic acid.* Such results, however, were never sufficiently consistent to warrant their adoption in normal horticultural practice. A comprehensive survey of early work with these various empirical stimulants will be found in the review by Pearse (1948).

### *Elucidation of the Action of Auxins*

The definite relationship between the presence of leaves on a cutting and its capacity to root naturally gave rise to speculation on its underlying causes. As early as 1880 the famous German botanist, Sachs, had postulated the formation of a specific rooting substance in leaves, but, in the years which immediately followed, botanical opinion tended to favour a nutritional rôle for leaves, and regarded the rooting factor coming from them as essentially a food supply. From then on, until the dawn of the "auxin era", opinions oscillated between one extreme and the other, neither being able to enlist the support of conclusive experimental evidence.

Our present understanding of the subject originates from the work of the Dutchman, van der Lek (1925), who made the first extensive investigation of the rôle of these internal factors in the rooting of cuttings. He demonstrated that in currant, willow, poplar and grape the presence of developing buds was a prerequisite for rooting, and that the intensity of root production was directly correlated with the rate of bud development. Cuttings with dormant buds (December or January) failed to root even under the most favourable conditions but in the spring, when the buds renewed their activity, rooting occurred. The removal of a ring of bark from a short section of stem below the buds also prevented rooting. To explain

* This may be due to acidity and not specifically to acetic acid itself, causing an increase in active auxin concentration in the treated portions of the cutting (see Chapter 3). The writer has found similar effects of acidity on the production of lateral roots by cress seedlings (Audus, 1948).

these phenomena, van der Lek suggested the formation of a hormone or hormones in the developing buds and its conduction in the bark to the base of the cutting, where it initiated rooting. This work was subsequently extended to show that leaves also were a source of this hormone. It was natural that, from these beginnings, attempts should have been made to isolate the hormone from leaves, and in the next few years extracts of such materials as paw-paw (*Carica papaya*, a tropical fruit) leaves, rice polishings and pollen were all shown to contain a substance stimulating root formation in cuttings.

By this time auxin had been isolated from urine, and when it, too, was tried a very marked root-forming activity was obtained. In 1934 Thimann and Went carried out a series of experiments in which they made a careful comparison of the properties of the root-forming hormone and of the auxins, and found them to coincide. This led (Thimann and Koepfli, 1935) to a direct testing of synthetic IAA and a clear proof that the root-forming hormone was indeed an auxin. Practical application followed rapidly on the heels of academic research, and in the same year Cooper showed that the application of lanolin containing small quantities of IAA to lemon cuttings was very effective in promoting rooting. In that year too, workers in the Boyce Thompson Institute in America demonstrated that similar responses could be evoked by other synthetic substances, viz. phenylacetic acid, α-phenylpropionic acid, IPA and NAA (Hitchcock, 1935; Zimmerman and Wilcoxon, 1935), and the career of the auxins as practical growth-regulators was well and truly launched. However, it is perhaps salutary to reflect that these were not really the first instances of the use of auxin in rooting. The old claim that better rooting could be obtained by inserting a germinating wheat grain in a slit made in a cutting, had its basis no doubt in a stimulation by an auxin from the grain.

## THE STIMULATION OF ROOTING WITH APPLIED AUXINS

### *Effective Compounds*

A question which immediately springs to the academic mind is whether all auxins, natural and synthetic, show this root-forming property and, furthermore, whether root-forming

activity runs parallel to the characteristic auxin activity of promoting stretching growth. The answers to such questions are also of great interest to the practical man anxious to find the best compound for treating his cuttings. To provide answers the plant physiologist developed a test involving uniform material from one plant species and carried out under strictly reproducible conditions. This test, also due to Went (1934), uses uniform lengths (about 4 inches long) of stem of pea seedlings grown in the dark (etiolated) and is called Went's pea test (not to be confused with the pea test for auxin activity; see p. 27). In essentials this test involves immersion of the slit tips of stem pieces in solutions of the compound under consideration, afterwards standing them with their basal ends in carefully purified 2 per cent. cane sugar solution in the dark for seven days. Any active substance absorbed by the cut apex is conducted to the base, where it initiates roots which develop at the expense of the sugar, the source of food. As a measure of activity the average number of roots formed per stem is taken. This test proved to be as important in the identification of these root-forming hormones as the oat test was in the isolation of auxin itself, for it formed the basis of Thimann and Went's (1934) experimental technique (see above). Subsequent work with it showed a close parallelism between rooting and auxin activities for a large range of compounds. A list of compounds, showing relative activities in growth and rooting tests, is given by Veldstra (1944).* Nevertheless, just as in the *Avena* test, secondary properties of the molecule may disturb the estimate of true rooting activity; for example, the low mobility of such compounds as indene-3-acetic acid and coumaryl-2-acetic acid necessitate a direct application to the base of the pea stem before the rooting action becomes apparent (Thimann, 1935).

Although of great use in a theoretical approach to the problem, the pea test has been of little value in the practical field, since it has long since been apparent that, within the more restricted range of relatively active compounds, the relative

* Statistical analysis of the data for the fourteen compounds for which activities in both the auxin test (pea test) and rooting test are recorded give a correlation coefficient of 0·98, which is highly significant.

effectiveness of auxins varies from species to species tested. These variations may well be due to secondary properties of the molecules such as relative ease of penetration of the tissue concerned and the speed of conduction in the plant, but in actual practice it means that a range of compounds have to be tested before one can be certain of obtaining the most effective substance for any given species. In addition, other unrelated properties of the molecule may further influence the choice. Thus above a certain concentration, toxic effects may set in to offset the root stimulation. In some species and with one particular compound the optimum rooting effect may lie below this toxic limit, whereas with other species or with other compounds it may not. There are no hard-and-fast rules that can be laid down, and the reaction of each species must be separately determined. There is no doubt, however, that these secondary properties of the molecule are of fundamental importance in deciding its value as a practical rooting stimulant. As we shall see later, comprehensive lists have been compiled showing the known reactions of a large number of species of horticultural importance and these can be consulted by the interested reader (see p. 377).

In spite of these variations, certain general points of interest concerning the relative effectiveness of compounds emerge from a perusal of the extensive literature and these will now be briefly considered.

In the first place, IAA, in contrast to what might be expected of it as a natural auxin, is not the most suitable compound to use in practice. Two others of equal rooting activity, $\gamma$-(indole-3)-butyric acid (IBA) and $\alpha$-naphthyleneacetic acid (NAA) are much superior because of their greater chemical stability and their low mobility in the plant. The first of these properties results in improved persistence and a correspondingly more prolonged action giving a better chance of success. The second property means that the compound is retained near its point of application and does not spread to other parts of the cutting and induce other growth responses. For example, IAA may spread into buds in this way and inhibit their early development and growth (see Chapter 8), a most undesirable effect. Such advantages as shown by NAA, however, are

offset by the narrow range of concentration over which this substance can be used. The toxic concentration limit of this compound approaches very closely to the optimum rooting concentration and great care is needed to ensure that this optimum concentration is not exceeded. Fortunately, IBA shows a much greater flexibility in this respect. For use in aqueous solutions, the sodium, potassium or ammonium salts of these compounds are to be preferred, since they are much more soluble than the free acids.

There have also been reports that the amides of IBA and NAA are more effective than either the free acids or their salts for the rooting of some species. α-naphthylacetamide (NAAm) in particular has not the high toxicity of its free acid and is correspondingly more flexible in its use. In view of the effectiveness of NAA, a wide range of its homologues have been tested, such as α-(1-naphthyl)-propionic acid, α-(2-naphthyl)-propionic acid, α-(1-naphthyl)-butyric acid and γ-(1-naphthyl)-butyric acid, etc., but none has proved to be superior. On the whole, the α-naphthyl- isomers are much more active than the β-isomers, and lengthening of the side chain results in lowered activity (Kruyt and Veldstra, 1947). It is of interest to note that whereas the *cis-* isomer of 1, 2, 3, 4-tetrahydronaphthylidene-1-acetic acid is extremely active, the *trans-* configuration gives a very low activity (Tincker, 1940). Incidentally, this is a very good example of the parallelism existing between rooting activities of these compounds and their basic auxin properties (*cf.* Chapter 3, page 70).

In the last few years a range of other effective compounds have come to the fore, mainly as the result of the work of Hitchcock and Zimmerman (1942) at the Boyce Thompson Institute in New York State. They compared with IBA and NAA a considerable number of the substituted phenoxyacetic acids. All the compounds were found to be active to some degree. The mono-halogen substituted derivatives were less active than IBA or NAA. The 2, 4-dichloro- and 2, 4-dibromo-derivatives showed either equal or greater activities depending on the nature of the side chain. Dimethyl- derivatives (xylenoxy acids) showed activities varying with the substitution position, the best being 2, 5-dimethylphenoxyacetic acid with an

activity equal to that of 2, 4-dichlorophenoxyacetic acid (2, 4-D). Still more recent reports (Hitchcock and Zimmerman, 1945; Osborne, Wain and Walker, 1952) indicate that the following compounds have activities comparable with or even greater than IBA and NAA:—

2, 4, 5-trichlorophenoxyacetic acid

α-(2, 4, 5-trichlorophenoxy)-propionic acid
α-(2, 4-dibromophenoxy)-propionic acid
α-(2, 4-dichlorophenoxy)-propionic acid
α-(2-chlorophenoxy)-propionic acid
α-(2-methyl-4-chlorophenoxy)-propionic acid

α-(2-chlorophenoxy)-*n*-butyric acid
α-(3-chlorophenoxy)-*n*-butyric acid
α-(2, 4-dichlorophenoxy)-*n*-butyric acid
α-(2-methyl-4-chlorophenoxy)-*n*-butyric acid
α-(2, 4, 5-trichlorophenoxy)-*n*-butyric acid

All these phenoxy compounds suffer from the disadvantage of having undesirable secondary properties that make their practical applications hazardous. Thus they are all prone to produce thick stunted roots and have a toxicity limit very near the optimum rooting concentration. Extreme care is therefore necessary in adjusting the concentration levels applied to avoid high cutting mortality. In addition the spread of these compounds up the shoot can affect its subsequent growth adversely. On the other hand, there is some promise that the trichlorophenoxyacetic acids in the above list, if used at very low application rates, are reasonably safe. Nevertheless, in view of their extreme potency and high associated toxicity, these compounds need much further study and much closer definition of practical concentration ranges before they can be recommended for general horticultural use.

Different compounds affect differently not only the quantity but also the quality of the roots they induce. For example, IBA treatment normally results in a few roots which rapidly become long and establish a strong fibrous root system. The phenoxyacetic acids, on the other hand, produce dense thick roots and a bushy and stunted root system. Much progress

has been achieved in regulating vigour and general structure of the root system by using appropriate mixtures of these compounds, since such mixtures induce roots with intermediate characteristics. In many cases a greater number of roots results from mixtures of IAA and NAA or IBA and NAA in equal proportions than from either compound alone at the same total concentration. IBA and NAA, separately or mixed, together with small amounts of 2, 4, 5-trichlorophenoxyacetic acid (2, 4, 5-T) (about 0·1 mg. per gm. of mixture) has proved to be a very effective combination. It is visualized that in the future we may be able to regulate precisely the exact size and structure of the induced root system by appropriate mixtures of suitable synthetic auxins.

*Standard Methods of Application* (Stem cuttings)

Various methods of application of the auxin have been evolved and these will be briefly discussed in the following sections:—

(*a*) Lanolin Paste Method.—Lanolin is a soft fat which is prepared from wool and which will dissolve auxins, and mixtures of this kind have been used by many experimenters for application to various plant organs. The paste, when applied to the base of the cutting, sticks firmly to it, does not dry out and thereby maintains a reasonably constant concentration of the auxin in contact with the treated part. Lanolin is completely without effect by itself, and IBA and NAA are dissolved in it at concentrations of 0·01 to 0·05 per cent. Nevertheless, the lanolin method is not so convenient in use as other techniques, and from the point of view of the practical man can be regarded as obsolete. It still finds valuable application, however, in the hands of the plant physiologist. Recently other, more fluid, proprietary wax mixtures have been suggested as vehicles for auxin applications, but they are unlikely to replace the better established methods which follow.

(*b*) Dilute Solution Method.—This is one of the earliest of the practical methods and is still regarded by many as the most effective. Concentrated stock solutions of the auxins are first made up in 95 per cent. alcohol (ethanol) and then diluted to

the appropriate strength in water. Concentrations ranging from 0·0005 per cent. to 0·01 per cent. are usually employed, depending on the species under treatment and the auxin chosen. The basal inch or so of the stem of the cutting is immersed in the solution for a period of hours in order that the required amount of the auxin should be taken up. This period will naturally vary with the type of cutting and, more particularly, with the environmental conditions during the soaking. In the case of leafy cuttings (softwood cuttings) the amount taken up will depend largely on the amount of water absorbed. This itself will be controlled by the amount of water lost from the leaves in transpiration, a process which increases in intensity with the dryness of the atmosphere. Thus under warm dry conditions excess of the auxin may easily be taken up and toxic concentration levels reached in the cutting. It is usual, therefore, to place these cuttings in a humid atmosphere during the soaking period to effect a much slower and more easily regulated uptake. For leafy cuttings of woody species of all types, 1 to 2 hours soak with a 0·01 per cent. solution or 10 to 24 hours soak with a 0·0005 per cent. solution respresents the average optimal conditions. With dormant hardwood cuttings taken in winter it is sometimes a practice to store them first at a low temperature (35–55° F.) for a month or two to allow their basal ends to form a good callus tissue * before treating with auxins. A similar soak method is then entirely satisfactory. Storage treatment of this kind has been found to be effective with holly, which is an evergreen.

Attempts have been severally made to accelerate the absorption of dilute solutions by an injection technique effected by exhaustion inside a vacuum chamber. The results obtained were not significantly better than those with the straightforward soak method, and therefore, in view of the special apparatus required, are of little interest to the practical man.

The concentrations of auxin given above are average. In practice the most favourable concentration to use will be determined by a number of factors of which the most important are the species from which the cutting is taken, the time of year

* Callus is an undifferentiated mass of wound tissue formed by the activity of the cambial cells near the wound. (See Chapter 6.)

it is struck and the compound used as a stimulant. Such details should be obtained from the many lists which exist summarizing the results of investigations on a large number of species. Failing this, a few experimental cuttings may have to be treated with a range of concentrations before venturing to treat the main batch of cuttings. It should not be necessary to stress at this point the extreme importance of keeping strictly to the directions which the makers send out with any of their proprietary preparations. The procedure they recommend is *optimal* and undue increase in the dosage may result in a complete loss of cuttings.

(*c*) Concentrated Dip Method.—A treatment which is rapidly gaining in favour is the application of the auxin in a concentrated solution in alcohol. The solution is made up in 50 per cent. ethyl alcohol of a concentration determined as above by species and conditions of striking. Concentrations of the order of 0·1 per cent. have been hitherto recommended, although it has been recently claimed (Hatcher and Garner, 1948) that, particularly under temperate conditions, much lower concentrations may be the best, and these are naturally more sparing of expensive auxins such as IBA. Cuttings, preferably done up in small bundles for ease of handling, are dipped to a depth of 1 inch or so in the strong solution, allowed to remain for 1 to 5 *seconds* and then removed and the alcohol allowed to evaporate in the air. In a short time only a very thin film of the growth substance is left covering the base of the cutting, which can then be planted immediately in the normal rooting medium. This method has two main advantages over the preceding one. In the first place, the long and often inconvenient soaking is avoided; secondly, the variable uptake, dependent on varying conditions during soaking, is also eliminated. In this way the amount of auxin applied per unit surface area of cutting base is constant and independent of external conditions. Hardwood apple cuttings were first induced to respond to auxins by this treatment (Hatcher and Garner, 1948) (see Fig. 16).

(*d*) Dust Method.—This is perhaps the simplest method of all and consists of applying to the base of the cutting a fine inert powder containing a small admixture of the solid auxin. Talc,

clay or finely powdered charcoal are most commonly used, and the order of concentration is 0·02 to 0·1 per cent. for herbaceous cuttings and about five times this concentration for hardwood cuttings. The dust mixtures are made by two methods. One is to grind the auxin crystals to a very fine powder and then mix them thoroughly with the carrier. This requires great care in ensuring uniform mixing. A better way is to soak the carrier thoroughly in an alcoholic solution of the active substance in suitable concentration. The alcohol is then dried off and the carrier re-ground if necessary. Treatment itself consists of moistening the basal $\frac{1}{2}$ to 1 inch of the end of the cutting with water, rolling it in the powder, gently tapping off the excess and planting immediately, taking care not to rub off the thin layer of powder when inserting in the soil. A small hole should therefore be made in the striking medium to allow of easy insertion.

This method offers the same advantages as the quick dip method in that there is no long soak period and the amounts of auxin applied are reasonably constant. However, it has the disadvantage that the physical properties of the carrier can greatly influence the effectiveness of the active compound, and different carriers may cause widely differing responses in the same plant even with the same concentration. Most workers are agreed that the best results are obtained when the powder is finely ground. It usually saves much time and trouble to use a good commercial brand of rooting powder, the effectiveness of which has been well and truly tested, and again makers' instructions must be followed implicitly. Particular care must be taken to see that all excess powder is removed from the cutting base before planting, or serious toxic effects may easily result. This is the critical point of the whole method. To avoid such excess, a small insufflator has been successfully used to supply the powder.

(*e*) Spray Technique.—Some success has been obtained by spraying leaves with dilute aqueous solutions of auxins. This treatment has been applied both to the mother plant before the removal of the cutting and to the cutting itself after insertion in the rooting medium.

Considerable success with certain evergreen shrub species

was obtained by spraying parent plants with 10–100 p.p.m. of 2, 4, 5-T or its sodium salt (Stoutemeyer and O'Rourke, 1945). Cuttings taken 9 to 40 days after this spraying showed essentially the same rooting response as cuttings treated by the more usual methods outlined above. Results of spraying plum layer-beds, however (Garner and Hatcher, 1945), or *Coleus* (Yeates, 1947), were not so promising.

Early tests on the effects of spraying cuttings during rooting were also not promising, but recent work in America (Mitchell and Marth, 1947) claimed good results with such plants as carnation, *Coleus* and marguerite. The addition of an emulsion of lanolin to the sprays is recommended for species requiring a long rooting period, as this is retained much longer on the foliage and allows a more prolonged action of the auxin. Much more work is required to be done with this spray technique before it can be adopted as a regular practice.

(*f*) Other Methods.—A variety of other methods have been tried all with varying degrees of success. A forty-eight hour leaf-soak method was unsuccessful. Direct insertion of auxin crystals under the bark or into the base of shoots was effective but was prone to give serious toxic effects. The insertion of toothpicks soaked in auxin has also been tried with success (*cf.* use in the transplanting of pecan tree seedlings, page 91). Attempts have also been made to improve the response by a second auxin treatment after a period in the rooting medium. Although success has been claimed for a number of cuttings that are normally difficult to root, it is by no means certain that the results justify the extra care and trouble needed for this double treatment. One manipulative technique which has proved to be of value in promoting auxin action is the splitting or cutting of the bark before dip or soak treatments. This may simply cause a more easy entry of the auxin, but it has been suggested that some part may be played by a natural wound hormone produced in response to tissue damage (see Chapter 6).

### *Auxin Treatments in other Propagation Methods*

We have considered in the previous section the methods that have been adopted for the auxin treatment of various types of

stem cutting. Let us now consider their possible use with other cutting types and in layering and allied techniques.

(*a*) Layering.—This is a method for inducing rooting from a stem before its removal from the parent plant. The selected portion is covered with a rooting medium (*e.g.* the soil in which the parent plant is growing) and, if conditions are favourable, roots will be produced from that portion of the stem. The stem can be bent over and pegged in a suitable position into the soil nearby, as in simple layering. A special layering technique has been developed for raising rootstocks such as plum in fruit-tree nurseries. The parent plants are set out in rows, and their shoots pegged down flat and lightly covered with soil so that the young shoots growing up from the buds are truly etiolated at their bases. The depth of soil is gradually increased as the new shoots grow, thus ensuring that the tips are never too deeply buried. Roots form at the bases of the shoots which, when dormant, are harvested, except for a few left to peg down for the next season's crop (Garner, 1943). Alternatively the parent plant can be cut back to a few inches above the ground and the stumps covered with soil. Numerous new shoots develop and root freely. This is called mound layering or stooling and is used commercially in a modified form for the propagation of apple rootstocks. In this technique the soil is not applied until after the young shoots have started to grow up so that the maximum yield of roots may be obtained. With simple layering it is often advantageous to remove a ring of bark about $\frac{1}{2}$ inch wide from the stem just below the region where roots are desired. This procedure will arrest the downward flow of food materials and auxins from the leaves above, causing their accumulation in the bark just above the ring, thereby stimulating rooting. When roots are well grown the shoot can be severed from the parent and established as a new plant. Little is known about the effects of auxin application to the rooting of layers. Application of the toothpick method has been reported as successful in the simple layering of pecans (*Carya* sp.) in America (Gossard, 1941). The spraying of plum rootstock layer beds with dilute auxin sprays at East Malling gave no promise of better rooting (Garner and Hatcher, 1945).

(*b*) Marcotting.—This is based on essentially the same principles as layering and indeed is sometimes called air-layering. The selected stem, instead of being bent down and pegged into the soil, is ringed and then wrapped just above the ring in a suitable rooting medium (e.g. *Sphagnum* moss or coconut fibre) and left to root, suitable precautions being taken to ensure that the medium is kept moist. Auxins have been used with considerable success in the promotion of rooting in marcots. The toothpick method proved effective in the marcotting of pecans. In India, 1–3 per cent. IAA in lanolin has been successful in stimulating rooting in young mango marcots. The application of concentrated solutions (0·5 per cent.) of IBA in alcohol to the ringed portions of marcots before covering with the *Sphagnum* rooting medium has been highly successful in quinine tree and cocoa tree propagation (Cooper, 1944).

(*c*) Leaf Cuttings.—In some plants isolated leaves, when placed in contact with a suitable medium, will develop buds and roots at certain well-defined spots on the veins, and new plants will develop from them. This is particularly true of species of *Begonia*, and the process is usually very much stimulated by previously cutting the main veins. The young plants then develop near to the cuts. Experiments in France have shown that soaking leaves in a 0·01 per cent. solution of IAA considerably hastened and improved root and shoot production in *Begonia* and in *Ficus elastica* (India-rubber plant). In other species, roots and buds develop from the base of the leaf-stalks when placed in a rooting medium. Auxins have been shown to stimulate root production in such leaf cuttings as African violet (*Saintpaulia*).

One method of propagating Easter lilies is to separate the old bulbs into their individual scale leaves, which, in a suitable rooting medium, will grow into a new plant. Workers in the U.S. Department of Plant Industry (Mitchell and Marth, 1947) have found that auxin greatly improves the rooting of the scale leaf cuttings, but there is usually a correlated increase in fungal rot. To avoid this, simultaneous treatment with a fungicide dust has been tried with great success. The most satisfactory treatment has been to dip the basal ends of the scale leaves in a

fungicide powder containing suitable concentrations of NAA and then plant in moist sterile sand to a depth of ¼ to ½ inch. The exposed portions of the scales were then covered with moist *Sphagnum* moss and left to root. A bud and roots developed at the base of each scale and a new plant grew up through the moss to be transplanted later into soil. A similar treatment with mixtures of auxin and fungicide have been found to be of great benefit in the propagation of narcissus bulbs and gladiolus corms.

(*d*) Leaf-bud Cuttings.—In such cuttings a leaf, with an axillary bud and a small portion of stem left attached, is separated from the parent plant. In a rooting medium roots will develop from the stem portion, and the bud will grow up to form a new plant. It is in actual fact a stem cutting. Successful stimulation of roots has been obtained in a number of species by application of auxin to the stem portion in dusts or dilute solutions by the usual methods. Species successfully treated in this way include *Rhododendron* spp., *Bougainvillea* spp., *Hibiscus* sp., *Ixora* sp., *Thunbergia* sp. and numerous broad-leaved evergreens. Successful application in the propagation of cabbage by such cuttings is also reported. Leaf-bud cuttings were soaked for 16 hours in 20–40 p.p.m. NAA or 40–80 p.p.m. IBA and then planted in sand. Prevention of fungal rot was effected by application of the auxin mixed into a fungicidal powder. The use of auxins in this method should not, however, be undertaken lightly, as it is very difficult to avoid complete suppression of bud growth (see Chapter 8).

(*e*) Root Cuttings.—Many plants can be very satisfactorily propagated by portions of root, from which new buds and roots will grow making a new plant. This has been of great value in the propagation and cultivation of the new Russian dandelion (*Taraxacum kok-saghyz*), which was grown as a war-time source of rubber (from its latex). A 16-hour soak treatment of such cuttings with 50 p.p.m. of a range of compounds (NAA and its amide, IBA and β-naphthoxyacetic acid (NoXA)) roughly doubled the percentage rooting (Marth and Hamner, 1943). Similar stimulation has been obtained with IAA-lanolin paste treatment of horse-radish (*Armoracea lapathifolia*) root segments. The objections raised to leaf-bud treatment

also apply here. For successful propagation, buds must develop as well as roots and it is by no means easy to stimulate the latter without completely inhibiting the former.

### *The Main Responses to Auxin Treatment*

In considering the responses of cuttings to auxin treatments we must first stress the fact that species of plant vary very widely in their capacity to propagate normally from cuttings. At one extreme we have the easy rooters such as willow and elder which root readily at almost any time of the year. At the other extreme we have plants like the conifers, pears and apples, that can as a rule be induced to root only with great difficulty. Obviously the most important use of auxin treatment would be to facilitate the propagation of the latter types not normally amenable to the cutting technique.

It is, however, generally found that the species which normally root with ease usually respond readily to auxins, whereas the poor- or non-rooters are, on the average, much less responsive. The positive response takes the form of an accelerated rate of initiation of root meristems and therefore the production of a greater number of roots. The quality of the root system eventually produced is therefore much better. There is also a corresponding rise in the percentage of the total number of cuttings producing roots. In the case of the shy rooters, a few remain quite unresponsive to auxin but the remainder show varying degrees of response. It is not thought desirable in a book of this type to go into specific details; full reports of treatment and responses of most of the species and varieties investigated can be found in lists prepared by Pearse, 1947; Avery and Johnson, 1947; Mitchell and Marth, 1947; and Thimann and Behnke, 1947. A condensation of these lists appears in Appendix A. Furthermore, in any one species the normal rooting capacity will vary throughout the year, and in those species so far studied in such detail the response to auxin is closely correlated with it. Thus in a typical example (willow) optimum rooting of normal cuttings occurs in July and March with minima in May and October. The stimulation of root production due to IAA applications ran strictly parallel

with this change, and in October, when cuttings will not normally root, IAA produced virtually no effect (Gumpelmeyer, 1949).

It is not surprising, therefore, that in recent years the use of auxins in the striking of cuttings has fallen somewhat into disrepute. It has been felt that treatment would only improve what was already good to start with, and fail the propagator where the need was most pressing in the striking of difficult rooters. That this is altogether too gloomy a view will be obvious if the lists noted above are consulted. But these lists are very extensive and it is not easy to form from them an accurate general picture of the behaviour of poor rooters as compared with fair or good rooters. In an attempt to dispel the above gloomy picture, the writer has taken the available data and analyzed them for presentation in a pictorial form so that the main characteristics may be readily appreciated. The data were taken from Thimann and Behnke's (1947) very comprehensive list for woody plants. These lists represent a complete summary of all investigations carried out up to June 1947. Many of the results are merely qualitative, but there still remains a very considerable amount of exact quantitative data suitable for such analysis. The selected criterion of response has been the percentage of cuttings which produced roots. Fig. 17 is a pictorial analysis of 1240 sets of experiments, including almost as many different species and varieties of cutting, in all of which the behaviour of auxin-treated and control untreated cuttings had been adequately recorded.

The base of this solid diagram is a grid of a hundred squares each representing a particular type of cutting behaviour. The height of the rectangular column standing on each square represents the percentage of the total number of species or varieties analyzed which showed that particular behaviour. Each of the ten rows of columns starting from the front left-hand and running to the back right-hand edge of the diagram represents different untreated rooting behaviour. Thus the row along the back left-hand edge is for virtual non-rooters (only 0–10 per cent. of untreated cuttings showing rooting) and that along the front right-hand edge represents very good normal rooting (90–100 per cent. rooting of untreated cuttings). Intermediate

rows are for intermediate rooting behaviour as shown by the juxtaposed scale. Each of the ten rows running in the other direction, *i.e.* from the back left-hand edge to the front right-hand edge, represents different rooting responses of treated cuttings. Thus the row on the front left-hand edge is for treated cuttings showing very poor rooting response (0–10 per cent.), whereas the opposite row on the right-hand back edge represents very good rooting responses (90–100 per cent.). Intermediate rows are again for intermediate reactions according to the corresponding right-hand scale. It is now possible to see at a glance how good rooters and poor rooters compare in their response to auxin treatments.

In the first place, by far the most imposing row of columns is that at the back left-hand edge, *i.e.* the normal non-rooters, representing about 42 per cent. of the total. But far from showing a lack of response to auxins, the species in these columns show a very marked response, indeed the highest column of the whole diagram is that for the normal non-rooters with 90–100 per cent. auxin response (column at rear corner) representing well over 8 per cent. of the total and nearly 20 per cent. of the non-rooters. Intermediate responses are reasonably uniformly spread throughout the non-rooting varieties, as shown by the heights of the intermediate columns, and only just over 10 per cent. of these non-rooters show no response whatever (left-hand corner column). For the better rooting types there is undoubtedly a higher percentage showing 90–100 per cent. rooting after treatment, and this gets more marked as we proceed from the poor to the good rooters (tall columns along back right-hand edge). For the last row (90–100 per cent. normal rooters), however, the diagram cannot distinguish a positive response, since all normally rooting cuttings will fall in the last column (right corner). A positive response of these latter cuttings, which, as we have seen, is usually well marked, is manifested by an increase in the number of roots per cutting and could only be demonstrated by a similar analysis of the relevant data. In fact the preceding analysis depicts the minimum response of the cuttings to auxin treatment since it does not include the usually correlated increase in root number. The columns with cross-hatched tops, which run from the left

to the right corner of the diagram, refer to those cuttings with identical treated and normal rooting behaviours. Leaving out the extreme right-hand column for the 90–100 per cent. rooters, which could not show a positive response in the analysis, these non-responders represent only about 8½ per cent. of the total. A further small 5½ per cent. is represented by those columns lying in front of this non-reacting diagonal and correspond to negative responses, *i.e.* inhibitions, due undoubtedly to the use of high toxic concentrations of auxin in the relevant experiments.

In addition to these two effects on percentage rooting and root number per cutting, another equally important advantage of auxin treatment is the improved *rate of root initiation* and hence a speedier establishment of the cutting. This very markedly reduces the mortality due to basal rots and any other causes aggravated by long striking periods. The net result is therefore a great improvement in the percentage strike, although, once established, there is little evidence that treated cuttings maintain more vigorous growth than non-treated.

### *Conditions affecting the Response*

(*a*) The Effect of the Condition of the Parent Plant.—As we have indicated in a previous section, the general state of activity and nutrition of the parent plant plays a very important part in determining the rooting response. Young plants tend to provide better rooting material, the rooting activity usually falling away with increasing maturity. The method of culture, *e.g.* closeness of individual plants in a bed, also has an effect. The age and vigour of the cutting and position on the parent plant from which it is taken is also of great importance. This factor has been clearly demonstrated in leaf cuttings of ivy and French bean (Gregory and Samantarai, 1950) where low rooting capacity was shown by both the youngest and the oldest leaves, maximum response being obtained in ivy with the ninth leaf from the apex of a shoot and in the bean with the second leaf from the apex. Again we may have with any one species a rhythm of rooting capacity throughout the year and this is closely correlated with maturity effects. The sensitivity to auxin treatment is found to run very closely parallel with the normal rooting capacity (see p. 115).

Superimposed on these maturity and cultural influences, we may also have effects due to the nutritional status of the parent. Thus excess nitrogen supply causes a reduction in rooting, and nitrogen starvation, down to a certain lower limit, will often result in better untreated cuttings and also a better response to auxin treatment. Other nutrients affecting rooting are potassium, phosphorus, magnesium and calcium. These in excess cause a depression of rooting, the severity of the effect increasing in the order given. Indeed, it has been claimed in grape and Mariana plum that the effects of this nutritional status completely dominate the hormone effect itself (Pearse, 1948).

It will be realized, therefore, that in addition to auxin there exists also a complex of other internal factors which require to be optimal for the best rooting. The precise nature of this complex is still very largely obscure, but the following résumé of our present knowledge may be considered pertinent.

(*b*) Consideration of the Nature and Rôle of Internal Factors. —One of the strongest pieces of evidence for the existence of internal factors comes from numerous observations that the presence of leaves and buds on cuttings not only promotes normal rooting but greatly increases the response to auxin applications. It is unlikely that this is due to a greater intake of the auxin by the leafy shoots, since responses have been obtained with cuttings treated either before or after the removal of the leaves. It is now widely accepted that this effect must be attributed to some essential substance or substances other than auxin moving down from the leaves to the region of root initiation, but it is still a moot point whether this factor is purely nutritional (*i.e.* organic food supply) or is another specific hormone. Earlier workers favoured the latter possibility. Thus, Cooper (1935, 1936, 1938) treated lemon and apple cuttings with IAA and, after roots had grown on the treated zone, cut off those portions and re-treated the new stumps with IAA. This second auxin application produced very little rooting response, although normal organic food supply from the leaves should still have been available. Cooper explained these results by postulating a limited supply of an essential

rooting factor complementary to auxin in these cuttings. Auxin application causes this to accumulate in the treated zone and it is therefore removed in cutting off the stump. This theoretical hormone rooting factor has been called *rhizocaline*. After a second auxin application, therefore, rooting is severely reduced by insufficiency or absence of this complementary factor. However, other workers subsequently obtained good responses to such second applications in a number of different plant species. These latter results do not necessarily disprove the rhizocaline theory, since they can be explained by postulating its continuous production in these particular cuttings or simply by slower mobilization into the auxin-treated zone. A limitation of rooting by an internal factor, apparently not nutritional, has also been shown in etiolated pea stems (Went, 1938). In this latter material rooting appears (Went and Thimann, 1937) to be controlled by a complex of three factors, *i.e.* the supply of auxin (IAA), of carbohydrate food (cane sugar) and the growth factor *biotin*. Biotin is an organic compound which was first isolated from yeast and which seems to be an essential growth substance, not only for the yeast organism itself but for many other micro-organisms and also for the higher green plant (see Chapter 13). Like the auxins, it is active in extreme dilutions. It was shown that in the presence of optimal auxin and sugar concentrations the rooting response could still be considerably increased by the application of biotin, suggesting that Went's internal factor could be biotin itself. These observations have yet to be repeated for other plant species. Still more recently, a second factor in addition to auxin has been claimed to limit the formation of lateral branches in excised pea roots growing in sterile nutrient media. This "rhizocaline" is presumed to be manufactured during the growth in length of the main root (Torrey, 1950). A similar root-initiating factor complementary to auxin seems to be present in asparagus (Galston, 1948). Sterile stem tips of this plant can be induced to grow indefinitely in a synthetic sterile agar medium containing sugar and mineral salts. Freshly isolated tips will form roots in the dark in response to IAA, but after long protracted subculture in the dark no such response is obtained, and this is thought to be due to the exhaustion of its "rhizocaline" supply.

Exposure to light restores the capacity to produce roots in response to IAA, presumably due to the synthesis of more "rhizocaline". It is interesting that one worker (Dettweiler, 1942) has claimed, on the results of direct assay using differentiation with acids and alkalies (see p. 52), that IAA is itself not a rooting hormone but merely mobilizes in the cutting the natural auxins (auxins A and B), which themselves are the rooting factors "rhizocaline". In view of the present uncertainty regarding auxins A and B, judgment on these views should naturally be suspended.

There are, moreover, other recent investigations which indicate that these additional internal factors may be purely nutritive. By far the most convincing were experiments on two varieties of *Hibiscus*, a red one that roots freely and a white one that hardly roots at all (van Overbeek and Gregory, 1945). Response to auxin application was closely correlated with normal rooting behaviour, showing again the existence of an internal limiting factor in the white variety. This was substantiated by grafting experiments. The easy-rooting red variety grafted on the non-rooting white variety did not enable the latter to root unaided but promoted a full rooting response to auxin. This showed conclusively that some influence had passed from the red scion to the base of the white cutting, completing with applied auxin the full complement of factors necessary for rooting. Subsequently it was shown that the effect of the leaves could be replaced entirely by feeding with appropriate organic nutrients. A very effective treatment consisted of soaking the base of the cutting for 24 hours in a solution of 4 per cent. cane sugar and 10 parts per million of the amino-acid arginine (an essential constituent of protein). More recently still, Gregory and Samantarai (1950) at Imperial College, London, have made a survey of factors influencing the rooting responses of detached ivy and French bean leaves. They have found that, when such leaves are kept in darkness, there is a progressive loss of auxin response which reaches zero after 25 days in ivy and after only 48 hours in bean. This decline is closely paralleled by the loss of sugar from the leaf, suggesting that when auxins are provided in sufficient amounts the supply of nutrients may limit the production of roots.

Nevertheless this loss of the power to respond to auxin was only partially restored by feeding with cane sugar and the amino-acid derivative asparagine. It is still an open question, therefore, whether some internal growth substances other than auxins and the raw materials for tissue synthesis are necessary for the initiation of roots in cuttings. If indeed such factors do exist, then it seems probable that great differences in their relative concentrations would occur not only between plant species, but also between different organs in the same species. Likewise, the optimal concentration requirements of different species would also be expected to vary. The apparent discrepancies in the limited results so far obtained might easily be explained by considerations such as these. We are clearly only at the beginning of our analysis of the problem.

### *The Effect of External Applications of other Substances on the Rooting Response to Auxins*

The very clear indications that internal factors, whether nutritional, hormonal or both, limit the rooting response of the plant to auxins have resulted in several attempts to augment such factors by the external supply of appropriate substances. These may be grouped as (*a*) carbohydrate nutrients, (*b*) nitrogen nutrients, (*c*) other nutrients, and (*d*) organic growth factors including vitamins. We will consider each of these in turn.

In the rooting of such starved material as etiolated pea stems in Went's assay technique, sucrose is a necessary component of the rooting medium. However, the vigorous cuttings used in normal propagation are in quite a different category. In both leafy softwood cuttings and dormant hardwood cuttings, an adequate internal carbohydrate supply is most probably present in both the leaves and the stem. The effects of such a supply and its exhaustion during starvation in leaf cuttings have already been discussed (Gregory and Samantarai, 1950). There is, indeed, considerable experimental evidence in the literature that one effect of applying auxins in high concentrations to tissues with such stores of starch is the rapid conversion of this starch to simpler transportable carbohydrates such as

sugars. Thus in cuttings treated with auxins in concentrations optimal for rooting, a mobilization of starch reserves takes place and sugars accumulate in the treated region (*e.g.*, stem cuttings of *Forsythia* and *Rhododendron* spp. (Doak, 1941) and leaf cuttings of ivy and French bean (Gregory and Samantarai, 1950)). These sugars slowly disappear as they are used up in the growth of the roots. It is not surprising, therefore, that the application of sugars to normal unstarved cuttings should cause little or no augmented response to auxins. Small positive responses to sugar solutions have been obtained in *Vitis* sp. (Evenari and Konis, 1938), Norway spruce (Grace *et al.*, 1940), Black locust (Stoutemeyer *et al.*, 1940) and *Rosa* spp. (Brandon, 1939). But from the point of view of the horticulturalist, little is to be gained by such feeding treatment, especially if care is taken to use cutting material either with an adequate store of carbohydrate or with the ability to manufacture it in a healthy leaf system.

Nitrogen compounds are also needed for the synthesis of new proteins during the growth of the roots. They often take the form of amino-acids and amides (*e.g.* asparagine and glutamine), produced in the breakdown of reserve proteins. Like the carbohydrates, there is evidence that one result of auxin treatment of cuttings is the mobilization of their nitrogen reserves into the treated portion, where they are re-formed into proteins in the growth of new roots. The picture here is more confusing than that for carbohydrate nutrition, especially when it is remembered that nitrogen starvation of the parent often results in a better cutting than does good nitrogenous manuring. Further complexities are introduced in nitrogen feeding experiments by the large range of compounds, both organic and inorganic, that can be used. The little systematic work so far done has given results that are somewhat confusing.

Among inorganic compounds, nitrates have been severally reported to increase auxin response in single bud cuttings of vines, leaf cuttings of bean and in stem cuttings of *Rhododendron* spp., while ammonium sulphate, though augmenting the response in *Rhododendron* spp. and defoliated *Hibiscus* cuttings, has inhibited it in leaf cuttings of bean (*Phaseolus vulgaris*). Turning to organic nitrogen sources, various amino-acids were

found to be without effect in the rooting of etiolated pea stem cuttings, but to increase the auxin response of normal stem cuttings of *Rhododendron* sp. and leaf cuttings of bean. Many other organic compounds have been tried with varying responses (see Pearse, 1948). The most systematic work has been that of Gregory and Samantarai (1950), who showed that asparagine augmented the auxin effect in both starved and normal leaf cuttings of bean and ivy, and that the response to low concentrations was largely additive to a similar response evoked by dilute cane-sugar solutions. The most favourable concentration of asparagine was different in the two species, a 0·5 per cent. solution producing a greater response than a 1·0 per cent. solution in bean but not in ivy. This is thought to be related to the normally much higher nitrogen content of bean leaves. It stresses the point made for carbohydrate feeding that the response to any external nutrient supply will depend on the internal nutritional status of the cutting. Very much more work like that on asparagine is needed if we are to form a true picture of the relationship between nitrogen nutrition and auxin response. For the present, the general addition of supplementary nitrogen nutrients to auxin-treated cuttings is too problematical to be recommended.

Little attention has been paid to the effects of other mineral nutrients on the rooting response. A few isolated observations suggest that a balanced solution of the major nutrients may increase the rooting of cuttings having a natural deficiency of such nutrients. A specific stimulation by manganese and magnesium has been reported in *Tradescantia* cuttings (Amlong and Naundorf, 1938), while in bean leaf cuttings calcium and magnesium may be inhibitory above certain levels. Two hundred parts per million of calcium nitrate give a slight and 82 parts per million of magnesium sulphate a complete inhibition (Thimann and Poutasse, 1941). It seems likely that in cuttings from healthy plants not suffering from actual deficiencies, the internal supply of such elements is adequate to provide for the early growth of the new roots.

The possible existence of a specific internal root-forming growth-factor (rhizocaline) has led various experimenters to observe the effect of known growth-factors on the cutting

response to auxins. We have already seen that biotin can limit root production in etiolated pea stem cuttings provided with optimal amounts of auxin and sugar, but this has not been shown for other plants. Vitamin $B_1$ (thiamin or aneurin, an essential constituent of one of the most important enzyme systems in all living cells, the carboxylase system) has received most attention. In 1938 it was claimed (Went, Bonner and Warner) that a soak treatment of lemon cuttings in 1 p.p.m. vitamin $B_1$ subsequent to auxin treatment doubled the root production, and even greater effects were claimed for leafy *Camellia* cuttings. It seemed that in such cuttings the normal Vitamin $B_1$ supply was suboptimal and therefore limiting root production. Other workers have been unable to obtain the same effect with other plant species and it is clear therefore that the situation needs careful systematic study. Another growth-factor, pyridoxine (Vitamin $B_6$), which is of definite importance in the growth of isolated root tissue (see Chapter 13), has been claimed to stimulate rooting in certain cuttings, both with and without added auxin (Stoutemeyer, 1940). In general, there seems little immediate promise of an advantage to be gained by using these growth-factors to supplement the auxins in promoting the rooting of cuttings. The wider question of the rôle of vitamins in the growth and development of the higher plant will be more fully discussed in a later chapter (Chapter 13).

## HINTS AND PRECAUTIONS IN USE

The most important point about the use of auxin preparations in the treatment of cuttings is that they should supplement and not in any way replace the art and skill of the propagator. All other conditions for the taking, preparing and planting of cuttings must be strictly followed, and there must be no relaxing from the care normally taken. Thus response to auxins throughout the year runs closely parallel with the normal rooting rhythms, and auxin treatment does not modify the natural optimal times for taking the cutting, although, as we have seen, auxin treatment may produce satisfactory rooting at unfavourable periods of the year and enables plants to be propagated at such times if particularly desired.

Great care must be taken to see that the most suitable auxin concentrations are used, and makers instructions must be carefully followed with all proprietary preparations. It should be noted that some of these preparations, *e.g.* those containing IBA, may lose their potency even when stored as a powder or in concentrated solution. The use of fresh compounds is therefore advised, but if only old preparations are available their effectiveness should first be checked. For this, a simple and efficient method, using leaf cuttings of tomato, has been evolved by Hitchcock and Zimmerman (1938) (see also Pearse, 1948). In no instance should the dilute solutions for direct application be left to stand for any length of time as their activity may then very quickly disappear. This applies particularly to the indole compounds, although other compounds such as the substituted phenoxyacetic acids are chemically very stable and remain unchanged for very long periods even in dilute solutions. For example, in dilute unsterilized solutions (of the order of 10 p.p.m.) all IAA may be destroyed in 24 hours and in solutions of 0·01 per cent. it may similarly disappear in 14 days. Solutions of NAA and 2, 4-D of the same strength remain unchanged for as long as a year, but they, too, disappear fairly rapidly if to the solution is added small traces of organic matter suitable for bacterial growth (Mes, 1951). Strong stock solutions of all compounds in alcohol can be kept more or less indefinitely.

CHAPTER 6

# AUXINS AS STIMULANTS OF CAMBIAL ACTIVITY: USE IN GRAFTING AND WOUND HEALING

## INTRODUCTION

AT the beginning of the preceding chapter we saw how, in a great proportion of higher plants, continued growth in girth of the stem and root took place as the result of the activity of a cell layer known as the cambium. By active cell division, layers of bark cells are produced towards the outside and layers of wood cells towards the inside, these cells later differentiating into the specialized conducting tubes for the transport of food and water, and into special thick-walled cells for the maintenance of rigidity in the growing shrub or tree. In trees of temperate regions where there are marked seasons there is a correlated rhythm of activity of this cambium (see Fig. 18). In winter it is dormant, but in spring, as the buds begin to swell, there is a rapid renewal of activity which persists until the following autumn. The tubular cells of the wood produced during the first flush of this activity are usually large and later-formed cells decrease in size as the season advances. This rhythmical growth is responsible for the grain of wood grown in such regions. In the tropics the less obvious seasons may have little regular effect on cambial activity, although rhythms, not obviously correlated with the seasons, are still observed.

It has been found that this rhythm is closely linked with the production of auxin by the expanding buds and is now believed to be largely controlled by it. This effect of developing buds on cambial activity is no new observation, having first been recorded by the German botanist Jost in 1891, and the idea of a specific chemical stimulant was suggested as early as 1910 (Keeble). Although several series of experiments were carried out in the interval, confirming the general truth of Jost's observations, it was not until 1933 that Snow at Oxford, as the result of some very elegant "grafting" experiments, established

the hormone nature of the stimulus, *i.e.* that it would pass from one plant to another across a water contact. Within the next two years he had demonstrated that IAA was very effective in evoking this response. Subsequent work both in America and Europe has correlated the wave of cambial activity in the spring with a similar wave of high auxin concentration moving down the cambium from expanding buds. It has been fairly widely accepted that the auxin flow is the immediate cause of the cambial activity. However, more recent work has indicated that this may not be the whole story since active cambial extracts give a far greater stimulation when applied experimentally to dormant cambium than solutions of synthetic IAA possessing the same auxin activity as the extracts (Söding, 1940). The presence of other cambial hormones (not auxins) immediately suggests itself, although no attempts have so far been made to isolate them. Observations on the stimulation of bean cambia by the vitamins ascorbic acid (vitamin C) and aneurin (vitamin $B_1$) (Künning and Söding, 1950) favour such a possibility. Consequently it has been suggested that the attainment of a certain threshold concentration of this vitamin-hormone mixture is necessary for starting cambial activity. The complexity of the situation is also exemplified by a series of experiments carried out in Holland (Gouwentak, 1941), where it was shown that dormant cambium must be in a reactive or sensitive condition before it can be stimulated into activity by auxin application. This sensitive state may be induced by ethylene gas which, by itself, has no action on cambial activity.

It is pertinent here to note the very important and far-reaching results obtained in the last few years in France by Gautheret and his colleagues, and by Nobécourt. These workers have developed a technique whereby isolated sterile fragments of mature plant tissue (*e.g.* storage roots of carrot, endive and chicory, and cambial tissue of elm) can be induced to grow indefinitely in the dark in a sterile solution containing sugars and mineral nutrients, if IAA or NAA is provided in certain limited concentrations. As a result of the inclusion of the growth-factor auxin, they have thereby achieved what Haberlandt had attempted nearly half a century ago, namely, plant tissue culture. A very interesting effect of auxin concentration

was observed and is of particular relevance here. Thus at low concentrations (one part in $10^7$) the main response was the induction and stimulation of cambial activity in these storage tissues and a correlated production of a voluminous callus. This furnished conclusive proof of the direct participation of auxins in this phenomenon, though it does not prove that they are the sole stimulatory agents. Further increases in concentration evoke successively the formation of roots, the inhibition of bud growth and, finally, the induction of tumour-like proliferations caused by the apparently uncontrolled expansion of cells in all directions. These latter responses will be considered in another chapter.

Apart from its main function in the growth in girth of woody plants, cambium plays another very important rôle. Thus, if a portion of bark is removed from a tree, such that an area of underlying wood is exposed, the cambium will usually produce a thick mass of tissue at its cut edge and, if the wound is not too large and the wood surface not too dry, this may eventually spread over the wound and effectively seal it off. This tissue is called wound callus and, in covering the exposed wood with healthy living tissue, prevents the entry of many potential disease-producing micro-organisms. An illustration of wound callus of this type is seen in Fig. 19. The facility with which callus is produced from cambium is also seasonal, running parallel with the normal activity of the cambium and, it might be thought, determined by prevailing auxin concentrations. It is, however, difficult to see why, if auxin is the only factor concerned, the mere act of wounding should cause such a marked local stimulation of cambial activity. Consequently another hormone, produced from the damaged tissue, has been postulated as the initial cambial stimulant. Here again the idea is not new, having been put forward over fifty years ago by Weisner. As we shall see later on in this chapter, claims have been made for the isolation of such a hormone from certain plant tissues. It has been called traumatic acid (Gr. *trauma* = a wound). It bears little resemblance to auxins in chemical structure and has no auxin activity.

The wound reaction has a very important practical application in horticulture in that it causes the union of the tissues of

scion and stock in grafting operations. In this century-old practice, buds or shoots of one variety of plant are induced to grow on the stem or rootstock of another, an intimate intermingling of the two tissues usually taking place at the junction. A successful graft results when the cambia of stock and scion are brought into contact, and the resulting callus tissues interpenetrate to perfect the junction. Subsequent differentiation of this callus into conducting tissue, etc., linking the corresponding tissues of stock and scion, turns the graft into a single functioning unit. Thus the success of grafting really relies on the wound response of the cambium.

## PRACTICAL APPLICATIONS OF AUXINS AS CAMBIAL STIMULANTS

The possible practical importance of this type of auxin activity has been considered both in Europe and America, but with little real promise of success. There are two main aspects that have been investigated and we will deal with each in turn.

*Wound Healing.*—It is clear that in such practices as pruning one of the greatest dangers is infection of the wound by pathogenic organisms, *e.g.* silver-leaf disease of apples. To reduce this risk the horticulturalist carries out his pruning activities at a time of year when cambial activity might be expected to be high enough to give adequate callusing. Nevertheless, promotion of activity by the application of auxins might, in some instances, be a great advantage, particularly after the removal of large branches.

The results of experiments in which IAA has been applied to such wounds are conflicting. Application at a concentration of 0·5 per cent. in lanolin to pruning cuts in apple, maple and linden, produced no significant response (Shear, 1936), but a similar paste containing 1·0 per cent. of IAA was reported to cause considerable stimulation in wounds of terminal shoots of peach, plum, apple and pear. Some initial stimulations were observed with 0·05 per cent. to 0·3 per cent. IBA in green ash trees in Canada (Oliver and Grace, 1940), but callus growth of the controls later caught up with the treated. More recently, the possible contribution of other factors has been investigated

(Davies, 1949 *a* and *b*). Not only several synthetic auxins, but also traumatic acid (see later) and a variety of other suspected stimulants have been applied to wounds of the sugar maple (*Acer saccharum*). They were treated in the summer when the normal spring flush of cambial activity was over and the substances applied either as dusts with talc as a carrier, or in lanolin paste. A wide range of concentrations of IBA, 2-chlorophenoxyacetic acid (2-CPA), 4-chlorophenoxyacetic acid (4-CPA), 2, 4-D and NAA, all failed to stimulate healing, and in some cases inhibitions of the normal callus growth were observed. Traumatic acid also failed to produce a response. Similar attempts to stimulate wound healing in ten species of forest tree in North America, using NAA, NAAm, IAA, IBA and NoXA, were all without success (McQuilkin, 1950). Some positive results have been produced by the substances cysteine hydrochloride * and glutathione * (Davies, 1949 *b*), but the significance of these effects is not at all clear.

The indications are that factors other than auxin supply are limiting in the process of natural wound healing, and the best treatment still seems to be simply to coat the wound with a suitable protective sealing paint or clay to prevent infection while normal healing processes get under way. There seems to be little advantage to be gained by the application of hormones to tree wounds to produce a more vigorous and extensive callus formation.

*The Promotion of Graft Unions.*—The grafting of grape vines has received much attention and most of the evidence suggests a significant stimulation of callus formation and an improvement in the rate of "taking" of the graft by the application of auxins. A strong even ring of callus is formed at the graft union (Müller-Stoll, 1938, 1940). Various methods of applying the auxin to the union were tried, and include soaking both stock and scion in dilute aqueous solutions of the auxin, painting the cut surfaces with a dilute solution before making the graft, spraying the scion before grafting and the application

* Cysteine is one of the most important natural amino-acids and is one of the few containing the element sulphur. It is an essential building brick of proteins. Glutathione is a more complex molecule composed of three amino-acids, one of which is cysteine. Its function in the living organism is as a component of many oxidizing systems.

of a lanolin auxin paste to the graft union. Soaking of the cut ends of both graft partners for 16 to 20 hours in a 0·05 per cent. solution of IAA was the most effective treatment, and solutions of IBA have a similar although smaller effect. The lanolin-paste method was ineffective. As might be expected, such treatment tended to bring about the initiation of roots in the soaked region, especially as the grafts were stored for a period in moist sawdust before planting out, but this was reduced by using drier storage conditions. It should be noted that not all investigators have obtained such satisfactory results and more confirmatory work needs to be done.

Satisfactory improvements in the grafting of apple and plum as a result of auxin treatment have also been reported, but cherry and pear have not yet proved responsive. In contrast to the grape vine results, lanolin-paste smears were effective. From this latter work with fruit trees it appeared that treatment may sometimes hinder union by the excessive production of callus which pushes stock and scion apart. There seems no doubt that a definite callus stimulation can be evoked by suitable hormone treatment and that, if its growth can be properly regulated, decided improvements in the success of grafting operations and in the strength of the resulting graft union may be expected. Nevertheless, the participation of other wound factors may not be ruled out and further research in this field might be expected to yield valuable results.

## SPECIFIC WOUND HORMONES

We must now turn to consider the specific wound-healing hormone first postulated by Wiesner at the end of the last century. Such a hormone would be generated by damaged tissue and, having diffused into neighbouring undamaged cells, would induce them to divide and produce the protective callus structures. Strong experimental evidence in favour of this suggestion was obtained by the famous German botanist, Haberlandt, as early as 1913, when he showed that extracts of damaged plant tissues could be applied to uninjured cells (*e.g.* cells dissected from leaves of succulent plants) and thereby induce division of those cells. Furthermore, in the kohl-rabi

root the wounding response could be prevented by washing the cut surface, presumably removing thereby a soluble chemical stimulant, and could be restored by applying crushed tissue containing that stimulant.

It is axiomatic that any advances in the isolation and identification of any unknown growth factor depend on the development of an adequate technique for biological assay, and further progress in this field of wound hormones did not come until it was shown that the interior lining of young kidney-bean pods was extremely reactive to this wound hormone (Wehnelt, 1927). Unfortunately, a large number of other compounds (*e.g.* sugars, salt solutions and even water itself) produces reactions, but they are small compared with that given by the specific hormone, and in the hands of Bonner and English (1938) a satisfactory method of assay was worked out. Thus a drop of solution of active material placed on the interior lining of a portion of bean pod caused, after a period of about two days under moist conditions, the growth of a small callus-like intumescence a millimetre or so in height. It was found that when allowance had been made for the small non-specific effects of water, etc., the height of the intumescence was, within certain limits, proportional to the concentration of the hormone in the solution. The height of the intumescence given by test solutions could therefore be used as a direct measure of hormone content. This assay technique allowed a wide range of plant sources to be studied and it was shown that the richest source of the active compound was the juice of the bean pod itself. A subsequent series of purification experiments led finally to the isolation of a highly active crystalline material which proved to be a long chain dibasic aliphatic acid of the following structure:

$$COOH{\cdot}CH : CH{\cdot}(CH_2)_8{\cdot}COOH$$

It was christened traumatic acid (English, Bonner and Haagen-Smit, 1939). It seems to be only one member of a complex of factors governing wound response, since a number of "co-factors" can greatly augment its action. One such factor is glutamic acid, which in concentrations of 0·25 per cent. can increase the activity of traumatic acid over ten times.

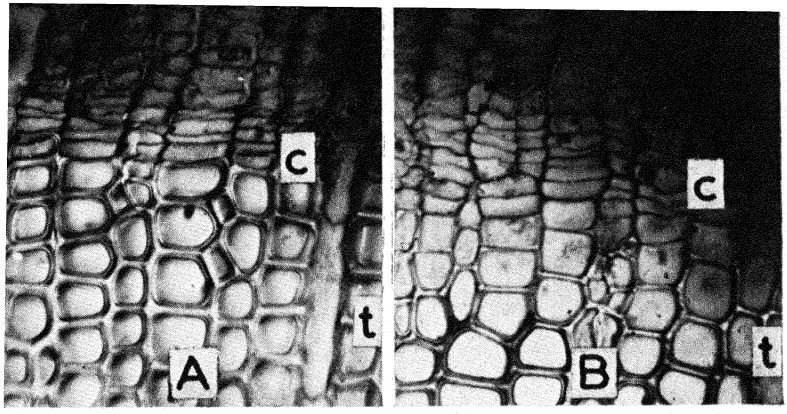



FIG. 18.—Photomicrographs of the cambial region of stems of Scots Pine (*Pinus sylvestris*) showing:—

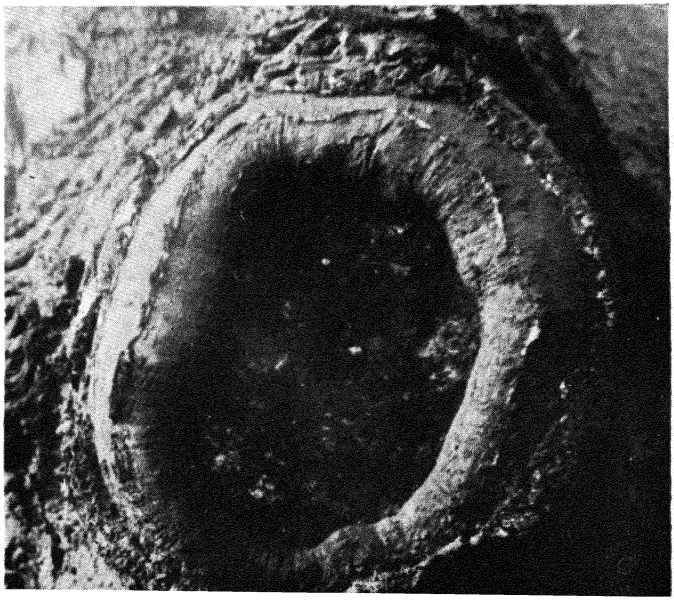
A. Inactive winter condition, and

B. Active spring and early summer condition.

Note that in A the mature thick-walled water-conducting cells (tracheids) of the wood (t) stretch right up to the inactive cambial layer (c). In B, between the mature conducting cells (t) and the active cambium (c) is a number of rows of thin-walled cells. This is the young, actively growing, immature spring wood tissue produced by the cambium. ($\times 300$.)

*Photographs by permission of Dr. P. F. Wareing, University of Manchester.*

FIG. 19.—Wound callus on plum tree. Here a branch has been sawn off and the callus ring has only partially covered the outer surface of the wood, leaving exposed wood still in the centre of the wound. This usually happens with large wounds.



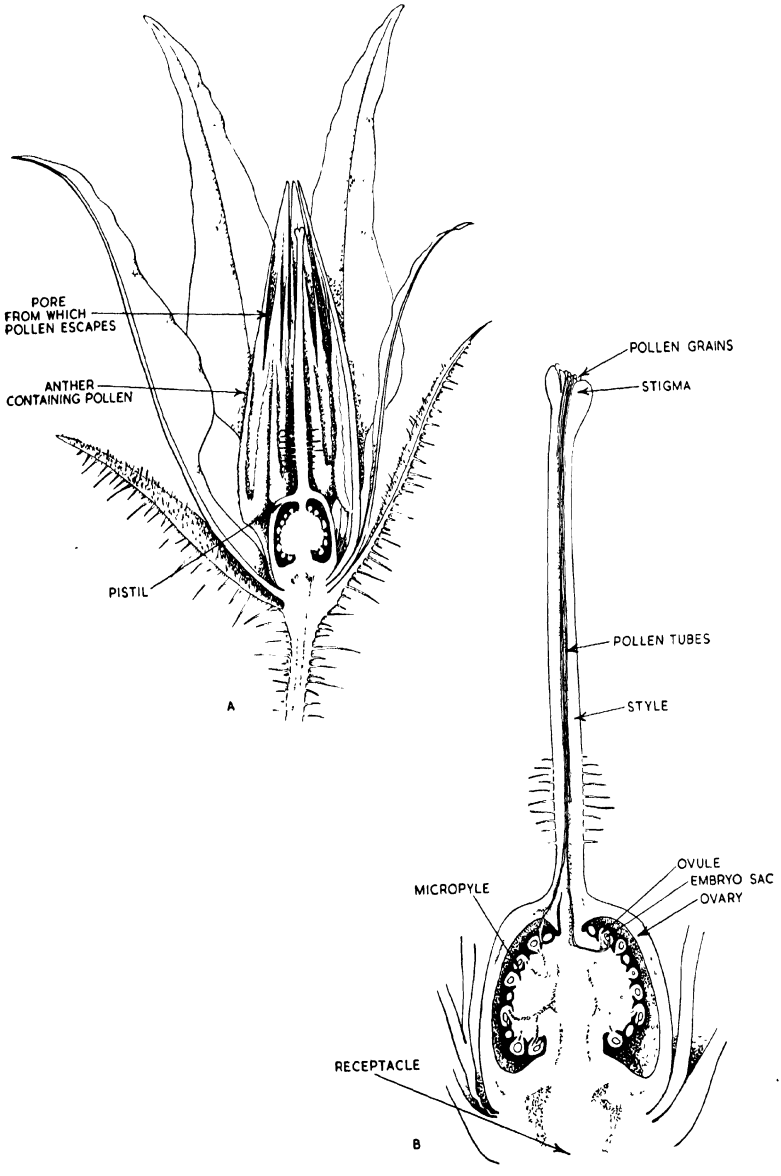



FIG. 20.—Pollination and fertilization in the flower of the tomato (*Lycopersicum esculentum*).

A. Vertical section of flower showing structure. The flower is at the stage when pollen is about to be shed. (×4.) B. Vertical section of pistil on a larger scale showing pollen tubes growing from the pollen grains on the stigma, down the long style and into the ovary chamber. One is shown passing through the micropyle of an ovule. *Note*: In this drawing the width of the pollen tube is somewhat exaggerated for clearness. (×8.)

Activity is not, however, restricted to this compound, since, as in the case of the auxins, the property is shared with a number of homologous synthetic molecules. All are long chain (6 to 13 carbon atoms) aliphatic dibasic acids with terminal carboxyl groups. Although some saturated acids of the type $COOH{\cdot}(CH_2)_n{\cdot}COOH$ are active, the maximum activity is attained with one unsaturated double bond in the chain.

The interrelations of a specific hormone and auxin in the wound healing of plant tissues still remains obscure. We have seen earlier in this chapter that it has not been found possible to demonstrate any effect of traumatic acid in stimulating the wound healing of sugar maple, and it has been intimated as a result that if this compound is indeed an active growth stimulant it may be specific for bean-pod tissue (Davies, 1949*b*). The writer is unaware of any work in which an effect has been demonstrated in other tissue, and in view of the relative paucity of our information on this proposed hormone, it would be premature to try to assess its importance in the general phenomenon of wound healing.

CHAPTER 7

# AUXINS AS INITIATORS AND STIMULATORS OF FRUIT DEVELOPMENT

## THE PHYSIOLOGY OF FRUIT DEVELOPMENT

IT is a fact of common knowledge that for fertile seed to be set in the higher plants, certain nuclei in a cell in the female part of a flower must fuse with other nuclei from suitable male cells (pollen grains) from the same or a closely related plant species. The cells resulting from such a fusion subsequently divide and develop into the embryonic tissues of the seed. Pollen grains are carried from the ripe open chambers (anthers) of the male organs (stamens) by one of a number of agents (*e.g.* wind, insects, etc.) to a special receptive surface (stigma) on the female organ (pistil) (see Fig. 20). This constitutes the process of pollination. Here the grains germinate, each producing a long thread-like projection which penetrates the tissues of the pistil and grows down into the flask-like ovary, which contains the potential seeds (ovules). This tube then passes through a small pore (micropyle) in the external covering of the ovule and thence into the special female reproductive cell (embryo sac). Here its end opens and two male nuclei are discharged into the embryo sac. One fuses with the female nucleus, or egg, and the other with two of the seven or so other nuclei present. These fusions constitute the process of fertilization and provide the necessary stimulus for the growth of the seed. From the former fused nuclei develops the young embryo, which will eventually become a plant of the next generation. From the product of the latter fusions develops a massive tissue serving to nourish the young embryo, and this tissue is called the endosperm.

Closely correlated with the growth of the pollen tube and nuclear fusions in the embryo sac are marked growth changes in the ovary and, in some plants, in the neighbouring tissue of the flower stalk (receptacle). Up to the time of fertilization the

growth of these organs is slow and takes place mostly by cell multiplication. After fertilization this growth-rate accelerates and is due in the latter stages mainly to cell enlargement. These tissues constitute the fruit. The fleshy edible part of the fruit may have various origins. In the tomato, for example, it comes entirely from the ovary wall. In apples and pears it is formed from the flower axis, which has fused with the ovary wall (see Fig. 21). In the plum, peach, etc., it is formed from the outer layers of the ovary wall, the inner layer giving rise to the hard stone surrounding the contained seed. The pineapple is a very complex structure, having been formed from many flowers growing in a spiral on a central axis. Such a collection of flowers is called an inflorescence. The axis itself, the ovaries and other flower parts (petals, etc.), and the leaves which grow under each flower all swell up and fuse into the fleshy cone-shaped fruit (see Chapter 12). In the majority of flowers, although not in all, if fertilization does not, for some reason, occur, the fruit does not develop, but instead a special loose cell-layer (abcission layer; see Chapter 10) is formed at the base of the flower stalk. This tissue soon breaks under the slightest tension and the flower is shed.

In view of this very close association of the act of fertilization with the start of fruit development, it might be thought feasible that the fusion of male and female nuclei constitutes the stimulus, not only for the formation of the seed, but also for the growth processes resulting in the fruit. This does not seem to be so, however, since plants of many families and species will produce fully formed fruit with no seed both in nature and under certain artificial cultural conditions; indeed, the production of such fruit has long been of interest to the plant breeder, the results of whose labours are so familiar to us in the form of seedless oranges, grapefruit, bananas and the like. This phenomenon of seedless fruit production is known as *parthenocarpy*. These facts strongly suggest that fruit can be initiated and come to maturity without the stimulus of fertilization. The work of the modern botanist has shown, however, that seedless fruit can arise in several ways. In a few plants, *e.g.* some varieties of grapefruit (*Citrus grandis*), avacado pear (*Persea gratissima*) and grape (*Vitis* spp.), etc., fertilization takes place, but the young

embryo aborts and a seed therefore never develops. This is not true parthenocarpy. In the vast majority of natural seedless fruits it seems that fertilization does not occur and that the stimulus to fruit formation has some other origin. Some of the best known examples of natural parthenocarpic fruits are the banana, pineapple, Washington navel orange, some species of fig, pear and grape (Corinth currant grape). We now know the causes underlying the formation of most of these natural seedless fruit and we shall return to this later. The interested reader should consult Gustafson (1942) for details. What interests us at this juncture is their production by artificial means.

The possibilities of such a cultural practice have long been of interest to the fruit-grower for various reasons. One is that seedless fruit are thought by some to be of better eating quality. Another is that many fruit varieties are self sterile and must consequently be grown with more than one variety in an orchard to ensure good crops. Several methods have proved effective in producing seedless fruits in different species. One consists in continued emasculation of flowers or the prevention of pollination by covering the stigma with a special antiseptic paint. This ultimately causes in some plants the production of parthenocarpic fruits, *e.g.* gooseberry and pear (Ewert, 1906). By far the most successful method is the application to the stigma of pollen which will not bring about fertilization, *e.g.* by self-pollinating self-sterile species (*e.g.* pineapples), or by pollinating with pollen from another plant species that will not hybridize (*e.g.* tobacco with *Azalea* pollen, etc.).

This last method strongly suggests that pollen performs at least two separate and independent functions in the processes leading to fruit and seed production. One is to provide the male partner in the fusion of sexual nuclei which is the starting-point for seed formation. There is evidence also from one species of plant (*i.e.* apple) that the final stage of development of the immature potential seed (ovule), prior to its fertilization by the male nucleus, is itself stimulated by the process of pollination (Bryant, 1935). Such a stimulation can best be explained in terms of a hormone coming from the pollen. A similar stimulation is the basis of the second well established

function of pollination, which is to initiate the swelling of the ovary and the formation of the fruit. That this latter stimulus exists and is apparently a purely chemical one was first established in Belgium, in 1902, by Massart, who showed that dead pollen grains could stimulate the swelling of the ovary in the orchid. A few years later Fitting in Java evoked the same response with water extracts of the pollen and thereby demonstrated for the first time the existence of a plant hormone. He called this extractable chemical stimulant "pollen hormone". This discovery lay dormant for over twenty years until, in fact, the auxins had been well and truly launched into the botanical world. It was then, in 1932, that Laibach, in Germany, showed that this pollen stimulant was extractable by ether, was active in the *Avena* test and was probably identical with auxin. Starting with the pioneer work of Gustafson (1936) in America, this has been subsequently confirmed by many workers in a wide variety of plants using IAA and various other synthetic auxins.

Although, as will be seen later, auxins may probably be the main controlling factors in the growth of fruits, yet the quantity introduced into the ovary tissue from the growing pollen-tubes is completely inadequate to account for its subsequent continued growth. This is shown by the fact that very few species of plant can produce fully formed parthenocarpic fruit after pollination with non-viable or incompatible pollen or single applications of pollen extracts. Thus we are driven to the conclusion that fertilization itself must provide the stimulus for an internal auxin production, which is responsible for the rapid growth of the fruit. That such a gush of auxin follows the union of sexual nuclei has been shown in the young maize grain (Wittwer, 1943). Detailed investigations on apples by Luckwill (1948) at Long Ashton, and on rye by Hatcher (1945) at Imperial College, London, have located the source of this hormone in the endosperm, the nutritive tissue surrounding the young embryo. It seems that the hormone is produced during the growth of this tissue, which, as we have seen, originates from the fusion of two female and one male nucleus in the embryo sac. These discoveries solve many hitherto unexplained enigmas of the physiology of fruit growth. They explain why in many fruits there is an intimate relationship

between fruit development and the number and distribution of normal seeds in the fruit. This is particularly well marked in those with few seeds and has been known for many years. The Swiss botanist, Müller-Thurgau, showed as early as 1898 that the size of the individual grape in each of five popular varieties was directly proportional to the number of seeds it possessed. In the apple, as can be easily verified, a lop-sided fruit is almost always correlated with an asymmetric development of the seeds (see Fig. 22). An elegant demonstration of these effects has recently been made by removing the "seeds" from selected areas of the immature strawberry (Nitsch, 1950), when growth of the fruit took place only in those areas possessing "seeds". A variety of queerly shaped strawberries were thereby obtained (see Fig. 23). That this was not due to injury to the growing tissues resulting from removal of the "seeds" was shown by treatment of the "seedless" areas with auxin. This reproduced the action of the "seeds" and allowed normal growth in the tissues of the receptacle. Distortions such as these are of common occurrence in nature when pollination is, for some reason, inadequate and few of the ovules are fertilized and develop. The origin of the auxin in the endosperm explains why, in many pome fruits (apple, pear, etc.), the pollen variety should influence the character and growth of the fruit (Luckwill, 1949). Since the fruit is derived entirely from the tissues of the maternal parent, it has been a great puzzle why its distinctive characteristics (*e.g.* chemical composition, time of ripening, etc.), should be affected by the variety of pollen used for fertilization. Since the endosperm arises as the result of a union of male and female nuclei, these characteristics will be partly determined by the variety of the pollen used, and this would be expected to influence, by the intensity and duration of its auxin production, the type of fruit produced.

We have still, however, to explain why a few species of plant will develop fully formed parthenocarpic fruit, either spontaneously (*e.g.* banana, Washington navel orange), after certain cultural treatments such as cincturing * (gooseberries, apples,

* Cincturing is a practice whereby the flow of food material in a plant organ such as a branch is impeded by restricting the channels of transport in the bark, usually by removing a narrow ring of bark from the base of the selected limb.

grapes) or by the use of suitable incompatible pollens (*e.g.* pineapples). In the case of spontaneous parthenocarpy, it is probable that the ovaries themselves are capable of considerable auxin production which apparently occurs in seedless oranges and lemons (Gustafson, 1939). With cincturing it is possible that the treatment may cause, above the point of the cut, an accumulation of auxin arriving from the leaves and that this may replace the supply which would normally come from the growing seed. The part played by incompatible pollen still remains obscure. It may be that the small dose of auxin from the pollen "triggers off" the growth of the ovary, which then acts as its own source of auxin (Gustafson, 1939*a*). Alternatively, an enzyme from the growing pollen-tube may liberate auxin from a precursor in the pistillate tissue (Muir, 1942). This interesting field of study still remains to be explored. An excellent review of the present state of our knowledge of hormones in the physiology of fruit development has recently been written by Nitsch (1952).

## THE USE OF SYNTHETIC HORMONES IN PRODUCING PARTHENOCARPIC FRUIT: GENERAL SURVEY

Although it was Yasuda in Japan, in 1934, who first produced a fully formed parthenocarpic fruit (in cucumber) by the injection of pollen extracts into the ovary, the pioneer work was carried out two years later by Gustafson (1936). By applying a 1·0 per cent. solution in lanolin of IAA, $\gamma$-(indole-3)-butyric acid (IBA), $\beta$-(indole-3)-propionic acid (IPA) and phenylacetic acid (PAA) to the stigma or cut surface of the style of tomato, pepper, crook-neck squash, aubergine, etc., he was able to induce the formation of seedless fruit. With other species, *e.g.* water-melon, summer and winter squash, pumpkin, etc., he was not so successful. These achievements were soon followed by a succession of claims from other workers. Thus similar treatments resulted in seedless fruits in *Gladiolus*, *Crinum* spp., *Fuchsia* spp., cucumber, water-melon, etc. Spraying the female flowers of American holly (*Ilex opaca*) with dilute solutions of NAA, IBA, and IPA, also produced parthenocarpic fruit (Gardner and Marth, 1937),

whereas spraying of apple sp. (*Malus apetala*) with similar solution of IAA and NAA failed to evoke a response (Varrelman, 1938). Much work since these early studies has confirmed the fact that some species of plant, notably the tomato, are very susceptible to auxin treatment and can be induced with ease to form fully developed parthenocarpic fruit from all flowers properly treated. On the other hand, certain other species have never responded despite repeated attempts. No purpose would be served at this point to produce full details of these investigations. A list of the most important species which so far have failed to respond is included in Appendix B. It is of interest in passing to mention that Lewis (1946) has pointed out that the single seeded fruits (*e.g.* plum, cherry, peach, etc.) fall into this group and has suggested that parthenocarpy can be induced only in many-seeded fruits. The present state of our knowledge does not justify such a wide generalization. In what follows, our attention will be mainly confined to those plants which show marked responses and in which auxin treatment might be expected to result in gain to the fruit-grower.

This brings us to the question of the possible economic benefits that might accrue from the exploitation of this phenomenon. Firstly, we have the advantages that might be attendant on the loss of the seed itself. In the parthenocarpic tomato this loss may be complete, and in the good fruit the gelatinous and acid-sweet pulp that fills the fruit cavity is fully developed. Although the flavour may not be greatly impaired, it is never improved, and little advantage, except perhaps to the manufacturers of fruit pulp for sauces, etc., seems to arise from the loss of the seeds. It must be mentioned, however, that in some species (*e.g.* grape, fig) seedless fruit have been reported as sweeter than the seeded, whereas in others (date, pear) the seedless were definitely inferior in flavour (see Gustafson, 1942). The effect of auxin treatment is not always as effective as it is in the tomato in preventing the development of the hard "pits". In many fruits the hard stony covering to the "seed" is really composed of the inner layers of the ovary wall, and these layers develop in both seeded and parthenocarpic fruit alike, although in these latter the "seeds" contain no embryo. This happens in holly (Gardner and

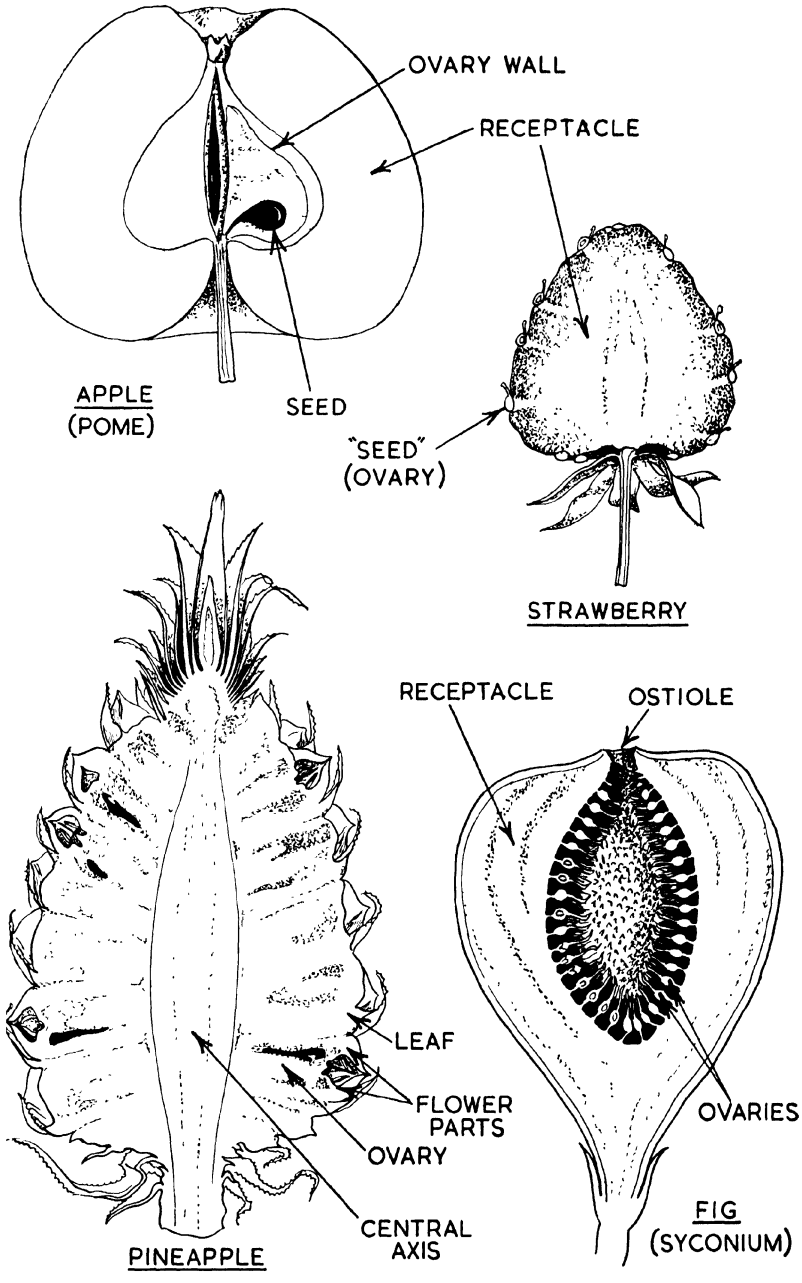



FIG. 21.—Vertical sections of four different false fruits showing their structure. (Apple $\times \frac{3}{4}$, strawberry $\times 1$, pineapple $\times \frac{1}{3}$, fig $\times 1$.)

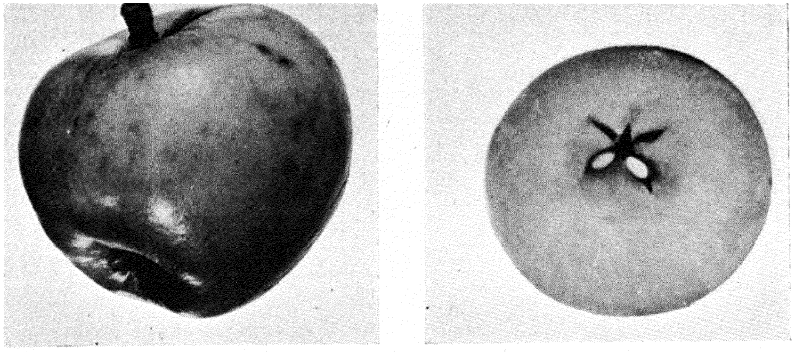


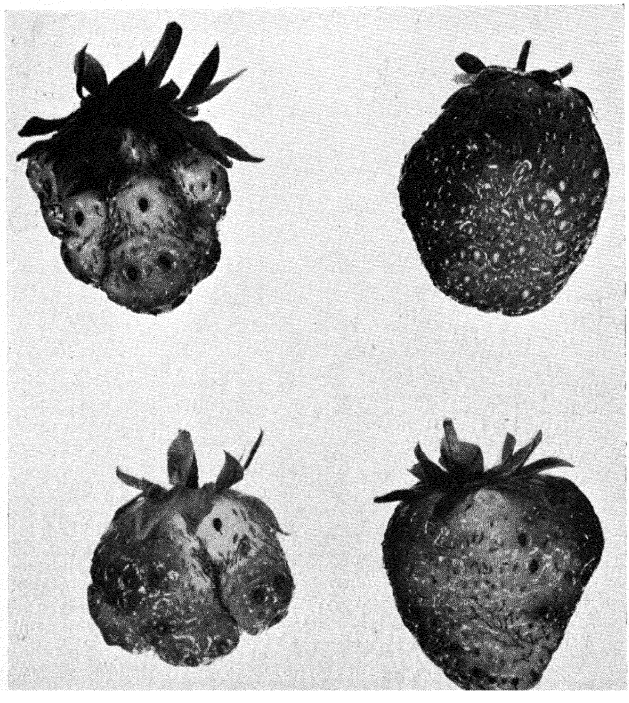
FIG. 22.—"Lop-sided" apple resulting from incomplete fertilization. The apple on the left is shown cut in half on the right. The close relationship between fruit growth and seed position is clearly shown.

*Photographs by permission of Dr. L. C. Luckwill and Long Ashton Research Station.*

FIG. 23.—Fruit development and pollination in the strawberry (Tardive de Leopold).

*Left:* Misshapen fruits resulting from inadequate pollination. Note that receptacle growth has taken place only under fully formed "seeds". This strawberry variety is male sterile.

*Right:* Similar fruit sprayed with 50 p.p.m. NoXA. Note the full development of the fruit in spite of the paucity of fully formed "seeds".



Kraus, 1937), blackberry (Marth and Meader, 1944) and strawberry (Gardner and Marth, 1937). A further complication arises in that the seed-coat (formed from the original covering to the ovule) may also develop and harden normally in artificially induced parthenocarpic fruits, although no embryo is developed inside. This occurs in the pumpkin (*Cucurbita pepo*) and watermelon (*Citrullus vulgaris*) (see Mann, 1948). Such results suggest that we may never be able to produce "pit-less" parthenocarpic fruits of cherry, plum, peach, etc. Conversely, in the Calymyrna fig, where the "pips" are whole fruits, one from each of a great number of female flowers contained in the hollow of the flask-shaped fleshy receptacle (see Fig. 21), treatments with IBA result in completely "seedless" fruits (Crane and Blondeau, 1949; Blondeau and Crane, 1950). Apparently treatment stimulates the growth of the receptacle only and does not affect the ovary walls, which remain soft and undeveloped. Natural parthenocarpic fruit of the same variety have fully developed pips, although of course without embryos. On the other hand, parthenocarpic fruits induced by 4-CPA caused slight hardening of the endocarp.* Thus the nature of the auxin used obviously has an effect, and holds out hopes that a substance suitable for inducing "pit-less" cherries, etc., may one day be found. At the moment, however, it would seem that, with the above exceptions, there is very little improvement to be expected in the quality or edibility of the fruit as a result of hormone treatment.

The great importance of such treatment, from the grower's point of view, comes from its use in supplementing normal pollination in the setting of fruit. We have seen that, except in a few rare cases of natural parthenocarpy, failure of pollination automatically results in a failure to set fruit, and the unpollinated flower soon falls. In those parts of the world not possessing an equable climate, where fruit has to be raised at periods of the year or under conditions unfavourable to effective pollination, such lack of fruit-set may be very serious economically. This is a particular problem in tomato-growing in Northern

* The wall of a true fruit can usually be divided into three distinct layers, an inner layer or endocarp, an outer layer or exocarp and an intermediate layer or mesocarp.

Europe and also in the Northern States of America and in Canada. Cool wet weather and particularly low night temperatures, when the flowers are coming to maturity, may greatly diminish natural fruit-set in the open, and this is most prone to happen on the lowest early-formed trusses. Attempts to grow tomatoes in greenhouses and at periods of the year when days are short and light intensity is low may also fail, since no viable pollen may be produced by the stamens. In any event, under such greenhouse conditions, lack of air currents and insects to bring about pollination necessitates the repeated tapping of trusses or some other tedious manual operation to ensure pollen distribution, and even then complete fruit-set cannot be guaranteed. Under these conditions the artificial setting and parthenocarpic development of tomatoes by the application of hormones should reduce considerably the labour employed and also bring about a very marked increase in the final crop yield. That such is indeed the case will be seen from the data presented in the following sections. A typical response is illustrated in Fig. 24.

## SYNTHETIC AUXINS AND ARTIFICIAL FRUIT-SET IN THE TOMATO

A far greater amount of effort has gone into research on the tomato than on all other species of plant combined, and a considerable amount is now known about its reactions to a wide range of synthetic auxins and treatment methods.

### *Methods of Application*

(*a*) Lanolin Paste.—In this method a paste of the auxin in lanolin at a concentration of 0·1 to 0·5 per cent. is applied, either to the receptive surface of the pistil (stigma) or to the stump of the decapitated style in an emasculated flower. This method formed the basic technique in all the early investigations, but, since it involves a separate micro-surgical operation for each flower, it is of little value to the large scale grower. Individual application of hormone to each flower is still, however, a most important technique in research. It has recently been adapted for the biological assay of fruit-setting hormones

(Luckwill, 1948*a*), where the response in the rate of growth of tomato ovaries was related very closely to the dose (*i.e.* total amount) of the auxin applied in aqueous solution.* The activity of test solutions can be compared with that of a standard strength NoXA solution and recorded in terms of NoXA equivalents. The method has already been successful in demonstrating and assaying the natural fruit-setting hormone from apple seeds (see p. 137).

(*b*) Water Sprays.—In this method the auxin is applied to the flowers as a dilute aqueous solution of about 50 parts per million, the absolute concentrations depending on the particular substances employed (see next section). A wetting agent is usually added to cause quick and easy spread of the solution over the sprayed parts. Particular care must be taken to avoid the growing apex of the plant, since severe stunting or even complete stoppage of growth may otherwise result. Sprayed parts will usually develop torsions, leaf-stalks will twist and blades curl, and the photosynthetic capacity of these leaves may be reduced. If the maximum of seedless fruit is required, flowers should be sprayed before they open. This is not, however, the usual concern of the tomato-grower. He desires to obtain the maximum fruit-set with the minimum expenditure of time and trouble. Luckwill (1946) recommends for this a spray when the last flowers on the truss are opening. This usually ensures that all the fruit on the truss set. In the case of very large trusses complete set may be obtained only by two successive sprayings at an interval (*e.g.* 10 to 14 days) determined by the rate of opening of the successive flowers. The flowers are sprayed in the open state and in such a way that the ovaries are thoroughly wetted. By spraying at such a late stage, some fruit on the truss will have set naturally and will have seeds. Dutch workers recommend for early cropping that the first and second trusses should be sprayed once a week as flowering begins, and all other trusses once when all the flowers on them are open (van Koot, 1947). Too early spraying, *i.e.* a week before pollen shedding, may result in a decrease of fruit-set and

* The curve relating growth response (calculated as the change in the relative growth-rate of the ovaries due to the hormone) to the logarithm of the hormone dose was S-shaped.

a reduction in final size of fruit (Hemphill, 1949). Pollen production in the immature anther may also be inhibited, thus preventing subsequent natural set and seed production.

In addition to the possibility of organ torsions (epinasty) and malformations, too lavish and concentrated a spray produces an increased tendency to blossom-end rot under conditions favouring that disease. In spite of these dangers, the water-spray method still seems to be the most reliable and consistent in its results, especially for field-grown fruit.

(*c*) Emulsion Sprays.—In America, some considerable success has been achieved by the use of emulsions of lanolin and water containing the same auxin concentrations as the water sprays. Presumably the function of the lanolin is to retain the auxin solution in contact with the flower parts, thereby making it effective for a more prolonged period. The final concentration of the lanolin is about $\frac{1}{4}$ per cent. and an emulsion stabilizer, such as ordinary household soap, oleic acid, triethanolamine stearate, etc., is usually included. Recipes for making such emulsions can be found in Avery and Johnson (1947) and Mitchell and Marth (1947). Trouble has been experienced in the clogging of nozzles in spray equipment, and attention is now being paid to the employment of non-fatty carriers, *e.g.* carbowax, which is a proprietary product consisting of polyethylene glycols. Mucillages, such as gum arabic, have also been used. It is claimed (Avery and Johnson, 1947) that both open flowers and well-developed buds will respond to these sprays, which are most successful when used in greenhouses. They do not appear to be of value with field-grown tomatoes.

(*d*) Vapour Method.—This method, first developed by Zimmerman and Hitchcock (1941), is suitable for use in greenhouses with good air-tight doors and shutters. The active compound, either dry or in alcoholic solution, is slowly vaporized from a flat container over an electric hot-plate, and the vapour dispersed throughout the greenhouse by a fan. The auxins are most conveniently used as the methyl or ethyl esters which are more volatile than the acids themselves (*cf.* treatment of stored potatoes with α-naphthylacetic acid methyl ester (MeNA) (Chapter 8). Since, by this technique, the whole of the plant is subjected to treatment, due attention must be paid

to the selection of a suitable compound and the adjustment of dosage so that adequate fruit-set is obtained without undue injury to the foliage or impairment of stem growth. NoXA and its ethyl ester have proved to be most effective: 250 mg. of NoXA per 1000 cubic feet of greenhouse space (Mitchell and Marth, 1947) and 10 mg. of the methyl ester per 1000 cubic feet (Strong, 1944) are the optimal concentrations. The methyl ester of 2, 4-D has also been successfully used at 1–10 mg. per 1000 cubic feet. After the vaporization is complete the greenhouses are usually kept closed for a minimum of 4 hours, although longer periods (15 hours) can be used. Two successive treatments at intervals of a few days may be more effective than a single application. So far, however, the fruit quality resulting from this treatment method is inferior to that from spray treatment, and further investigations are needed before any precise recommendations can be made.

(*e*) Aerosols.—A hormone application, which is gaining in popularity in America, especially for use in greenhouses, is the use of "aerosols". A solution of the hormone is made in a solvent of very low boiling-point, usually a gas under pressure in a cylinder. This solution is then released from the cylinder through a small nozzle. The liquefied gas carrier immediately evaporates, leaving the growth substance suspended in the air as a very fine mist of particles. The cylinder usually holds 1–2 lb. of solution, and is fitted with an easily adjusted needle-valve for rapid action. In use, individual trusses may be accurately treated to a "puff" of aerosol, an operation which is much more quickly performed than spraying with aqueous solutions or emulsions, or a whole greenhouse can be filled with the mist from a large cylinder. Such a mist may persist for as long as 5 hours and, of course, the whole plant is subjected to its action. In a wide range of trials in the laboratories of the U.S. Department of Agriculture, highly satisfactory results have been obtained in some instances, equal to the best from water or emulsion sprays. On the whole, however, the response is not so consistent as with sprays, and many trials have produced coarse and malformed fruit (Howlett and Marth, 1946). There is little data available to indicate the value of this method in the open, although Zimmerman

and Hitchcock (1944 and 1944 *b*) obtained a 100 per cent. set of field tomatoes using an aerosol of 0·1 per cent. α-(2-chlorophenoxy)-propionic acid in Freon,* and express high hopes of the future practical value of this technique.

(*f*) Other Methods.—Several other methods have been tried with varying degrees of success. One involving the injection, by hypodermic needle, of small quantities of dilute auxin solution direct into the ovary, and another the application of crystals on to the stigma, may be of use in experimental work with small numbers of plants, but are unlikely to be of any value in commercial tomato-growing. The possibility of using talc as an auxin carrier has been explored, but the increase in set is somewhat offset by the aggravation of blossom-end rot in field-grown fruit. Miscellaneous physiologically inert viscous fluids such as triganine, morpholine, cotton-seed oil, Glycopon AA, etc., have been used as carriers for IBA with considerable success (Strong, 1941). Attempts have also been made to increase set by application of dilute solutions of active compounds to the soil, in the hope that they might be absorbed by the roots and translocated to the opening flowers. Preliminary experiments (Zimmerman, 1942 *b*) with various substituted phenoxyacetic acids gave some positive results, and more recently it has been reported (Randhawa and Thompson, 1949) that applications of 2, 5-dichlorobenzoic acid † to the soil increases the size of the fruit produced.

### *Active Compounds and their Relative Effects on Fruit and Plant*

In the last decade, the period over which this practical aspect of auxin physiology has been developing, a very considerable number of chemical compounds have been tested. A large list of active compounds was first published by Zimmerman and Hitchcock (1944 *a*), and this has been much supplemented since then. Another list is given by Avery and Johnson (1947). The most recent list of 75 aryloxyalkylcarboxylic acids,

* Freon = Dichlorodifluoromethane.

† 2, 5-dichlorobenzoic acid has not, as far as the writer is aware, been shown to be an auxin, although Bentley (1950) has demonstrated auxin activity for 2, 3, 6-trichlorobenzoic acid (see p. 62).

together with their relative effectivenesses, has been compiled by Osborne, Wain and Walker (1952). By no means all of the compounds that are active have any practical application, since they either require to be applied at very high concentration levels or have undesirable toxic actions in addition to their fruit-setting properties. In Appendix B appear all the most important of these compounds so far reported to be of definite practical value. Here the effective dosage ranges for four different application techniques are recorded in four separate columns. The table contains by no means all the data available, but the writer has selected from the literature those treatments appearing to be of the greatest practical value. Those compounds which seem to be, by a general consensus of opinion, the most promising have been starred, and the corresponding number in brackets gives the average optimal concentrations.

All these compounds and treatments result in a considerable increase in the total number of fruit set per truss, under the most favourable conditions a set of 100 per cent. being obtained. Since, by careful choice of treatment times, all fruit on a truss can be induced to start their development within a very short time-period, the resultant crop tends to be of a much more uniform size. An earlier start to development also means somewhat larger fruit, which often come to maturity at a much earlier date. Under optimal growth conditions and when the best growth substances are used, the parthenocarpic tomatoes differ very little from those set by pollination, except of course in that they have no seed. They have the same shape and colour, their flesh and gelatinous pulp is of the same consistency and flavour, and there seems to be no important changes in the various chemical constituents that have so far been analyzed. The acidity, total solids and vitamin C content are not altered (Mitchell and Whitehead,1942). Slightly higher sugar content may bestow a somewhat insipid flavour in some instances (Pollard, Kieser and Steedman, 1945). Such ideal results can most easily be achieved by suitable concentrations of IBA, but this compound has of recent times fallen into disfavour owing to the very high concentrations needed. A compound which is probably the most reliable single substance

for inducing fruit-set, and one which, in addition, works at a low concentration, is NoXA. In early work on this compound, using the spray technique, considerable toxic actions were reported (Swarbrick, 1942). The growth of the plant was considerably checked and the sprayed parts developed, in the course of a few hours, marked epinastic * curvatures and leaf disturbances. Such toxic effects can be avoided if suitable low concentrations (*e.g.* 50 p.p.m. in an aqueous spray) are employed. Similar disturbances of the growth of the plant accompanied by undesirable structural and internal chemical changes are much more frequently obtained by the use of the very active phenoxyacetic acids. In those compounds the lower concentration limits of toxicity lie much closer to the optimal concentration for fruit-set than in IBA and NoXA (*cf.* their rooting activities in cuttings). This is particularly well marked with 2, 4-D and 2, 4, 5-T. Even under optimal concentrations for fruit-set, some disturbance of plant growth may be expected and a percentage of the fruit will be of very poor quality. This loss of quality, occasionally resulting in completely unsaleable fruit, is due to several causes. One of the commonest features is a loss of the gelatinous pulp and the development of a hollow fruit with a tough skin and puffy insipid flesh (see Fig. 25). The fruit is also very prone to elongate and become beaked, thereby resembling in shape the large red pepper or paprika (*Capsicum annuum*). The red colouring may also fail to develop internally in regions where the seeds are attached and thus the whole fruit may be streaked with green. A milder reaction results in watery pulp, woolly insipid flesh and thick skins. An extreme reaction has been seen when 4-CPA was applied direct to the ovary wall. Concentrations above the toxic limit suppressed the development of the fruit wall and resulted in the exposure of a naked strawberry-like placenta (Zalik, Hobbs and Leopold, 1951). Although, therefore, their activity is so very high, the latter compounds are still inferior to NoXA. But it should not be forgotten that NoXA can also provoke the same symptoms with excessive dosing, which seems more prone to occur in greenhouses than in

* An epinastic curvature of a plant organ is caused by a more rapid growth on the upper side than on the lower.

outdoor-grown tomatoes (Wain, 1950). Nevertheless, great promise is shown in the results of treatment with mixtures of active compounds. Three combinations that have so far been tried appear at the bottom of the table in Appendix B.

With the aerosol method of treatment (Howlett and Marth, 1946) the mixture of the three compounds NoXA, IBA and 4-CPA gave much better results than either NoXA or IBA singly or the other two combinations. Encouraging results have been obtained in England (Wain, 1950) where the action of the following sprays were compared:—

| | |
|---|---|
| 40 p.p.m. | α-(2-chlorophenoxy)-propionic acid |
| 60 p.p.m. | β-naphthoxyacetic acid (NoXA) |
| 100 p.p.m. | α-(2-naphthoxy)-propionic acid |
| 40 p.p.m. | NoXA + 10 p.p.m. 4-CPA |

all as the methylglucamine salts.* Experiments were conducted on field-grown tomatoes at two localities, Canterbury and Wye College. Not only was fruit production followed throughout the whole growing season, but the quality of the fruit (*i.e.* its shape, evenness of ripening, interior appearance and flavour) was carefully assessed from random samples by a panel of seven persons. In the Canterbury trials the mixture (NoXA + 4-CPA) produced by far the greater weight of crop (1·59 times the unsprayed), whereas in the Wye trials it was bettered only by α-(2-chlorophenoxy)-propionic acid alone (4·16 as compared with 4·48 times the untreated). Comparisons of fruit quality on the other hand showed that, on the average, the mixture gave the best and the α-(2-chlorophenoxy)-propionic acid the poorest results, the naphthoxy compounds occupying a position slightly inferior to the mixture. Only in two assessments did the treated fruit gain marks superior to those of untreated fruit. This was for interior appearance of fruit sprayed with NoXA and the mixture at Wye. Such results give us reason to hope that further research will eventually find a combination of compounds that will give us 100 per cent. fruit-set with no imperfections in fruit quality. An illustration of differences in quality of normal tomatoes and those set

* Methylglucamine has the formula $CH_3{\cdot}NH{\cdot}CH_2{\cdot}(CHOH)_4{\cdot}CH_2OH$.

by NoXA (good quality) and 2, 4-D (poor quality) is seen in Fig. 25.

*Economic Aspects*

As we have noted previously, the economic advantages to be expected from auxin treatment of this kind lie in increased set and the correlated gain in crop and not in the production of seedless fruit, which, it is generally agreed, tends at the moment to be somewhat inferior in quality to naturally seeded fruit. One would therefore not expect any advantage to accrue from treatment of plants grown in parts of the world and at times of the year favourable to high natural rates of fruit-setting. Thus, in California, treatment of field-grown tomatoes with water sprays, dusts and aerosols of NoXA, 4-CPA and 2, 4-D all indicated that, although yield was shifted to an earlier part of the season, there was no increase in the total yielding capacity of plants (Mann and Minges, 1949). Under conditions of low natural set (see p. 142) in greenhouses or in the open, treatment may produce a manifold increase in the total crop. To quote an example, Howlett, and Marth (1946), applying hormones by the aerosol treatment to greenhouse tomatoes, increased the yield per plant by 20 to 40 ounces, and the total crop by about 5 to 10 tons per acre. Recently Wain (1950) has shown that similar results can be obtained from outdoor tomatoes in England under conditions of poor natural set. At Wye, in 1948, a very poor setting year, natural set gave a yield of approximately 3·3 ounces per truss, whereas treatment raised the total yield to 11·5 to 15 ounces per truss depending on the particular compound used. In the same year at Canterbury, better setting conditions gave a natural crop of approximately 9·5 ounces per truss and a treated crop of 12·5 to 15 ounces per truss. In 1947 at Canterbury, however, using the same variety of tomato (Harbinger) during very good natural setting weather, a control yield of 16·5 ounces per truss had been obtained and treatment increased this by only 4·6 to 5·5 ounces. The advantages of spraying come therefore purely from the increased fruit-set and are more marked the poorer the natural setting conditions. Although there are indications that the earlier onset of fruit growth so induced may result

finally in larger individual fruits, it seems unlikely that the hormones by themselves induce a greater growth-rate. It is most probable that rate of growth is limited by prevailing conditions of light intensity, temperature, rainfall, mineral nutrition, etc., which will determine the rate of synthesis of food materials. This stresses the need for the maintenance of optimal cultural conditions at all times. There is little advantage to be gained by ensuring the set of the fruit if it is not subsequently given every encouragement to grow. The remarks made on the rooting of cuttings are also pertinent here; auxin treatment *supplements* but does *not replace* the normal cultural practices.

## THE USE OF AUXINS IN THE PRODUCTION OF PARTHENOCARPIC FRUIT OF OTHER SPECIES

### *Pome Fruits.* (Apples and Pears)

We have already seen that there seems to be a definite relationship between the growth of the apple and the number and distribution of its seeds, suggesting control by a hormone arising in those seeds (see Fig. 22). Analysis of the auxin content of apple seeds throughout the development of the fruit shows that marked changes occur in the total amounts (Luckwill, 1948) (see Chapter 10), but that the gross growth of the apple is in no way correlated with them. In addition, several investigators, who explored the possibility of increasing the set of apples by auxin sprays, obtained negative results. Lanolin pastes, water and emulsion sprays and even the injection of dilute solutions into branches, produced either no response or injury. In one series of experiments on pears and apples (Stuivenberg, 1943) the ovaries were removed from unopened frosted flowers, and the hollow receptacle, from which the fruit is formed, filled with lanolin containing various auxins, in particular NAA at 100 $\gamma$ per gm.* In the pear variety, Précoce de Trévoux, such drastic treatment with NAA and its sodium salt induced considerable fruit-set, *i.e.* about 20 per cent. of those treated. IAA was inactive.

Subsequent modifications of the spray technique, however,

* $\gamma$ is the symbol used for one microgramme $= 1/1000$ mg. $= 10^{-6}$ gm.

have produced small but significant increases in the set of both apples and pears, with corresponding improvement of crop yield. Thus a mixture of auxins (5 p.p.m. IBA + 5 p.p.m. NAA + 5 p.p.m. 2, 4-D + 60 p.p.m. NoXA), applied as a water spray to young fruit damaged by frost (Swarbrick, 1945), produced 100 per cent. of parthenocarpic fruit in Miller's Seedling, but was without effect on Cox's Orange Pippin. Although these results are not from normal but from frost-damaged apples, yet there is a promise in them that growth substance mixtures may perhaps prove effective where simple substances have failed (*cf.* results for tomato, p. 149). Why pome fruits should respond more readily to the application of hormones after exposure to frost presents an intriguing problem, although it is a suggestive fact that frost itself will sometimes induce parthenocarpy in some varieties, *i.e.* Conference pear and Wealthy apples (Luckwill: Personal communication).

A succession of applications of dilute solution sprays, first to the flower, then 3 or 4 weeks later at the time of fruit-set, and subsequently at 10- to 14-day intervals for three further sprayings, has been claimed to be very effective in ensuring fruit-set in this type of fruit. In pears a most effective compound is the sodium salt of α-(2-naphthoxy)-propionic acid applied at 100 p.p.m. (Osborne, 1949). A single spraying of emasculated flowers caused rapid initial growth, but this ceased and the young fruit fell after about 4 weeks. Regular sprays of the same compound at three-day intervals prevented this, and in the variety Pitmaston Duchess a 40 per cent. set of fully grown parthenocarpic fruit resulted. This represents a crop almost twice the size of that from normally fertilized trees. A similar effect of successive sprayings of emasculated flowers was obtained in the variety Dr. Jules Guyot, but here the final crop was much smaller than the normally fertilized crop (Osborne and Wain, 1950, 1951). This suggests that pears, unlike tomatoes, need, for full parthenocarpic development, a sustained supply of hormone. But in America a single spraying of the variety Bartlett at the pink bud stage with 100 p.p.m. solutions of 2, 4, 5-trichlorophenoxypropionic acid caused a greatly increased set of fruit, which were smaller and more apple-shaped than normal. In other varieties (D'Anjou, Hardy

and Winter Nelis) similar treatment caused a reduction in set, illustrating the wide varietal differences in response to be expected from this fruit (Griggs, Iwakiri and DeTar, 1951). Apples are even less sensitive than pears, since most are completely insensitive under normal conditions, even to successive sprays. As far as the writer is aware, the only normal (unfrosted) apple brought to full parthenocarpic development by successive sprays is Bramley Seedling treated as above with 100 p.p.m. of α-phenoxypropionic acid (Osborne and Wain, 1951). Repeated sprayings with appropriate mixtures of auxins may well be of great future value in increasing the set of pome fruits, provided an economical technique can be worked out.

### *Plums and Plum-like Fruit*

There is no evidence with the compounds so far tried that parthenocarpy can be induced in such fruit as plum, cherry, apricot, peach, etc. In fact, as pointed out by Lewis (1946), only species with many-seeded fruit have so far shown themselves sensitive to treatment. He suggests that the reason for this may be that the main stimulus to fruit development in these fruits is the auxin from the single developing seed, and that this may completely dominate the small auxin contribution that came in with the pollen. As we have seen, however, it seems likely that this latter stimulus from pollen auxin merely acts as a trigger to internal auxin production in both single- and many-seeded fruits (see p. 139), and the difference in behaviour cannot be so easily explained. In addition, in view of results from other plant species (*e.g.* holly, blackberry, strawberry, etc.—see p. 141), where parthenocarpic fruits possess hard "pips" without embryos, we may find it impossible to get rid of the hard "pit" in such fruit, even though we may succeed in inducing parthenocarpic development.

It is relevant to mention at this point that a number of other commercial fruit-trees not strictly belonging under this heading have been subjected to hormone treatment without worthwhile gain in fruit-set. Species which fall into this category are the citrus fruits (orange and grapefruit), mangoes, avacados and pecan nuts (see Gardner, 1951).

## *Figs*

The fig is a unique fruit as regards its structure. The bodies popularly known as seeds are, in reality, fruits, since they are produced one from each of the ovaries of the numerous female flowers which line the inside of the fleshy flask-shaped receptacle forming the false fruit, called here a *syconium* (see Fig. 21). In the wild figs and those cultivated varieties known as the Smyrna and Calimyrna figs, the latter being extensively grown in California, pollination, and consequently fruit-set, depend on a small wasp, *Blastophaga psenes* L. These wasps breed by laying their eggs inside the syconia of the fig, which they enter via a small opening at the apex (*ostiole*, see Fig. 21). The male flowers are not borne in the same syconia as the female flowers, but in separate structures known as "caprifigs" which themselves grow on separate male trees. Pollination occurs when pollen from such caprifigs is transported by the visiting wasp to the female syconium on the female tree. Thus, in the cultivation of Smyrna and Calimyrna figs, the fruit-grower must maintain caprifig trees in his orchard in order to ensure adequate pollination of his figs. Not only is this expensive, but the trees occupy orchard space and yield no crop themselves. There is, in addition, a considerable risk that the male and female figs may not reach the same stage of development at the same time, and this may reduce or even prevent pollination. There are also dangers of over-pollination, which results in splitting and a high incidence of a rot disease (due to the fungus *Fusarium* sp.) spread by the wasp. Thus it will be seen that the process of "caprification", as it is called, not only involves much labour and expense, but its satisfactory completion requires a considerable amount of experience and skill on the part of the grower, the guiding principles being almost entirely empirical and not susceptible of precise standardization. Is there any wonder, therefore, that the possibility of eliminating this caprification process by the use of auxins to induce set has recently received much attention?

Parthenocarpic figs are by no means unknown. Indeed, three out of the four most important of the varieties grown in California are naturally parthenocarpic and are never pollinated.

They contain at maturity embryo-less "seeds". The fourth main variety, the Calimyrna, is the most popular variety for drying, by virtue of its thin rind.

Three methods of application of the auxins have been tried. Treatment of the young syconium with a lanolin-paste preparation (Stewart and Condit, 1949), or by the injection of a dilute solution through the ostiole (Crane and Blondeau, 1949), was ineffective. On the other hand, both water and emulsion sprays applied to the whole branch bearing the immature syconia have proved highly effective, the parthenocarpic "seedless" fruit produced being equal to the caprified controls in both quality and size. No significant differences could be observed in either flavour, colour or consistency, although parthenocarpic fruit contain slightly more sugar than caprified, *i.e.* 79 per cent. as compared with 74·5 per cent. (Crane and Blondeau, 1951), and the flesh (receptacle) was much thicker. Drying and packing tests also showed the parthenocarpic fruit to be as good as the normal caprified fruit, although they became somewhat milder in taste (Blondeau and Crane, 1950). In addition to inducing parthenocarpy, the hormones also greatly accelerated the rate of maturation of the fruit, resulting, with some treatments, in ripening a month earlier. As might be expected, the useful concentrations were rather critical, since overdosing the branch resulted in leaf yellowing (chlorosis), and in one instance (25 p.p.m. of 2, 4, 5-T) in death of the branch after one month (Crane and Blondeau, 1949).

The most effective compounds so far reported are the following (Crane and Blondeau, 1949 and 1951; Blondeau and Crane, 1950):—

(*a*) IBA in concentrations ranging from 200 to 2670 p.p.m. Toxic symptoms are observed over 600 p.p.m. The resulting fruit is of excellent quality.

(*b*) NAA at 25 to 250 p.p.m. with toxic symptoms appearing at 50 p.p.m.

(*c*) 2, 4, 5-T at 10 to 100 p.p.m. This compound also greatly accelerates fruit ripening, which occurred nearly two months earlier than in the caprified controls. Toxicity sets in at a low level and death may result at 75 p.p.m.

(*d*) 4-CPA at 40 to 80 p.p.m. gives excellent parthenocarpic

fruit, which develop at about the same rate as the caprified. Another interesting feature is that the endocarp of the fruit ("seed") may become partially hardened with this compound, giving "ghost" pips (Baskaya and Crane, 1950). Lack of toxic effects at these concentration levels recommends it for practical use.

(*e*) NoXA and 2, 4-D are relatively inactive and are not recommended by the investigators (Blondeau and Crane, 1950).

This list illustrates the growing complexity of the problems facing the plant physiologists in their studies on chemical structure and physiological activity of auxins, when it is remembered that NoXA and 2, 4-D are among the compounds with the highest auxin activity. Are these differences due to secondary properties of the molecule or to their basic action in inducing fruit development?

These results offer high hopes for the successful use of auxins in the fig-growing industry, although a warning note against too great optimism has been sounded from Australia where much less satisfactory responses have as yet been obtained with the Smyrna fig (Harris, 1950).

In the holly, too, we have a similar example of a plant with distinct male and female trees, and the grower who is interested in getting a plentiful supply of berries on his female trees must ensure fruit-set, *i.e.* by interplanting with a certain proportion of male trees. In this species, too, such interplanting can be dispensed with if the female flowers are sprayed with dilute hormone solutions. A number of compounds have been found effective, but the most satisfactory ones at present seem to be NAA, at about 60 p.p.m., or NAAm at 50 to 100 p.p.m., the latter apparently being more suitable for outdoor treatment (Avery and Johnson, 1947).

### *Strawberries*

Like the apple and the fig, the strawberry is also a false fruit since the fleshy edible portion is produced by the growth of the receptacle (see Fig. 21). The "pips" are really the true fruits. The growth of this fleshy receptacle is controlled by the seeds inside these pips, presumably by the production of auxin (Nitsch, 1950) (see page 138 and Fig. 23).

The parthenocarpic development of strawberries can be induced in unpollinated but otherwise normal flowers by spraying with dilute auxin solutions, or by exposing the whole plant to a suitable volatile compound (*i.e.* MeNA). Both IBA and NAA have been reported effective, but NAA causes considerable distortion of foliage. The most striking results have been obtained with NoXA at concentrations of 20 p.p.m., where little damage is done to foliage. Both Tardive de Léopold and Royal Sovereign varieties gave greatly increased yields after drenching spray treatment during flowering. The increase in yield amounted to nearly half a ton per acre and was largely due to an increase in the size of the fruit (Swarbrick, 1944). Such parthenocarpic fruit usually possess a few scattered pips, produced in the main by partial pollination occurring before spraying, although embryo-less achenes (pips) can also be produced by the action of IAA alone (Gardner and Marth, 1937) (*cf.* the action of 4-CPA in figs).

*Beans*

The bean pod, having been produced from the ovary of the bean flower, is, like the tomato, a true fruit. It is given, with that of the pea, the particular name of *legume*. In the broad bean (*Vicia faba*) and in the garden pea (*Pisum sativum*), the crop is the seed inside the pod, and pollination is therefore an indispensable factor in its production. In the runner bean (*Phaseolus multiflorus*), French bean (*Phaseolus vulgaris*), etc., it is the young pod which is eaten. Since in these latter beans the pollination and therefore fruit-set is as dependent on prevailing weather conditions at flower maturity as it is in outdoor tomato, one might expect auxin applications to increase the crop. In the beans it is hot dry weather at this time which usually results in extensive failure of pod-set. A number of experiments have been conducted in America on snap beans (*Phaseolus vulgaris* var.), and they have shown that spraying with dilute auxin solutions (*i.e.* 5–25 p.p.m. of NAAm or NoXA and 2 p.p.m. of 2-CPA or 4-CPA) brought about great increases in set under conditions unfavourable to normal pollination. There was a correlated increase in pod size and uniformity. Very much smaller or negligible increases were observed under favourable

natural setting conditions, as would be expected (Murneek, Wittwer and Hemphill, 1944; Wittwer and Murneek, 1946). A very effective treatment consisted in spraying with these dilute solutions at successive intervals of two days. It is still too early for any specific recommendations to be made. Even under conditions favourable to set, hormone treatments may give increased yield by the stimulation of the growth of the pod tissue and the resulting increase in pod size (Wittwer, 1951). In contrast to this, hormone spray treatment of lima bean (*Phaseolus lunatus*) and dry shell beans, where the seed itself is the economic crop, has resulted on the whole in a decrease in yield, even though the growth of the pod tissue may have been stimulated (see Wittwer, 1951). Likewise, no benefit can be expected from the treatment of other similar legumes, such as the garden pea and the broad bean.

### *Other Fruits of Economic Importance*

*Blackberry.*—Aqueous spray and aerosol treatments have been successful in greatly increasing the fruit size in the blackberry, a doubling being observed in some favourable cases. Effective compounds so far recorded are IAA, IBA, NoXA, 4-CPA, 2-CPA and 2, 4-D and certain mixtures of these compounds at concentrations of the order of 50 p.p.m. The relatively high toxicity of 2, 4-D is also apparent in these treatments.

*Grape.*—Dilute solution sprays of NAA (2·5–40 p.p.m.) have been successfully applied to unopened flowers of grape varieties that normally show a high percentage of flower drop. By emasculating the flower and spraying the whole branch with dilute hormone solutions, a high percentage of seedless grapes could be obtained from a normally seeded variety (Brevieri, 1948). There are, however, naturally seedless-grape varieties such as the Corinth (currant) grape. Recent work with a wide range of synthetic auxins has shown that an increase in the number and size of fruits in a bunch can be produced either by dipping the flower cluster into the dilute solutions at full bloom, or by similarly dipping the young immature grapes. It is a very interesting point that one

compound (4-CPA at 5 p.p.m.) actually induced the formation of seed-like structures in what would normally have been seedless grapes (Weaver and Williams, 1950).

Many other species of plant have at one time or another been tested for their sensitivity to applied auxins. Few as yet show any promise of commercial advantage to be had from such treatment. The most important responses are tabulated in Appendix C.

## THE POSSIBLE USE OF AUXINS IN PLANT BREEDING

One of the most important botanical research activities to-day is plant breeding. One aspect of such work is the development of pure lines of plant species and varieties, necessitating prolonged inbreeding and selection of the progeny for the desired characteristics. Such a procedure necessitates the self-pollination of the plant species concerned. An equally important aspect of such breeding is the crossing of two pure lines, each having one or more different characteristics which it is desired to combine and perpetuate in a hybrid. In crop plants such desirable characteristics would be resistance to disease, high cropping potential, general growth vigour, high food value, etc. The flower-breeder would be concerned with other more æsthetic features. The success of all such work depends primarily on pollination being followed by successful fertilization of the ovule and the production of viable seed. The ease with which this is brought about in cross pollinations, *i.e.* pollination of a flower by pollen from another variety of the same species or by that of an allied species, depends very largely on the nearness of the relationship between them. Very closely related varieties usually cross with great ease and are said to be compatible. As a general rule, as the relationship gap widens, successful crossing becomes more and more difficult. Thus it is very rare indeed that two species from different genera will cross and produce viable hybrid seeds. These plants are said to be incompatible, and the failure to produce seed may be due to a variety of causes, the most important of which, in closely related plants, is the difference in the constitution of the male and female nuclei. Even if the nuclei fuse in fertilization, the

state of unbalance in the resulting fusion nucleus resulting from such differences is sufficient to prevent seed development from it. Incompatibility is not uncommon between very nearly related varieties of the same species, and, indeed, some species are self-incompatible, *i.e.* they cannot normally be successfully fertilized by their own pollen. This kind of incompatibility has quite a different origin. In some cases it is due to the failure of the pollen to germinate on the stigma. In others it is due to such a very slow growth of the pollen-tube down the style, that either the flower as a whole or the style itself is shed and falls before fertilization can take place. It is in overcoming this latter type of incompatibility that growth hormones have a possible use. If, by the timely application of a suitable auxin solution at the time of pollination, abscission of the flower or style could be prevented, this fertilization might be expected eventually to take place and viable seed to be produced.

Arguing along these lines, a number of botanists have attempted to overcome cross- and self-incompatibility in selected plants, and have met with some small degree of success. For example, self-sterile *Petunias* sprayed with a 10 p.p.m. NAAm solution after pollination formed viable seeds in the fruit (Eyster, 1941). Similar success in reducing incompatibility in certain self-sterile races of marigold (*Tagetes erecta*), cabbage (*Brassica oleracea*) and red clover (*Trifolium pratense*), has been reported. Otherwise incompatible cross-pollinations between several lily varieties have responded to auxin treatment and produced viable seed (Emsweller & Stuart, 1948), and increased seed yield has been reported after auxin treatment of different experimental crosses of cantaloupes (*Cucumis Melo*) made for the production of disease-resistant strains (Wester and Marth, 1949). We are still, however, uncertain of the true underlying reasons for these successes. In the *Petunia* it has been suggested that, in view of the small number of seed produced, fertilization of one or two ovules may invariably take place but that this may be insufficient to stimulate ovary growth. Auxin treatment does this, and the few seeds are then allowed to develop (Lewis, 1949). In addition also to the possible prevention of flower or style

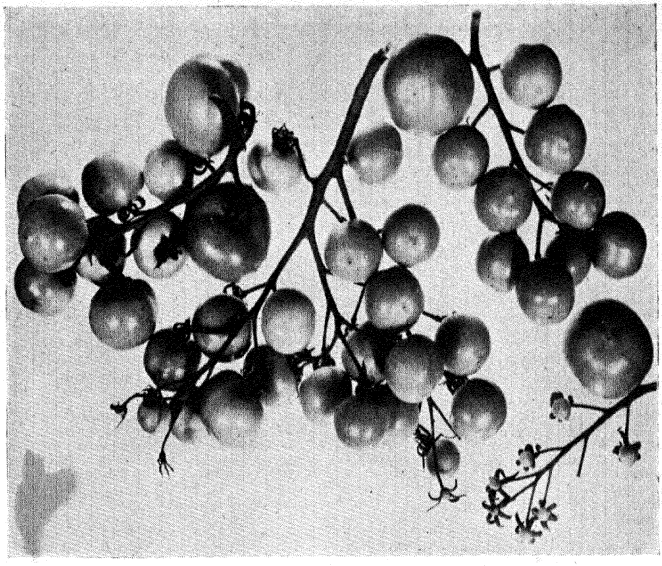


FIG. 24.—Set in greenhouse-grown tomatoes induced by spraying flowers with 50 p.p.m. NoXA.

*Bottom* right-hand corner. Unsprayed truss in which only one tomato has set, a common condition in greenhouse-grown tomatoes in early spring.

*Top*. Three typical sprayed trusses showing complete set.

*Photographs by permission of Dr. L. C. Luckwill and Long Ashton Research Station.*

FIG. 25.—Slices of tomato fruit (cleared in lacto-phenol reagent) showing structure differences between normal and parthenocarpic fruit.

1. Normal tomato with full formation of seeds.
2. Good quality parthenocarpic tomato induced by NoXA spray. Note the absence of seeds, but otherwise normal pulp content.
3. Poor quality "puffy" parthenocarpic fruit induced by 2, 4-D spray.

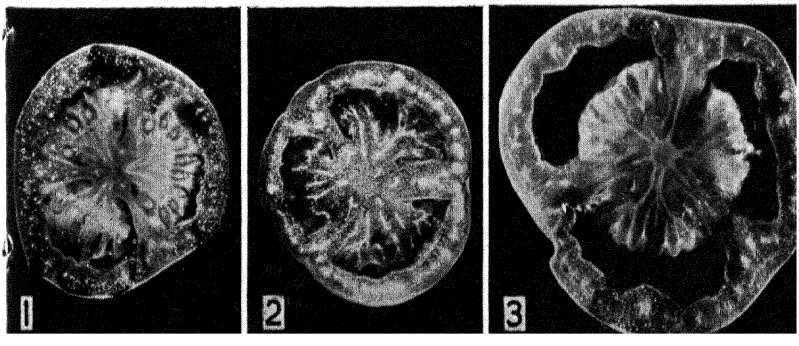
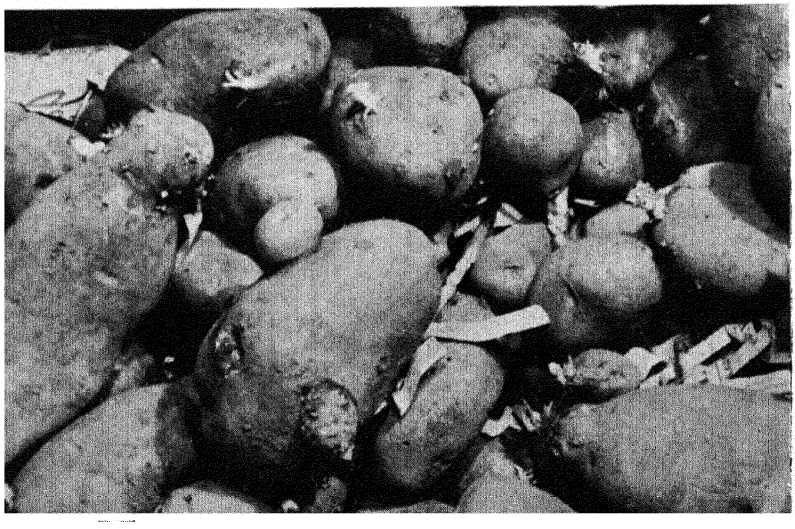




FIG. 26.—Untreated potatoes (var. Majestic) stored until June. These tubers show excessive sprouting and shrivelling.

FIG. 27.—Potatoes (var. Majestic) stored until June after treatment with MeNA. The tubers were mixed with strips of newspaper impregnated with the compound. The shredded paper can be seen in the photograph still mixed with the tubers. Note the very slight sprouting and complete absence of any shrivelling.

*Photographs by permission of Dr. L. C. Luckwill and Long Ashton Research Station.*



abscission, it has been suggested that the auxin may have a direct stimulating effect on the rate of pollen-tube growth (Mann, 1948), thus causing an increase in the number of ovules fertilized. No such growth stimulation was observed, however, in cherry and plum pollen-growing in the styles under the action of low concentrations of NAA (Lewis, 1946).

Although the ultimate mechanism underlying the positive auxin effects is still obscure, these few results hold out some promise for the future. Not only may auxins serve as a means of solving the riddle of these particular incompatibilities, but they may also be of great practical value to the plant breeder in enabling him to increase greatly the number of new plant varieties and hybrids so necessary for the improvement of our agriculture. Although there are grounds for some optimism, we must, however, recognize that in some species incompatibility will never be overcome in this way. Thus the application of 20 p.p.m. NAA solution to ovaries of cherry (*Prunus avium*), plum (*Prunus domestica*) and *Œnothera organensis*, where incompatibility is due to the slow growth of the pollen-tube down the style, failed to promote the production of viable seed after pollination. Although style abscission was delayed by about 2 days, yet the pollen-tubes stopped growing and no fertilization resulted (Lewis, 1946). In order to overcome incompatibility here, it would be necessary to prevent the stoppage of pollen-tube growth in the style as well as delaying style abscission.

CHAPTER 8

# AUXINS AS GROWTH INHIBITORS: I. BUD-GROWTH AND INDUCED DORMANCY

## INTRODUCTION

In Chapter 2, when the general physiological properties of the natural auxins were being discussed, it was pointed out that the stimulation of growth that they brought about in the plant had a maximum at a certain optimal hormone concentration, and that, above this level, growth was inhibited progressively as the concentration further increased. It was seen that this optimal concentration was lowest in roots and highest in shoots and intermediate in the case of buds, and that these differences most probably underlie the control of organ development and shape in the plant. We have so far considered only those practical aspects of auxin action that are concerned with the *stimulation* of plant growth in one form or another. In the next two chapters we shall be considering the practical applications of auxins at concentrations above the optimum, whereby growth inhibitions of organs or whole plants may be achieved. The subject falls naturally into two parts.

Firstly, we have those inhibitions that are brought about by the application of physiological concentrations of the auxin, *i.e.* concentrations approximating to those occurring under natural conditions in the plant under treatment. Such applications are almost entirely concerned with the suppression of bud growth and will be dealt with in the present chapter. Secondly, we have the inhibitions that result from treatments with very high concentrations never met in the normal plant, at which level the action of the auxin is one of general toxicity. The concentration at which such toxicity is reached varies from species to species and indeed from organ to organ in the same species. We shall see in the next chapter that this allows synthetic auxins to be used to kill certain species of plant in a selective manner, viz. in the control of weeds.

## BUD-GROWTH AND THE RÔLE OF THE NATURAL AUXINS

Just as a whole plant goes through a succession of discrete and well-marked stages in its development from seed to full maturity, so we can distinguish a similar set of stages in the formation and development of a bud. Buds are indeed small embryonic shoots and are composed of stem, leaves and, in flowering buds, a complete flower or collection of flowers (inflorescence) in embryo. Such a bud starts its life either from an apical meristem (apical bud) or from a small lateral meristem in the angle that a leaf makes with the main stem (lateral or axillary bud) (see Fig. 2). The initiation of such buds takes place at a very early stage, *e.g.* when the branch bearing it may itself be a bud. Indeed, it is often possible by dissection to make out the bud meristem in the axil of an embryonic leaf in an unopened bud. The development of the second generation bud from this bud meristem commences as soon as the parent bud bursts and starts its growth into a new branch. It finally reaches its mature size and development when the new branch is fully grown. During the development period it has been subjected to a considerable complex of influences, both environmental and internal, which have controlled its development and regulated the number and kind of rudimentary organ formed from its meristem. Thus it may have become a purely vegetative bud that, upon bursting the following season, will produce nothing but stem, leaves and a further complement of apical and lateral buds. It may, on the other hand, produce stem, leaves and flowers. These influences have been much studied but the physiological principles which underlie them are only now beginning to be understood. Evidence is fast accumulating that a special group of hormones may be playing a vital part in this control of organ initiation in the bud. Consideration of these substances must be left to another chapter (Chapter 12).

When buds have reached this mature stage, further development stops and the bud becomes dormant. The renewal of development or the "breaking" of the dormancy depends on exposure, at some season of the year, to certain environmental conditions, *e.g.* cold in the winter months. Exposure to certain

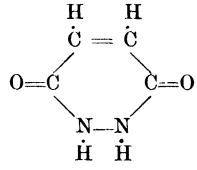
chemicals (see later) may also produce the same effect. In addition, the position of the bud on the branch has an important influence on its subsequent behaviour. For example, terminal buds invariably develop into new branches in the spring (*e.g.* leafy branches in the apple, flowering branches in the horse-chestnut, etc.). Lateral buds, however, show little growth, and it is not until the terminal bud, or the shoot developed from it, is removed, either artificially (*e.g.* pruning in fruit trees) or naturally (falling of horse-chestnut inflorescence "candles" in the early summer) that such lateral buds will themselves grow out into branches. Thus, as a general rule, we may say that the uppermost developing bud on a shoot inhibits the growth of buds beneath it on the same shoot. The plant physiologist has given this phenomenon the name of *apical dominance.*

It is because removal of the uppermost bud on a pruned shoot will release the growth of lower buds that the horticulturalist is able to control the shape of his tree. The other functions of pruning, *i.e.* the promotion of flower initials in lateral buds and the correlated stimulation of fruiting, are probably very different phenomena and are at present far from being properly understood. One is tempted to think that they too may be induced by the disturbance (by removal of apical buds) of some hormone balance resulting in a switch from vegetative to reproductive growth (see Chapter 12).

The fundamental causes underlying this phenomenon of apical dominance have long been a subject for debate among botanists, and early theories again favoured an "internal secretion" or hormone as the effective correlating agent (Errera, 1904). Opposing theorists, however, preferred a nutritional explanation, the vigorous apical bud being presumed to rob the laterals of the available food supply and thus inhibit their growth. General botanical opinion continued to waver between these two theories until 1925, when Snow at Oxford resolved this controversy by a convincing demonstration of the existence of such a hormone. He showed that it was produced by the young growing parts of runner-bean seedlings, and would diffuse through a water layer into a part of the seedling isolated by a cut, and there inhibit, by direct action, the growth

of a bud in the axil of a leaf. The experiment was so designed, with careful controls, that nutritive effects could be discounted. The principal conclusions of these experiments were soon confirmed by others, but it was not until well after the discovery and isolation of auxin that the true nature of this inhibiting hormone was revealed. Thimann and Skoog (1933, 1934), struck by considerable similarities between the behaviour of auxin and that of the bud inhibitor and by the fact that apical buds usually have the highest auxin concentration of any bud on the shoot, performed a simple experiment which strongly indicated that these two hormones were identical. They removed the apical bud of a number of etiolated garden pea (*Pisum sativum*) seedlings. The normal result of this treatment was the immediate growth of lateral buds on the main stem. Application of suitable concentrations of IAA in lanolin paste to the cut end of such decapitated seedlings entirely prevented this bud growth, *i.e.* similated completely the action of the terminal bud and the inhibitor arising from it. Although the general validity of these conclusions has been confirmed over and over again in the last fifteen years, we still seem, in spite of the rapidly accumulating data, to be no nearer understanding the exact mechanism by which auxin exerts this action. The enigmatic situation which has been testing the ingenuity of plant physiologists ever since these early experiments is that auxin should inhibit the growth of the lateral bud but have no apparent action on the apical bud itself, although it is present there in much higher concentrations. Very briefly, the following are the various theories that have been propounded to explain this anomaly. The earliest fell back on the older ideas of a differential distribution of food, and suggested that the topmost bud, by virtue of its high auxin content, somehow diverted to itself either the food supply or certain accessory growth factors (Went, 1936). Later, with the discovery of the auxin precursor, a diversion of this to the apical bud was suggested as the cause of the inhibition. These theories, and several variations on them, all have the very serious objection that direct application of auxin to lateral buds gives the same inhibitions. A very reasonable theory, first suggested by Thimann (1937), is that the growth of buds bears the same sort

of relationship to auxin concentration as that of shoots or roots, *i.e.* the single-peaked optimum curve of Fig. 11. The lateral buds, having their concentration optimum at a much lower level than that of the apical bud, are inhibited by concentrations equal to or much lower than those which stimulate the growth of the apical bud. Even if this is true, we still have the much more difficult task of explaining why buds should so differ merely by virtue of their position on the twig or perhaps by their slight difference in age. That such differences do exist in apical and lateral buds has become apparent as a result of the discovery of a new synthetic plant growth inhibitor, maleic hydrazide, which has the following formula (XXXIX):—



XXXIX

This compound, when applied in a 0·05 per cent. solution spray, has been shown (Naylor, 1950) to suppress apical dominance in all plants studied, and this was apparently due to a much greater sensitivity of the apical bud. This inhibitor will be further discussed later (p. 169). More recently, with the discovery of naturally occurring plant growth inhibitors (see Chapter 14), it has been suggested that high auxin concentrations might bring about the formation of a special inhibiting substance, but it is then difficult, without formulating more elaborate theories, to explain why such an inhibitor is not active in the apical bud itself. A comprehensive survey and assessment of the value of these theories is given by Thimann (1939).

However, in the last year or so, very interesting work in Sweden (Hemberg, 1949 and 1949 *a*) has shown that natural bud dormancy in the potato and in ash (*Fraxinus excelsior* L. var. *pendula* Ait.) is closely correlated with high content of special inhibiting substances (tested on the growth of oat coleoptiles) although the auxin content is very low. These substances, the constitutions of which are unknown, rapidly disappeared when dormancy was broken either naturally or as a result of

treatment with chemicals (ethylenechlorhydrin). Whether or not such an inhibitor may enter into the apical dominance story, remains for future research to elucidate.

## PRACTICAL APPLICATIONS

A number of possible uses of this bud-inhibiting action of auxins have suggested themselves to research workers in the applied field, and some have proved to be successful. These will now be considered separately.

### *Retarding Bud Opening and Lateral Shoot Production on Plants in the Open*

Fruit-growers in particular, experience some of their most anxious moments in the early spring when flower-buds are just bursting on the trees. One late frost may destroy most of the flowers and ruin a whole crop. Such a tragedy would be avoided if the opening of the flower-buds could be delayed for a week or two till all danger of such late frosts had gone. With this end in view, several workers, both in England and America, have treated a number of varieties of fruit-tree with auxin at various times before expected fruit-bud break. If the treatment is carried out in spring only a short time before buds begin to swell, the results are of doubtful practical value. Although in America the spraying of trees with solutions of NAA was claimed to delay flowering in peach, etc., similar experiments at East Malling on Cox's Orange Pippin were disappointing. Concentrated solutions (up to 860 p.p.m.) killed the fruit-buds, but lower, non-toxic concentrations (down to 1 p.p.m.) brought about no delay in flowering. No intermediate concentration was found that would be high enough to delay flowering and yet produce no toxic effects. Even a succession of sprays with dilute solutions produced no effect. A similar state of affairs was shown (Sell and co-workers, 1942, 1944) for vegetative bud-break in tung * trees in America. Direct application of a $\frac{1}{4}$ per cent. solution of NAAm or IAA in a lanolin emulsion to the buds brought about a delay of about

* Tung tree or "chinese varnish tree" (*Aleurites* spp.) is grown for the oil (wood oil) which is extracted from the seed.

a fortnight in bursting, but this was correlated with a high bud-mortality of up to 60 per cent. in some instances. Two successive applications at 30 and 15 days before expected bud-break were found to be most effective. In view of the closely correlated toxic effect, the investigators were not able to evolve any satisfactory recommendations for practical use in commercial orchards. Similar toxic effects, completely offsetting any delay in bud-break, have been noted for 2, 4-D in grape vines (Corbaz and Duperrex, 1947).

Much more promising possibilities of treatment arise from observations made in the Boyce Thompson Institute (Hitchcock and Zimmerman, 1943), that an auxin application to fruit-trees in the summer or early autumn produced a considerable delay in bud-break the following spring. Sprays of 0·02 to 0·08 per cent. of the potassium salt of NAA were applied during July, August and September to apple, cherry, peach, pear and plum, and delays in bud-burst up to 14 days for flower-buds and 19 days for leaf-buds resulted. Later in the same institution, other synthetic compounds were shown to be equally effective (viz. 2, 4-D and 2, 4, 5-T). It was further demonstrated in Montmorency cherry that the later the application the higher the concentration necessary to produce the same response the following spring. For example, 0·02 per cent. of the potassium salt of NAA as a July spray was sufficient to cause a delay but the effective concentration rose to 0·08 per cent. for September treatment bringing with it a closely correlated increase in severe damage to twig, leaves and buds. 100 to 200 p.p.m. sprays in early August were tentatively recommended for the best results. As might be expected, weather conditions play a very important part in the reactions of the trees and make hard-and-fast rules for treatment difficult to draw up. Furthermore, not all investigators have obtained delays by this summer spray treatment, and much more intensive work is needed before specific recommendations can be made for its general use.

Dilute solutions (1/10 to 10 p.p.m. of 2, 4-D and 2, 4, 5-T) have been used for spraying red kidney beans in the field (Stromme and Hamner, 1948; Rice, 1949) and have resulted, in addition to a variety of other symptoms, in delays in flowering

and have also retarded the onset of ageing and yellowing in the leaves. The net result of this treatment has been a considerable delay of the harvest, which was not adversely affected in any way. Indeed, there was a suggestion of a slightly higher yield with spray concentrations of 10 p.p.m.

In tobacco-growing it is the custom to remove, at an appropriate stage, the apex of the plant and with it the flower-buds so that flowering is prevented. This is known as "topping". The result is a more vigorous vegetative growth, including the development of strong lateral branches. These laterals have also to be removed at intervals over a period of time, depending on the variety of tobacco, and this improves the quality of the leaf and, in some sorts, increases the yield. Experiments have recently been made (Steinberg, 1947 and 1950) to study the possibility of replacing this time-consuming manual "suckering" by treatment with auxins in order to suppress the growth of these side-shoots. The experimental procedure adopted was to apply to the apical stump of the plant, after removal of the flowering parts, either solutions (5 per cent. and over) or the pure crystals of one of a large number of synthetic auxins, *e.g.* IBA, NAA and 2, 4-D and derivatives. Suppression of lateral shoot-growth was always obtained and this was paralleled by an increase in the leaf yield as with manual suckering. The most effective compounds were NAA and derivatives (particularly MeNA) and the least effective 2, 4-D and derivatives, which gave growth abnormalities. These effects, however, do not persist indefinitely, and obviously field experiments will have to be done to see whether the method has any real practical advantage to offer over manual suckering.

It is of interest to mention here the recently discovered non-auxin plant growth inhibitor which is characterized by having marked but transitory inhibitory effects on bud development and growth. This compound, the growth-inhibiting properties of which were first described by Schoene and Hoffmann (1949), is maleic hydrazide, already mentioned a few pages previously. We saw there that one of its characteristic actions is the destruction of apical dominance owing to the greater sensitivity of the apical bud to its inhibiting action (Naylor, 1950). Its possible use in delaying the flowering

of fruit-trees has already been investigated (White and Kennard, 1950) by spraying various fruit plants with 0·1 to 0·3 per cent. solutions in spring before fruit-bud break. No positive response has so far been obtained with apple and grape. Temporary stoppage of flowering was obtained with strawberry, and in Bristol Black raspberry plants a 24- to 36-day delay was obtained, although at the higher concentrations (0·3 per cent. and 36-day delay) some foliage damage occurred. There was a corresponding delay (16 to 23 days) in the maturation of the fruit. These results are only preliminary but are very promising.

It is advisable, however, at this point to echo a recent warning (Darlington and McLeish, 1951) on the use of maleic hydrazide in any practice directly connected with food production. It has been demonstrated that one of the causes of the profound growth disturbances induced by this compound is a fragmentation of the chromosomes * similar to, although not identical with, what may be produced by X-rays and high velocity atomic particles. The workers announcing these effects make the statement that "Since nearly all chromosome-breaking agents have so far proved to be cancer-producing as well, we must hope that the agricultural use of this new agent will not be encouraged before suitable tests are made". No further comment is necessary.

### *Retarding Shoot-Growth of Trees in Storage*

It is the practice with many nurserymen to dig up and store, for varying periods during the winter, nursery trees of various species. The storage period may be only for weeks prior to dispatch in the spring, or it may be for a much longer period during a considerable part of the winter and early spring. During this storage period it is common for a certain percentage of the buds to fail to remain dormant and so grow out into etiolated (soft, spindly and yellow) shoots. Such fragile premature shoots are easily damaged or destroyed during transport and transplanting, and the establishment growth of the seedling may be correspondingly hampered. In view of the great advantages to be had from the suppression of this premature bud-break, the possibility of using auxins as bud-

* Chromosomes: the small, thread-like bodies of specialized protoplasm in the nuclei of all cells. They are the bearers of hereditary characteristics.

growth inhibitors has been explored in stored rose bushes (Marth, 1942, 1943). Out of a total of seventeen substances tried, NAA and its derivatives (the methyl and ethyl esters and the acetonitrile) were found to be most effective. The best results were obtained by two methods. The first was the application of any of the above compounds at concentrations of 50 to 100 p.p.m. in a wax-emulsion spray to the tops of the stored plants before storage. The second was the application of the esters to the whole plant in the form of vapour. The most effective way of doing this was to evaporate them from a solution in 95 per cent. alcohol in a dish over a small electric hot-plate. The exposure had to be made in a small sealed space, and a fan used to ensure rapid and effective distribution of the vapour. It also proved to be an advantage to place the plants as near as possible to the source of the vapour (*i.e.* within 12 feet), as it tended to condense rapidly. This naturally limited its spread. Optimal dosages with the ester were from 0·3 to 0·4 gm. ($= \frac{1}{100}$ to $\frac{1}{70}$ oz.) per 1000 cubic feet of space for periods of 16 hours at 32° F., 4 hours at 40° F. or 1 hour at 70° F. Such dosages prevented bud-growth for periods up to 40 to 60 days in the most favourable cases. Higher concentrations of 0·5 gm. per 1000 cubic feet caused considerable injury to the canes, while lower values (0·1 per cent. gm. per 1000 cu. ft.) tended to accelerate rather than inhibit bud-growth. This is interesting in view of Thimann's theory of bud inhibition by auxins at supra-optimal concentrations (see p. 165). As was to be expected, there were considerable differences in response in the several varieties tested and also differences in the same variety due to the state of maturity of the seedling when treated. Those varieties that showed the greatest tendency to break dormancy during storage were the least affected by auxin treatment. In any one variety the success of treatment increased with the maturity of the seedling. Since this was correlated with a much higher starch content of the cane, and also since auxin treatment retarded starch breakdown it was thought that the subsequent increased vigour of the treated cane on transplantation was in a large part due to the much higher food (starch) reserves it contained. Not only was shoot and root production much improved, but

it was claimed that the bushes produced much better blooms.

Subsequent experiments have extended the list of successfully treated species to apple, cherry, pear, peach, persimmon, mock orange and Japanese maple. Attention has also been given to stored forest trees, also with satisfactory results. Vapour treatment at 0·3 to 0·5 gm. per 1000 cubic feet in an enclosed space, or spray at 100 to 600 p.p.m. in a commercial wax emulsion using MeNA or a mixture of equal parts of NAA, NAAm, NoXA and IBA, were generally effective in preventing the development of etiolated shoots in white ash in store (Ostrom, 1945). There was a correlated improvement in the percentage survival of transplanted trees, particularly if treated late in the season (June). The treatment was also effective for trees stored at 32° F., but storage of untreated trees at this temperature gave better results than treatment with auxins and storage at ordinary temperatures. It would seem that the latter practice is not likely to replace storage at low temperatures where these trees are concerned (Way and Maki, 1946). Black locust, Eastern red oak, loblolly and pitch pines also responded to treatment, although the pines were very much more sensitive and liable to injury than the hardwoods. The results of this work with forest trees suggest that the benefits to be derived by treatment are much less certain than those for rose canes.

## INHIBITION OF SPROUTING OF POTATO TUBERS AND OTHER VEGETABLES IN STORE

(a) *Introduction.*—As a rule, buds, after a period of active development, enter on a dormant stage when growth stops, even under temperature conditions favourable to growth. We have already commented on this phenomenon in tree-buds in the summer where the dormancy may be due in some measure to a high auxin content provided by synthesis in the leaves. In the newly formed potato a similar dormancy is observed in the buds (eyes) and this is correlated with the appearance of a non-auxin chemical growth inhibitor in the skin (Hemberg, 1949 and 1949 *a*). Such potatoes will not sprout immediately, even under favourable growth conditions, but must wait until the disappearance of the inhibitor allows the buds to develop.

This breaking of dormancy usually takes place only after a certain definite period of time, unless it can be brought on by exposure to artificial dormancy-breaking treatment. In most species of plants in temperate parts of the world this break in dormancy is usually induced by exposure to a few weeks or months of low temperatures during the winter, followed of course by suitable conditions for growth in the spring. The cold stimulus sets in motion a chain of chemical processes which terminate in a removal of the inhibiting conditions (*e.g.* a specific chemical substance). We are only just beginning to explore this field of study from the biochemical point of view, and very little is yet known about this complex chemical reaction sequence. In many warm parts of the world these natural dormancy-breaking cold periods may not be adequate for the practical needs of the grower, and artificial methods are then desirable. Thus in the southern states of America, in Palestine and South Africa, continued warm weather in winter may prevent dormancy break in such fruit-trees as orange, peach, apple, etc. Again, in other warm parts of the world, it would be possible to obtain two potato crops in one season if the dormancy of new potatoes could be broken so that they could be used as "seed" for the second crop. Much empirical work has been done since the beginning of the present century in an attempt to find suitable chemical substances for breaking dormancy, and this culminated in 1926 when Denny described the results of tests on over two hundred compounds, many of which were highly effective. The most valuable compound has proved to be ethylene chlorhydrin, a highly poisonous derivative of the gas ethylene,* and this has been much used

* Ethylene is a natural plant product and may cause, in addition to this dormancy-breaking effect, other marked growth responses in plant tissues. Considerable epinastic responses, in which stems and leaves of plants become distorted and misshapen, can arise from the presence of this gas in dilutions as low as one part in ten millions of air. Ripening fruits such as apples are the most prolific, although not the only producers of this compound, and it is regarded as a by-product of their metabolism. It has the additional property of accelerating the rate of ripening of such fruits. Its action on growth is regarded by most physiologists as indirect, presumably affecting in some way the concentration and distribution of auxin, although there is very little clue to the mechanism involved. It is not certain that ethylene plays any rôle in the normal growth of the plant. It should not yet be included, therefore, in the list of natural plant growth substances.

for dormancy breaking in potato tubers, *Gladiolus* corms, numerous tree species and even in certain recalcitrant seeds. The interested reader will find a comprehensive review of the literature, and summaries of available practical methods for dormancy breaking, in Avery and Johnson (1947).

In the food potato, however, stored in clamps or storehouses, it is the *early break* of dormancy that can be the greatest source of trouble to the grower. Extensive growth of sprouts of such stored potatoes results in a rapid loss of weight accompanied by wilting and a considerable decrease in the starch (*i.e.* food) content. In extreme cases the potato becomes unsaleable. In this respect a method for *prolonging* dormancy is what is wanted. Such a method was discovered by Guthrie (1938), when he demonstrated that by soaking the cut base of a portion of tuber for one to ten days in dilute IAA solutions (250–1000 p.p.m.) such dormancy could be induced. A highly effective modification of this treatment, making it eminently suitable for practical storage use, was the exposure of whole tubers at room temperature to the vapour of the very volatile MeNA. Such vapour could be easily absorbed through the skin of the potato, thereby obviating any cutting of the tuber. Much work has since been done both in America and in Holland in an attempt to determine the economic advantages of treatment and to evolve practical methods of application to tubers in bulk storage. The most important points which emerge from these researches will now be briefly considered.

(b) *Methods of Application and their Relative Efficiencies.*— The vapour method devised by Guthrie consisted essentially of soaking strips of filter paper in an acetone solution of MeNA, allowing the acetone to evaporate and then mixing the tubers with the filter-paper and placing them in closed containers. Storage in small paper bags impregnated with the hormone was also highly effective. In an extension of this to bulk storage treatment, Denny (1942) experimented with storage in wooden boxes or bins and in small (50 lb.) bags. He found that, although MeNA-impregnated paper strips mixed intimately with the tubers gave complete sprouting inhibition, merely covering or lining the sacks or bins with the paper

induced dormancy only in its immediate vicinity, showing that an even distribution of vapour through the whole volume of the stored tubers was necessary for effective control. This was to be expected. The amount of substance needed was 100 mg. per kilogramme of tubers, and only 5 per cent. of this was absorbed by the tubers themselves. For very intimate mixing of the tubers and the impregnated paper, it has been found advisable to use the latter in the form of confetti. Figs. 26 and 27 show the effect of MeNA on the sprouting of tubers of Majestic potatoes stored until June. In this particular treatment the MeNA was carried on strips of newspaper.

Many experimenters have used talc as a carrier of the hormone, and this can be easily and uniformly distributed throughout a large bulk of tubers. The concentration of hormone needed is somewhat lower than for the impregnated paper method. With MeNA, a rate of 25 mg. per kg. of tubers has been found suitable for use with a talc carrier. The white dust covering tubers, as a result of this talc treatment, tends to detract from their market value, and it has been suggested that fine dry soil should be used as an alternative.

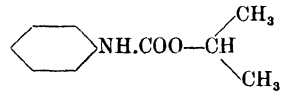
The Dutch workers van Stuivenberg and Veldstra (1942) tried a spray treatment with a 2 per cent. solution of MeNA in 95 per cent. alcohol (methylated spirit). The solution was applied at the rate of 0·35 gm. per bushel of potatoes, which were covered as quickly as possible to prevent loss of hormone by evaporation. They were stored both in clamps and in cellars. This treatment also was highly effective, although it gave a rather higher percentage of rotten tubers than in the untreated controls, and this was attributed to the slight dampness left after the spraying. They therefore recommended the dusting technique. Some workers (Bradley and Dean, 1949) have dipped tubers in dilute aqueous solutions and dried them at 70° F. to 135° F. for 20 minutes, with highly satisfactory results.

One recent set of experiments in America (Ellison and Smith, 1948) has indicated that spraying potato plants in the field before harvesting may greatly reduce subsequent sprouting of tubers in store. Spraying with MeNA solutions in July and August reduced the sprouting significantly. Although

the July treatment was the more effective, it brought about a reduction in the size of the crop, whereas the August treatment had no such effect. This method needs further exploration.

(c) *Effective Compounds.*—In view of the very great effectiveness of MeNA, most of the applied research work on this particular use of the auxins has been confined to it. Other derivatives of NAA (*i.e.* the sodium salt and the free acid itself) have been shown to be equally effective. Being non-volatile they have to be applied either in talc or, better still, in an aqueous dip (Bradley and Dean, 1949). The few observations that have been made on other auxins show that they are much less active. Guthrie showed that IAA had an activity of only a tenth that of MeNA. NoXA and various of its derivatives gave no control at all.

In the last year British workers (Rhodes, Sexton, Spencer and Templeman, 1950) have announced that the synthetic compound isopropylphenylcarbamate is very much more efficient in suppressing storage sprouting than MeNA itself. They claim that extensive field and laboratory trials with thirteen varieties of potato show that this compound in a 1 per cent. mixture, with an inert dust carrier applied at the rate of 2·68 gm. per kg. of tubers, gives complete sprouting inhibition. Four times this rate of application of MeNA still allowed considerable sprouting in some varieties. This compound, which has the following formula (XL), will be seen to bear no chemical relationship to the auxins and, as would be expected, does not share their physiological actions. It was




XL

first developed and used in the agricultural field as a selective weed-killer (see Chapter 9), but the above results suggest its great potential value in potato storage.

Other non-auxin sprouting inhibitors that show considerable promise are 2, 3, 5, 6-tetrachloronitrobenzene (Brown, 1947;

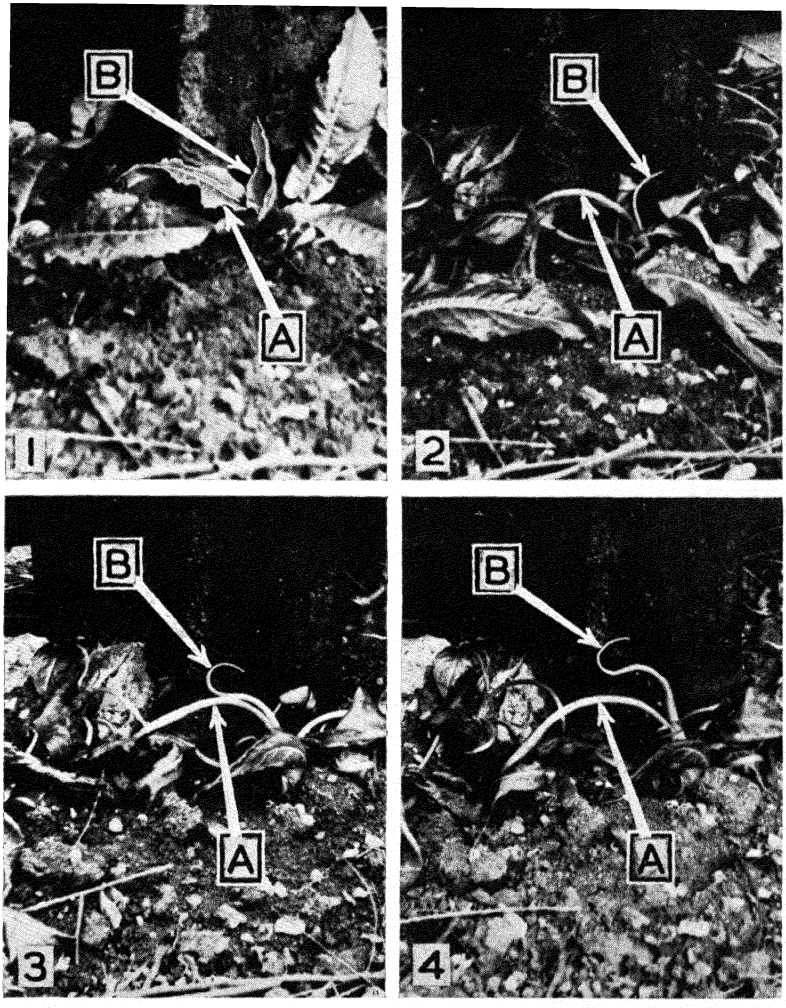



FIG. 28.—Photographs showing the reactions of plants of dock (*Rumex* sp.) to spraying with 0·2 per cent. 2, 4-D solutions.

1 shows the plants at the time of spraying (early on a bright April morning) with a 0·2 per cent. solution of the sodium salt of 2, 4-D in water containing a small quantity of household detergent. 2, 3 and 4 show the condition of the plant after 1, 4 and 7 days respectively. The main responses are the immediate crumpling up of the leaf blade, most marked in the youngest leaves, and a very great stimulation of the growth of the leaf stalks and young stems, both of which become hooked and twisted. This is best seen in the two leaves (A and B) of the plant in the centre of the photographs. Note that in B after 7 days the whole of the lamina has withered away leaving at the tip only the curled whip-like mid-vein. This distorted growth continues for a fortnight or more before death occurs.

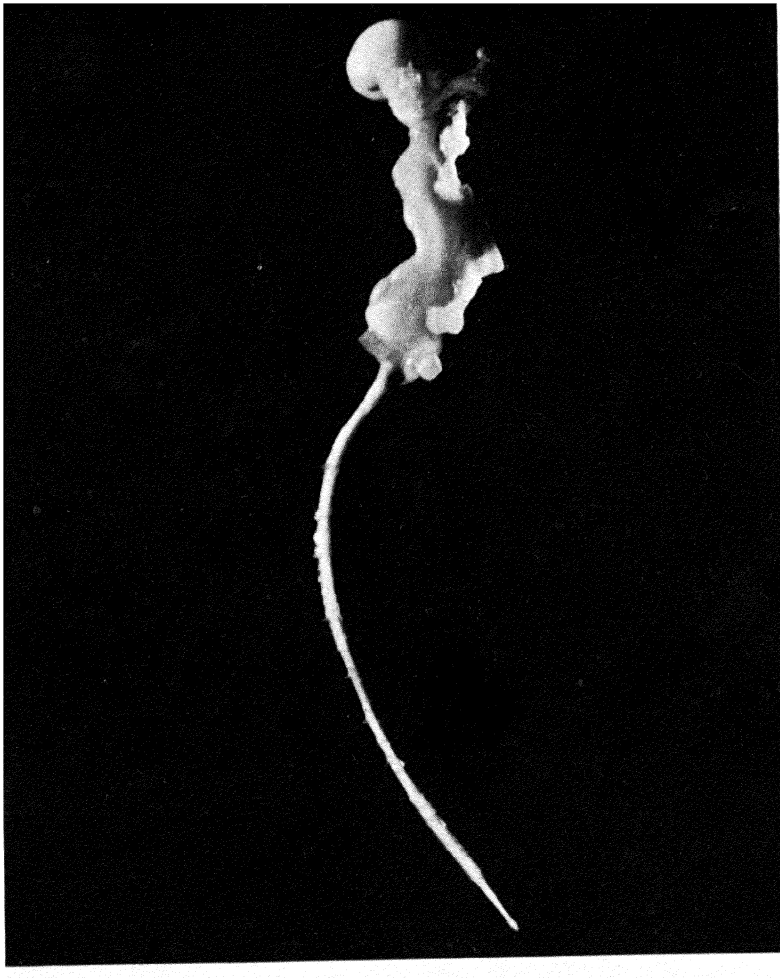
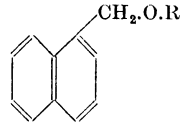


FIG. 29.—Seedling of garden pea (*Pisum sativum*) showing reaction to treatment with 2, 4-D. The root was placed for 24 hours in a solution of 1 p.p.m. when the seedling was 3 days old. It was then allowed to continue growth in tap water and the photograph was taken after a further week. The apical meristem has continued to grow normally. The treated portion behind the tip has produced three rows of lateral roots (two rows only can be seen in the photograph) so densely packed that they have emerged as a solid sheet. The cortex of this portion of the root has sloughed off completely.

Kruyt and Veldstra, 1951) and the α-(alkoxymethyl)-naphthalenes (XLI) (Kruyt and Veldstra, 1951).




XLI

Of the latter compounds the most active are those with R = $-CH_3$ and $-C_2H_5$. All three of these compounds are more effective than MeNA in high concentrations (60 mg. per kg. of tubers), but the situation is reversed in low concentrations (15 mg. per kg. tubers). The toxicity of these latter compounds to mammals has, as far as the writer is aware, not been studied.

(d) *Effect of Time of Treatment.*—The Dutch workers, van Stuivenberg and Veldstra, found that the date of treatment greatly affected its efficiency. Treatment in December was too early and a second treatment was needed in March to keep the tubers dormant. If treatment were delayed until the spring, when sprouts were about to grow, dormancy could be maintained till July. Dormancy could also be maintained by early treatments (*e.g.* October), but only at the expense of a much heavier application of hormone.

(e) *Effect of Temperature of Storage on Treatment Efficiency.*—With a rate of application of NAA of 0·4 gm. per kg. of tubers it has been found (Denny, Guthrie and Thornton, 1942) that storage at 10° C. prevented sprouting and shrinkage of tubers for at least a year. Increasing storage temperatures gave a progressive shortening of the storage life and also increased the degree of wilting. At 23° C. the storage life was reduced to 3–6 months.

(f) *Economic Advantages of Hormone Treatments.*—Under normal storage conditions, sprouts produced by March can amount to 3 to 6 per cent. of the total fresh weight of the tuber, depending on the variety (van Stuivenberg and Veldstra, 1942), but this does not represent the full extent of the loss of weight of such potatoes. Much more food has been used in this new growth than appears as new shoot, since much more has been

burnt away in respiration processes to provide the necessary energy and this represents a loss of food value (dry weight loss) of up to 6 per cent. Loss of water in wilting may be extensive. Up to 15 per cent. of the total fresh weight of the tuber can be lost in this way. These are the losses that can be prevented by hormone treatment (Stelzner, 1944; van Stuivenberg, 1946). It has been estimated (van Stuivenberg, 1946) that if such treatment were universally adopted throughout the Netherlands, an annual saving of from thirty to one hundred thousand tons of potatoes would result. Such a gain far outweights the cost involved in treatment.

(g) *Effect of Treatment on the Subsequent Use of Tubers.*—The question now presents itself whether treatment affects in any way the quality of the potato as an article of food, or whether there is any danger of the synthetic auxins present in the tuber causing any toxic symptoms in the consumer. This does not seem impossible *a priori*, since NAA and some of its derivatives are to some degree toxic when injected into animals, and also since it is known that MeNA is retained in tubers to the extent of 5 mg. per kg. of tuber. Eighty per cent. of this is in the peel and is usually removed in preparation for cooking, but 1 mg. per kg. still remains (Denny, 1942). Careful tests were made in Holland (van Stuivenberg and Veldstra, 1942) with MeNA treated and untreated samples of various varieties, using half a score of voluntary "tasters". No differences could be detected in the cooking qualities, colour, consistency and flavour of treated and normal tubers that had been stored either in clamps or in cellars. They also carried out experiments on toxicity and found that MeNA had the same order of effect as NAA when injected into mice, *i.e.* a lethal dose of about 0·1 mg. per kg. of body-weight. Applications in food, however, were much less toxic, viz., the lethal dose was 1·5 gm. per kg. body-weight (*i.e.* 1500 times as great). Thus for the average man the lethal dose taken by mouth would be about 100 gm. (=3 oz.). Since the maximum uptake by man is not likely to exceed 5 mg. per day, and this will be almost completely excreted, any toxic effects are not to be expected. The position for the newcomer, isopropylphenylcarbamate, is still uncertain and extensive experiments on the above aspects of its use have

still to be completed. As far as the available evidence goes, there seems to be no effect of treatment with the hormone inhibitors on the starch, protein, sugar and vitamin C content of stored tubers (van Stuivenberg and Veldstra, 1942).

The dormancy produced by hormone applications is much deeper and more lasting than natural dormancy, and tubers in such a state may give inadequate sprouting if used as "seed". Treated tubers show a much-delayed emergence and a correlated decrease in the number of stems per tuber (up to 50 per cent.). The final crop-yield is correspondingly reduced (up to 20 per cent.) (Gandarillas and Nylund, 1949). It is fortunate that this induced dormancy can be broken by ethylene chlorhydrin and other similar agents, as we have seen for normal dormancy, but the inhibiting effect of the hormone is never wholly removed (Denny, 1942; Gandarillas and Nylund, 1949). The use of hormones in the storage of seed potatoes should, therefore, not be undertaken lightly.

(h) *Use in the Storage of other Vegetables.*—Although little work has been done, some results promise that MeNA may be useful in maintaining dormancy in other vegetables that have to be stored for greater or shorter periods. It has been shown (van Stuivenberg, 1946) that the sprouting of stored onions could be prevented, but much higher concentrations were needed than for potatoes. Since the degree of rotting was also increased, more investigations were thought advisable before making definite recommendations. In addition, the quality of the onion may be seriously impaired by the initiation of swellings at the base of the bulb and the formation of basal "blow-out" (Wittwer, Sharma, Weller and Sell, 1950). Stored carrots, too, respond to MeNA by showing reduced sprouting, but storage life is shortened by treatment as a result of the stimulation of callus formation and the production of lateral roots.

Attempts have also been made to increase the storage life of potatoes, carrots and onions by spraying the foliage of the plants before harvest with various growth inhibitors. The possible advantages of this pre-harvest treatment would be greater convenience in application, and presumably a more rapid and efficient translocation of the effective compound to

the growing-points of the tuber sprouts (Wittwer and Sharma, 1950). Considerable success has been obtained with potato using 50 p.p.m. of 2, 4, 5-T derivatives applied just before flowering, these being much more effective than the sodium salt of NAA. No reduction in tuber-yield was observed by this treatment (Ellison and Ora Smith, 1949). 500 to 5000 p.p.m. of NAA and 25 to 250 p.p.m. of 2, 4, 5-T were without effect on carrot and onion, but the new inhibitor, maleic hydrazide (XXXIX), and a second compound with somewhat similar properties, benzothiazole-2-oxyacetic acid applied in a $\frac{1}{4}$ per cent. solution, were very effective (Wittwer, Sharma, Weller and Sell, 1950). These latter growth inhibitors, since they are not auxins, do not stimulate the growth of callus and lateral roots, and the prolongation they cause in the storage life of these vegetables by sprout inhibition was therefore not offset by such secondary effects. Their use cannot yet be recommended for reasons given earlier in the chapter.

An intriguing feature of the physiological action of maleic hydrazide is that it appears to be a direct inhibitor of the action of auxins in promoting extension growth (Leopold and Klein, 1952) and this may account for its suppression of apical dominance (see p. 164). But in these responses maleic hydrazide is presumably acting like auxin itself, *i.e.* as a dormancy promoter.

It has recently been reported from America (Acker, 1949) that the use of MeNA in the storage of Estate, Croft and Regal lilies at low temperatures (34° F.) resulted in their flowering two weeks earlier than the untreated bulbs. The effective concentrations were 0·062 and 0·62 gm. MeNA per cubic foot of storage space. A stimulation of the production of roots was a consistent feature of the results.

CHAPTER 9

# AUXINS AS GROWTH INHIBITORS: II. GENERAL TOXICITY AND USE AS SELECTIVE WEED-KILLERS

## INTRODUCTION

In the general introductory remarks on the action of auxins we saw that the growth of all plant organs, so far as has been investigated, is inhibited by concentrations in excess of a certain optimum, which varies widely from organ to organ. We have also seen in the preceding chapter how such auxin inhibitions of bud-growth may have a great practical use in prolonging dormancy in certain plant organs, *e.g.* potato tubers. It is very remarkable that, in spite of the widespread knowledge of these inhibitions among plant physiologists, for a long time no one thought of using the auxins as general growth inhibitors, *i.e.* in the control of weeds. It was not indeed until the early days of the Second World War, when blockaded Britain was intensifying her agricultural research in an effort to grow more food, that the practical potentialities of this aspect of auxin action were realized. At that time Slade, Templeman and Sexton, working at the Jealott's Hill Research Station of Imperial Chemical Industries Limited, were investigating the probable part played by auxins in the beneficial effect of organic matter in soils. In the course of experiments in which oats grown in boxes in the open were sprayed with dilute NAA solutions at the rate of 25 lb. per acre, the discovery was made that seedlings of the weed yellow charlock (*Sinapis arvensis*) were killed by the concentrations of auxin applied, whereas the oats were unharmed. This indication, that synthetic auxins could exert a selective toxic action on plants when applied at these high concentrations, led to a series of planned experiments with a variety of plants, when it soon became apparent that, as a general rule, cereals were relatively insensitive, whereas broad-leaved weeds (dicotyledons) were very sensitive to such treatment.

Here, then, was a method of controlling the growth of broad-leaved weeds in cereal crops by the use of appropriate differentiating concentrations of hormones. These very promising results stimulated the search for other substances that might be more effective than NAA as selective weed-killers. The result was the discovery that 2-methyl, 4-chlorophenoxyacetic acid (MCPA) or "Methoxone", as it was subsequently called, was one of the most active of compounds and could kill such weeds as yellow charlock at rates of application as low as 1 lb. per acre. These experiments were started late in 1940 and the results communicated to the Agricultural Research Council at the end of 1942 (Templeman and Halliday, 1950), but owing to the need for security in war-time it was not possible to make them public until 1945 (Slade, Templeman and Sexton, 1945).

Over the same period Nutman, Thornton and Quastel, working in another part of the country at Rothamsted, were investigating the mechanism of entry into the clover root of the organism responsible for nodule formation in those roots.* In similar experiments with applied auxins, they demonstrated the great susceptibility of red clover and sugar-beet to auxin inhibition and the much lower sensitivity of wheat. Furthermore, they demonstrated that IAA and NAA toxicity was rapidly lost in unsterilized soil, whereas that of the highly effective and selective 2, 4-D was not so lost (Nutman, Thornton and Quastel, 1945). This persistence of 2, 4-D (and incidentally MCPA) in soil is fundamental to their success as practical herbicides when applied as soil dressings, owing to the fact that their toxic action on susceptible plants is not immediate, and the seed or seedling needs to be exposed to them for a considerable time in order to be permanently affected. Early in 1943 the result of this preliminary work by these British teams was communicated to a third team under G. E. Blackman, who initiated a programme of systematic field trials on the

* Small swellings are formed on the roots of members of the clover family by a bacterium called *Rhizobium*. These organisms, which live in the swellings, convert nitrogen gas from the air into nitrogenous compounds for use in protein synthesis. The host plant naturally benefits from these unusual capabilities of its guest. These researches were started since certain features of the invasion of the host root by the bacteria suggested that IAA secretion was an essential part of the process.

relative merits of 2, 4-D and MCPA. The results of these investigations first appeared with those of the other two teams (Blackman, 1945).

Although it is to Zimmerman and Hitchcock of the Boyce Thompson Institute that credit is usually given for the demonstration in 1942 that 2, 4-D possesses all the properties of an auxin, yet it was not until 1944 in America that Mitchell and Hamner suggested its possible use as a selective herbicide, in view of the very wide variations in response obtained from different plant species. Direct experiments, carried out by Hamner and Tukey in 1944 to test such a possibility, succeeded in showing that both 2, 4-D and 2, 4, 5-T were very toxic to bindweed (*Convolvulus arvensis*). Following these early publications came a frenzy of research activity on the new application of synthetic auxins, and the relevant scientific literature of the last few years has become largely dominated by this subject.

Although roots are so sensitive to auxins that their growth can be stopped by concentrations of the hormone which normally occur in the shoot, yet, in order for applied hormones to be effective as herbicides, considerably greater concentrations than these have to be applied in practice. For a susceptible plant such as garden cress, for example, the application to the seedling root of a solution of one part in 100 million of 2, 4-D will cause some growth inhibition, but this concentration has to be increased to one part in a million to stop all root-growth. At this concentration there is some inhibition of germination, and a further increase to 10 parts per million is necessary to suppress germination completely in this species. If, however, we wish to kill mature plants by the application of a hormone spray to the leaves, concentrations of the order of 100 parts per million, or even one part per 1000, will have to be used. At these concentrations we are no longer dealing with the normal physiological inhibition of auxins: we have entered a concentration region in which auxin is acting as a cell poison. The growth process or processes in which auxin normally plays a fundamental rôle are presumably clogged by these relatively vast excesses of hormone, and a completely chaotic jumbling of the delicately interwoven pattern of normal growth processes is the result.

According to the species of plant concerned, the method of application and the concentration of applied hormone herbicide, so a variety of reactions and toxic symptoms are observed. A general feature of all these plant reactions is, as would be expected from their close relationships to the natural hormone IAA, that they are not confined to areas near the point of application of the auxin. The compound is absorbed at this point of application and may be transported to other parts of the plant where interference with growth takes place. In this way application to leaves may result in a rapid translocation to roots or underground creeping stems (e.g. *Convolvulus arvensis* = bindweed), which can in that way be killed. This is the great advantage that these particular compounds have over old type weed-killers, which are mainly contact poisons and kill only those tissues at or near the actual site of application of the chemical. Thus in the case of the troublesome weed *Convolvulus arvensis*, spraying with these latter chemicals would only kill the leaves and not prevent the white underground stems (rhizomes) from continuing their growth and producing new buds and leaves. The milder symptoms of this hormone "poisoning" in susceptible species takes the form of a wide variety of growth distortions. From twisting of stems and leaves, stunted growth, or in some cases greatly accelerated growth (as in the leaf-stalks of the dock, *Rumex crispus*) (see Fig. 28), we may get considerable changes in the shape of the affected organs. In beans grown from seed containing 2, 4-D derived from treated parent plants, young seedlings produced leaves which were crisped as if infected by a virus disease, and in some cases toothed and fused leaflets were obtained (Pridham, 1947).*

In the more severe symptoms the root-forming activities of these compounds may become apparent. Treated main tap-roots may develop a mace-like outline of densely packed stunted laterals. A pea seedling affected in this way is seen in Fig. 29. Similar bunches of roots may burst through the outer tissues (cortex) of treated stems. These reactions are usually accompanied by marked proliferation of bark cells,

* The normal bean leaf has three free lobes (leaflets) with entire (not toothed) margins.

and the resultant disorganized rupture of outer tissues by these two growth reactions allows, especially in subterranean organs, an unimpeded invasion by soil rot-producing organisms. These may be an important contributory cause of the final death of the plant. Cell proliferations may also result in the disruption of normal food translocation by blocking or even destroying completely the channels of transport in the bark (Eames, 1950). The green colouring matter chlorophyll, an essential component of the food manufacturing system in the leaves, may be seriously depleted, thus greatly reducing the food supply. This can be further aggravated by other chemical changes which we shall consider later when we are discussing the mechanism of this herbicidal action. Many of these symptoms, particularly those involving the active stimulation of cell proliferation, are characteristic of these hormone herbicides. Another feature of these compounds is their relatively slow action. Since they are not direct contact poisons or corrosive agents, but exert their action by upsetting the delicate balance of vital processes involved in growth, it may take some time, *e.g.* a few weeks, before the treated plants die, although growth distortions may usually be expected to appear in susceptible species within a few days.

Before we turn to consider these compounds in more detail, it should be stressed that they are by no means the only selective herbicides. Inorganic compounds such as various salts of copper, sulphuric acid, ammonium sulphate, etc., have all been used from time to time for the destruction of annual broad-leaved weeds in cereal crops. Indeed, the use of copper sulphate dates back to the end of the last century. In more recent years certain organic compounds such as 2, 4-dinitrophenol and 3, 5-dinitro*ortho*cresol have proved to have markedly selective toxic actions (Blackman, Holly and Roberts, 1949). These latter organic compounds are not auxins and do not therefore evoke the characteristic growth responses noted above. The toxic action does, nevertheless, resemble that of the hormones in being slow and in not being confined to the point of application. Much of what follows in this chapter concerning the general principles of the use of synthetic auxins as weed-killers also holds for these other organic

molecules. Since they cannot, by any stretch of the imagination, be regarded as related to any naturally occurring growth substances, it is not the intention here to go into specific details of their properties and uses. A small section dealing in outline with some of them concludes the present chaper. Recent reviews covering these compounds are by Norman, Minarik and Weintraub (1950), and Blackman (1951).

## THE COMPARATIVE SENSITIVITY OF SPECIES TO HORMONE HERBICIDES

The general contrast between sensitive broad-leaved weeds on the one hand and insensitive cereal crops on the other, which was apparent from the early pioneers' work, led to a broad classification into sensitive dicotyledons * and insensitive mónocotyledons.* As the number of species studied has increased, so this has shown itself to be an over-simplification of the facts, since there is now an imposing list of broad-leaved dicotyledonous plants that are highly resistant and a number of monocotyledons that are relatively easily injured by these compounds. This will be obvious if the list of species in Appendix D is consulted, when it will be seen that many troublesome dicotyledonous weeds, such as cleavers (*Galium aparine*), corn marigold (*Chrysanthemum segetum*), stinking mayweed (*Anthemis cotula*) and the speedwells (*Veronica* spp.), are highly resistant, whereas some monocotyledonous crops such as onion are easily damaged. This thorough sharing of resistant, susceptible and intermediate species by monocotyledons and dicotyledons has been well shown up by a recent survey of over 500 weed, crop and ornamental plant species taken from 17 families of monocotyledon and 74 families of dicotyledons (Bertossi and Tomaselli, 1950). Plants were placed in six arbitrary sensitivity grades from highly sensitive to highly resistant, and the results obtained have been drawn

* The kingdom of the flowering plants is divided into two great groups, the dicotyledons, usually with broad net-veined leaves and flower parts in fours and fives, and the monocotyledons with long, narrow, parallel-veined leaves and flower parts in threes. Grasses and cereals belong to this second group.

in graphical form in Fig. 31. The distributions show that both groups have their fair share of highly resistant and highly sensitive species with the majority falling in intermediate grades. They also show that the monocotyledonous families possess a higher proportion of resistant species than do the dicotyledonous families, where the majority are in the intermediate and susceptible grades. Although these broad general differences between the two plant groups are very real,* they are merely of academic interest since, in practice, we are concerned with the responses of individual species, between which one may find considerable differences even within the same genus. This is well marked in the buttercups for example, where we find the bulbous buttercup (*Ranunculus bulbosus*) is much more resistant than the bitter buttercup (*Ranunculus acris*) or the creeping buttercup (*Ranunculus repens*), which is due partly to its greater powers of regeneration. Indeed, very recent work has demonstrated that similar wide differences may be observed between *varieties* of the same species, *e.g.* the grass *Agrostis palustris* (Albrecht, 1947), spring oats (Hagsand and Väärtnöu, 1949), linseed (Blackman, 1950). In linseed the resistance of the plant to herbicide treatment has been shown to be under genetic control, and indicates the possibility in the future of breeding strains of crops that are resistant to one or more of these weed-killers. It is an equally great possibility that a similar automatic selection of resistant weed varieties may go on over a period of years as a result of herbicide treatment in the field. This aspect of selectivity will be further discussed in a later section. It is found, however, in compiling any list of species reactions, that various workers often report conflictingly upon the sensitivity of any one species, and this makes it impossible to allocate a definite sensitivity level to that species in such a list. Conflicting results of this kind no doubt arise because the sensitivity of any one species is not a constant but a variable quantity, and is determined primarily by the age of the plant when treated, by the mode of application of the herbicide, and, to a lesser extent, by the nature of the herbicide itself. Differences in environmental and climatic conditions

* Statistical analysis using a $\chi^2$ test shows that the differences between these two distributions are very highly significant.

during tests may also account for many of the widely reported differences in plant response, particularly those between two continents, *e.g.* Europe and North America. Many years of intensive and carefully planned research is needed before full details of sensitivity variations and their controlling factors can be included in such a list of species.

The effect of treatment method, nature of herbicide, etc., will be dealt with in the relevant sections, but there is one general feature of sensitivity variation which should be considered at this point. This is the effect of age of the plant. It has already been noted several times that the root, particularly the young primary radicle, is the part of the plant most sensitive to hormone action. There is now a considerable amount of evidence (Blackman, Holly and Roberts, 1949; Templeman and Halliday, 1950; Åberg, 1949; Weaver, Swanson, Ennis and Boyd, 1946) that the plant as a whole is most sensitive in the earliest stages of its development, and that a greater degree of resistance sets in as it becomes mature. The whole question of this ageing effect has been systematically studied in a wide variety of weeds and crop plants by Blackman, working at Oxford. The results obtained have been of extreme complexity, but a number of general features can be distinguished. Fig. 32 shows the relationship between age and sensitivity, to a 0·2 per cent. MCPA spray, of a number of plants at various stages of development. The great variation in response is at once obvious. At one extreme we have the weed *Thlaspe arvense* (pennycress), which is completely killed at any stage in its development. On the other hand, we have wheat which is slightly sensitive during the germinating stages, rapidly becoming resistant as the first leaf stage is reached. *Linum* (flax) is very sensitive at germination and rapidly becomes resistant at the first leaf stage. Other species such as *Dactylis glomerata* (cock's-foot grass), *Polygonum aviculare* (knotgrass), and *Papaver* (poppy) show a later and later appearance of this resistance, and finally *Sinapis arvensis* (yellow charlock) becomes resistant only in the flowering stage. These facts have an obvious bearing on the most suitable time for the application of herbicides. Thus from the curves it is seen that cock's-foot can be controlled in wheat or flax if all are at the cotyledon stage,

but not at a later seedling stage. Similar considerations hold for other annual species as far as has at present been studied. With perennial weeds, the age effect is not quite so simple, but is still closely linked with the annual cycle of development. Thus, in field bindweed (*Convolvulus arvensis*), and field thistle (*Cirsium arvense*), treatment in the spring at the time of leaf emergence gives a small degree of killing, but susceptibility rises to a maximum when the leaves are fully expanded, and falls again as the flowering stage is approached. It seems likely that the low kill in the early spring is related to the small leaf area and the correspondingly small amounts of herbicides that can be absorbed by the plant. Again, the stage of maximum sensitivity may vary with the compound employed. In *Cardaria draba* (hoary pepperwort) the sodium salt of MCPA is most effective when the flower-buds are forming, but for 2, 4-D, sensitivity is at a maximum at full flower (Blackman and Holly, 1949).

## EFFECTIVE COMPOUNDS

Most synthetic auxins, when applied in sufficiently high concentrations, act as selective herbicides, and since the discovery of this fact many thousands of compounds in the same chemical family have been synthesized and tested for herbicidal activity. For this work, laboratory assay methods have been very largely employed to screen, for the most active molecules, the flow of compounds coming from the chemists before actual field tests are made. All such methods of biological assay in the laboratory depend on what may be called the direct action of the herbicide, and usually involve the measurement of growth inhibition of seedling organs in sensitive species. Root-growth inhibition was first employed by the British pioneers (Nutman, Thornton and Quastel, 1945) using red clover seedlings. Subsequently accurate bio-assay techniques have been developed using primary root-growth in *Zea Mais* (Swanson, 1946) and cress (*Lepidium sativum*) (Audus, 1950/51). As a further criterion, shoot- as well as root-growth has been used in another assay method with cucumber seedlings (Ready and Grant, 1947). Other tests have been devised using plants at the more

mature stage of development. The substance to be tested is applied in water or oil solution as a single drop to one leaflet of a seedling plant of kidney bean (*Phaseolus vulgaris*), subsequent growth inhibition in the young shoot being used as an index of activity (Thompson *et al.*, 1946). One method involves the insertion into the test plant of a thread moistened with the herbicide (Felber, 1947) and its subsequent use as a wick for a continuous supply to the plant. A very reproducible and convenient toxicity assay technique has been developed in the Agricultural Research Council's Unit of Experimental Agronomy at Oxford, viz. determining the rate of killing of samples of *Lemna* (duckweed) growing under carefully controlled conditions in standard culture media. Of course, all these methods can be of use in early screening tests only, and many secondary properties of the molecule, such as its persistence in the soil, its ease of absorption by and translocation through plants, etc., could make or mar its use as a practical selective herbicide. The final evaluation comes, therefore, from extensive field experiments. The phytotoxicity of a herbicide is therefore not to be accurately assessed on the basis of a few casual tests with one test plant. A range of species of such plant needs to be investigated under a number of treatment conditions and the data from carefully planned and replicated experiments subjected to thorough analysis by the methods of statistics. Thoroughness such as this is only now being introduced into herbicide testing (Blackman, 1952).

Already in the first year or so of research on these compounds, many thousands had been screened in this preliminary manner, both in England and in America (Slade, Templeman and Sexton, 1945; Thompson, Swanson and Norman, 1946). From this and much subsequent work it has appeared that the most effective compounds are the substituted phenoxyacetic acids, and that their effectiveness runs very largely parallel to their activity as auxins. In Fig. 33 a kind of family tree has been constructed of the chloro- and methyl-substituted phenoxyacetic acids, in which the height of the relevant compound above the base line represents its basic herbicide activity. The chemist who is concerned with the relative physiological activities of related organic molecules may find this simple picture of interest.

Thus, phenoxyacetic acid itself has a very low activity, but mono-substitution in the benzene ring brings about a great increase in effectiveness. The complexity of the relationship between structure and activity is indicated by comparison of the monochloro- and monomethyl-substitutions. In the chloro-compounds, substitution in the 4 position is much more effective than substitution in the 2 position, whereas with the methyl compounds the reverse is the case. A second substitution may increase or decrease the activity, but it is not yet possible, in spite of considerable study, to formulate any definite rules relating activity to substitution position or nature of substituent groups in these molecules. We see that the original compounds of the two British teams, *i.e.* 2, 4-D and MCPA, occupy the topmost point of this tree, and they are still unsurpassed at the present time. Of these two compounds, 2, 4-D has become the most popular in America, while MCPA has received most attention in England. A third compound of rather lower general toxicity has become popular because of certain aspects of its selectivity (see later, p. 218). This is 2, 4, 5-trichlorophenoxyacetic acid (2, 4, 5-T). It is interesting here again to note the effect of substitution position on effectiveness, since 2, 4, 6-trichlorophenoxyacetic has a negligible activity (Hansch, Muir and Gallup), and it has consequently been suggested that at least one free ortho position is a *sine qua non* of activity. A systematic study has been made recently of all the possible chlorinated phenoxyacetic acids (Leaper and Bishop, 1951), with the result that all compounds showing high activity were those with at least one pair of unsubstituted para positions in the benzene ring. It is suggested that these compounds may be oxidized in the plant to para-quinone derivatives, which may be the true toxic molecules, or even that the benzene nucleus may subsequently be split to give chloromaleic acids which may be the toxic agents. Such a theory can hardly account for the toxicity of IAA in similar high concentrations.

A series of substituted phenoxyacetic acids which show no activity are those with an isobutyric acid side-chain (Wain, 1951). This lack of effect in the higher plants, coupled with a distinct toxicity to pathogenic fungi, has suggested the use of

both isobutyric acid compounds and also the 2, 6 substituted phenoxyacetic acids as systemic fungicides (Wain, 1951). Thus those compounds should be translocated rapidly through the treated plant, and it is hoped that the development of the fungus parasite may be prevented without serious damage to the plant. Results of preliminary experiments along these lines with 2, 4, 6-T and the chocolate spot of beans (due to *Botrytis cinerea* or *Botrytis fabæ*) are very promising (Crowdy and Wain, 1950).

In practice the three most important herbicides, 2, 4-D, MCPA and 2, 4, 5-T, are not always applied as the free acids, but in various other forms. They may be made up in aqueous solutions as salts of sodium and ammonia, or of such organic bases as ethanolamine or morpholine or as amines. They may be applied as ethyl, butyl or propyl esters in oil or ordinary emulsions. The reactions of any one plant to these various derivatives, applied in their several ways, will depend on such secondary properties as degree of retention and ease of absorption by the foliage, etc. In this way, the reactions of plants to these three herbicides under field conditions may not follow their relative susceptibilities to these compounds found in laboratory assays. This state of affairs is well illustrated by reference to the use of the relative newcomer, 2, 4, 5-T. This compound, although having a lower basic toxicity than 2, 4-D or MCPA in the laboratory assay, is found to be much more effective for the eradication of woody plants, and is being used more and more in the U.S.A. for this purpose. Again, MCPA has been shown, in a wide range of laboratory assay tests (i.e. *Avena* test, pea test, root initiation test, root inhibition test, *Lemna* toxicity test, etc.) to be definitely more active than 2, 4-D, but in long-term toxicity tests in the field the latter compound is, on the whole, more effective as a weed-killer and also more toxic to cereal crops (Woodford, 1952). But even in practice such differences do not always remain clear cut. For instance, MCPA seems more toxic than 2, 4-D when applied at low rates to very susceptible plants, but 2, 4-D becomes much the more toxic molecule at high application rates. If we compare the sodium salts of 2, 4-D and MCPA we find that the former easily crystallizes out on the leaf as the

spray solution evaporates and this prevents absorption of the compound, while the MCPA salt is not so easily crystallized and is therefore more toxic. This may account for the more rapid loss of activity of the 2, 4-D salt after rain. Since European rainfall is, on the average, much higher than U.S.A. rainfall in the corresponding growing season, this may also account for the fact that much higher rates of 2, 4-D application seem to be necessary in Europe than in the U.S.A. (Ripper, 1952). These effects are therefore apparently purely solubility effects, determining the speed of entry of the herbicide molecule.

In most of the large scale field trials, however, there is one factor which may affect the relative activity of the compounds tested and which may account for some of the discrepancies in the results of different observers. This is the presence in commercial preparations of the hormone herbicides of relatively large amounts of other related organic compounds that may markedly modify the activity of the herbicide itself. Thus one commercial sample of MCPA has been shown to contain only about 50 per cent. of the pure herbicide, and impurities such as 2-methyl-6-chlorophenoxyacetic acid and 2-methyl-4-chlorophenol to the extent of about 17 per cent. and 6 per cent. respectively. Such impurities may augment or lower the effectiveness of the herbicide itself (Hansen, 1951). Obviously such factors must be taken into account in future comparisons of herbicide activity in field trials with impure commercial products.

Although it is possible to observe a few examples of consistent behaviour, yet the majority of reports concerning the relative toxicity of these compounds and their derivatives to various plants are not susceptible of generalization. On the whole, the toxicity to herbaceous weeds follows the general order, 2, 4-D and MCPA esters > 2, 4-D and MCPA acids and salts > 2, 4, 5-T compounds, although there are some notable exceptions. The high toxicity of the simple esters is offset by their high volatility, which is a source of danger in that they may easily spread to neighbouring susceptible crop plants (*e.g.* in American plantations). This has been largely overcome by the development of esters of low vapour pressure (*i.e.* low volatility), which are just as effective and do not vaporize from

the treated plants in dangerous amounts. The polypropylene-glycol-butyl esters (Mullinson, Coulter and Barrons, 1951) and the polyethylene-glycol di-esters (King and Kramer, 1951) of 2, 4-D and 2, 4, 5-T are compounds of this type.

The relative toxicities of corresponding derivatives of 2, 4-D and MCPA vary considerably from one plant species to another, and the literature contains many scattered observations on that point. A few carefully conducted comparisons have been made. For example, *Galeopsis tetrahit* (hemp nettle), *Raphanus raphanistrum* (wild radish) and *Scandix pecten-veneris* (shepherd's needle) are all much more susceptible to MCPA than to 2, 4-D, whereas *Scleranthus annuus* (knawel) and *Anthemis cotula* (stinking may-weed) are more easily killed by 2, 4-D (Ripper, 1952). This is a very simple picture compared with the true state of affairs, since relative susceptibilities will vary with conditions and with the stage of development of the plant. It is not possible or desirable in such a book as this to go into full details concerning the relative toxicity of different compounds under different conditions, but a reference to some recent work by Blackman (1950) will illustrate the complexity of the situation. For instance experiments on the weed hoary pepperwort (*Cardaria draba*) show that pre-flowering treatment with 2, 4-D and MCPA caused a percentage kill of 46·9 per cent. and 77 per cent. respectively. At flowering, however, the order of toxicity was reversed, 2, 4-D giving 69·4 per cent. and MCPA 58·2 per cent. kill. Similar results were obtained with the dandelion (*Taraxacum officinale*). In April, during flowering, MCPA gave approximately 50 per cent. more kill. In May and August there was little significant difference between them, but in October the early position had been reversed and 2, 4-D was more than twice as toxic as MCPA. It will be many years before sufficient data of this kind has accumulated to allow generalizations to be made and the underlying causes of these complexities to be seen.

Up to the present time MCPA has been most widely used in England, mainly as its sodium salt, whereas in America 2, 4-D compounds are used almost exclusively. Since the sodium salt of 2, 4-D is sparingly soluble in water, amine and ester recipes have been widely adopted because they are readily



FIG. 30.—Control of charlock (*Sinapis arvensis*) in oats by 2, 4-D. The oat-field illustrated has been treated with 2, 4-D, except for strip running across the centre. In this untreated strip a prolific crop of charlock has grown up, but this weed is completely absent fro the remainder of the field.

*Photograph by permission of Plant Protection, Ltd.*

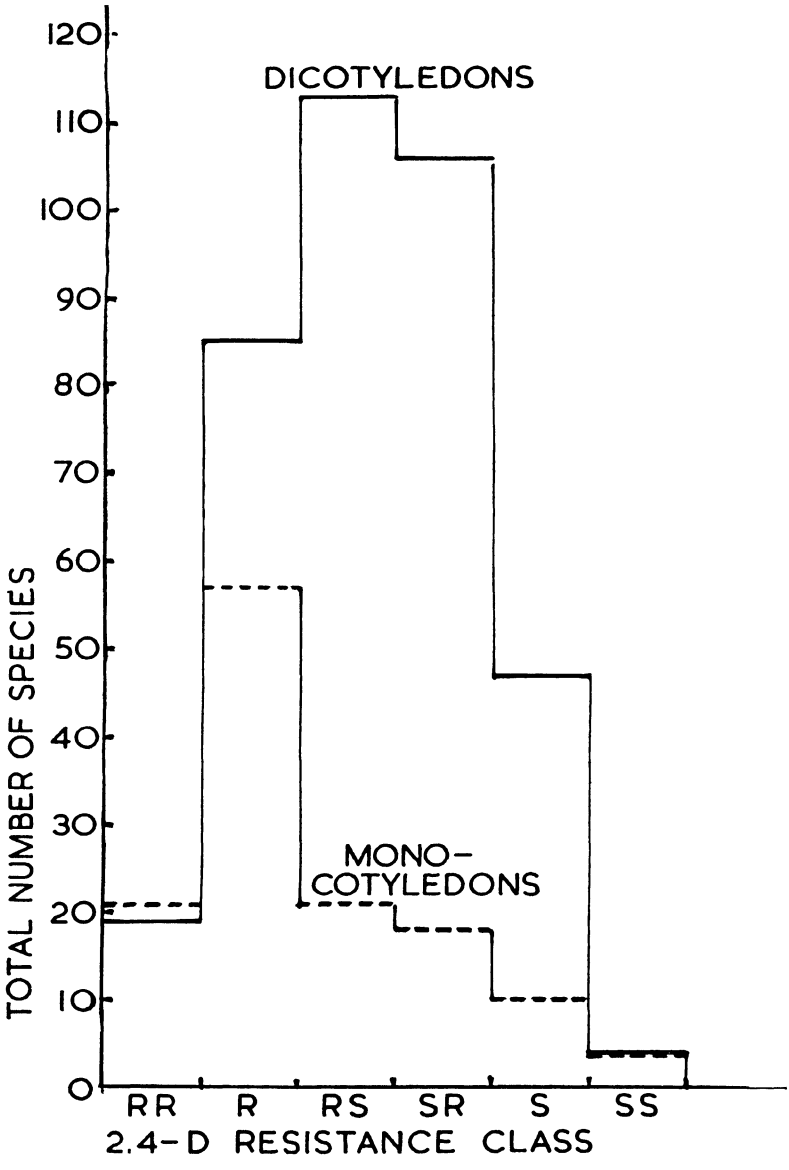



FIG. 31.—Graphs showing the distribution of 2, 4-D-resistant and 2, 4-D-susceptible species in the two main plant groups, monocotyledons and dicotyledons. The resistance-susceptibility classes are: RR, Highly resistant. Plants unaffected. R, Moderately resistant. Plants slightly injured. RS, Slightly resistant. Plants injured but not killed. SR, Slightly susceptible. Some plants killed. S, Moderately susceptible. Most plants killed. SS, Highly susceptible. All plants killed.

*From the data of Bertossi and Thomaselli*, 1950.

diluted with water. As we have seen, there is little to choose between these two molecules in their general herbicidal activity, although there is considerable evidence that MCPA is much less toxic to cereals. This may allow higher concentrations of this compound to be used in cereal crops to give a better weed control than 2, 4-D. Mixtures of two hormone herbicides are also being tested, and there are reports that they may be more effective than either compound acting alone. A mixture of 2, 4-D and 2, 4, 5-T has proved very efficient in eradicating woody plants in America.

## METHODS OF APPLICATION

*Spraying.*—The most widely used method of applying these herbicides has been by spraying in water solution at a suitable concentration. The sodium or ammonium salts, being reasonably soluble in water, have usually been preferred. The free acids are much less soluble, and when these are employed a solvent such as "Carbowax", a polyethylene-glycol, is used to make the initial solution of the acid, and this is then added to the water with thorough stirring to give a uniform fine dispersion of the herbicide. The concentration of the glycol used is usually about ½ per cent. and the herbicide 1000 to 5000 p.p.m. (0·1 to 0·5 per cent.). The carbowax also acts as a spreading and wetting agent so that efficient coverage of the foliage is obtained.

The precise quantities and concentrations of solution to be used will depend, amongst other things, on the weeds to be controlled, the compound employed, the crop itself, etc., and this book is not the place for such details. Instructions of this kind are easily available (*e.g.* Ministry of Agriculture Advisory Leaflet No. 315, etc.). They are very largely empirical, and very little detailed work has yet been done to establish the sound general principles underlying spray efficiency. Most of the work so far has been with high-volume, low-concentration sprays in which quantities of from ½ to 2 lb. per acre have been applied in a volume of 100 gallons. Little is known of the effect of droplet size on the efficiency. The scanty data that are available suggests that large droplets are more effective than

small (Smith, 1946). A recent development has been the adoption of low-volume fan-jet spraying, whereby as little as 6 gallons of solution can be applied per acre. This has become widely used in America, although there is little experimental data on its efficiency *vis-à-vis* the high-volume sprays (Blackman, 1950). The reduction in the volume of the spray means a corresponding increase in the concentration, if the same total rates of application are to be maintained, and in addition there must be a corresponding decrease in droplet size if the same efficiency of coverage is to be ensured. With such small droplets, much may remain suspended as an aerosol (see p. 145 and later), and will therefore be effectively lost, but there will be much less run-off than with high volume sprays. The few direct experiments that have been performed in England (Blackman, 1950) show that the optimum spraying volume per acre depends very much on the species of plant to be killed. This is well illustrated by the results of the first critical experiments, as far as the writer is aware, on the relationships between spray volume and solution retention on the plant. The test plants were sprayed with dye solutions instead of hormones at various rates of application and with concentrations so adjusted that, at all rates, constant amounts of dye were applied per unit area. Spray retention was subsequently determined by washing the dye off the leaves and determining the quantity of it by a colorimetric technique. Two test-plant species were used. *Brassica alba* (white mustard), a susceptible broad-leaved species, had leaves which were easily wetted and became covered with a spray film even at low-volume applications. Higher rates over a wide range gave no further retention since the excess apparently ran off. Barley, the resistant cereal, had leaves which were very difficult to wet, and showed a steadily increasing retention as spray volumes were increased, reaching a maximum only at the highest rates. Obviously, for the best control of white mustard in barley, one should use the lowest spray volume to give the maximum retention on the mustard but a very small retention on barley (Holly, 1952). It is obvious that all types of intermediate behaviour will be shown to occur when other weed and crop plants have been adequately studied in this way. It is not possible to estimate, without careful

experiment, how easily plants may be wetted by different spray volumes and different droplet sizes. There are even some indications that this wettability may vary in the same leaf throughout the day. It is obvious therefore that very extensive experiments must be carried out on a wide range of weeds and crops before precise recommendations for spray volumes can be made.

The hormone herbicide is essentially slow in acting, thus differing from the contact poisons, such as sulphuric acid and copper salts. It has first to be absorbed into the leaves or stems of the plant and is thence transported to the growing-points where its action is mainly exerted. It is obvious, therefore, that the conditions affecting absorption of the herbicide will be very important in determining the efficiency of the treatment. Little or no absorption takes place when the solution is dry on the leaf (Rice, 1948), and therefore the addition of a hygroscopic agent such as glycerine, which prevents drying, will increase the total amount of herbicide absorbed (Hopp and Linder, 1946). The addition of surface-active compounds (*e.g.* detergents) may produce better wetting and thereby a greater retention of spray and a higher degree of spread over the plant surfaces (Staniforth and Loomis, 1949; Hitchcock and Zimmerman, 1948). The use of hard water when making up spray solutions may result in precipitation of the acid as a calcium salt, reducing the effective concentration of herbicide, and may produce clogging of the spraying gear. This can be avoided by adding to the solution the compound ethylenediamine tetra-acetate which retains the calcium in solution as an unionized soluble salt (Zussman, 1949). Many other possible additives have been tested from time to time for their ability to increase the toxic action of hormone herbicide sprays. Contact weed-killing agents, such as chlorinated benzenes and ammonium sulphamates, when mixed with 2, 4-D, produce a more effective solution than any of the components used individually (Hitchcock and Zimmerman, 1948), and it seems possible that these compounds may increase the rate of 2, 4-D penetration. Even 5 per cent. common salt is claimed to "activate" 2, 4-D solutions (Hitchcock and Zimmerman, 1948) supposedly by increasing the

permeability of the leaf cells (tomato in these experiments), and therefore causing a speedier killing.

In areas subjected to heavy rainfall, aqueous solution sprays can soon be washed off the leaves (Weaver, Minarik and Boyd, 1946) and their effectiveness lost. Under these conditions it is of advantage to use solutions or emulsions in oil. The phenoxy-acetic acids are not directly soluble in oil, however, but may be caused to dissolve in the presence of a second solvent. A very efficient supplementary solvent of this type is tributyl-phosphate (Ennis, Thompson and Smith, 1946), which has the doubtful advantage of being itself somewhat toxic.

In the case of many resistant weeds a series of sprays at carefully timed intervals may be necessary to provide complete control. In the Japanese honeysuckle (*Lonicera japonica*) a second spray of 2, 4-D, 44 days after the first, is much more effective than the first spray alone (Hitchcock and Zimmerman, 1948*a*). Ribwort plantain (*Plantago lanceolata*) can be eradicated by much lower concentrations of 2, 4-D if two successive sprays are applied (Marth and Mitchell, 1944). A much more effective control of plantain (*Plantago media*) and daisy (*Bellis perennis*) has been obtained by three successive sprays each at the rate of 1 lb. per acre than by one spray at the rate of 3 lb. per acre. The percentage killing of daisy, for example, was 83 per cent. for the triple and only 62 per cent. for the single spraying (Blackman, Holly and Roberts, 1949). Many applications may be necessary to eradicate dandelion (*Taraxacum officale*) (Hagsand and Väärtnöu, 1949), or ragwort (*Senecio Jacobea*) (Halliday and Templeman, 1951). With such very recalcitrant species as the horsetail (*Equisetum arvense*), spraying in three successive years is necessary to kill the underground stems (rhizomes) completely (Jackman and Tincker, 1949).

The spraying can be carried out by hand over small areas or by mobile power spray with a multiple jet boom (see Mitchell and Marth, 1947) for large fields, etc. For even larger areas, such as the cattle ranges of the Southern Great Plains of America, the aeroplane and the helicopter have been employed for the control of such undesirable range weeds as the sand sagebrush (*Artemisia filifolia*) (McIlvain and Savage, 1949; Offord, 1949). Low-volume sprays are then used.

It is desirable to indicate at this point the great care which must be exercised in the cleaning of all spraying equipment which is subsequently to be used for other purposes. All such equipment must be thoroughly washed with the solvent employed for the herbicide spray (*i.e.* water or oil as the case may be). The smallest trace of these compounds in other sprays may produce disastrous effects on the growth of any crop plants subjected to them. If expense is no object, it is desirable to set aside spraying equipment solely for use with herbicides.

*Aerosols.*—We have already seen that aerosols, fine mists of the hormone in certain solvents, can be satisfactorily used for inducing fruit-set in tomatoes. A similar technique has been successfully used for the application of herbicides, particularly 2, 4-D (Marth and Mitchell, 1944; Hamner and Tukey, 1944 *a*; Stewart and Gammon, 1947). No doubt due to the simplicity and effectiveness of other methods, it shows little sign of becoming regular practice.

*Dusts.*—Dust carriers, such as powdered chalk, have been used to apply these hormone herbicides to weeds in the field. The rates of application have to be higher than in spraying treatment to obtain the same degree of weed control, *e.g.* where spraying would require 1 lb. per acre in 100 gallons of water, the dusting would require 2 lb. of herbicide in 2 cwt. of carrier (Templeman, 1946). As would be expected from what has been said before, the addition of hygroscopic agents to the dust, bringing about the absorption of water by the dust, results in a more rapid absorption of the herbicide and a more effective kill. The polyalkylene glycols or glycerine are very effective in this way, but, strangely enough, calcium chloride, a very hygroscopic salt, is far inferior (Marth, Davis and Mitchell, 1945). Great care must be exercised in the application of these dusts, as they can so easily drift to neighbouring cultivated plants. Their main uses have been in application to soils, particularly in pre-emergence treatment methods (see next section).

*Application to Soils.*—The application of hormone herbicides to soils was a method first adopted by the English pioneers, Slade, Templeman and Sexton, who showed that the germina-

tion of broad-leaved weeds could be prevented by such treatments and that cereal seeds were very much less sensitive. Subsequently, a rate of soil application of MCPA suitable for effective weed control in oats or barley was worked out by Templeman, in 1946, at 2 lb. per acre. However, much subsequent work, particularly in America, has shown that germination (emergence from soil) of grasses can be seriously repressed or even prevented completely by treatment of soil with 2, 4-D (see Akamine, 1948). This is to be expected if application levels are on the high side since, as we have already seen from the work of Blackman, even the most resistant plants are to some extent sensitive in the early stages of development.

In spite of this fact, however, the application of hormones to soils, either as sprays or dusts, before the crop plant emerges is becoming more and more widely used as a method of weed control. The compound can be applied before planting, at planting time, just before the crop is due to emerge or at any intermediate time, depending on the nature of the crop and the weeds and the stage of development of the latter. Such "pre-emergence" treatment has on the whole been very successful with cereals, but is of doubtful value with such broad-leaved crops as potatoes and beans.

There seems to be no precise agreement on the best time of application. In England, for pre-emergence treatment of kale, mangolds, lettuce, onions, beans, peas and sugar-beet, application is recommended at 1 lb. per acre three to four weeks before sowing, when no damage results to the crops (Templeman and Wright, 1950). In America, treatment seven days after sowing has been recommended for peas, flax, sunflowers, beans, etc. (Slife, 1949). It seems agreed that the soil should be sufficiently moist when the herbicide is applied, so that seeds can germinate, since it is only the germinating seed and growing seedling and not the dormant seed which is killed by the herbicide. The type of soil may also to a large extent decide the success or failure of pre-emergence treatments. For example, danger to crop seedlings seems to be much greater in soils poor in organic matter than in peat soils (Hernandez and Warren, 1949 and 1950). It is thought that this is due to a retention of the herbicide by colloidal adsorption in the upper

layers of the high humus soils, whereas in the soils poor in humus it is leached downwards in sufficient quantities to injure the lower-lying roots of the crop seedlings. Further consideration will be given to the relative merits of this method in the next section, where different crops are considered separately.

Successful application has also been made to composts and farmyard manure (Hamner, Moulton and Tukey, 1946). All toxicity disappeared from such treated manures in about a month.

A new approach to the method of weed control by soil application has come from the discovery that sodium 2, 4-dichlorophenoxy-ethyl sulphate is without effect when applied to certain plants (*e.g.* tomato) in aqueous sprays (10–1000 p.p.m.), but is very effective in controlling weeds when applied to soil even at rates as low as 1½ lb. per acre (King, Lambrecht and Finn, 1950). It is probable that this compound, not itself an auxin herbicide, is rapidly converted into one, most likely 2, 4-D itself, by micro-organisms in the soil (Audus, 1952). If applications are made so that only the surface layers of the soil receive this compound, then germinating weed seeds and seedlings will be controlled. Since there is apparently no risk of damage to crops by direct contact of the foliage with this compound, it can be used as a spray for weed control in sensitive crop plants. So far it has been used successfully in asparagus, strawberry, potato, alfalfa and sugar-cane plantings.

## SPECIFIC APPLICATIONS OF HERBICIDES AND THEIR EFFECTS ON THE RELEVANT CROP PLANTS

### *The Control of Weeds in Monocotyledonous Crops*

(a) *Temperate Cereals.*—Wheat, Oats, Barley, etc. The very considerable amount of research that has been carried out in England (Blackman, 1945; Blackman and Holly, 1948; Templeman and Halliday, 1950), Sweden (Osvald, 1947; Åberg, 1949) and Finland (Hilli, 1947) has established that a wide range of weeds can be controlled in such cereal crops in Europe by spraying with 2, 4-D or MCPA solutions. The most suitable concentration of 2, 4-D is from 0·1 to 0·2 per cent. (1 to 2 lb. in 100 gallons of water) and of MCPA 0·2 per cent.

at 100 gallons per acre. Although the weeds should be sprayed as early as possible in their development, yet the cereals themselves are at their maximum sensitivity one or two weeks after emergence, and it has become customary to spray only when they begin to tiller actively (*i.e.* throw up lateral shoots), and treatment is not normally given after the plant has reached a foot in height. Such common weeds as yellow charlock (*Sinapis arvensis*), pennycress (*Thlaspi arvense*), treacle mustard (*Erysmium cheiranthoides*), poppy (*Papaver rhœas*), fat hen (*Chenopodium album*), etc. can be easily controlled in this way. Fig. 30 shows the control of charlock in oats by the use of 2, 4-D. Others such as chamomile (*Matricaria chamomilla*), cleavers (*Galium Aparine*) and knotgrass (*Polygonum aviculare*) are resistant. A full list of such weeds with their reactions is to be found in Appendix D.

In spite of the very high resistance of these cereals, there are many reports of herbicide effects on subsequent grain yield and germination (Hagsand and Väärtnöu, 1949). Treatment in the danger period up to three weeks after emergence gives an appreciable number of malformations and growth disturbances. Thus wheat may show club-shaped, twisted and sometimes branched ears with an irregular arrangement of spikelets. The glumes may be fused and yellowed. The ears may become very elongated in barley and the spikelets blasted. Tubular leaves closely resembling onion may develop, and tillering and heading may be affected in oats. In all species, flower abortion may occur with considerable disturbances in number of spikelets per group on the main rachis (stem axis of the ear). Some malformations of this type are seen in Figs. 34 to 37. 2, 4-D is the most toxic, giving up to 19 per cent. of abnormal plants when applied as the ammonium salt at 1 kilogramme per hectare (about 2 lb. per acre) (Åberg, 1949 *a*). The ethyl ester is particularly toxic. MCPA on the other hand, while being as toxic as 2, 4-D to the weeds, seems to be much less so to cereals, it being possible sometimes to use even the esters against highly resistant weeds without serious damage to yield of oats and barley (Templeman and Wright, 1951). As would be expected, different species of cereals differ in their tolerance to these herbicides. As a general rule, wheat is the least and

oats the most susceptible, while barley occupies an intermediate position. In this latter cereal there are two sensitive growth periods, the first from emergence up to the five-leaf stage and the second during the early heading stages, the intervening period being one of relative tolerance (Derscheid, 1952). After heading, the plant becomes most resistant. The periods of greatest susceptibility or "danger periods" are also much longer in oats than in barley and very much longer than in wheat. Later spraying causes no abnormalities, but some reduction in total yield and germinative capacity of the grain has been claimed. Many workers, however, have reported no such harmful effects (Blackman, Holly and Roberts, 1947; Marth, Toole and Toole, 1948; Schellenberger, Phillips, Johnson and Miller, 1950). There also seems to be no detectable adverse effect, at least in the hard red winter wheats of North America, on the protein yield (total protein per acre), mineral content or the milling or baking properties of grain from plants subjected to hormone herbicide treatment (Schellenberger *et al.*, 1950). Indeed, Swedish workers report that 2, 4-D sprays during June may cause an increase in the protein content of wheat by as much as 2 per cent. (Åberg, Hagsand and Väärtnöu, 1948). It is also highly encouraging to note that no adverse genetic effects have been observed on the progeny of cereal plants treated with the normal spray concentrations. This was tested specifically by Åberg and Denward (1947), who followed two generations of barley plants from sprayed parents showing ear abnormalities. No trace of inherited malformations was observed. In cereals one possible aspect of herbicide effects, which may not be apparent except under certain unfavourable weather conditions, is an altered susceptibility to disease. Fortunately, there are no indications at present that this is likely to be of any great magnitude, although one report from France announces a marked aggravation of the incidence of smut and also of ergot in certain varieties of winter wheat, apparently resulting from the use of the ethyl ester of 2, 4-D as a herbicide (Longchamp, Roy and Gautheret, 1951).

It is not easy to make any definite statements on the economic aspects of hormone herbicide treatment of cereals, since the extent of the increased yield due, of course, to reduced weed

competition, will depend on a wide variety of factors. A recent estimate for cereal crops in Great Britain put the average expected increase at 20 per cent. for MCPA treatment at 1·7 lb. per acre (Holmes, 1949). For the present national average yield of 18½ cwt. per acre this gives a gross increase of just over 3½ cwt. per acre. This is a very considerable amount when viewed in terms of total national production. At the then current (1949) price and wage levels an increase return of £4 per acre was estimated. Investigations in Germany and Austria show that for winter rye the net financial gain occurring from 2, 4-D treatment was 2 to 2½ times the outlay for herbicide and the labour employed in applying it; for winter wheat it was about 3 times, and for oats it varied from just over one to 5½ times, depending on the degree of weed infestation (Linser, 1951). The corresponding increases in grain yield were about 18 per cent. for rye, 19 per cent. for wheat and 7–25 per cent. for oats.

(b) *Tropical and Sub-tropical Cereals.*—Maize (*Zea Mays*), or corn as it is known in America, is also relatively insensitive to the hormone herbicides, which can therefore be used to control weeds in this crop. In America, where 2, 4-D is the compound at present preferred, rates of ½ lb. per acre of the ammonium and triethanolamine salts have been claimed to give increased yield of up to 1 ton per acre (Dearborn, Sweet and Havis, 1948). As in the case of the temperate cereals, spray treatments during the early stages of growth after emergence tend to give growth abnormalities and considerable reduction in total crop yield. There are wide differences in tolerance of the various varieties grown. There is also evidence that early sprays may reduce the yield of subsequent generations from treated parents by acting through the vitality of the progeny and causing a greatly reduced stand of mature plants (Rossman and Staniforth, 1949; Rossman and Sprague, 1949). As would be expected, application to roots is very much more harmful than application to the aerial parts, but pre-emergence spray treatment of soil has proved highly satisfactory in effecting weed control. One remarkable result reported for this treatment is the considerable stimulation of root development, not only of a strong "feeding" root system below ground, but also of lateral

roots rising at the base of the main stem above ground. These latter roots grew down thence into the soil, forming a system of struts ("prop roots" is the botanical term) at the base of the plant, thereby maintaining the plant in a vertical position and preventing the so-called "lodging" (Hamner, Tukey and Carlson, 1947). But closely associated with this in susceptible varieties may come a great increase in stem brittleness and the young stems may be easily broken off just above this point (Rodgers, 1952).

*Sorghum* (Millet) is also resistant, and 2, 4-D at $\frac{1}{2}$ lb. per acre can be used to control a wide variety of its weeds (Elder, 1949).

Rice is a plant which likes plenty of water, especially in the early stages of its growth, although there are varieties that prefer drier conditions. In the cultivation of rice, seeds are sown in the ordinary soil and then, when the seedlings have become established, they are either flooded *in situ* or transplanted into specially flooded fields, the "padi" fields of the East Indies. Later, when the plant comes to maturity, the fields are allowed to dry up. The important weeds of rice crops, therefore, are those which compete with it during these early stages of development during flooding, and are therefore aquatic plants. Considerable success has been obtained in the control of such weeds by appropriate 2, 4-D applications both in Italy (Ferrario, 1949), in California (Pryor, 1948) and in Louisiana (Ryker, 1947). An interesting feature of this control is that the main weeds of the Italian crops are predominantly monocotyledons, *i.e.* species of *Scirpus* (sedge), *Cyperus* and *Heleocharis* (club-rush). These are controlled by 0·1 per cent. solutions of the sodium salt of 2, 4-D applied at the rate of 10 litres per 100 sq. metres (*i.e.* approximately 100 gallons per acre). In California, low-volume sprays have controlled susceptible weeds at $1\frac{1}{2}$ lb. per acre. Dusts are also effective (Ryker, 1947). In Malaya, however, although rushes (*Scirpus* spp.) and broad-leaved weeds, which send up leaves above the water surface (e.g. *Jussieua* spp., *Sagittaria* sp.), can be controlled, yet grasses (e.g. *Hymenachne myuros*) and submerged weeds (e.g. *Utricularia flexuosa*, *Enhydrias angustipetala*) are completely unaffected by treatment (Buckley, 1951). The

optimum time for treatment would seem to be the first week or so after flooding, but although very young plants can suffer damage, applications at heading time may also cause malformations known as blasting. Nevertheless, very considerable increases in grain yield have been reported as a result of treatment (Ryker, 1947).

The sugar-cane, *Saccharum officinarum*, is grown exclusively in the tropics. It is a large bamboo-like grass, and the sugar is stored in the soft central pith of the stems from which it is extracted by crushing. Propagation of the sugar-cane is usually by cuttings, and one of the major problems of their cultivation in the hot humid regions so favourable to rapid plant growth has been the constant weeding that is necessary. This is particularly so in the early stages, to prevent stifling of the young plant, and at the stage of harvesting to leave clean surfaces under the canes, so that the cane-cutters can work close to the ground and avoid loss of valuable basal stalk. Certain weeds, e.g. *Stizolobium pruritum*, with stinging hairs on the fruits, are very painful to the labourers and hinder operations. Labour at the rate of 150 man hours per acre is needed for hand hoeing (White and Mangual, 1948). With the emancipation of the native has come greatly increased labour costs, and therefore 2, 4-D has found great favour as a weed-killer with sugar planters, since the labour involved in spraying is less than a quarter that needed for hand-hoeing (White and Mangual, 1948). The cane itself seems to be amongst the most resistant of all the grasses to 2, 4-D action. Even spray concentrations as high as 0·3 per cent. have been shown to be without effect, and toxic symptoms only appear at 0·5 per cent., and with oil soluble 2, 4-D esters. This high resistance also applies to the early stages of growth from the cutting and it has been suggested that this might be due to the fact that such cuttings, and the shoots developing from the lateral buds, are, physiologically speaking, much older than seedlings would be and therefore possess the higher resistance of the mature plant (van Overbeek, 1947). Whatever may be the ultimate causes, this high resistance of young canes means that it is relatively easy to control susceptible weeds by hormone sprays and dusts. In some recent experiments in Cuba, it has been claimed that low rates

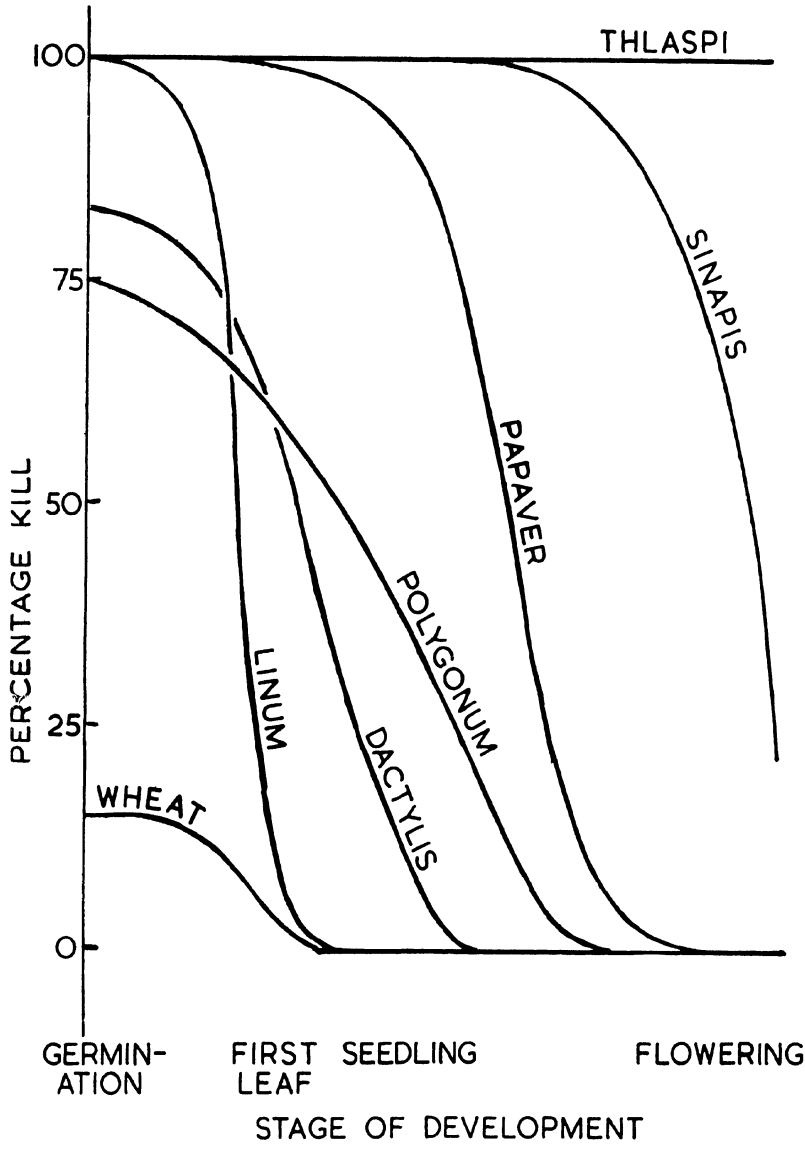



FIG. 32.—Graphs showing the different relationships between sensitivity to 0·2 per cent. MCPA spray and stage of development in a number of plants of economic importance.

*After Blackman, Holly and Roberts* (1949); *S.E.B. Symposium, No.* 3.

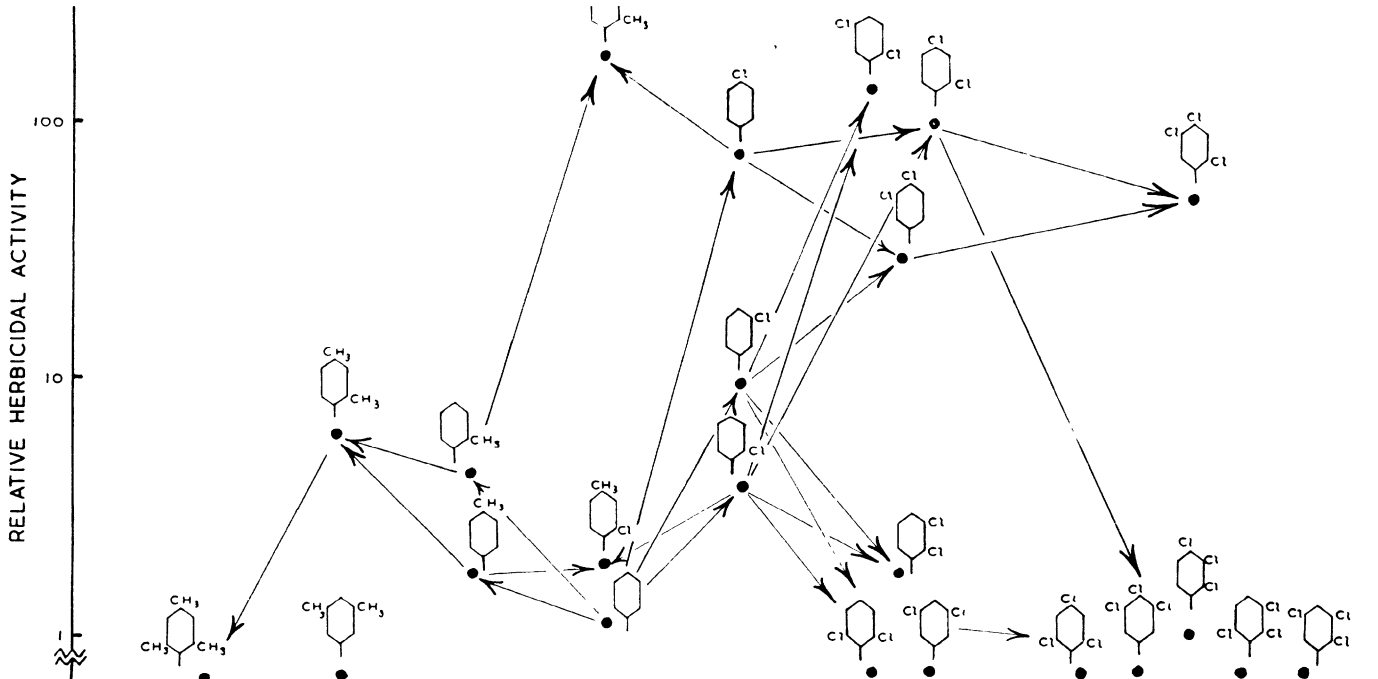



FIG. 33.—Graphical representation of the relationships between structure and herbicidal activity in chloro- and methyl- substituted phenoxyacetic acids. The formulæ of the compounds are given only in skeleton, the —O—$CH_2COOH$ groups being represented as a single free bond pointing downwards. Against each compound is a dot, the height of which above the base line is a measure of that compound's herbicidal activity. (*N.B.* The left-hand scale giving activity is a logarithmic one.)

of 2, 4-D application towards the end of the growing season may even cause an increase in the sugar content, amounting to rather more than 1 per cent. of the total cane weight (Beauchamp, 1950). This would be a further benefit to be derived from 2, 4-D treatment if this increase proves to be consistent. Such very common weeds as alligator weed * (*Alternanthera phylloxeroides*), nut grass (*Cyperus rotundus*), *Commelina* spp., morning glory (*Ipomœa purpurea*), bird's eye (*Caperonia castanæfolia*), etc., are among the many so controlled. It is unfortunate, however, that many of the weeds of sugar-cane are themselves resistant grasses, e.g. *Digitaria* spp., *Cynodon dactylon*, *Panicum maximum*. The removal of competition by herbicide sprays may bring about an actual increase in these grass species. But most of them are small species and are soon overshadowed by the growing canes. It does mean, however, that 2, 4-D will not give complete control, and that some supplementary weeding is necessary, either by mechanical means or by some non-selective contact weed-killers, if complete weed eradication is desired.

(c) *Lawns and Grasslands.*—The use of 2, 4-D and MCPA to eradicate broad-leaved weeds in lawns has proved highly successful and is capable of producing the most spectacular results, since the majority of lawn grasses are highly resistant to these compounds, while the common weeds of lawns (plantain, dandelion, daisy, hawkweed, buttercup) are all relatively sensitive. Application can be made by spraying with an aqueous solution of one of the water-soluble hormone compounds at the concentration and rate usually applied to field crops or in the form of a dust, using an inert carrier of some kind. The precise rate of application will vary depending on the weed to be killed. There is some evidence that spraying is, on the whole, more effective than dust applications.

Both in Europe and in North America the chief lawn weeds are perennials and include the plantains (*Plantago major*, *Plantago media* and *Plantago lanceolata*) and the dandelion (*Taraxacum officinale*). Extensive tests have shown that these can both be eradicated by spray applications of 0·1 per cent. 2, 4-D or MCPA. Single applications, however, may not be

* These are the common names used for these plants in the U.S.A.

completely effective, especially with the dandelion, since seeds which have been previously shed may germinate later and partially killed tap-roots may re-sprout. A second spray after a suitable interval, when this new growth has appeared, *e.g.* one application in the spring and one in the autumn (Hitchcock and Zimmerman, 1947), is usually recommended for complete control. Similar repetitive spraying may also be necessary for such annual weeds as chickweed (*Stellara media*), which produce large amounts of seed.

The rate of application to such susceptible weeds is usually 2 lb. per acre. There are, however, some common lawn weeds, *e.g.* clover (*Trifolium repens*), sheep's sorrel (*Rumex acetosella*) and weeds of salt-marsh turf, often used for golf greens in Great Britain, *e.g.* sea milkwort (*Glaux maritima*) and sea plantain (*Plantago maritima*) (Dawson and Escritt, 1948) which are resistant and need much higher rates of application up to 6 lb. per acre with MCPA and 4 lb. per acre with 2, 4-D. With these resistant species, too, a succession of sprays at a low rate of application (2 lb. per acre) may be as effective as one spray at the higher rate. By such a series of low-rate treatments less damage may be done to relatively susceptible grass species. With resistant weeds in small lawns spot spraying with high concentrations is very effective but obviously involves too much time to be employed for large areas.

That there is a considerable variation in sensitivity between several genera and species of grass has become obvious from the work of Mitchell and Marth (1945), who showed that the germination and early growth of white bent (American = red top) (*Agrostis alba*) was checked to a much greater degree than that of red fescue (*Festuca rubra*) or meadow grass (Kentucky blue grass) (*Poa pratensis*) by applications of 2, 4-D at $\frac{3}{4}$ to $1\frac{1}{2}$ lb. per acre. This variation in susceptibility of the various species of bents has been confirmed by much subsequent work, and similar variations have even been recorded between varieties of the same species, i.e. *Agrostis palustris* (Albrecht, 1947). It is unfortunate that these bents are commonly grown as turf grasses in American golf greens, and so great caution must be employed in the use of 2, 4-D in controlling weeds in such greens. It is equally unfortunate that such coarse

weed-grasses as cock's-foot (*Dactylis glomerata*) are highly resistant and cannot be eradicated by the hormone herbicides.

It must be remembered that all grasses, like the cereals, are relatively sensitive in the early seedling stages, and it is generally regarded as unwise to treat newly sown lawns until the grass is at least 2 inches high. For the same reason the bare patches, appearing on the lawn after the death of weeds due to hormone spraying, should not be re-sown until sufficient time has elapsed for the herbicide to have disappeared from the soil (see later, p. 227).

In order to offset any retarding effects of treatment on the growth of the grass, nitrogenous fertilizers can be applied to the lawns mixed with the hormone material to act as a general growth stimulant. Urea dissolved in the hormone spray has been used successfully at the rate of 60 lb. per acre (Marth and Mitchell, 1946). Rates of 90–120 lb. per acre bring about scorching of the grass. In a recent modification of this technique, 2, 4-D has been successfully supplied as a dust using dry commercial fertilizer as a carrier (Mitchell and Marth, 1947). Both in autumn and spring applications, very good control of weeds and a considerable stimulation of grass growth were obtained. Such dry mixtures can be stored for periods of up to ten months, even at temperatures as high as 60° C., without any loss of herbicide activity (Marth, Hardesty and Mitchell, 1949).

There should be no need to stress here, in view of what has been said before, the need for great care in the application of hormone mixtures to avoid drift to neighbouring ornamental plants, of which all but a few are highly sensitive. (See Appendix D, p. 388.) Too heavy treatment of lawns bordering or surrounding artificial ponds may result in draining of the herbicide into the water with somewhat startling results on the plants growing in it. For example, the leaf stalks of the water lily (*Nymphæa* spp.) may be stimulated to elongate and crawl out on to the offending lawn.

Developing from their use on lawns, hormone herbicide treatments have shown great promise for the control of perennial weeds in parks, pastures and grassland, both in Europe and America. Here the position is somewhat more complicated in

that some of the coarse undesirable grass species are highly resistant, while some of the sensitive broad-leaved plants such as chicory (*Cichorium Intybus*), yarrow (*Achillea millefolium*) and ribgrass (*Plantago lanceolata*) may be desirable in grazing land in that they provide a varied and balanced diet for the animal. However, there are a number of unpalatable and even poisonous weeds that need attention. Examples are ragwort (*Senecio jacobea*) in England, and wild onion (*Allium* spp.) in America (Marth and Mitchell, 1947), the latter being particularly troublesome, since even small degrees of contamination will taint the milk of grazing cows. Halliday and Templeman (1951), in England, have shown that MCPA, both as a spray (at rates of the order of 4 lb. per acre) and as dusts (at roughly twice the spraying rates), could effectively control weeds such as creeping or field thistle (*Cirsium arvense*) and ragwort (*Senecio jacobea*). Buttercups (with the exception of the bulbous buttercup, *Ranunculus bulbosus*) were controlled by sprays or dusts at 2 lb. per acre, and the docks and sorrels (*Rumex* spp.) could be kept down by early treatment in the seedling stage with applications of 4 lb. per acre. In a series of field trials in established leys and permanent grassland at various centres spread over a wide area in England, MCPA applications at 2 or 4 lb. per acre in the autumn significantly increased the yields of useful herbage (grasses and clovers) when harvested next year at the hay stage. The increase for the 2 lb. rate was equivalent on the average to about 5 cwt. of dry matter per acre. Treatments in spring, although affording good weed control, resulted in no significant increases in yield, probably because this late application left insufficient time for the grasses to take full advantage of the reduced weed competition (Halliday and Templeman, 1951*a*). Unfortunately, the greatest pest of all in some areas, bracken (*Pteridium aquilinum*), is resistant even to concentrations as high as 20 lb. per acre. A list of the susceptibilities of the common British pasture weeds is given in the original paper (Halliday and Templeman, 1951) and these have been included in Appendix D. In some American National Parks, such troublesome weeds as the ragweeds (*Artemisia* spp.), poison ivy (*Rhus toxicodendron*) and honeysuckles (*Lonicera* spp.) can be eradicated by

appropriate 2, 4-D treatments, although there is a danger of damage to susceptible trees. Birch is particularly sensitive, especially in the early growth stages (Curran, 1948; Wester, 1949). Hormone-dry fertilizer mixtures have been reported to be particularly effective with such pasture weeds (Mitchell and Marth, 1947).

On the great cattle and sheep ranges of America, particularly in the south, the fight against the advance of weeds is one of the major pre-occupations of the range managers. Some weeds may be highly poisonous to animals, such, for instance, as the bitterweed (*Actinia odorata*) on the Texas ranges and orange sneeze-weed (*Helenium* spp.) in Colorado, but their greatest depredations arise from their competition with the grasses and their great reduction of the carrying capacity of the range. Weed control can in some cases be effected by mowing practices as in the control of sand sagebrush (*Artemisia filifolia*) of the Southern Great Plains (McIlvain and Savage, 1949), but even with this plant the much more economical and rapid method using low volume 2, 4-D oil-emulsion sprays from aeroplanes has proved most effective. Rates of the order of 1 lb. of the sodium salt of 2, 4-D or its ester in 1 gallon of Diesel oil per acre are sufficient. A wide range of weeds can thus be controlled, including the sagebrushes (*Artemisia* spp.), wild currants (*Ribes* spp.) (Offord, 1949), bitterweed (*Actinea odorata*) (Sperry, 1949), and, of course, most of the annual range weeds are completely eradicated. Animals themselves may be responsible for the spread of some such weeds. Thus in Texas and Arizona, the mesquite (*Prosopsis* spp.) may rapidly invade grassland, which consequently reverts to woodland. The fleshy pods of this plant are eaten by cattle and sheep, and the seeds are spread in the animals' fæces. Attempts to reduce the viability of these seeds while in the animal's alimentary canal by adding 100 parts per million of 2, 4-D to their drinking water has not proved successful (Glendenning and Paulsen, 1950). There are reports that animals show a preference for grazing hormone-treated grass plots, and it has been suggested that this may not be due entirely to the elimination of unpalatable weeds. Whether or not this is true, there seems little doubt that the animals suffer no ill-effects from the

hormone herbicide swallowed with the grass they eat (see later, p. 241).

These herbicides show great promise in eradicating weeds from grasses grown for seed. Both the grasses and the common weeds have very small seeds, and the cleaning of contaminated grass seed presents grave problems for both the grower and the seed tester, whose certificate of purity and percentage germination is required for all seed offered for sale. Applications of 2, 4-D at 1½ to 4 lb. per acre in dry fertilizer and also as ·01 per cent. aqueous spray have been shown to reduce greatly the weed populations of timothy (*Phleum pratense*) and meadow grass (*Poa pratensis*) with no effect on the germinative capacity of the grass seeds produced (Mitchell and Marth, 1947). MCPA at 1–2 lb. per acre has been used to control weeds in red and meadow fescue without affecting seed-yield or quality, but cock's-foot, on the other hand, shows much reduced growth even at rates as low as 1 lb. per acre (Halliday and Templeman, 1951 *a*).

(d) *Gladiolus Beds.*—2, 4–D was first used in America in pre-emergence treatment of *Gladiolus* beds for the control of a wide range of weeds (Krone and Hamner, 1947). Up to 20 lb. per acre could be applied three weeks before planting the corms without apparently harmful effects. Even resistant grasses such as crab grass (*Digitaria sanguinalis*) and goose grass (*Eleusine indica*) could be controlled by these high levels of application (Jenkins, 1949). Rates up to 5 lb. per acre could be applied as late as one to two weeks after planting to improve control. Spraying of weeds when the young *Gladiolus* plants have emerged is not recommended, as the latter are relatively sensitive. In general, caution is to be urged. Weed control in daffodil and Dutch iris beds can similarly be effected by pre-emergence hormone treatment.

(e) *Asparagus Beds.*—This is another monocotyledonous crop plant of medium sensitivity to these herbicides in which weed control can be effectively obtained by pre-emergence treatment with up to 4 lb. per acre of 2, 4-D and its salts (Warren and Hernandez, 1948). Spot treatment of perennial weeds such as *Cirsium arvense* and *Convolvulus* spp. has also proved successful in mature beds (Blackman, 1948).

(f) *Onions.*—The onion is one of the sensitive monocotyledonous crop plants, and weed control by these herbicides is not always possible without causing a considerable degree of damage to the crop. Complete control of a range of broad-leaved weeds has been claimed for soil-spraying treatments with 2, 4-D two days before the onions emerge, but only on well-drained peaty soil. On badly drained soil similar treatments resulted in severe injury to the crop (Warren and Hernandez, 1948).

### *Control of Weeds in Dicotyledonous Crops*

In dicotyledonous crops we are dealing mainly with species sensitive to the hormone herbicide, and control of sensitive weeds, let alone resistant ones, becomes a very much more difficult matter.

(a) *Vegetables.*—Most vegetables are sensitive, but a few are sufficiently resistant to suggest that weed control by hormone herbicides may be at least partially effective. Such a species is the potato (*Solanum tuberosum*), of which some varieties appear not to be damaged by 2, 4-D sprays, while others are somewhat more sensitive. A resistant variety in America is Katahdin, whereas Irish Cobbler is sensitive (Thompson and Shuel, 1948). In the former 1·2 to 1·6 lb. per acre of the triethanolamine salt of 2, 4-D gave complete control of fat hen (*Chenopodium album*) and a number of other common weeds without any deleterious effect on the yield of tubers or their cooking quality. With sensitive varieties such as Irish Cobbler, pre-emergence application has controlled weeds like chickweed (*Stellaria media*) without affecting the subsequent potato yield, but the later summer weeds were, of course, not affected. Spraying sensitive varieties when about 6 inches high could give as much as 50 per cent. reduction of the yield (Danielson, 1949). All varieties so far listed, however, seem to be sensitive to 2, 4, 5-T (Ennis, Swanson, Allard and Boyd, 1946).

Carrots, parsnips, radishes, pulses, brassicas, beets, lettuces, spinach, in fact the majority of broad-leaved vegetables are very sensitive, and weed control even by pre-emergence treatment is a very chancy affair. Some little success has been claimed for spinach when sowing was left for 12 days after spraying (Danielson, 1948). A suggestion has been put forward that

control of large weeds like thistle in peas could be carried out by "spot-spraying" with herbicides (Barrons and Grigsby, 1945). Even though the vegetables may show few or no symptoms themselves when sprayed at maturity, the herbicides may be observed to have accumulated in appreciable quantities in the seed. The writer, when on a recent visit to the Cambridge University Seed Testing Station, had his attention drawn to peas from plants that had been sprayed with dilute MCPA. The plants themselves, and the peas from them, had appeared completely normal, but when the seed germinated, most of them developed stunted roots, typical of MCPA toxicity (see Fig. 28). Such seedlings could not, of course, establish themselves and therefore died. Since these symptoms could be effected in an individual pea seedling by as little as $\cdot 01\gamma$ ($10^{-8}$ gm.) of the herbicide, the necessity of taking extreme care when dealing with sensitive species grown for seed production needs no stressing. Hormone herbicides should never be allowed anywhere near such plants.

One way in which sensitive plants might be protected against these herbicides is by use of an adsorbing powder such as activated charcoal preparations, or with a cation exchange resin powder. These substances have the power to adsorb relatively large amounts of herbicide compounds (Weaver, 1948). This fact has been used successfully to allow the planting-out of slips of the sensitive sweet potato (*Ipomœa batatas*) into soil treated with 2, 4-D. The roots of the slips were moistened and dipped into Norit A (an activated charcoal preparation) before being planted directly in the soil (Arle, Leonard and Harris, 1947).

Great promise for weed control in sensitive vegetable crops is held out by the compound sodium 2, 4-dichlorophenoxyethyl sulphate, which is without effect when applied to the foliage even of sensitive plants, but is converted to a very active compound in the surface layers of the soil, where it controls weed seedlings (see p. 201).

(b) *Soft Fruits.*—The strawberry is a relatively resistant species and it has been suggested that control of its broad-leaved weeds is possible by spraying with 0·1 per cent. 2, 4-D solution (Carlson, 1947 and 1949). Such sprays should be applied either in

the first year when no fruit is required or after the fruit has been picked, since blossom is particularly prone to damage by the spray. Damage to plants can be avoided if soil treatment (2–3 lb. per acre) is given about two weeks before planting, but weed control is then correspondingly poor (Wilson and Stamper, 1950).

The American blueberry (*Vaccinium* sp.) is somewhat sensitive, but by using low concentrations of the ammonium salt of 2, 4-D (*i.e.* ·03 to ·05 per cent.), control of most sensitive weeds (*e.g.* sweet fern, *Comptonia peregrina*) has been claimed (Smith, Hodgson and Russel, 1947).

The use of the sodium salt of 2, 4-D for weed control in vineyards is not to be recommended. Although weeds are killed by spraying under vines, yet enough herbicide falls on the soil and is absorbed by the roots to cause serious injury to the vine in the following season. Growth is greatly reduced, leaves malformed, flowering delayed and there is an increased drop of the grapes (Chabrolin and Thellot, 1948).

(c) *Various Field Crops*

(i) *Linseed and Flax (Linum usitatissimum).*—This plant, which is not unduly sensitive to the hormone herbicides, is peculiar in that it is grown for two quite different products, the seed for the extraction of linseed oil and the fibres of the main stem for flax. Because it is much more sensitive than cereals, and because of the relative ease with which malformations may be caused, much time has been devoted in Sweden (Granhall and Zienkiewicz, 1948) and particularly in England (Blackman, Holly and Roberts, 1949) to working out exact permissible hormone concentrations for control of its weeds. It has been found that, for MCPA, concentrations of up to 0·2 per cent. of the sodium salt can be used at 100 gallons per acre, without having any effect on seed production, although considerable stunting and morphological abnormalities may be produced in the plant at this concentration level. In fact the Swedish workers report an increase in seed yield as a result of a more highly branched but stunted plant. Higher concentrations not only reduce seed-yield, but may also reduce the oil-content of the seed as well (Blackman, Holly and Roberts, 1949). To

avoid these morphological effects, which greatly reduce the fibre-yield, MCPA concentrations must not rise above 0·075 per cent. 2, 4-D is much more toxic and can be used only at a concentration of 0·1 per cent. for linseed production and not at all for flax production. Under no conditions should oil-soluble esters be used in oil sprays, as these are particularly toxic and may kill the crop completely. The sensitivity also varies markedly with the age of the plant and it is recommended that treatment should not be applied until the plants are at least 4 inches high. The earliest possible treatment is advisable as the weeds should be caught in their sensitive juvenile state (Blackman and Holly, 1948).

(ii) *Cotton.*—Cotton is extremely sensitive to the hormone herbicides at all stages of its growth, and their use to control weeds in this crop is therefore completely precluded. Indeed, there exists a great danger that spray drifts from the treatment of neighbouring crops may produce serious damage and this applies particularly to the drift of the vapour of volatile ester sprays (Staten, 1946). Some idea of this danger can be formed from the calculation that 1 oz. of 2, 4-D uniformly distributed could cause serious damage to 35 acres of cotton (Ergle and Dunlap, 1949). The main deleterious effects are suppression of flower production and hence seed- and cotton-yield.

(iii) *Clover.*—Clover is among the more resistant of the broad-leaved crops, but can easily be damaged or killed by high concentrations of hormone sprays, especially in the early stages of its growth. Clover can be eradicated in lawns by such sprays (Mitchell and Marth, 1947). It has been regarded as dangerous practice to use these weed-killers in crops where clover has been sown as an under-crop. This is quite true in the early stages where clover, crop and weeds are all seedlings and of the same size. Later, when weeds and crop have outgrown the smaller clover and afford protection from the spray, these herbicides can be satisfactorily employed to kill the weeds (Holmes, 1949 *b*).

### *Use in Nurseries, Orchards, etc.*

Great caution should be exercised in the use of these weed-killers in nurseries, since the majority of broad-leaved orna-

mental plants are, like the majority of broad-leaved vegetables, sensitive to them. There are, however, some that are sufficiently resistant to permit a restricted use of these compounds. Some of the more important species are listed with their sensitivities in Appendix D.

The use of hormone sprays to control weeds under orchard trees would seem to be without danger, provided great care is taken to avoid drift on to the trunks and particularly the leaves of the fruit-trees themselves. In America one of the most troublesome weeds of orchards is the poison ivy (*Rhus toxicodendron*), which may seriously compete with the fruit-trees for water and nutrients from the soil. This weed can be controlled by spraying the young leaves with 2, 4-D solutions, but the underground portions are apparently not killed if the plants are not growing in shade (Avery and Johnson, 1947) (see also p. 225). There are indications that the esters of 2, 4-D are more effective under these conditions, but there is consequently more danger of the spread of these volatile compounds to the fruit-trees themselves.

The coffee tree (*Coffea arabica*) is very resistant to aqueous 2, 4-D sprays both in the seedling as well as the mature state. Great use, therefore, is being made of this herbicide in killing many of the harmful weeds of coffee plantations in South America (van Overbeek, 1947). *Clerodendron* spp., which threatens to overshadow young plantings, and *Ipomoea* spp., which clamber over the crowns of mature trees, are both easily controlled; so also are the giant nettles, *Urera* spp., which may make working in the plantations very difficult. This is of great economic value since these weeds are not easy to control by the traditional methods. It is also fortunate that *Erythrina* species, which are planted as shade trees * in these plantations, are resistant to 2, 4-D (van Overbeek, 1947).

A very sensitive monocotyledonous fruit-tree is the banana (*Musa* sp.). Concentrations of 2, 4-D as low as $2\frac{1}{2}$ to 5 p.p.m., corresponding to a dosage of $\frac{3}{4}$ to $1\frac{1}{2}$ gm. per acre, can cause

* Shade trees are quick-growing species that are often planted between the rows of young saplings of such trees as coffee and rubber in the tropics, in order to provide some protection from the intense radiation of the sun during the early stages of development when the seedling tree is easily damaged.

abnormal growth in sensitive plants. Drift of weed-control sprays over distances of a few hundred feet from sugar-cane plantings, etc., can cause stem-splitting, toppling of plants, and distortions and premature ripening of fruit. Extensive killing of plants is achieved only by application of normal herbicidal concentrations (*i.e.* 0·1 per cent.) (Hendrix, 1952).

*Eradication of Woody Plants and Weed Trees*

In some parts of the world shrubby plants establish themselves as weeds in agricultural land and become a very serious problem. They may form dense impenetrable thickets, and repeated cutting may not eradicate them. The invasion of cattle-range grassland by such shrubs has already been discussed. Here the hormone herbicides show promise of being of great use. The majority of woody species tested can be killed, although, as with herbaceous plants, there are some species that are resistant. One of these is the juniper (*Juniperus communis*) (Hamner and Tukey, 1946). As a rule, higher concentrations and rates of application than are used for the herbaceous weeds are necessary. Esters are more effective than the acids or the salts, and there are indications that 2, 4, 5-T often has a stronger toxic action than 2, 4-D (Glendenning, 1949). In woody plants, too, young growth is much more susceptible than mature, and suckers and stump sprouts are easily killed by spraying. In the control of poison ivy (*Rhus radicans*) cutting experiments have shown a much improved control by spraying the young regenerating shoots than by treating the mature plants. Thus, uncut plants treated with a ·01 per cent. 2, 4-D spray showed only a 27 per cent. reduction in stand. Treatment of the young growth from plants cut the previous year gave a 40 per cent. kill, and when spraying followed cutting in the same year, a 83 per cent. kill was obtained (Minshall, 1951). On the other hand, treatment of trees when dormant can still be very effective, although the final death of the tree may be considerably delayed (Southwick, 1950). Application to the cut stumps of shrubby or woody plants seems to result in little or no translocation downwards into the roots (Torrey and Thimann, 1949). This downward translocation seems to be closely associated with the normal downward transport of

organic food materials from the leaves in which they are synthesized (see later, p. 226). Maximum toxic action is therefore to be expected if intact plants are sprayed under conditions when transport is at its height, *i.e.* during or after a period of active photosynthesis in the light. Any residual vitality left in the roots and manifesting itself in the production of new shoots or suckers from the stumps, can be dealt with by a subsequent spray. In this way, roots not actually killed by the hormone will eventually die as a result of exhaustion of reserves (starch, etc.). Nevertheless, stump application has proved successful in preventing subsequent sprouting of a wide range of felled shrubs and trees in Connecticut plant communities (Egler, 1949), and presumably works by killing around the cut the living tissues which would give rise to the regenerating sprouts. Such treatment, however, requires very high concentrations (25 to 30 per cent.) of the esters (butyl-, isopropyl-) of 2, 4-D or 2, 4, 5-T. Another very effective method with shrubs and even with large trees is the painting of the bark or trunk wounds with concentrated solutions of this kind. In the forests of Central Louisiana, where heavy cutting and fires give rise to a great increase in the proportion of unwanted hardwoods (*e.g.* blackjack oak) in softwood forests, the quickest and most economic way of killing these "weed" trees is by poison solutions inserted in axe cuts in the lower trunks. Although not so effective as the contact poison "Ammate" (sodium sulphamate) in high concentrations (32·4 per cent.), a 2 per cent. solution of the ammonium salt of 2, 4-D can produce nearly complete killing when applied to "frills" (gutter-shaped circumscribing cuts) round the bottom of the trunks.

Treatment of individual plants of selected shrub and tree species with hormone herbicides by all these various methods is now being used to study long-term ecological effects on natural vegetation. In Connecticut, investigations have been under way since 1946 in various types of plant community, viz., forest trails, pine forest, grasslands and shrublands (Egler, 1949 and 1950), with very marked effects on the natural equilibria in these communities. By such "spot" treatments it appears that shrublands can be converted to and maintained as stable

grasslands, stable pinewoods produced from mixed woods, etc. The usefulness of the hormone herbicide as a tool for controlling the make-up of vegetation lies apparently in the relative ease with which the whole plant is killed, thereby preventing regeneration from root and stump as is usually the case when the offending plant is merely cut back to ground level. The ease with which woody plants can be destroyed by spraying with a suitable hormone herbicide makes it possible to use these compounds in maintaining power lines and fire lanes in afforested areas. Similarly, all weeds, including shrubby plants and seedling trees, can be controlled alongside railways and on roadsides, particularly in those parts of the world where other control methods involve much time and labour.

An interesting possibility in the application of the selective properties of 2, 4-D to woody plants has been described from Australia where the semi-parasitic mistletoe (*Loranthus* spp.) can be a serious pest. It has been shown that the 2, 4-D solution can be injected into the trunks of the host (*Eucalyptus* spp.) whence it is transported into the branches. The mistletoe, being very sensitive, is killed, whereas the host plant is relatively unharmed (Greenham, Fielding, Hamilton and Nicholson, 1951).

### *Destruction of Water Weeds*

The use of chemical phytotoxic agents for the control of aquatic plant weeds from lakes and inland water-ways offers its own peculiar problems. Emergent plants that have their leaves borne well above the water surface can be sprayed with a solution containing appropriate wetting and sticking agents, and susceptible species can then be killed. Even the monocotyledonous aquatic bullrush (*Typha latifolia*), arrowhead (*Sagittaria latifolia*) and lotus lily (*Nelumbo lutea*) can be killed by a ·01 per cent. ester spray of 2, 4-D. Even those with floating leaves like the water lily (*Nuphar adrena*) can be killed by spraying, although some pond weeds (*Potamogeton* spp.) and duckweed (*Lemna minor*) seem to be resistant (Bauman, 1947). Water weeds that are completely submerged cannot be reached by sprays and would need impossibly large amounts of herbicides added to the water of the lake or river to produce toxic concentration levels.

One of the greatest pests of inland water-ways in many tropical areas of the old and the new world is the beautiful water hyacinth (*Eichornia crassipes*). This plant grows floating on the surface of water, where it is maintained by its rosette of leaves with their swollen hollow stalks that act as floating bladders; the roots hang down freely into the water and are not normally anchored to the underlying soil. The phenomenally rapid spread of this plant over vast areas of water is due to its method of vegetative propagation by side-shoots, which rapidly establish themselves as daughter plants in rather the same way as strawberry runners. At the height of its growth activity, one plant can produce nearly two thousand offspring in this way in three months (Hitchcock, Zimmerman, Kirkpatrick and Earle, 1949). Since also the plants float so high in the water, detached portions can be easily and quickly blown by the wind and this accelerates spread. The worst sufferers from this rapacious weed are the United States coastal areas round the Gulf of Florida, Ceylon, Australia, Java, Fiji, etc. Control can be effected by mechanical clearing, but this is very costly both in money and manpower.

The effectiveness of 2, 4-D sprays against this very troublesome weed was first reported in 1946 (Hildebrand), and since then considerable success has been reported from workers in the Gulf of Florida and in Fiji (Mercer, 1948).

The most extensive experiments so far carried out were done in Louisiana (Hitchcock, Zimmerman, Kirkpatrick and Earle, 1949). Optimum treatments worked out in these experiments involved high concentration sprays, either of the esters (isopropyl, butyl) or the alkanolamine salts of 2, 4-D, which were quickly effective. Similar compounds of 2, 4, 5-T were not so active. Complete killing occurred with solutions of 0·3 per cent. and over, applied at rates of 6–150 gallons per acre, and best results were obtained when treatment was applied after the period of optimum growth when the leaves had become mature. This greater sensitivity of mature leaves contrasts strongly with the findings for land plants (see p. 188). An effective spray cover was shown to be desirable, since translocation of the herbicide from parent to attached off-shoot was small. Although the lower range of concentrations killed

the plant, the higher concentrations were necessary to cause the killed plants to sink, a very desirable state of affairs. The action of 2, 4-D in causing sinking is not yet understood. Any survivors of this treatment were easily controlled by later sprays.

The troublesome alligator weed (*Alternanthera phylloxeroides*) is often associated with the water hyacinth in America and is much more resistant to 2, 4-D. Complete control of this was obtained by spraying with a 17·5 per cent. solution of the isopropyl ester of 2, 4, 5-T at the rate of 8 lb. per acre (Hitchcock, Zimmerman, Kirkpatrick and Earle, 1949).

### *Control of Pollen Production*

It has been found that water sprays of 2, 4-D at suitable concentrations, when applied at an early flowering stage to ragweed (*Ambrosia* spp.) in America, inhibit the production of pollen (Grigsby, 1945; Smith, Hamner and Carlson, 1946) without killing the plant. This may prove to be of great practical importance, since ragweed, while being on the one hand a very active soil binder, and therefore valuable in preventing soil erosion, yet produces prolific pollen, which causes much distress from hay-fever. The use of 2, 4-D may resolve these difficulties.

In concluding this section on the various applications of hormone herbicides, it is relevant to point out that apprehension is being felt in some circles, which include eminent botanists, that man's selective control of plant growth in the various plant communities, natural and artificial, of economic importance to him, may result, as did the introduction of the rabbit into Australia, in a gross unbalance of nature, and ultimate effects more harmful than beneficial. Thus the eradication of one set of *sensitive* weeds may merely make way for the establishment of equally vigorous and harmful *resistant* species that cannot be localized or touched by the herbicides. In answer to this, we may say that, with the rapidly increasing number of compounds showing considerable dovetailing in their selectivities (see p. 194), the farmer should be able to avoid this state of affairs by "ringing the changes" with his herbicides. A second fear is that sensitive weeds may develop resistant

strains which, by the selective action of the herbicide, may gradually oust their sensitive progenitors and form a new resistant population not controllable by the toxicant, in the same way as strains of pathogenic bacteria, resistant to the sulphonamides and to penicillin, are becoming disturbingly of more frequent occurrence. So far there is little sign that this is happening (Blackman, 1950), although there is one isolated and disturbing report that weed plants surviving 2, 4-D treatment in sugar plantations in Louisiana yielded seed, which produced offspring with a much higher resistance to 2, 4-D than the normal (see Linser, 1951, p. 209). If this prove true, then obviously we are dealing here with a selective action of the herbicide allowing the survival of only the resistant strains, which can serve as the parents of a new resistant population. Nevertheless it must also be remembered that the higher plants multiply extremely slowly compared with bacteria, and so the rate of appearance of resistant strains would be correspondingly slower. If at some future date the increase in resistant strains becomes serious, we should by that time have a large number of compounds with a sufficiently wide variety of selective actions to be able to overcome such an acquired resistance to any particular molecule.

## THE EFFECT OF ENVIRONMENTAL CONDITIONS ON HERBICIDE ACTIVITY

*Introduction.*—Naturally a question which is of great concern to the agriculturalist is how far environmental conditions such as the weather, nature of the soil, etc., will modify the response of his weeds and his crops to these herbicides. As we have seen, the great advantage of the hormone compounds is that their action is largely systemic, *i.e.* they eventually become distributed throughout the whole plant, virtually irrespective of their point of application, and so ensure the death of the whole plant structure. It is obvious, therefore, that any factors effecting the absorption or the translocation of the hormone herbicide will considerably modify the final result; in fact, such effects dominate the picture and far outweigh any small variations induced by the environment on the ultimate toxic

action in the cell. It should not be necessary to point out that the effect of any particular environmental factor will be closely bound up with the mode of herbicide application; indeed, the selection of the latter is often dependent on prevailing weather conditions at the time of treatment. Thus, in the use of pre-emergence soil treatments, the overriding factors will be the structure, situation and condition of the soil itself. A consideration of these will be left until a later section. With applications to the aerial parts of growing plants, however, soil conditions will have no effect, except in so far as they control the general vigour of the plant, and here the important factors will be the prevailing atmospheric and weather conditions. It is with these latter conditions that we are concerned in this section.

*Temperature.*—There is a considerable amount of evidence that spray treatments may be much more effective in the summer months than in the spring or autumn. This has been shown for bindweed (Hamner and Tukey, 1944 *a*), and also for dandelion (Klingman, 1946; Hitchcock and Zimmerman, 1947). The greater susceptibility of young plants (see earlier section) is here swamped by summer conditions, of which a high temperature would seem to be the most important. Similar field experiments in Sweden under various soil and air-temperature conditions have shown that the corn sow-thistle (*Sonchus arvensis*), colt's-foot (*Tussilago farfara*) and yarrow (*Achillea millefolium*) are all killed more quickly at air temperatures of 25° C. and soil temperatures of 20° C. than when these temperature conditions are 5° C. lower (Åslander, 1950). A marked effect of temperature on effectiveness has been demonstrated by direct experimentation on several species grown under constant temperature conditions in greenhouses (Marth and Davis, 1945). Effectiveness increases with rising temperature. In early winter cress (*Draba verna*), for example, at 75°–90° F., complete kill was obtained in 24 days with ·15 per cent. 2, 4-D spray, where at 50°–65° F. between 34 and 44 days were necessary, and at 32°–40° F. about 30 per cent. injury resulted after 44 days. Temperature treatment subsequent to spraying with salts of 2, 4-D and MCPA has been shown to effect greatly the response of flax. A much greater

response was obtained when spraying was carried out at 85° F. than at 50° F., and this difference was preserved whatever the later temperature conditions for growth. The greatest response was for plants sprayed at 85° F. and grown subsequently at 50° F. (Dunham, 1951). There is also some indication that different species behaved differently in relation to temperature, but no systematic work has been done on this point.

It has been suggested that this effect of high temperature is to cause a higher growth-rate of the plant, and that this is correlated with a greater susceptibility. Absorption and translocation rates, however, may come into the picture; some absorption at least has been shown to be increased with rising temperature (Rice, 1948). That these are not the only factors is suggested by temperature pre-treatment experiments in which kidney bean plants were kept at different temperatures (5°, 15° and 25° C.) for a week before spraying with 2, 4-D at a common temperature of 15° C. Subsequent responses showed the plants previously kept at 25° C. to be the most susceptible, indicating a direct action of temperature on the cell reactivity (Kelly, 1949).

*Light Intensity.*—There seems little doubt that the light conditions during spring greatly influence the effectiveness of the herbicide. Early work on bean plants indicated that overall toxicity was higher in bright light than in dark or shade (Mitchell and Brown, 1946; Weaver and de Rose, 1946), but later, other workers were not able to disclose such an effect of light conditions on the same plant (Penfound and Minyard, 1947). It is possible that these discrepancies may be due to the use of different carriers for the 2, 4-D in the two instances, the latter using kerosine and the former water. Nevertheless, conflicting results have been obtained from other plants. Thus, ester treatment of a wide range of species in Connecticut showed no light effect (Egler, 1949), whereas the water hyacinth (Penfound and Minyard, 1947) and japanese honeysuckle (Hitchcock and Zimmerman, 1948 *a*) seem to be more easily killed in shade.

It is obvious that here we have the possibility of light action at several points in the sequence of events associated with hormone herbicide action. Firstly, it may be on the absorption process.

For instance, entry of the solution might be thought to take place partly through the stomata, those small pores on the leaf which allow the interchange of gases and the evaporation of water, and which may open and close in relation to external light conditions. Experiments, however, show that entry does not occur via stomata, and cannot then be controlled by light intensity in this way (Weaver and de Rose, 1946; Thimann, 1948; Fogg, 1948). Highly volatile compounds, *e.g.* esters, may enter by diffusion through stomata, but this is likely to represent only a fraction of the total substance penetrating (Blackman, 1950 *a*). In contrast, absorption of the ammonium salt of 2, 4-D by bean leaves from a solution containing carbowax, to keep it from drying up, has been shown to be greater in the dark than in the light (Rice, 1948).

Secondly, an indirect action of light in accelerating the removal of hormone from the leaves, and its distribution throughout the plant is fairly well established. There seems little doubt that conditions favouring a rapid movement of food materials from the leaves, during and after a period of rapid synthesis in the light, also favour the transport of 2, 4-D, and that when such rapid food transport ceases from darkened leaves, so also does outward movement of herbicide (Mitchell and Brown, 1946; Weaver and de Rose, 1946; Rice, 1948). That movement is directly associated with food transport and takes place with it, is supported by experiments in which sugars as well as various herbicides have been introduced into starved darkened leaves of beans, and transport of herbicide into the main stem has been thereby induced (Rohrbaugh and Rice, 1949; Weintraub and Brown, 1950). This movement takes place, as does the movement of the organic food materials, only in the living cells of the bark (Weintraub and Brown, 1950), probably in those elements known as the sieve tubes. It seems, however, that under certain conditions the hormone herbicide can be conducted upwards in the dead water-conducting elements of the wood, *e.g.* after application to the plant roots or when high local concentrations in the bark may cause leakage out into the wood. There is no indication whether this conduction is influenced by light or not. Thirdly, the possible effect of light on the toxicity of the herbicide in the

cell has not escaped attention. Some workers have claimed to have "activitated" 2, 4-D compounds and MCPA in the laboratory by using ultra-violet light, and suggest that the action of light on plant responses to herbicide may be explained by some similar "activating" process (Payne and Fults, 1947). On the other hand, the claim has been made that, in the presence of the co-enzyme, riboflavin,* in the light 2, 4-D inhibition of corn-root growth can be greatly reduced, suggesting a light "inactivation" phenomenon (Carroll, 1949). But quite apart from any direct effect on the activity of the herbicide molecule, light also seems to modify the sensitivity of the cell itself, since the toxicity of 2, 4-D to *Lemna* is dependent on the *previous* light treatment of the plant, the lower light intensities being associated with higher mortality on subsequent treatment with 2, 4-D (Robertson-Cuninghame and Blackman, 1952).

This mass of empirical and very highly conflicting data leaves little of conclusive practical value to be abstracted. It seems fairly clear that behaviour will vary greatly from species to species, and that there may well be interactions between the light effect and a variety of other environmental and treatment conditions. The full elucidation of such interactions and species effects is a task for the future.

## THE FATE OF THE HORMONE HERBICIDES IN SOIL

The ultimate fate of these herbicides when applied to soil is obviously of immense practical importance, since crop plants could be greatly effected in their later growth by residual toxicity in the soil. Fortunately, under normal conditions of moisture in cultivated soils, the toxicity disappears completely within a reasonable period of time, which varies from a fortnight to a few months, depending on conditions. Only in very dry soils may the applied compounds persist for a year or more (Mitchell and Marth, 1946).

* An enzyme is not a simple substance but a complex consisting of a protein portion, and, very often, of a substance of relatively small molecular complexity called the co-enzyme. Neither portion has any catalytic action without the other. As far as we can see, the vitamins are all co-enzymes in different enzyme systems. Riboflavin is a co-enzyme closely associated with respiration in living tissues.

The speed with which these compounds disappear depends on a very wide variety of soil conditions. We have already noted the effect of soil moisture, which has been confirmed by several series of laboratory experiments involving the incubation of soils of various moisture contents with 2, 4-D (Mitchell and Marth, 1946; Brown and Mitchell, 1948). As might be expected, soil temperatures also have an effect, the rate of disappearance being higher the higher the temperature (Brown and Mitchell, 1948; Jorgensen and Hamner, 1948; Akamine, 1951). Heavily manured soils with a high content of organic matter also detoxicate much more rapidly than sandy or loamy soils poor in such constituents (Krone and Hamner, 1947; Kries, 1947). Another soil component which seems to affect detoxication adversely is lime (Kries, 1947), and it has been suggested that for this reason caution should be exercised when using herbicides in very chalky soils. More work needs to be done on this, since one report claims that low acidity favours breakdown (Akamine, 1951).

This disappearance of herbicide from the soil has a number of possible causes. Under conditions of relatively high rainfall there is no doubt that much is lost by leaching or washing out, and this runs away in the drainage water. Direct experiments show that 2, 4-D can rapidly be removed by leaching from all types of soil (Hanks, 1946), even when heavily limed. Leaching will not, however, remove all the herbicide (Nutman, Thornton and Quastel, 1945), since a small but definite amount of the order of 0·1 mg. per gm. of dry soil seems to be adsorbed and retained after treating with 0·1 per cent. solutions of 2, 4-D, 2, 4, 5-T and MCPA (Audus, 1951). These adsorptions occur immediately on addition of the compound to the soil and do not in any way account for the delayed detoxication of the remaining bulk of the herbicide. This adsorbed herbicide is still capable of inhibiting the germination of seeds sown in the soil (Audus, 1951). The degree of this adsorption is directly related to the humus content of the soil, and it has been shown that soils poor in organic matter ($\frac{1}{4}$ to 2 per cent.) are leached much more rapidly by rain than those rich in such matter (9 per cent.). These differences may greatly influence the success of pre-emergence treatments for some crops, since,

in the former type of soil, rapid washing of the herbicide from the upper layers, where it is effective on weed seedlings, to the lower layers may seriously damage the more deeply rooting crops (Hernandez and Warren, 1949).

Both slow chemical decomposition and breakdown by soil micro-organisms have been suggested as causes of detoxication where there is no leaching, and support for the latter was first obtained from experiments on autoclaved (sterilized by steam under pressure) soil in which this detoxication was very greatly retarded (Brown and Mitchell, 1948). The high rate of breakdown in heavily manured soils, with their high bacterial content, could also be explained along these lines. Further proof that the breakdown was entirely due to living micro-organisms that decompose both 2, 4-D and MCPA, was subsequently obtained by the writer (Audus, 1951), who demonstrated that detoxication could be completely prevented by killing off the active microbe population in the soil with the poison sodium azide ($NaN_3$). Subsequently the bacteria responsible for 2, 4-D breakdown were isolated and grown in pure culture (Audus, 1950). Thus the sequence of events when 2, 4-D or MCPA is added to the soil is first an immediate small uptake and retention on the soil colloidal particles. Secondly, in response to the presence of the new foreign compound, a bacterial flora starts to grow up capable of utilizing the compound for growth. Their growth is slow at first; then, as in all populations, it increases in proportion to the population size, and eventually becomes large enough to remove the herbicide rapidly. Such a soil containing these "educated" bacteria will detoxicate fresh additions of 2, 4-D in the course of a day or so. These soils may have to be left to allow this new soil population to lose its high detoxicating capacity, presumably by the death of the "educated" bacteria, before herbicides can be again expected to persist long enough to prevent seedling growth. For 2, 4-D a detoxicating bacterial flora can be built up in 14 to 20 days, but for MCPA the time needed is 60 to 80 days. For 2, 4, 5-T a very much longer time (over a year) would seem to be necessary (Audus, 1951). It has been shown that cultivation (ploughing or digging) accelerates the detoxication process (Weaver, 1948), presumably by favouring the proliferation of the detoxicating

bacteria which require a plentiful oxygen supply for growth (Audus, 1950).

It is interesting to note at this point that these herbicides, as far as they have been studied, seem to have little toxicity for soil micro-organisms except at relatively high concentrations. According to one set of experiments, concentrations up to 100 p.p.m. in loam and 500 p.p.m. in sandy soil have no effect on the total number of bacteria, fungi, actinomycetes * and protozoa * in the soil (Smith, Dawson and Wenzel, 1945), although soil-nitrifying * bacteria were inhibited at some concentrations (Smith, Dawson and Wenzel, 1945; Newman, 1947). Some damage may, however, result in acid soils, since 2, 4-D is much more toxic to soil organisms under acid than under alkaline conditions of growth (Martin, 1946). Such an effect of acidity has been confirmed for MCPA and 2, 4, 5-T (Newman, 1947). On the other hand, the leguminous nodule-forming nitrogen-fixing organisms (see footnote, p. 182) seem to be very sensitive to these compounds; rates of application of 2, 4-D as low as ·009 lb. per acre (Fults and Payne, 1947), and concentrations as low as ·007 p.p.m. (Carlyle and Thorpe, 1947) can depress nodulation in peas, beans, etc.

## THE MECHANISM OF ACTION OF THE HORMONE HERBICIDES AND THE NATURE OF THEIR SELECTIVITY

Much attention has been paid to the possible mechanism of action of these herbicides, and it soon became apparent that their toxicity was due to a gross disturbance of the normal biochemical processes (metabolism) going on during growth. All auxins in high concentrations bring about these disturbances, but by no means all show sufficiently high phytotoxicity to be of use as herbicides. IAA and NAA are of this latter type. A possible reason for this is that the herbicidal auxins (*e.g.* 2, 4-D, etc.) are indeed much more active in the higher concentration ranges than IAA or NAA. Although at low

* Actinomycetes are exclusively soil organisms. In structure they occupy a position somewhat between the bacteria and the fungi. Protozoa are very primitive unicellular animals. Nitrifying bacteria, which are very important inhabitants of fertile soils, are responsible for the conversion of ammonia to nitrous and nitric acids.

concentration levels (0·01 p.p.m.) all these auxins are, molecule for molecule, of about the same activity, yet the effectiveness of 2, 4-D rises much more rapidly with concentration from this point than that of IAA, etc., and at concentrations still well below toxic levels 2, 4-D attains an activity many thousands of times greater than that of IAA (Overbeek, Blondeau and Horne, 1951). It has been suggested that the metabolic disturbances set up by these very effective herbicide molecules may give rise to the accumulation of natural growth-inhibitors (*e.g.* coumarin * derivatives) that are the true toxic agents. Although our knowledge is as yet very sketchy, still it is not impossible that auxin action in the cell in these high toxic concentrations is therefore on a system different from the one on which it acts in normal growth. We know so little about the fundamentals of either aspect at the moment.

There are two main sources of data which throw light on this herbicide mechanism; one comprises investigations on the changes induced by treatment in the structure and organization of cells and tissues seen under the microscope; the other involves studies of changes in the physiological behaviour and chemical constitution of the plant, organ or tissue concerned.

A very considerable mass of data has been accumulated on structural responses. Such responses are not confined to the region of application, a natural result of the ease with which these compounds are translocated. Naturally they can be evoked by all auxins that are easily transported, but the effectiveness of such compounds as 2, 4-D depends very largely on the fact that, unlike indole compounds, which can be rapidly broken down in plant tissues (Tang and Bonner, 1947; Wagenknecht and Burris, 1949), they are not so labile, but remain in growing cells to exert a progressively toxic action for long periods. The presence of 2, 4-D as such has been demonstrated in tomato and peas for as long as 26 days after treatment (Dhillon and Lucas, 1950). They may, indeed, persist in mature seeds and produce responses on seedlings growing from the seeds in the next season. This has been observed in

* Coumarin is a compound occurring naturally in many plants, giving them the characteristic scent of new-mown hay. It is a very active inhibitor of seed germination (see Chapter 14).

dandelion, where seeds from plants treated with 2, 4, 5-T produced a second generation that showed a great delay in flowering (Hitchcock and Zimmerman, 1947 *a*). A transmission of a similar effect to the progeny of treated lettuce (Clark and Wittwer, 1949), cotton (Dunlap, 1948) and red kidney bean (Pridham, 1947) plants is also claimed. Previous mention has been made (p. 214) of germination tests on peas, obtained from plants in plots where MCPA had been used to control weeds, in which a retention of the herbicide had subsequently caused great stunting and malformation of the young radicle, so that the seedlings failed to become established. It has been shown in cotton that the degree to which seeds may carry over 2, 4-D effects to the next generation depends on the time of application to the parent plant, relative to the setting of the seed in the bolls. Applications of 0·1 to 10 mg. per plant during and after seed-set all resulted in such a carry-over. Even in bolls formed eight weeks after treatment some seeds were affected. It seems that the length of time after application during which seeds may be affected in cotton is determined by the growth vigour of the parent plant. The more active the vegetative growth of the plant, the shorter the period after treatment during which young developing embryos may be damaged. It is suggested that during vegetative growth "most of the stimulus will be exhausted in the vegetative meristems" (McIlrath, Ergle and Dunlap, 1951).

That the ultimate death of the cell is due to a prolonged disturbance of growth equilibria, and not to a direct killing of the cell by the compound, is indicated by experiments on pea roots inhibited by 2, 4-D (Audus, 1948). The tissues that respond to these compounds are those that are growing or capable of being stimulated to grow. At the mild end of the scale the responses are merely manifested by an accelerated growth in length of the organ, *e.g.* the main axis of young dock plants, or in curvatures of such organs as leaf-stalks (epinastic responses). The next grade of response involves the partial or complete inhibition of organ expansion, particularly that of the leaves and roots. Cessation of growth in the latter will, of course, seriously affect the uptake of water and mineral nutrients from the soil. Such partial growth-inhibitions in particular

portions of the leaf-blade can give rise to symptoms closely resembling the attack of a disease organism, *e.g.* virus diseases in the tomato (Zimmerman, 1943). The inhibition of the growth (increase in area) of young bean leaves has been used as a biological assay method for 2,4-D (Brown and Weintraub, 1950). The most marked responses, and those which, in most instances, result in death of the plant, are those caused by rapid and often disorganized proliferation of cells in certain tissues. We have already seen in Chapter 5 how auxins stimulate the production of roots in cuttings. Herbicides may produce the same response in stems of treated plants, when adventitious roots in dense clusters may force their way through the outer layers of the stem (cortex), having been initiated in the cells of the inner layers of the bark (percycle and endodermis). Similar proliferation of outer bark cells gives rise to unorganized tumorous growths, the cells produced rounding off and forming a loose spongy mass which ruptures the outer skin of the stem (epidermis, cork layer) and exposes the inner tissue to the outer environment. When such a disorganization and rupture of tissues takes place in the underground portions of the plant, the way is open for the entry of pathogenic organisms from the soil, and these play an important part in bringing about the death of the plant (Tukey, Hamner and Imhoff, 1945). In addition, there may be proliferation of cells in the food-conducting channels of the stem (phloem), and the impeded transport this causes can very seriously hinder growth (Tukey, Hamner and Imhoff, 1945; Eames, 1950). The starvation of the roots as a result of the blockage of food transport from the leaves may cause the death of some species of plant. In view of these marked and striking responses, it is not surprising that it should have been said that these herbicides cause plants to "proliferate themselves to death" (Gilbert, 1946).

This would seem to be an over-simplification, since the herbicide induces marked changes in the biochemical make-up of the cell, and they have no obvious relationship to this proliferation. These changes cover a considerable variety of plant constituent and are not sufficiently consistent to allow one to put forward a general theory of toxicity based on them.

One of the most marked effects is the rapid depletion of carbohydrate food reserves, such as starch and sugars. This has been observed in the morning glory (*Ipomœa lacunosa*) (Mitchell and Brown, 1945), bindweed (Smith, Hamner and Carlson, 1947), dandelion (Rasmussen, 1947) and bean (Brown, 1946), and a direct action of the hormone on the controlling enzyme systems has been suspected, but by no means proved. This is very largely correlated with an increase in the rate of respiration of the affected tissues (Brown, 1946; Smith, Hamner and Carlson, 1946; Rasmussen, 1947). Most of the reserves may have been oxidized away therefore to carbon dioxide and water by this augmented respiration. We have seen in the early part of the book that part of the carbohydrate food manufactured by plants in the light is subsequently oxidized in respiration, releasing energy for use in building up the remaining food into the constituents of the plant body (protoplasm, cell-wall, etc.). The utilization of the respiration energy in synthesis has been shown to be under the control of a special system of cell catalysts (enzymes) involving organic phosphorus compounds.* It has been suggested (van Overbeek, 1947) that 2, 4-D and other hormone herbicides may exert their toxic action by interfering in these energy coupling systems, successfully preventing the utilization of the respiratory energy in growth. There is, however, very little experimental evidence for this theory; it is even doubtful whether starvation of the plant as a result of reserve exhaustion plays a major rôle in the toxic action of these herbicides, especially in view of some reports in which 2, 4-D treatment had little effect on starch content (*e.g.* in cotton leaves) (Ergle and Dunlap, 1949) or induced a rise (*e.g.* buckwheat stems) (Wort, 1949). Nevertheless the discrepant nature of these reports on carbohydrate behaviour may be bound up with the nutritional status of the experimental plants. For example, in tomato, MCPA spray treatment has been shown to bring about no depletion of available carbohydrates (starch and sugars) of plants as a whole, although it brought about a reduction in the net rate of accumulation of these substances. Such depletion was observed only in roots

* Chemists interested in this aspect of the growth and metabolism of organisms should consult Baldwin (1952) and Street (1950 *a*).

growing under conditions of adequate nitrogen nutrition (Rhodes, 1952). It was suggested that this was due to the dual effect of MCPA increasing the utilization of carbohydrate under these good nutrient conditions and at the same time stopping transport from the leaves by blocking the conducting channels (see p. 233). It was thought possible that the consequent root starvation might be the basis of the killing action of MCPA.

One marked change involves an alteration of cell-wall properties such that the affected organs, *e.g.* stems, are very easily snapped. The underlying causes of this change are not yet understood.

In addition to these effects on the carbohydrate metabolism, other biochemical changes are induced. The amount and distribution of various nitrogen constituents in the plant may be affected by hormone applications. A varied series of investigations has demonstrated that where local or general applications of relatively high concentrations of auxins have resulted in localized initiation of cell proliferation and root production, this is accompanied by a parallel local increase in the content of nitrogenous constituents, presumably protein. The underlying cause of this redistribution in response to auxin application remains obscure. In some cases, *e.g.* accumulation in roots, impeded transport through damaged conducting systems might account for the altered distribution. These latter effects would seem to be the result and not the cause of the growth responses. Again, plants susceptible to hormone herbicides may show a considerable disturbance of potassium metabolism as a result of treatment (Rhodes, Templeman and Thruston, 1950). In a susceptible species such as rape (*Brassica campestris*), where normally growth is paralleled by a considerable uptake of potassium into tops, treatment completely prevented this accumulation, whereas in resistant species such as oats (*Avena sativa*) no such effect could be observed. On the other hand, there was no observable effects on phosphorus uptake. In tomato, a susceptible species, non-accumulation of potassium in the tops in treated plants was accompanied by an accumulation in the roots, although the net uptake by the whole plant was reduced. Unfortunately these results could not be repeated later (Rhodes, 1952). In

sugar beet, treatment may result in a twenty-fold increase in the nitrate content of the leaves (Stahler and Whitehead, 1950). Again, experiments on soya bean (*Glycine soya*) (Wolf *et al.*, 1950) have demonstrated that sensitivity is closely correlated with the nitrogen nutrition of the plant, those plants receiving high rates being killed by 2, 4-D applications to the roots much more easily than those on a low nitrate diet (*cf.* results of Rhodes (1952), p. 235). Correlated with this heightened sensitivity was decreased uptake of phosphorus and sulphur. In addition to a reduction in starch and sugar in the plant, treatment resulted in a considerable increase in hemicelluloses.

A disturbance of the natural auxin relationships of growing cells has also been suggested as the cause of 2, 4-D toxicity (Goldacre, 1949, 1951). The experimental support for this suggestion was the observation that the destruction of IAA by an oxidizing enzyme system (peroxidase) isolated from etiolated stems of pea seedlings was markedly increased by 2, 4-D. In addition, the action of certain natural inhibitors of this destruction (*e.g.* onion juice) was counteracted by it. While agreeing that 2, 4-D obviously may disturb natural auxin relationships, it is difficult to see how the above findings could explain the characteristic responses of the plant to 2, 4-D, since many of these responses are produced by the application of IAA itself.

With this welter of histological and metabolic effects of toxic concentrations of the hormone herbicides, it is not to be wondered at that no comprehensive theory of action has yet emerged. It seems probable that the primary action is not on any one of the systems so far mentioned, but on some as yet unidentified "master system", the disturbance of which brings all these various metabolic responses in its train. Evidence is now accumulating, from studies of 2, 4-D preparations containing radio-active carbon atoms, that considerable breakdown of this herbicide takes place during the period of its action in the plant, both the carboxyl carbon and the $—CH_2—$ carbon in the side-chain being lost as carbon-dioxide (Weintraub, Brown, Fields and Rohan, 1950). Other as yet unidentified compounds may be formed (Holley, Boyle and Hand, 1950). It has even been suggested (Leaper *et al.*, 1951), on a basis of

the comparison of the toxicity of all the possible chlorinated phenoxyacetic acid homologues, that the benzene ring is oxidized in the plant to give para-quinones, or even ruptured with the formation of chlorinated maleic acids, which are the real toxic agents. On the other hand, we have seen that some herbicide may persist as such for very long periods in treated plants (see p. 214), and it seems unlikely that breakdown products of 2, 4-D are the true toxic agents.

Similarly, our knowledge of the mechanism of selective action is by no means complete, although we have a certain amount of data on the various factors effecting selectivity. We have seen that there is a broad distinction between susceptible dicotyledonous and resistant monocotyledonous plants, although the overlap is considerable. In addition, attempts have recently been made to relate the sensitivity of species (to 2, 4-D), not to their natural family affinities but to their general form and structure, in particular to the way in which their resting buds may be borne and protected, *i.e.*, on branches above ground, between rosettes of leaves at ground level, in bulbs, etc. underground, or not at all as in many annual plants (Bertossi and Tomaselli, 1950). The basis of this approach is the assumption that the ease with which these vital spots can be reached by the herbicide might determine their ultimate sensitivity. Over five hundred species from more than ninety families of monocotyledonous and dicotyledonous plants were classified in this way, and their relative sensitivities to 2, 4-D noted. Statistical analysis * shows that, although these several classes of plant do exhibit real differences in the pattern of response to 2, 4-D, yet the differences cannot be related in any obvious or rational way to the mode of protection of the resting buds.

The broad general differences in susceptibility between classes of plant (*e.g.* monocotyledon and dicotyledon) seem to be very largely due to a fundamental difference in the ultimate sensitivity of the growing cell to toxic action, since it can be demonstrated in the inhibition of root-growth in water culture when the herbicide is applied directly to the growing cells. Such basic differences of toxicity at cell-level have recently been confirmed (Mitchell, Wood, Wolfe and Irving, 1947) by experi-

* By the writer.

ments with a radioactive * herbicide (2-iodo-3-nitrobenzoic acid with labelled iodine). Identical concentrations of this compound in young leaves of bean and oat greatly reduced the growth of the bean leaf, but did not affect that of the oat. That this difference is merely one of degree and not of kind has been shown by experiments in which very high concentrations of hormones have been applied to various monocotyledonous plant organs. The responses then obtained, *i.e.* initiation of adventitious roots, cell proliferations, etc., have been very similar to those in susceptible dicotyledonous plants (Eames, 1949). Such differences in cell sensitivity are those determining to a large extent the selective action of herbicides when applied as pre-emergence treatments in soils. If the hormones are applied only to the surface of the soil, the depth of planting of the seed and the form of the root system may determine the reactions of the plant. Shallow-rooting surface-germinating plants will obviously suffer more than deep-rooting plants germinating well below the surface (Crafts, 1946; Blackman, 1950*a*).

Although great differences do exist between plants, as regards the various aspects of their general metabolism, *e.g.* the storage of sugars in the sugar-beet and of starch in the potato, there is very little data indicating a consistent biochemical difference between species susceptible to the hormone herbicides. One pointer has come from comparisons of the susceptible castor oil (*Ricinus communis*) and the resistant wheat. Preparations, from the seeds of these two species, of the enzyme lipase, which is responsible for the splitting of oils and fats into their constituent fatty acids and glycerine, were inhibited by 2, 4-D solutions, but the lipase from the castor-oil seed was four hundred times more sensitive to inhibition than that from the wheat (Kvamme, Clagett and Treumann, 1949). This is indeed a beginning.

* One of the most important properties of the radioactive elements now being produced in the atomic pile is that extremely small quantities of them can be detected and measured by the use of relatively simple electronic instruments. If, therefore, we incorporate an appropriate radioactive "tracer" element into a chemical compound when it is made, the compound is said to be "labelled" and its fate when used in very small quantities in biological experiments can then be easily followed usually when the classical chemical methods would be completely out of the question. (See excellent review article by Arrol, W. J. (1952); *Endeavour* **11 (42)**, 61.)

When, however, we turn to consider the sensitivity of species treated by sprays or dusts, then we see that factors other than the above enter into the picture. Such factors have been admirably reviewed by Blackman (1950 *a*), from whose article the following remarks are very largely taken.

The first important factor is the ease of retention of the spray on the leaf. It has already been seen that this retention factor may be very important in deciding the efficiency of various carriers. Surface-wetting agents added to the sprays and hygroscopic substances to dusts greatly aid this retention. The form and arrangement of the leaves and the nature of their surfaces, whether waxy, rough or hairy, are all important factors in determining the susceptibility of the species. For example, the erect cylindrical leaves of the onion will retain much less of the spray than the flat horizontally disposed leaves of dicotyledonous weeds. The nature of the leaf surface will also determine the degree to which the spray may spread over that surface and this may eventually determine the amount of herbicide absorbed. A certain number of observations have been made on the "wettability" of leaf-surfaces of different plant species by measuring the contact angle between leaf-surface and water-drops upon it. The spread of the herbicide solution and its retention on a leaf is directly correlated with this wettability, which therefore determines the amount of toxic agent absorbed by the leaf. Comparative measurements of contact angle in a number of species show that there is a rough positive correlation between wettability of the leaves and susceptibility to the hormone herbicides. For example, young wheat, oats and maize and mature lupin leaves all have a large contact angle, are not easily wetted and are resistant to the hormone herbicides. On the other hand, the susceptible tobacco and charlock have a much smaller contact angle and are much more easily wetted (Linskens, 1950). A systematic and comprehensive study of this kind might reveal the precise contribution that the leaf-surface factor makes to the determination of overall sensitivity to the herbicide. An associated factor is the disposition of the most sensitive regions of the plant (usually the growing-points), and this may, in some cases, effect response. In the sensitive dicotyledon this is usually

the exposed apical bud, but in the monocotyledon the apical meristem, at least in the vegetative plant, is usually buried inside ensheathing layers of leaves.

The next important factor is the ease of penetration of the herbicide into the treated organs, of which the most important are the leaves. We have already seen that the stomata are suspected to be of little consequence in aiding herbicide entry. Most of the entry must be through the thin pellicle of "varnish" that covers all leaves and which is called the cuticle. Entry can be effected only from solutions, and it has been noted above that activity of sprays, etc., can be greatly enhanced by the addition of hygroscopic agents that prevent drying up of droplets adhering to leaves. Little, however, is known of the facility with which different cuticles can be penetrated by these compounds, although great variations would be expected from species to species, indeed from leaf to leaf on the same plant. The use of 2, 4-dichloro-5-iodophenoxyacetic acid containing radioactive iodine has enabled penetration to be followed in different leaves on the same plant, and it had been demonstrated that the very young leaves absorb most actively, due, one would imagine, to the much thinner cuticular "varnish" (Mitchell and Linder, 1950). It is only recently, however, that 2, 4-D itself, labelled in its methylene carbon, has been studied, and the much more rapid penetration into young leaves of bean (*Phaseolus vulgaris*) verified for this practical herbicide (Fang *et al.*, 1951). Similar work has been carried out with 2-iodo-3-nitrobenzoic acid labelled with radioactive iodine. It was found that this compound penetrated into the young leaves of the sensitive bean plant roughly twice as fast as it penetrated those of the resistant barley (Wood, Mitchell and Irving, 1947). In experiments on soya bean, which possesses hairy leaves, and kidney bean, which has smooth leaves, the spreading and sticking agent carbowax was shown to increase 2, 4-D activity in the latter but not in the former. This was thought to be due to the differing hairiness. Thus, in the soya bean, entry of the herbicide may be effected immediately through the feebly cuticularized hairs, whence it travels into the interior of the leaf. In the bean, such points of easy access are not present and so a much longer contact of

the solution with the external leaf-cells, brought about by the presence of the carbowax, would be expected to increase effectiveness (Innes and Boyd, 1946). It has been suggested that epidermal hairs may be a normal point of entry for toxic agents.

The rate and extent of transport through the plant, once entry has been effected, will also influence the overall toxicity of the herbicide. Although something is known of the conditions affecting this transport in the plant (see p. 226), there is no evidence that it plays any part in determining the relative sensitivity of the species.

## THE EFFECTS OF HORMONE HERBICIDES ON ANIMALS AND MAN

Naturally the extensive use of these compounds in weed control raises the question of their possible effects on animals fed on treated plants and on man, who may eat products from treated plants (*e.g.* potatoes, wheat, maize, etc.). Cows and sheep grazed on pastures treated with liberal applications of 2, 4-D have suffered no loss of health or performance (Mitchell, Hodgson and Gaetjens, 1946). No pathological symptoms could be detected even in a cow which had received $5\frac{1}{2}$ gm. of 2, 4-D daily for 106 days. Neither was any 2, 4-D to be found in the milk, although at the end of this period 8 p.p.m. of this compound could be demonstrated in the blood-stream. At Jealott's Hill, cows have been given doses as high as 18 gm. per day without tainting the milk or affecting the health of the animals (Halliday and Templeman, 1951 *a*). These doses far exceed the amounts likely to be picked up by animals grazing on herbicide-treated pastures. It is reported that the human male can take orally as much as $\frac{1}{2}$ gm. of 2, 4-D daily for three months without showing any ill-effects (Mitchell, Hodgson and Gaetjens, 1946). As with MeNA (see Chapter 8), injections direct into the blood-stream are much more toxic, 280 mg. per kg. of body-weight being lethal in mice. Experiments have also been carried out on the toxicity of 2, 4-D to fresh-water fish (bream, bass, etc.) and it seems that concentrations up to 100 p.p.m. are likely to do little harm.

Indirect effects may arise from the accumulation of decaying weeds that have been so killed, in that they may restrict the supply of dissolved oxygen in the water (King and Penfound, 1946).

Some uneasiness might be felt that useful insects, such as bees, might be harmed by feeding from plants treated with hormone weed-killers. Direct experiments show that such uneasiness would be unfounded, since bees can take up to 0·1 per cent. solutions of the sodium salt of 2, 4-D in sugar solutions for a whole day without harmful effects. In any case, weeds are usually sprayed long before flowering, so that there is little risk of bees feeding on contaminated nectar.

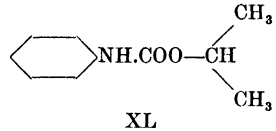
Toxic effects may, however, arise when animals browse on plants treated with sublethal doses of 2, 4-D. Experiments on sugar-beet (Stahler and Whitehead, 1950) indicate that these small doses give a great increase in nitrate content. This nitrate is not toxic in itself, but is converted into nitrite by micro-organisms in the rumen of the cattle. This nitrite, when absorbed into the blood, converts the hæmoglobin into methæmoglobin, which is ineffective as an oxygen-carrier and the animal may quickly die of anoxia. The very few doubtful cases of cattle-poisoning reported as resulting from 2, 4-D treatment of pastures, may have been due to the animals eating resistant weeds which accumulated nitrates in this way.

## OTHER SELECTIVE HERBICIDES NOT OF THE AUXIN TYPE

Many other selective herbicides not of the auxin type are in wide general use. They include copper salts, nitrated phenols and cresols, particularly 2, 4-dinitrophenol (DNP), dinitro*ortho*cresol (DNOC), dinitro*secondary*butyl-phenol (DNSBP) and pentachlorophenol, cyanates, salts of trichloro-acetic acid, ammonium sulphamate, etc. (see p. 185). The selective actions of these compounds on different species vary widely and differ, of course, from those of the auxin herbicides. This diversity of action is one of the most promising features of this rapidly developing subject, and as the number of active compounds grow, we look forward to the day when perhaps particular weeds in particular crops may be

controlled by their own specific herbicides. The general principles underlying selectivity in these compounds are the same as those already outlined for the auxin herbicides. Space does not permit a detailed discussion of the individual characteristics or of the mode of action of these toxicants, and the interested reader is referred to the comprehensive reviews of Blackman (1950 *a* and 1951) and Norman *et al.* (1950).

Some consideration should, however, be given to the compound isopropylphenylcarbamate (IPPC) (XL), since it promises to be a selective herbicide with an action very largely complementary to that of the hormone group, *i.e.* it is much more toxic to cereals and grasses than to the broad-leaved plants. It has the formula




XL

Although the toxic action of low concentrations of this class of chemical compound had been known since 1929 (Friesen), it was not until 1940, in the early days of the Second World War, that their selective herbicidal properties were demonstrated in England (Templeman and Sexton, 1945). The compound ethylphenylcarbamate was shown to be highly toxic to oat seedlings, but was ineffective on charlock (*Sinapis alba*). This led to the testing of over fifty related compounds, of which IPPC proved to be the most effective. Subsequent extensive experiments both in England and America confirm its general toxicity for cereals and grasses, and the insensitiveness of dicotyledons. As we found with the hormones, however, there were exceptions to this general rule, some grasses, *e.g.* Bermuda grass (*Cynodon dactylon*) and millet (*Sorghum vulgare*), were relatively resistant (Mitchell and Marth, 1947 *a*). Mixtures with 2, 4-D or MCPA have been shown to be of great use as general herbicides, since both monocotyledons and dicotyledons are thereby controlled. Some resistant weeds, *e.g.* chickweed (*Stellaric media*) and goose grass (*Galium aparine*) are damaged more by the mixture than by either of the two herbicides acting separately

(Templeman and Wright, 1950). IPPC is also stable in soils and is slowly broken down, presumably by micro-organisms, over periods ranging from twelve days to two months, depending, as in the case of 2, 4-D, on the organic and moisture content, etc., of the soil. This compound acts only on seeds and seedlings, except when used in very high concentrations, and would seem therefore to be much more effective in soils than when applied as sprays to the leaves. This may be due mainly to the ease with which the growing-points are reached by the compound in each treatment. The action, though in nowise resembling that of the hormone herbicides, is essentially one of a disturbance of growth processes, studies on roots showing that certain aspects of cell division are completely dislocated (Doxey, 1949; Ivens and Blackman, 1949). The compound therefore comes into the category of mitotic * poisons.

A number of other synthetic plant "growth regulators" have been shown to possess selective herbicidal properties when used in high concentrations. Thus, maleic hydrazide (see p. 166) seems to have a higher toxicity for grasses than for certain broad-leaved species, *e.g.* cotton (Currier and Crafts, 1950). A synthetic compound 3-(α-iminoethyl)-5-methyltetronic acid,† which is related to the naturally occurring plant growth inhibitor coumarin (see Chapter 14), in that it has an unsaturated lactone structure, has been shown to inhibit the formation of the green photosynthetic pigment chlorophyll (Hamner and Tukey, 1951). This results in arrested development and eventual death of the plant. Preliminary reports suggest that monocotyledonous plants are particularly sensitive, indicating that here may be another selective herbicide.

* Mitosis, from which the adjective "mitotic" is derived, is the name given to the complicated sequence of changes which takes place when the nucleus of a vegetative cell divides into two exactly similar daughter nuclei, a process essentially for the maintenance of the hereditary make-up of the cell.

† The formula for 3-(α-iminoethyl)-5-methyltetronic acid is

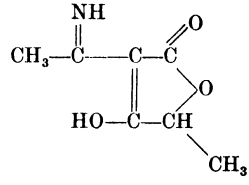





FIG. 34.—Malformations in wheat ears produced by high doses of 2, 4-D.

1. Stem deformity and head enclosed in leaf sheath. 2. Double or bunched head. 3. Opposite head. 4. Normal untreated head.




FIG. 35.—Malformations in barley ears produced by high doses of 2, 4-D applied by spray *prior to tiller formation.*

1. Double or bunched head. 2. Opposite head. 3. Normal hea with one naturally occurring sterile glume.

*Photographs by permission of Professor G. E. Blackman.*

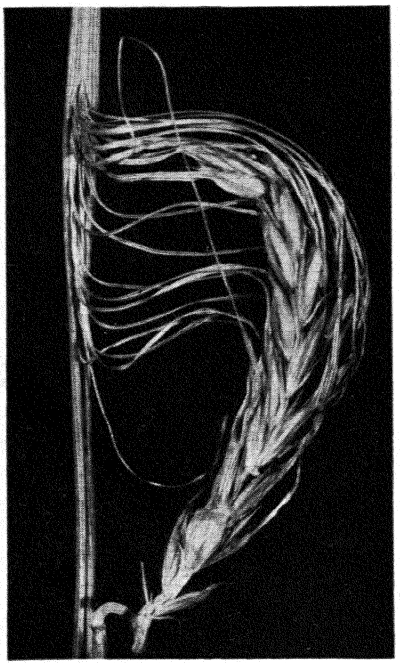


FIG. 36.—Barley ear. Stem deformity with awns trapped in tubular leaf sheath caused by an excessive dosage of 2, 4-D applied *prior to tiller production.*

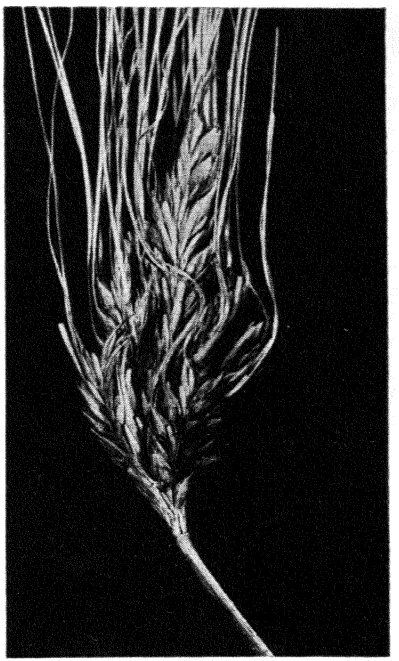


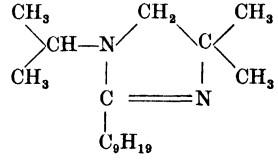
FIG. 37. — Barley ear. Multiple branching of the rachis caused by treatment with an excessive dose of 2, 4-D applied *prior to tiller production.*

*Photographs by permission of Professor G. E. Blackman*

Similar claims of selective action have been made for certain imidazoline derivatives (Allen and Skoog, 1951) of which 1-isopropyl-2-nonyl-4, 4-dimethyl-2-imidazoline * is one of the most toxic. A 90 per cent. control of broad-leaved weeds in maize, with no damage to the crop, was obtained by pre-emergence treatment with 2 lb. per acre. Post-emergence treatment caused damage to the crop. One striking difference between this and the auxins is that it is immediately inactivated in soil presumably by adsorption on to colloids. This may be an asset in pre-emergence treatments for sensitive crops which would otherwise be damaged by action on the roots as with the hormone herbicides.

All these new classes of compound are still virtually in the laboratory phase of investigation, and will need the kind of intensive testing under field conditions received by the hormone herbicides and the substituted phenols before they can be considered for general use as weed-killers. We see, therefore, from the rapidly accumulating literature, that a large number of synthetic organic chemicals are being subjected, in a purely empirical manner, to suitable screening tests for growth inhibition activity. A number of highly effective molecules, quite unrelated to the auxins either chemically or in their physiological action, are emerging (Templeman and Halliday, 1951). Since all these herbicides, including the auxins, have widely differing selectivities, it is natural that the use of mixtures has been contemplated in order to increase the range of weeds controlled by one treatment. The advisability of this procedure still remains a controversial subject (Templeman, 1951). The hormone herbicides and IPPC homologues, for example, are completely compatible, and weeds susceptible to both these groups of chemical can be controlled by such mixtures, particularly in pre-emergence treatments (Templeman, 1951). On the other hand, it is inadvisable to use mixtures of the

* The formula for 1-isopropyl-2-nonyl-4, 4-dimethyl-2-imidazoline is



delayed-action internally-mobile herbicides, of which auxins are the best example, with purely contact poisons, such as oils, etc. These latter kill the tissues with which they come into contact, and since the active translocation of the former can take place only in living cells, their action on the whole plant is very severely restricted, if not prevented entirely.

CHAPTER 10

# AUXINS AND TISSUE DIFFERENTIATION

## INTRODUCTION

In Chapter 6 reference was made to the revolutionary technique of the two French workers, Gautheret and Nobécourt, who demonstrated that auxin ($\beta$-indolylacetic acid) was essential for the growth of isolated plant fragments in tissue culture. In these cultures it was shown that auxin induced, in an ascending concentration series, firstly cambial activity and callus formation at one part in ten millions, then the initiation of roots, followed by the inhibition of buds. At the highest concentration of ten parts per million, growth was rapid and unregulated and produced tumours. This multiple influence of auxin, exerted in a wide range of cell and tissue growth phenomena, has recently been extended by the discovery by a colleague of Gautheret (Camus, 1949) that some auxins may determine the *differentiation* of certain specialized cell types, particularly conducting tissues such as wood and phloem,* in the tissues of storage roots of endive and chicory. Using a technique in which buds were grafted on to root fragments in various kinds of storage tissue under sterile conditions, he showed that in the course of a few days an "influence" had spread from the bud scion into the underlying tissue of the stock causing a "de-differentiation", *i.e.*, resumption of the capacity to divide with the subsequent formation of a cambium, and re-differentiation into conducting tissue. In certain tissues, only isolated conducting cells were produced, whereas in others a cambium was formed between the vascular system of the stock and that of the grafted bud, and from the cambium wood was formed

* Wood cells are mainly concerned with the transport of water. Phloem is the name given to a specialized tissue, usually the innermost layer of the bark in trees, concerned mainly with the transport of organic food materials. The cells in which this transport takes place are greatly elongated in the direction of the long axis of the conducting strand itself and, in contrast to the empty dead elements of the wood, are full of active living protoplasm.

on one side and phloem on the other. The hormone nature of this "influence" was demonstrated by its passage through a cellophane film inserted between bud and rootstock. Subsequently, IBA and IAA applied to such root tissue cubes, produced a response identical with that caused by buds, although other auxins (*e.g.* NAA, 2, 4-D, etc.) produced only slight responses. These preliminary results suggest that an additional action of auxin may be the control, at least in part, of cell differentiation, although the full extent of that control has still to be elucidated.

Whatever the rôle of auxins may be in this aspect of tissue development, it has been known for some time that these hormones may prevent certain tissue changes associated with the shedding of leaves, flowers and fruit. Such changes, particularly those concerned in the fall of fruit like the apple, are naturally of considerable concern to the fruit-grower, and their prevention by the application of hormones forms the subject of this chapter.

## ORGAN ABSCISSION AND NATURAL HORMONES

In the broad-leaved trees and in the gymnosperms (pines, larches, etc.) leaves are shed either regularly every autumn, as in the deciduous trees of the temperate zones, or at a definite age, as in evergreens. Leaf fall is brought about by the differentiation at the base of the leaf-stalk (petiole), just where it joins the main stem, of a cell layer known as the separation layer. This layer may be formed direct from mature existing cells of the leaf-stalk or from new tissue arising as a result of meristematic activity resumed in that region. This narrow plate of cells, which extends over the whole of the cross-sectional area of the stalk, except for the central dead water-conducting elements, is composed of somewhat smaller cells than the rest of the stalk. The walls of these cells undergo a marked chemical transformation in that the internal layers cementing the individual cells together become soft and gelatinous due to the formation of pectic materials.* The cells

* Pectins, the carbohydrates of fruit which cause the setting of jams, belong here.

therefore lose their adherence, and the slightest air movement is enough to rupture the leaf-stalk at this layer and the leaf is shed. These phenomena are illustrated in Fig. 38. It is called abscission by the botanist. Either just before or just after this shedding, other changes in the cells on the stem side of the abscission layer cause this tissue to form a waterproof protective barrier effectively sealing off the scar surface. The precise conditions determining the inception of this process have still to be explored. Leaves are not, however, the only organs to show this phenomenon. Flowers, and particularly fruit such as pomes and stone fruits, may develop similar abscission layers in their stalks in response to a variety of stimuli. In these plants the shedding of blossom and very young fruits can have various causes. It can be due to defects caused by frost or spray injury, or to failure of pollination resulting from unfavourable weather. It may be due to the failure of fertilization, even though the flowers have been pollinated, or even to the abortion of young embryos. Subsequently, there may be waves of fruit dropping during the early development of the fruit. In the apple two major peaks, the "first drop" and the "June drop", have been widely recognized. In the stone fruits three drops would seem to be more usual.

The "first drop" occurs just as the young fruit of apple or plum begins to swell, whereas "June drop" occurs when the fruit is about the size of a walnut. There is a marked tendency for the intensity of these two drops to be inversely correlated. In any event, some drop is desirable if the fruit-set is at all heavy, since there is a definite optimum number of fruit per tree for maximum cropping. Too large a number will give small inferior fruit and the tree may bear badly in the following season. Similarly, too small a number may induce a subsequent heavy-cropping year. Both will tend to produce the undesirable biennial rhythm of alternate heavy and light crops. It is often necessary, therefore, to thin the fruit-spurs artificially, either by hand or chemically. Hormones have been used for this purpose (see Chapter 11).

In these early abscissions, the separation layer develops from relatively immature tissue and seems to be associated with a

certain amount of cell-division. Later on, at the end of the season, there occurs in these orchard fruits the drop of most consequence to the fruit-grower. This is the "pre-harvest drop." This shedding of almost mature fruit just before picking-time must not be confused with that caused by insect pests like the codlin moth, since all the apples which fall are sound. Due to some as yet unknown biochemical changes as the fruit nears ripeness, the auxin balance, as we shall see presently, is altered, cells at the base of the fruit-stalk develop into an abscission layer and the fruit falls prematurely. In the apple this pre-harvest drop can sometimes account in extreme cases for the whole of the crop. Pre-harvest drop is therefore usually by far the most serious economically. Fruit which falls in this way, although sound, comes into the category of wind-falls and is usually of little value in the quality market, even if it can be salvaged. Of course this drop, being the outcome of normal physiological drifts inside the apple, is naturally greatly affected by weather conditions, by climate and even to some extent by cultural treatments. Similar varieties will show considerable differences both in intensity and duration of the drop. Such details are, however, outside the scope of this book, and interested readers will find a full discussion in the monograph by Vyvyan (1946).

It was not until 1933 that it was suspected that auxins played a part in the phenomenon of organ abscission (Laibach, 1933), but, soon after, it was demonstrated that the application of various synthetic auxins delayed leaf-fall in the decorative plant *Coleus* (La Rue, 1936). Later, in an investigation primarily concerned with the induction of parthenocarpy in holly, independent confirmation of the effect was obtained for both the leaves and the berries of that plant (Gardner and Marth, 1937 *a*). It was not until 1939, in America, that extensive experiments on apples in the field resulted in the successful control of pre-harvest drop (Gardner, Marth and Batjer, 1939, 1940). A vast amount of confirmatory research since that date has demonstrated the almost universal effect of hormones on the inhibition or delay of abscission. Until recently, however, the part played by natural hormones in abscission phenomena in fruit was only suspected, but in the last year or

so investigations by Luckwill at Long Ashton Research Station, have brought out strongly the close relationships that exist in the apple between hormone content of the seed and incidence of abscission and fruit-fall (Luckwill, 1948). We have already noted in a previous chapter (Chapter 7) that the apple-seed is a centre of auxin production. The site of this production is a layer of nutritive tissue (the endosperm) which coats the young embryo inside the seed. Luckwill has followed the changes in the hormone content of this tissue during the growth of the apple and finds that it bears a close relation to the various "drops". In the early stage of seed development after fertilization, no hormone is produced by the endosperm and this is the period of the "first drop". After three or four weeks the endosperm starts its rapid growth, with a corresponding increase in hormone production. This corresponds to a period of "sticking" in which no fruit-fall occurs. Subsequently, the embryo starts rapid growth, partially at the expense of the endosperm, and the consequent reduction in the hormone content is closely correlated with "June drop" in some of the varieties studied. Pre-harvest drop is also closely correlated with very low hormone content of the seed. Some of Luckwill's results are shown in Fig. 39. This interesting and important work is still in its early stages and promises to throw much light on the physiology of natural fruit-fall. There is also considerable evidence to suggest that normal leaf-fall is also associated with decline in the natural auxin supply from the leaf-blade (Shoji, Addicott and Swets, 1951).

## THE USE OF SYNTHETIC AUXINS IN THE CONTROL OF ABSCISSION PHENOMENA

### *Fruit Drop in Apples.*

#### (a) *Introduction*

The pioneer work on the application of synthetic auxins to the prevention of fruit-drop in commercial orchards was that of Gardner, Marth and Batjer (1939, 1940), who made a very comprehensive survey of a wide variety of compounds and treatments, and on a number of apple varieties. These experi-

ments were so successful that control of drop by this method was immediately and widely adopted as an important horticultural practice. The considerable volume of research, in the ten or so years which have elapsed since these early details were worked out, has added little in the way of new techniques or ideas, although it has confirmed its usefulness with other species such as the pear and in parts of the world other than America. Attention has been paid almost exclusively to pre-harvest dropping, since this has paid the most rapid and marked dividends. June drop has been little studied, partly because early hormone application experiments on it were not successful (Gardner *et al.*, 1939, 1940) and partly because, as we have seen, some drop is an advantage in the regulation of cropping rhythms. In this chapter, therefore, we shall be concerned almost entirely with the control of pre-harvest drop, but a small section on June drop is included at the end. In England, most of the work on the subject has been done by Vyvyan and his colleagues at East Malling Research Station. One of his major contributions to the subject is the devising of a rate of dropping index whereby an accurate estimate can be made of the relative effects of hormone sprays under the various experimental conditions (Vyvyan, 1946 *a*). Thus the absolute rate of dropping of apples at any stage is obviously proportional, all other conditions being equal, to the total number of apples on the tree at that time. In order, therefore, to obtain, for all trees, figures which are strictly comparable one with the other, the rate should be calculated for a standard number of fruit on the tree. This offers difficulties, since dropping can only be measured over a period, and this raises the question whether drop should be related to the apples on the tree at the beginning, end or middle of the period. Vyvyan has found that the most accurate measure of this standardized rate of dropping, or dropping "index", is given by the expression:—

$$Q = \frac{\log_e X_1 - \log_e X_2}{t_2 - t_1} \times 100$$

where $X_1$ and $X_2$ are the numbers of fruit on the tree at times $t_1$ and $t_2$. From this expression the results of hormone treatment

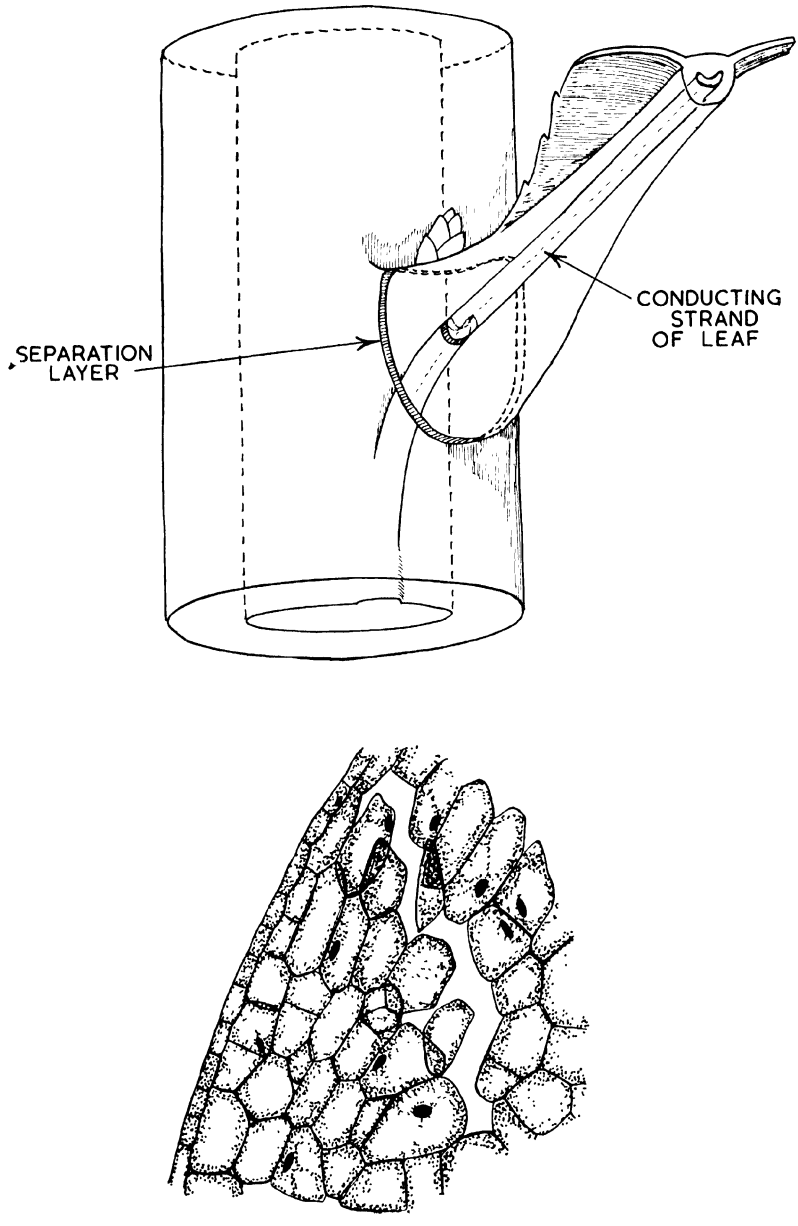



FIG. 38.—Abscission of the leaf.

*Top.* Diagram showing position of the separation layer at the base of the leaf stalk.

*Bottom.* Drawing of section through the separation layer, showing cells coming apart during abscission. ( × 150.)

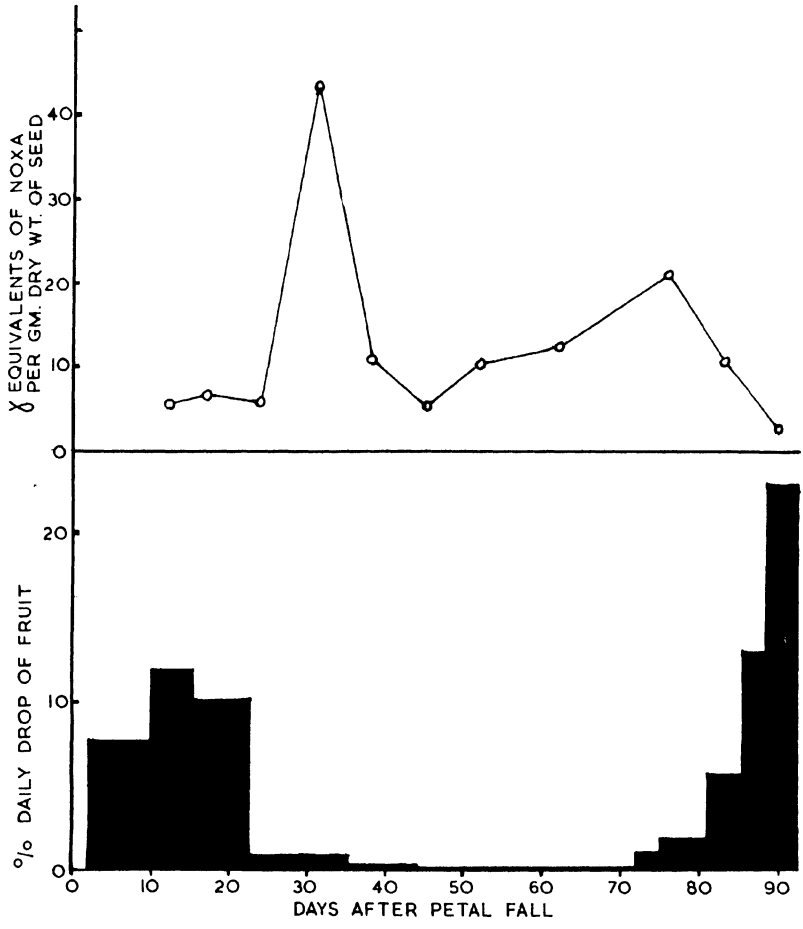



FIG. 39.—Graphs showing the relationship between hormone content of the seed and fruit drop in Beauty of Bath apple from petal fall to fruit maturity. Note that the cessation of early drop coincides with the first rapid increase in hormone content, while the onset of pre-harvest drop is closely correlated with a marked fall in hormone content.

*After L. C. Luckwill, J. hort. Sci.*, 1948.

can easily be assessed by finding for each period the value of—

$$q = 100 \times \frac{Q_c - Q_s}{Q_c}$$

where $Q_s$ and $Q_c$ are the rates of dropping indices for the treated and the control trees respectively. "$q$" is an estimate, therefore, of the reduction in the rate of dropping caused by hormone treatment over the relevant period. The striking effects that are obtained by these hormone treatments can best be illustrated by some of Vyvyan's own results expressed in terms of this dropping index. In Fig. 40 are reproduced results obtained with Beauty of Bath, an early dessert apple, which is marketed as soon as picked, but which tends to ripen unevenly and drop heavily as it does so, and Miller's Seedling, a variety with normally fairly heavy drop. In the former, spraying took place on 24th July, when dropping had already begun. Although this treatment somewhat increased the drop in the first three days, the subsequent peak-dropping was very considerably reduced, on the average by 60 per cent. In other experiments in which the spray was applied before dropping began, a gain of about 300 bushels of apples per acre was obtained. In Miller's Seedling, sprayed with 5–10 p.p.m. NAA on 7th August, a higher percentage reduction in rate of dropping was maintained over the whole of the month or so before plucking, even in the period of normally rapid drop just prior to picking. Estimated gains from such experiments were of the order of 140 bushels per acre. These results are typical of the majority of experiments with these two varieties. Photographs of an experiment are shown in Fig. 41.

It is an interesting fact that although these hormone effects were discovered from studies of leaf-fall phenomena in *Coleus*, yet there seems to be little or no effect of fruit-drop sprays on the subsequent shedding of the foliage on the treated apple tree (Gardner, 1940). This enigma offers intriguing possibilities for future research.

(b) *Effective Compounds*

In the pioneer work in America, the compounds investigated were IAA, IBA, IPA and NAA applied as a dilute aqueous

spray to the variety King David (Gardner *et al.*, 1939, 1940). The order of effectiveness was—

| | | | | |
|---|---|---|---|---|
| NAA | 79 | per cent. | reduction | in accumulated drop. |
| IAA and IBA | 36 | ,, | ,, | ,, |
| IPA | 15 | ,, | ,, | ,, |

In view of their low effectiveness and considerably greater cost, the indole compounds have not subsequently been used, and NAA and its various derivatives were used almost exclusively up to 1945. The compounds that have been studied include the acid itself, its sodium, potassium, calcium and ammonium salts, the acid-amide and the methyl and ethyl esters. The salts appear to be as effective as the free acid, although it has been suggested that the action of the salts is rather more transient than that of the acid (Batjer and Marth, 1941). Considerable attention has been paid to the relative intensity of the action of the free acid and the amide, and the latter compound has been severally reported to have an effectiveness greater than, smaller than and equal to that of the free acid. Recent investigations on the inhibition of abscission of defruited stalks of Cox's Orange Pippin and Bramley Seedling showed that no significant differences could be detected between these two compounds (Barlow, 1948). The esters, which have been little used, seem to be somewhat less effective (Garner, Marth and Batjer, 1940).

Naphthoxy acids such as $\beta$-naphthoxyacetic acid (NoXA) and $\beta$-(l-naphthoxy)-propionic acid, also have an action which is not, however, as marked as the NAA compounds (Batjer and Marth, 1941; Swarbrick, 1943).

In the last five years or so, the chlorinated phenoxyacetic acids (in particular, 2, 4-D) have been consistently studied: their action seems to be much more erratic than that of NAA. In America, two varieties, Stayman's Winesap and Winesap, have always responded well (Moon, Regeimbal and Harley, 1948; Harley, Moon and Regeimbal, 1948), whereas no profitable response has been obtained from such varieties as Delicious, Golden Delicious and McIntosh (Harley, Moon, Regeimbal and Green, 1946; Edgerton and Hoffman, 1948). The English varieties, Worcester Pearmain (Swarbrick, 1945 *a*)

and Bramley Seedling (Vyvyan and Barlow, 1948 *a*), also seem to be insensitive. At the same time the high toxicity of 2, 4-D and its derivatives also shows up in these applications as it does in those for fruit-set (Chapter 7). The butyl esters are particularly prone to produce damage both to the fruit and to the tree itself, and this may even extend to the year following the treatment when reduction in crop may result (Moon, Regeimbal and Harley, 1948). A recent newcomer which promises to be more effective than NAA, even with such insensitive varieties as McIntosh, is α-(2, 4, 5-trichlorophenoxy)-propionic acid (Edgerton and Hoffman, 1951).

All this comparative work has been carried out in the orchard mainly by spraying whole trees and comparing drop with that in control trees. Owing to the very large variations found between individual trees, the difficulties attendant on carrying out experiments with numbers of trees sufficiently large to give significant results have led to a modification of technique whereby half a tree, or even one limb of a tree, is treated, and the remaining part left as unsprayed control with which to compare subsequent reaction to spraying. Even with these refinements, it is cumbersome to use such gross methods for a comparison of effectiveness of large numbers of compounds, and for this purpose a technique involving a simple de-fruited stalk of apple has been evolved at East Malling (Barlow, 1948). The hormone, made up in a lanolin paste, or as a solution in 50 per cent. alcohol, is applied to the cut surface of a stalk still attached to the main stem. Control de-fruited stalks fall off in about a week, and the degree to which the abscission is put off by the hormone is taken as a measure of effectiveness. Checks by spraying single branches have shown that this simpler assay method gives reasonably accurate results.

Investigations using this assay showed clearly that the effectiveness of any one compound may vary considerably from variety to variety, as already forecast by the different behaviour of American varieties to 2, 4-D. In preliminary investigations, eight compounds were tried on Cox's Orange Pippin and Bramley's Seedling. There is, of course, considerable overlap in degree of response to the different compounds and it is not possible with the figures obtained to arrange

them strictly in order of effectiveness, especially since this varies somewhat with the concentration applied. The approximate orders, starting with the most effective, are:—

*Cox's Orange Pippin*

(1) α-(2-chlorophenoxy)-propionic acid
(2) 2-methyl-4-chlorophenoxyacetic acid (MCPA)
α-(2, 4-dichlorophenoxy)-propionic acid
Methyl ester of α-naphthylacetic acid (MeNA)
(3) α-naphthylacetic acid (NAA)
(4) 2, 4-dichlorophenoxyacetic acid (2, 4-D)
α-naphthylacetamide (NAAm)
γ-(indole-3)-butyric acid (IBA).

*Bramley's Seedling*

(1) 2, 4-dichlorophenoxyacetic acid (2, 4-D)
2-methyl-4-chlorophenoxyacetic acid (MCPA)
(2) α-(2, 4-dichlorophenoxy)-propionic acid
(3) α-(2-chlorophenoxy)-propionic acid
(4) Methyl ester of α-naphthylacetic acid (MeNA)
α-naphthylacetic acid (NAA)
(5) α-naphthylacetamide (NAAm)
γ-(indole-3)-butyric acid (IBA)

These results illustrate the complexity to be expected in the responses of the different varieties to a range of compounds and indicate the vast field of applied research still to be covered in this subject.

(c) *Effective Concentrations*

The effect of auxins in preventing fruit-drop is exerted in very low concentrations, ten parts per million in a water spray (=0·001 per cent.) being most frequently used. This is the "standard strength" of most workers. However, very considerable control has been claimed in some sensitive varieties (*e.g.* King David) by concentrations as low as 1 p.p.m. of NAA (Gardner, Marth and Batjer, 1940), although the majority of varieties are not affected by it. On the other hand, a somewhat higher concentration of 2½ p.p.m. has been shown to give a marked reduction in most varieties of apples tried. Con-

centrations higher than the standard strength (up to 20 p.p.m.) have also been used, but may give rise to certain disadvantages such as too firm "sticking" of the ripe fruit during plucking. It has been suggested that in some varieties even the standard strength of 10 p.p.m. may produce this effect, and result in broken spurs and stalks pulled from the fruit (Batjer, 1941). Between these two extremes lies a range of effective concentrations with no clear-cut optimum. Out of a considerable mass of experiments, quite a few have compared the effectiveness of two, and sometimes three, different concentrations. This bulky literature has been analysed and tabulated by Vyvyan (1946) in an attempt to reveal any consistent relationship between concentration and reduction in drop. As would be expected from such widely diverse sets of experiments, results are very conflicting. In some experiments, marked progressive improvement in control was claimed as concentrations were increased even up to 40 p.p.m. (Southwick, 1940), but in others no such effects were observed. In Bramley's Seedling, 10 p.p.m. was even less effective than 6 and $7\frac{1}{2}$ p.p.m. (Vyvyan, 1946). A close scrutiny of Vyvyan's tables does show that in the majority of instances 10 p.p.m. was somewhat more effective than 5 p.p.m., the improvement in drop control being of the order of 0–50 per cent. No consistent differences are discernable between $2\frac{1}{2}$ and 5 p.p.m., although sometimes (*e.g.*, in variety U.S.A.D.A. 9) (Gardner *et al.*, 1940) the latter concentration gave much better control. Vyvyan concluded that on the whole it is best to use the "standard strength" of 10 p.p.m. Weaker solutions (*i.e.*, 5 p.p.m.) could profitably be used for certain sensitive varieties (*e.g.* Williams' Early Red, Delicious, Beauty of Bath) especially in warm weather (see section (i)), thereby reducing cost of materials. Some of the compounds, *e.g.* α-(2, 4, 5-trichlorophenoxy)-propionic acid, are recommended to be used at 20 p.p.m., at which concentration there seems to be no damage to the tree and its foliage.

Very recently the concentration effect has been investigated by the defruited stalk abscission technique with NAA and 2, 4-D compounds in Cox's Orange Pippin (Barlow, 1950). Examination of the results of these experiments indicates that for each

compound there is a definite threshold concentration below which no significant action is observed. Above this threshold value, increase in the concentration applied results in proportionate delay in abscission up to the maximum concentrations employed. The value of this threshold varies very considerably with the conditions of the test, thus indicating the origins of the wide response variation in tree-spraying experiments. For NAA this threshold varied between 1 and 10 p.p.m., whereas for 2, 4-D it seems to be of the order of 50 p.p.m. Interesting as these results are, they are of little immediate practical value, since over the range of spray concentrations effective in controlling fruit-drop, little delay is caused in de-fruited stalks abscission.

(d) *The Nature of the Receptor*

The ultimate action of the hormone is the prevention of the biochemical changes taking place in the separation layer of the stalk, and it is widely assumed that the action is a direct one, *i.e.*, the hormone has actually to reach the layer in order to exert its action. It is, therefore, of considerable academic, as well as practical, interest to know where to apply the hormone in order for it to reach this point of action most effectively. The obvious place for such an application is the stalk itself, and there is evidence to suggest that the most rapid and complete response is achieved when solutions are applied direct to that organ (Gardner, Marth and Batjer, 1940). There seems little doubt, however, that other parts of the plant can absorb the hormone and transmit it to the stalk. Application to the calyx end of the apple produces an appreciable reduction in drop (Gardner, Marth and Batjer, 1940). It has also been noted that a larger response is obtained when the whole tree is sprayed than when the fruit alone is treated (Overholser, Overley and Allmendinger, 1943), suggesting that the leaves and shoots may also absorb and transmit the hormone. In fact, some workers claim that the leaves on the fruit-spurs are the main receptors of the hormone (Batjer and Thompson, 1948). The subsequent action is exerted on the fruit-stalk on the same spur and there is no transmission to neighbouring spurs on the same branch, or even from branch to branch of a compound spur.

In one series of recent experiments (Barlow, 1950) the hormone NAA was shown not to spread even from one fruit stalk to a neighbouring one on the same spur, although such transmission was observed with very high concentrations of 2, 4-D (1000 p.p.m.). Although the picture is by no means clear, it seems that for effective action the hormone must be applied very near to its site of action, *i.e.* to the stalk itself, to the neighbouring parts of the fruit, or to the leaves on the same spur. NAA, the most suitable compound so far investigated, seems not to be transmitted, except for very short distances, when absorbed from externally applied sprays. There is, on the other hand, evidence that it can move all over the plant if absorbed by the roots, since some slight control of drop has been claimed from application of NAA to apple-tree roots (Gardner, Marth and Batjer, 1940). Such transmission must be in the water-stream (transpiration stream) moving up the tree in the wood, as confirmed by the direct injection experiments mentioned later (Edgerton and Hoffman, 1948) (p. 261).

(e) *Methods of Application*

(1) *Sprays.*—The hormones are usually applied to the tree in the form of water-sprays using solutions of 10 p.p.m. In view of the fact that the growth-substance action is very localized, it is obvious that the spray should be so applied that the stalk itself or the closely adjacent organs are thoroughly wetted by the solution. Thus, unlike the spraying methods for the control of codlin moth, where the spray is directed upwards to wet the calyx of the fruit, fruit-drop sprays should be directed from above downwards, so that the stalk and stalk-cavity is completely wetted. For the same reason, spraying should be thorough, so that all fruit receives the hormone application, and it has been recommended (Batjer, 1941) that the tree should be sprayed both from the inside as well as the outside. Indeed, in order to ensure thorough and complete cover, it may be of advantage to halve the concentration and double the quantity of spray used for the tree. Similarly, it has been pointed out that the apples at the top of the tree should receive full attention, since they are usually the best fruit and could, in falling, knock off other lower fruit. It has been said

that incomplete spraying is worse than no spraying at all, and it would seem advisable to err on the generous side when making these applications. Applications to large orchards by spraying from aeroplanes have been tried in America with some success (Scamen, 1944). Smaller volumes of more concentrated sprays are then used, but the dangers of incomplete coverage would seem to be inherent in this method.

The quantities to be applied have been estimated according to the age of the tree and also on the basis of the expected crop. The former, which has been used in America, allows half a gallon for each year of the tree's age, whereas the latter allows 1 to 1¼ imperial gallons per bushel of expected crop. The latter estimate is recommended by Vyvyan (1946) for English conditions where trees of the same age may differ widely in size and bear according to the root-stock on which they have been worked.

(2) *Dusts.*—Dusts have been tried extensively and are recommended by some workers as being easier and quicker of application, and the necessary apparatus less likely to cause damage to trees and crops. Vyvyan (1946) has summarized the data on the relative efficiency of sprays and dusts, and points out that for dusts it is much less easy to gauge the amounts deposited on the tree. In addition, the weather conditions suitable for dusting are rather more critical than for spraying. The best time would seem to be the early morning, when the dew still on the leaves retains the dust particles and facilitates the penetration of the auxin. From a comparison of the results of dusting and spraying from a number of investigations, he concludes that effectiveness varies widely with location and conditions, and that the method has not yet been tested sufficiently. Spraying is certainly at the moment the more suitable method.

(3) *Aerosols.*—Aerosols have also been used successfully by a few workers (Tukey and Hamner, 1945; Mitchell, Toenjes and Hamner, 1948). Effective concentrations of NAA were 37·5 to 100 p.p.m. at rates of 34 mg. per bushel of fruit (1–1¼ gallons of spray per bushel at 10 p.p.m. is equivalent to 55–70 mg. per bushel). This method, too, needs further testing, as the dangers of incomplete coverage seem to be present.

There is also the danger of damage by excessive local deposits. With water sprays this risk does not occur, because a leaf cannot be more than completely wetted, all excess spray dropping off.

(4) *Other Methods.*—The pioneer work (Gardner, Marth and Batjer, 1940) included injection trials by boring holes in the trunks of the trees and allowing the tree to draw in 5 litres of 100 p.p.m. NAA solution, or by filling the holes with a 5 per cent. solution in lanolin paste. No success attended these experiments. More recent work on the variety McIntosh, which is insensitive to 2, 4-D sprays, gave marked reduction in drop when the tree was injected just before harvest with a 10 p.p.m. solution of that compound. It is interesting to note that no injury to the tree was observed as a result of this treatment (Edgerton and Hoffman, 1948). Other tests have been made by applying 2½ to 10 p.p.m. solutions to tree roots in quantities of 20 gallons, and some slight reduction in drop was recorded (Gardner, Marth and Batjer, 1940). These experiments have not since been repeated as far as the writer is aware.

(5) *The Use of Spreaders.*—The addition of spreading or wetting agents, as in the fruit-setting sprays, has been widely used in fruit-drop sprays, but a warning is issued by Vyvyan (1946) that care should be exercised until more is known about the action of these compounds. He points out that there is already evidence that wetting agents may reduce very considerably the storage life of Bon Chretien pears and may damage the natural waxy protective layer covering the fruit.

In the early experiments on drop control, oil was added to the hormone sprays with the hope that it might aid retention of the hormone in a thin oil film on the tree and so increase total penetration. Both in this work and in a number of other subsequent investigations, addition of oil at the rate of ⅛ to 1 per cent. gave definite indication of decrease in drop as compared with the hormone treatment without the inclusion of oil. Such beneficial results have not been obtained consistently, and no definite practical recommendations can therefore be made. The danger that oils, too, may impair fruit quality advises caution in their use.

(f) *Compatibility with Fungicides and Insecticides*

Fruit-drop sprays are usually applied much later in the season than the routine fungicide and insecticide sprays, and it is therefore not practicable, as a general rule, to save labour by using a combined spray. Such combined sprays might be used for early varieties or, as suggested by Vyvyan, with late oil or lime sprays in England to control red spider. They might thus be used for June drop in such combinations. In any event, pest control sprays often themselves promote fruit-drop, and it might be thought that the addition of a suitable concentration of auxin might counteract this effect. It is, therefore, of importance to know to what extent these hormones are compatible with the fungicide or insecticide preparations in common use.

Experiments by Hatcher at East Malling (Vyvyan, 1946) have shown that neither lime sulphur, Bordeaux mixture or lead arsenate had any effect on the hormone activity of NAA as measured by the *Avena* test. Experiments in the field have confirmed the complete compatibility of lead arsenate (Overholser *et al.*, 1943; Vyvyan and Barlow, 1948) and sulphur (Vyvyan and Barlow, 1948), but the situation with lime-containing mixtures is not so clear cut. The earlier workers (Gardner, March and Batjer, 1940) held that lime had no effect on hormone action in preventing drop, but subsequently a very serious reduction in effectiveness has been indicated for various lime compounds (Bordeaux mixture, lime-sulphur) with NAA (Kadow and Hopperstead, 1941) and for Bordeaux mixture with 2, 4-D (Harley, Moon and Regeimbal, 1948). The application of hormone sprays to trees with well-weathered lime-sulphur or Bordeaux mixture spray residues, results in a normal action on drop (Gardner, Marth and Batjer, 1940; Kadow and Hopperstead, 1941). This suggests that the inhibiting action of the fresh lime sprays on growth-substance action is due to the presence of excess lime which acts by maintaining alkaline conditions. There is, indeed, very considerable experimental evidence that the entry of the weak auxin acids into plant cells is favoured by acid and greatly retarded by alkaline conditions. There is even some suggestion

that acidification of pre-harvest hormone sprays with citric acid increases drop control (Gardner, Marth and Batjer, 1940). Summer oil is also compatible with NAA drop sprays, but there is no evidence that the increased drop caused by such oil sprays can be counteracted by the addition to them of NAA (Vyvyan and Barlow, 1948).

(g) *The Duration of the Hormone Effect*

The rapidity with which treatment may be expected to exert an effect on the tree, and the total period over which the effect of one spray may last, are obviously points of great importance in relation to the exact timing of the spraying. Unfortunately, the available data shows that varieties vary widely in these respects, and weather factors further complicate the picture. There are claims that an appreciable reduction in drop can be observed only a day after treatment, and others that recorded no effect until after a week. Usually, however, the maximum effect is reached at somewhat intermediate times (*e.g.* 5 to 6 days), which may be greatly modified by weather, particularly the temperature. This is illustrated by work on McIntosh in America, where high temperatures resulted in marked response in 1 to 2 days, whereas low temperatures delayed the effect for a further 3 days. Such effects, coupled with varietal differences, presumably account for the rather widely variable reports (Gardner, 1940).

Records for the total duration of effect show similar wide varietal and other differences. In the rather exceptional varieties, McIntosh and Wealthy (Southwick and Shaw, 1941), the effect lasts 8 or 9 days and then rapidly disappears. This period is somewhat longer under cooler conditions. In many other varieties the effect may persist for a very considerable period such as a month, and, indeed, fruit may remain on the tree when overripe and rot without falling off. This may happen with Beauty of Bath (Swarbrick, 1945 *a*). When such fruits are picked, the apple is most likely to part company with the stalk, leaving the latter still attached to the spur, no abscission layer having been formed. Most of the varieties show an intermediate duration of response and this too is somewhat modified by weather conditions, the full effects of

which have still to be worked out. Naturally, both these aspects of the response to hormones have an important bearing on the timing of the application, and it should occasion no surprise therefore, in view of the varietal differences observed, that such timing is still a subject for considerable discussion.

(h) *The Time of Application*

In view of the fact that the total duration of the effect is so uncertain in most varieties, it is obvious that the time of spraying must be chosen with great care. If treatment is too early, the effect may have passed off before the main pre-harvest drop has started. On the other hand, if application is made when dropping is well under way, it may have little result. In fact, more apples may be knocked off the tree by the spraying operations than are saved by the spray itself. There seems no doubt from accumulated results of ten years of research since the first reports appeared, that the early workers were right in claiming that "timing is probably the most important single factor in the success of harvest sprays" (Batjer, 1942).

This timing is not, however, a matter of choosing a prescribed date on the calendar for any particular variety, since, as the fruit-grower is only too well aware, the rates and times of maturation and ripening of the fruit will vary considerably from season to season. The experienced grower will therefore need to make a reasonably accurate estimate of the state of maturity of his crop, and base his spraying-time on this. Two main methods of timing have been employed. The first depends on a close watch being kept on falling fruit. When sound ripe fruit, free from any worm-holes or skin damage, first appear under the trees, spraying should be started immediately. Under some conditions, however (*e.g.* Victoria, Australia (Cole and McAlpin, 1941)), heavy drops may come suddenly and with little warning, and much loss may be sustained before the spray becomes effective. Here then the second method is advised, namely, to calculate as nearly as possible the date on which the drop is expected and arrange to spray at a prescribed interval before that date, the exact time depending on the duration of the effect on the variety

concerned. Vyvyan (1946) recommends this method for all those varieties that tend to show sudden drop just before harvest. There are, however, some in which dropping may continue over a long interval of time, and heavy drops may even occur several weeks before the optimum picking-time. In these varieties the period of effectiveness of the spray may well be exceeded by the long dropping period. Here it might seem profitable to spray more than once, at the first signs of dropping and later when the effects of the first spray are wearing off. The literature on the effects of such repeated spray applications has been thoroughly analysed by Vyvyan (1946). He has come to the conclusion that the available data do not allow it to be decided whether the treatment is advantageous or not. The main function of such sprays is, of course, to prolong the period of control, and it seems doubtful whether this has been generally achieved. There are strong indications that the duration of the effectiveness of subsequent sprays is very much shorter than that of the first spray, a second applied up to five days after the first giving protection for only an additional two days (Batjer and Marth, 1941). It is generally felt that repeated sprays are of little value provided that the first one has been correctly timed. Nevertheless, there does seem to be some hope of our extending the total period of effectiveness by using hormones other than NAA. There are signs from American work that this period is very much longer with 2, 4-D for the variety Winesap and Stayman's Winesap. Even greater hope comes from work on α-(2, 4, 5-trichlorophenoxy)-propionic acid, which, at 20 p.p.m. applied a fortnight before the expected commencement of pre-harvest drop, brought about an even greater reduction of that drop than NAA applied when dropping commenced (Edgerton and Hoffman, 1951) or two sprays of NAA timed to give the best control with that compound. With the variety Delicious, which we have seen is relatively insensitive to 2, 4-D, one spray with a 10 p.p.m. solution caused the fruit to "hold" for 47 days before harvesting (Thompson, 1951). If these results prove to be universally applicable, such compounds could be applied much earlier and the exact timing would not therefore be so critical.

(i) *The Effect of Weather Conditions*

There are two aspects of the relationships of weather to the effectiveness of hormone spraying, the first concerned with the conditions at the time of application and immediately following, and the second with conditions over the period from treatment up to harvest; the latter is of minor importance compared with the former. Thus the temperature during the control period seems to have little effect on the intensity of spray effects, but may influence its duration only. It should be noted that high temperatures increase the rate of normal dropping. One point of interest concerning the weather during this control period is the considerable protection that is afforded against falls in high wind. Both in Europe (v. Stuivenberg, 1941; Vyvyan, 1946) and in America (Gardner, 1940) heavy winds after spraying removed much less fruit from treated than from control trees. In those varieties where such windfall prevention was not particularly marked, viz., Edward VII in England (Berry and Swarbrick, 1942) and McIntosh in America (Hilton, 1944), the variety is, in any event, relatively insensitive to the hormone sprays.

On the other hand, weather conditions at the time of spray application and just after may have a great effect on the subsequent response. Temperature is a most important factor, warm weather facilitating a much greater response than cool. To obtain the same response in cool weather as in warm, much higher concentrations may have to be applied. These effects may be seen even in treatments carried out at different times of the day, the warmer midday conditions evoking a much better response than the cooler early mornings or late afternoons (Batjer, 1942 *a*). This better response is probably due to a more rapid absorption of the hormone at the higher temperature, in spite of the fact that the spray dries much more quickly. Too rapid evaporation in *very* hot dry weather may reduce the absorption and account for the much smaller response. But although the response may be smaller, even low temperatures do not stop it completely, and good control has been reported for applications made during a period when day temperatures did not rise above 40° F., and night temperatures were well

below freezing (Roberts and Struckmeyer, 1943). A very adverse weather factor is rain immediately following the spray application, when much of the hormone may be washed off the tree before it has had time to penetrate. Direct experiments in America with the variety Delicious, in which artificial rain was applied to trees at set intervals after the spray application, indicate that the first two hours after treatment are critical and that rain after this period has little effect (Overholser *et al.*, 1943). This shows that, in this variety, two hours may have been sufficient for the amount of hormone requisite for full control to have been absorbed by the tree. Similar evidence for the effects of rain has been obtained for the apple variety Sunset, in England (Barlow, 1951).

(j) *Varietal Differences in Response*

One of the most obvious features of all the investigations which have dealt with more than one variety is that there are consistent and marked differences in their responses. In America, Williams' Early Red and Delicious, and in England Beauty of Bath and Worcester Pearmain, always give a substantial reduction in drop when treated. On the other hand, Grimes Golden in America, and Bramley's Seedling in England, show very little or no response. A table, condensed mainly from Vyvyan's comprehensive survey (1946), and showing the average behaviour, under normal conditions, of thirty-three American and European varieties, illustrates this range of response.

The causes underlying these varietal differences are partly morphological, *i.e.*, due to the structure of the fruit itself and the ease with which its stalk can be hit by the spray, and partly physiological, *i.e.*, connected with the intrinsic reactivity of the fruit to the hormone. The two sensitive American varieties mentioned above are both characterized by having long fruit-stalks so that the fruits are pendulous. The stalks are, therefore, easily hit by the spray, any slight excess of which, instead of falling to the ground, may collect in the cavity where the stalk joins the apple, and so extend the period of absorption. The universal sensitivity of pear varieties could easily be due to the accessibility of their stalks to the spray. The insensitive and

intractable varieties of apple, such as McIntosh and York Imperial in America, have short stable stalks that hold the fruit rigid in an inclined position. Not only is the short stalk correspondingly difficult to hit with the spray, but excess solution will not be retained in the stalk cavity as in the pendulous varieties. In the insensitive variety (Bramley's Seedling in England) another factor, *i.e.*, more than one fruit per spur, provides an added impediment to the penetration of hormone to the stalk. Usually two fruits are so closely pressed together at the stalk end that access to the stalks is virtually cut off. A correlated effect of clustering of fruit on single spurs is that during growth they may push one another off. Since this is a purely mechanical effect and not associated with the function of a separation layer in the stalk, hormones will not prevent it.

From the physiological aspect it is noteworthy that early maturing varieties of fruit give a much better response than late varieties (Blair, 1940; Batjer, 1942; Southwick and Shaw, 1941), although there are exceptions, *e.g.* Delicious in America. This correlation might be explained by the fact that spray is applied to the early varieties at a time when the weather is still warm, and either absorption is correspondingly faster or the stalk tissues more active physiologically: the fruit reacts, therefore, more strongly to the applied hormone.

(k) *Effects of Spraying on Fruit Quality*

In addition to the total quantity of sound, marketable fruit that we can save by means of suitably timed hormone sprays, the fruit-grower is very much concerned with the overall quality of his crop, and it is thus germane at this point to consider the several possible effects on it of hormone fruit-drop sprays. The importance of any one aspect of fruit quality will vary from variety to variety. In those destined for immediate sale, colour, size, flavour, etc. will be of outstanding importance, whereas, with a keeping variety, storage properties and resistance to mechanical damage in transport will be of major concern to the grower.

Hormone sprays can affect these various aspects of fruit quality either directly or indirectly, and in practice it is often very difficult to disentangle the two types of effect. This is due

to the fact that fruits retained as a result of treatment may have completed their development under conditions quite different from those of normally retained apples. Thus the apples in the heavier crop, which have been treated, will have to compete more actively with each other for available food materials and this may restrict growth. However, in making comparisons, there is some danger that apparent effects may appear as a result of selective dropping and give a distorted picture. If, as is usually the case, the largest apples are the ones to drop, it might seem that spraying had increased the size of the fruit. Similarly, since the ripest fruits tend to drop first, spraying might give the appearance of having hastened the ripening of the fruit.

The most important indirect effects that have been recorded are those due to postponement of picking. These will be considered shortly. When such effects are eliminated by picking at normal harvest-time, then the majority of the available evidence points to a complete absence of any direct effects. In America (Overholser *et al.*, 1942; Haller, 1943), in England (Vyvyan, 1949) and in Holland (v. Stuivenberg, 1941), no significant changes could be observed in the maturity of a wide range of varieties, and their storage qualities (resistance to development of decay, breakdown, scald or lenticel spot) were in no way impaired. One interesting experiment was carried out on McIntosh in America (Christopher and Pieniazek, 1943) in which the stalks of unsprayed fruit were bound with adhesive tape to prevent drop and these apples were then compared, after normal harvesting, with apples retained by spraying. Both types of apple had had the same period of development on the tree and any differences in quality might have been due to the direct action of the growth substance. No such differences were observed.

Sprays of a higher concentration than normally used for drop control, *i.e.* 50 to 200 p.p.m., may, however, cause an improvement of colour and a direct acceleration of ripening that is quite marked in fruit picked at normal harvest-time. This was first shown in Holland (v. Stuivenberg, 1942) for the varieties Sterappel and Yellow Transparent, the latter becoming fully ripe long before reaching full size and some individuals

actually burst. Results of a similar nature have been recorded in America (Gardner, 1940) and England (Vyvyan, 1942). This effect of concentrated hormone solution has been exploited recently for inducing a more rapid and uniform ripening of stored fruits. This aspect of the use of hormones will be considered later (Chapter 11).

One of the great advantages of hormone control of drop is that fruit can be left on the tree for much longer periods, even after normal harvest-time, without considerable loss by dropping. This results, in many varieties, in a larger and more mature fruit with a corresponding improvement in colour and eating qualities. For example, the English variety Worcester Pearmain is relatively immature and still growing rapidly at its commercial gathering date, whereas Bramley is usually fully mature (Archbold, 1932). In America, one set of measurements on McIntosh showed a 40 per cent. increase in volume during this extra period on the tree (Southwick, 1940). The great advantage of increased maturity of the whole crop brought about by delayed picking is that a much higher percentage of the crop can be placed in the top marketing grades. This means not only an increase in the total crop marketed, but a higher price for each bushel. An added advantage is that the picking period can be greatly extended, which is of particular value to the grower who has a large acreage all of one particular variety (*e.g.*, Worcester Pearmain). The period of delay will, of course, vary considerably with the variety. In the English varieties, Beauty of Bath and Worcester Permain, a delay period of 5 and 14 days respectively has resulted in all fruit, not damaged in picking, being of first "tray" quality (Swarbrick, 1945 *a*). Warnings of the dangers of over-maturity, and its attendant fruit disorders resulting from too long a delay, should be given at this point. The usual test of fitness for picking, *i.e.*, seeing whether the fruit comes away from the spur when it is given a slight twist, may not be applicable here, since the hormone treatment will have inhibited the formation of the separation layer. This danger of overripeness is more acute in spells of warm weather. In those varieties that are destined for storage, a too advanced state of maturity is to be avoided, as keeping qualities may be impaired. But even here

some delay would seem to be an advantage, since several reports have shown that in the varieties McIntosh, Worcester Pearmain and Cox's Orange Pippin, mature fruits picked after a delay of this kind showed keeping qualities which were equal to those of fruits gathered at the usual harvest-time. In addition, maturity at picking means that the fruit is perfect for eating at any time during its storage life and, as was shown for Cox's Orange Pippin, of better quality than the fruit picked normally (Vyvyan, West and Barlow, 1949). Most pears, however, suffer considerably if picking is delayed, due presumably to the much greater speed with which the maturation and ripening cycle takes place in that fruit.

In the varieties so far considered, the whole crop matures at the same rate and can therefore be harvested at one picking when in an optimum condition. There are, however, some varieties (*e.g.* Williams' Early Red, Early McIntosh) in which the fruits ripen individually over a considerable period of time. Since each fruit is in perfect condition for a few days only, this means a succession of pickings at regular time-intervals. Although hormone sprays will prevent fall of this ripe fruit, gathering cannot be delayed for long, and successive pickings are still necessary. Treatment may still be of advantage notwithstanding, since it could reduce the number of immature fruits knocked off the tree during the early pickings.

(l) *June Drop*

The early American workers (Gardner *et al.*, 1939) failed to effect any appreciable control of this early immature fruit-drop and little attention has subsequently been paid to it, as the thinning it produces is often of value (see page 249). It has been pointed out (Vyvyan, 1949) that early thinning could be carried out with greater confidence and precision if this subsequent drop could be eliminated. But it seems that the abscission phenomena which give rise to June drop are closely associated with cell divisions in the abscission layer and not with simple changes in the cell-walls. It has been suggested that this type of abscission is not affected by auxins (Lewis, 1946). In spite of this pessimistic forecast, experiments were carried out on Cox's Orange Pippin at East Malling

using 10 p.p.m. NAA alone, and in mixtures with routine lead arsenate and dispersable sulphur sprays (Vyvyan and Barlow, 1947 and 1948). In two very different seasons considerable control was obtained, whether the hormone was used alone or with the routine spray. The effect in some experiments persisted for a period of several months. 2, 4-D at 10 p.p.m. gave no control by itself, but did not affect the control of NAA when mixed with it. So far, worthwhile control has not been reported for any other varieties. It should be noted that very early sprays, applied for example in the second week in May, may actually increase the rate of dropping (Vyvyan and Trowell, 1951).

*The Control of Drop in Other Fruit*

(a) *Pears.*—It has already been noted that the majority of pear varieties so far studied show consistently good response to hormone sprays of those concentrations effective for apples. This includes Bartlett pears in America, Conference pears in England, Bon Chretien, a heavy dropper, in New South Wales, and Comtesse and St Rémy in Holland. These sprays have no direct effect on the quality or storage properties of the fruit, and in Conference pears picking may even be delayed for a week after the normal date without impairing storage quality. The eating quality of the pear is thereby improved (Vyvyan, West and Barlow, 1948). The danger of damage from over-ripeness due to delayed picking is, however, very much greater in pears than in apples.

(b) *Stone Fruit.*—This type of fruit seems to be relatively insensitive to hormone treatment. Slight reductions in drop have been observed in plum and apricot but are not sufficiently marked to make spraying an economic proposition. No response has yet been evoked in peaches. In cherry, while almost complete control has been reported by the use of ·01 per cent. NAA, its use is not recommended, since the cherries subsequently remain too firmly attached to the tree for convenient picking, and small red fruit, normally destined to drop early, remain on the tree to lower the quality of the gathered crop (Wurgler, 1947).

(c) *Citrus Fruits.*—Considerable success has been achieved

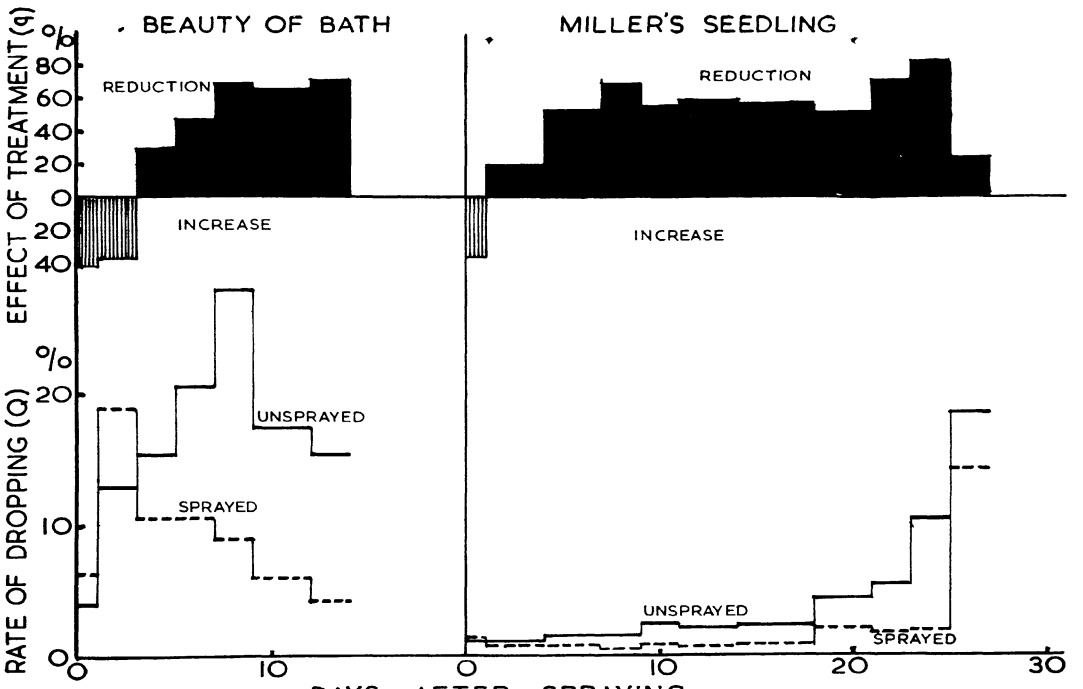



FIG. 40.—Graph showing the effect of auxin sprays on subsequent pre-harvest drop in Beauty of Bath and Miller's Seedling apples. The bottom graphs show the rate of dropping (Vyvyan's index Q) in sprayed and unsprayed trees. The top graphs show the effects of treatment (q). The black areas above the lines show a positive effect (*i.e.* reduction in dropping rate.)



FIG. 41.—Effects of spraying Beauty of Bath apple trees with 10 p.p.m. NAA on pre-harvest fruit drop. The trees were sprayed on 20th July 1951 and photographed on 9th August. Starting from the left, the first and third trees were sprayed while the second and fourth were untreated.

*Photograph by G. F. Trowell by permission of East Malling Research Station.*

by the use of 2, 4-D sprays in both orange and grapefruit. In California, an 8 p.p.m. spray gave a 30 to 60 per cent. reduction of drop in Washington and Valencia navel oranges (Stewart, Klotz and Hield, 1947). Higher concentrations of 25 p.p.m. gave almost complete control but induced leaf distortions. It was found that, to be effective, sprays had to wet the calyx. Applied to young fruit, however, they may also produce serious fruit deformation such as thick warty rind and large protruding navels (Stewart and Klotz, 1947). As would be expected, the degree of control varies considerably with season and the age and nutritional status of the tree (Stewart, Klotz and Hield, 1947 *a*). A remarkable characteristic of this 2, 4-D treatment is the duration of effect, which can last from an application date in October to the date of harvesting in May. Low volume "fog" applications from helicopters have proved most successful, but dusts are not very effective (Stewart, Klotz and Hield, 1951). Similar control is reported for grapefruit in California, where 5 to 25 p.p.m. were most effective (Stewart and Parker, 1947), and in Victoria, Australia, where 22 p.p.m. of the sodium salt were used with summer oil as a spreader (McAlpin and Merrett, 1949). In Florida, on the other hand, results have not been so satisfactory and this indicates that such practical details as hormone concentrations, times of application, etc., may have to be worked out separately for each citrus-growing region (Gardner, 1951). More work needs to be done before any definite recommendations can be made.

(d) *Decorative Evergreens.*—Dipping cut branches of decorative evergreens such as holly and spindle tree (*Euonymus* species) into hormone solutions, to prevent leaf and berry abscission during transit and use, has been reported as giving considerable success (Avery and Johnson, 1947). The whole branch is dipped into a NAA solution of 20 to 30 p.p.m., and the effects of such treatment lasts from 10 to 14 days.

(e) *Cotton.*—Cotton is a plant which does not normally suffer from fruit-drop and is moreover a very prolific cropper. The fine, white, cellulose hairs surrounding the seeds in the seed-pod (boll) and forming the cotton of commerce can amount to 60 per cent. of the final dry weight of the plant.

No advantage can be gained by improving fruit-set here, since more and smaller bolls might result in a smaller total crop. Nevertheless, some good hybrids fruit poorly, and artificial setting by hormones might be an advantage. Applications of 0·1 per cent. NAA and 0·01 per cent. of the sodium salt of 4-CPA have been used without success in an attempt to reduce boll-shedding (Eaton, 1950). Earlier, weekly sprays of 20 p.p.m. effectively reduced the number of bolls per plant, illustrating the great sensitivity of this species, noted in Chapter 9.

*The Prevention of Blossom Drop*

Beans are prone, under adverse weather conditions or as a result of heavy insect attack, to shed their flowers and flower-buds prematurely, and this reduces the crop. Attempts have been made to prevent this by the application of hormone preparations. In North Wisconsin, experiments in the laboratory indicate that considerable increases in the crop may result from NAA applications at 40 to 80 p.p.m. (Fisher, 1946). Similar flower-drops in hot, dry weather gave rise to great difficulties in obtaining successful experimental crosses of lima bean varieties in Maryland (Wester and Marth, 1949). Spraying emasculated hand-pollinated flowers with 0·8 per cent. IBA and 0·2 per cent. 4-CPA in 3 per cent. glycerol produced no significant effect, but the application of a similar mixture in lanolin paste to a scratch made at the base of the pedicel resulted in a 2 to 3 days' delay in flower-drop and a correlated increase in the percentage of successful crosses (18·7 to 28·8 per cent.), and a 50 per cent. increase in the number of seeds per pod. Tomato flower-abscission can also be prevented by 25 p.p.m. NAA sprays (Gouwentak and Bing, 1948).

The abscission of individual flowers from inflorescences greatly limits the value of certain species as cut flowers. This is true of the lupin, but there is promise that this drop may be controlled at appropriate applications of auxins. Both IAA and NAA sprays in concentrations of 0·04 per cent. to 0·08 per cent., applied a few hours after cutting the inflorescence, have been shown to reduce the drop in the first three days to a mere fraction (about 10 to 15 per cent.) of the drop in untreated plants (Warne, 1947). In pot flowers, under some conditions,

the same excessive bud- and flower-drop may occur, *e.g.*, in *Begonias* in the winter. This also has been reduced by over a half by NAA spray applications at concentrations of 5 to 12½ p.p.m. No harmful effect has been noted on plant growth at this concentration. It is claimed that even open flowers last longer with this treatment (Wasscher, 1947).

CHAPTER 11

# MISCELLANEOUS APPLICATIONS OF AUXINS

BEFORE we leave the subject of the auxins and pass on to consider the other growth substances of the plant, there are a few miscellaneous effects of sufficient practical interest to warrant mention. These auxin actions are not related in any particular way, and this chapter is concerned merely with the tidying up of odds and ends. The physiological background of these applied aspects of hormone actions has not been investigated and it is not known whether these actions have any counterparts in the normal physiology of the several organs concerned.

## FRUIT RIPENING

We have already seen in the preceding chapter that the indirect effects of dilute hormone sprays on such fruits as apples and pears, arising from prolonged attachment to the tree, may be very considerable, but that no direct action on fruit quality could be detected. Concentrations higher than optimal (*e.g.* 50 p.p.m.) applied before harvest can subsequently give rise to an acceleration of colour formation and ripening in apples (see Vyvyan, 1946; Pearse, 1948). Attention has been given in the last few years to the possibility of storage ripening control by applying hormone preparations *after* harvest, either before or during storage. Attempts of this kind were first made in 1938 (Traub) with a range of subtropical fruits, including passion-fruit (*Passiflora* spp.), tree tomato (*Cyphomandra betacea*), orange (*Citrus sinensis*), lemon (*Citrus limonia*) and lime (*Citrus aurantifolia*). The fruits were immersed for a day in water solutions of the hormones IAA, IBA and NAA, or covered with a thin lanolin film containing those compounds. It was found that dilute solutions (10 to 100 p.p.m.) retarded ripening in all of the above, whereas higher concentrations (500 p.p.m. and over) accelerated it. The ripen-

ing effect of NAA was subsequently shown to occur in apples and pears in Holland (van Stuivenberg, 1946). The variety Sterappel ripened much more quickly at 5° C. if it were first dipped in 50 to 100 p.p.m. NAA solution. The pear (Beurre de Mérode) stored at 20° C. also responded in the same way, but when stored at 1° C. the acceleration of ripening depended on the time of plucking, the attainment of a certain stage of maturation on the tree apparently being necessary before the hormone could take effect.

With the advent of the substituted phenoxyacetic acids, 2, 4-D was investigated for its ripening effect in a variety of fruit (Mitchell and Marth, 1944). Both dipping into an aqueous solution containing carbowax as a spreader and treatment with an aerosol, accelerated the ripening of apples, pears and bananas: effective concentrations ranged from 100 to 500 p.p.m. One very great advantage of the treatment was that ripening set in much more uniformly throughout the treated sample. Such uniformity adds much to the market value of the fruit and avoids the additional expense of sorting which may be necessary in some species (*e.g.* pears). Peaches (Southwick, 1946) and figs (Stewart and Condit, 1949) also react in the same way to 2, 4-D applications, but no effect of this compound has so far been recorded on tomato, persimmon and pepper (Mitchell and Marth, 1944). During the accelerated ripening of bananas, starch was rapidly broken down and sugar accumulated. The above three insensitive species of fruit, however, have no starch reserve, and it has been suggested that this accounts for the lack of action of the growth substances (Mitchell and Marth, 1944). But starch breakdown is the result, not the cause of the protoplasmic ripening processes, presumably triggered off by the hormones, and the absence of response in these starch-free fruits probably has some more deeply seated origin. The homologous compound 2, 4, 5-T also accelerates ripening in apples (200 p.p.m.), peaches (25 to 75 p.p.m.) (Marth, Harley and Havis, 1950) and prunes (20 to 60 p.p.m.) (Zielinski, Marth and Prince, 1951). The lower concentrations (20 to 40 p.p.m.) applied to prunes produce sweeter fruit.

Another of the striking biochemical changes which takes

place during the ripening of many fruits is the production of the gas ethylene ($C_2H_4$). This is particularly marked in apples and pears. The synthetic gas has itself a remarkable ripening action on fruits of all kinds, and is widely used in the control of ripening of bananas, apples and citrus fruits in various parts of the world, a concentration of 0·1 per cent. in air being applied in sealed storage spaces. This is not, however, the place for a discussion of this very wide subject, and an excellent and pertinent résumé will be found elsewhere (Marth and Mitchell, 1947). 2, 4-D applied as a solution in 1 per cent. carbowax has been shown to stimulate by 30 per cent. the respiration rate of Bartlett pears and to increase the ethylene production to 3½ times (Hansen, 1946). Although, as in the case of starch breakdown, this increased production may be only the result of ripening processes, it does raise the possibility that hormones may in some way trigger off the production of ethylene, which is the immediate ripening stimulant. An intimate connection in the plant between ethylene action and the auxin system has often been suggested (see Borgström, 1939). In lemons, however, in contrast to their behaviour to applied ethylene, both 2, 4-D and 2, 4, 5-T, applied as wax-ester emulsions at 500 p.p.m. and 100 p.p.m. respectively, do not accelerate but retard yellowing, and might thereby be used to increase the storage life of these fruits (Stewart and Palmer, 1950). This slowing down of the "physiological ageing" resulted in reduction in rot due to the fungus *Alternaria* sp. and reduced the condition known as "black buttons". Decay has also been reduced in short-period post-harvest storage of limes in Florida by dipping in concentrated (100 to 500 p.p.m.) 2, 4-D solution (Gates, 1949). This retardation of physiological ageing also occurs when citrus fruits are sprayed on the tree with low concentrations of 2, 4-D (10 to 40 p.p.m.) during growth. Correlated with this is an accelerated growth-rate resulting in larger harvested fruits (Stewart and Hield, 1950) in Valencia oranges, and grapefruit in California. Sprays of increasing strengths have been recommended to be given at monthly intervals during the growth of the fruits, starting with 16 p.p.m. and rising to 400 p.p.m., since the fruit becomes less sensitive to the auxin as it grows. No adverse effects on fruit

quality have been noted at these levels, but at the higher concentrations distortions of shape and development of a thick rough rind with enlarged oil glands may result. A gain of 8 boxes of marketable fruit per 100 trees is claimed.

It would seem that the auxin-type hormones have a rather more restricted action than ethylene in the ripening of fruit, but their use is becoming more widespread in those fruits which are sensitive to them. One of their chief advantages is the relative ease of application and their chemical inertness compared with ethylene, which is an inflammable gas.

## THE INITIATION OF FLOWERING

We are all familiar with the fact that there is a definite rhythm of growth and development in the plant. A succession of distinct phases follow one another in a definite order, the embryonic dormant phase in the seed, the vigorous growth phase after germination resulting in the rapid production of stems, roots and leaves—the vegetative phase as it is called—and then the phase of flower production culminating in the production of seed. In a perennial plant, such as a tree, vegetative and reproductive phases alternate in a regular annual cycle. In the next chapter we shall consider in more detail the various factors, both intrinsic and environmental, that determine and modify the characteristics of this rhythm and the possible part played by hormones in the phenomena. Data are rapidly accumulating to indicate that the auxins themselves may be a component in the complex of factors determining this "phasic development", and particularly the initiation of the flowering phase. We shall not deal here, however, with this aspect of auxin action, but shall leave it for detailed consideration in the next chapter when flowering phenomena have been discussed.

## PROLONGING OF FLOWER BLOOMING

Various synthetic auxins (NAA, NoXA, 4-CPA) in low concentrations (5 to 80 p.p.m.) have been used as sprays in an attempt to prolong the blooming of ornamental plants under conditions (*i.e.* abnormally warm spring weather) which cause

rapid blossom fall. Some success has been claimed for oriental cherries (*Prunus serratula*) with NAA, where 25 to 80 per cent. of the blossom was retained for 3 to 10 days longer, and for dogwood (*Cornus florida*) with 4-CPA, where an extension of 4 to 6 days was obtained (Wester and Marth, 1950). A number of other species including *Azalea*, flowering almond, lilac and *Magnolia*, were unresponsive.

### USE IN BLOSSOM THINNING

We have seen in Chapter 10 that the apple is prone to bear fruit with a biennial rhythm of alternating heavy and light cropping years. This rhythm, once established, tends to continue and is economically very undesirable. It can be prevented if crop size is carefully regulated by early thinning operations, and for this reason natural thinning by early and June drops is regarded as not entirely undesirable. Hand thinning, however, can be a laborious and costly operation, and in recent years the possibility of thinning by the application of toxic chemicals has been subjected to considerable experimental investigation. Most of the compounds which have been employed are synthetic organic chemicals bearing no relationship whatever to the plant hormones. Some of the most successful have been 2, 4-dinitro*ortho*cresol, 2, 4-dinitro-*ortho*cyclohexylphenol and various tar-oil distillates; these are applied at concentrations of the order of $\frac{1}{2}$ per cent. and result in a killing of a suitable percentage of the flowers. It has been found that the time of application is very critical, unopened and fertilized flowers not being killed by the treatment. Maximum sensitivity seems to occur at full bloom, and the success of the sprays may depend on gauging the time of application to a single day. Such timing should aim at a stage of development in which one flower per spur is fertilized and is therefore insensitive, while the remaining flowers are just opening and are therefore easily killed by the correct concentration of toxic compound. Naturally there are very wide varietal differences in response, but it is not intended to go into further details here, as these wholly lethal compounds could hardly be classed as plant growth substances. The interested

reader is referred to a comprehensive review in the book by Avery and Johnson (1947).

All these compounds cause leaf injury which may be severe in some species, and for this reason certain synthetic auxins, which have a similar blossom-thinning action but no associated effects on leaves, are tending to replace these contact poisons.

This thinning action of synthetic auxins was first discovered during attempts to increase apple-set in "off" years (Schneider and Enzie, 1943) in order to break the biennial-bearing rhythm. Instead, the set was reduced to *nil* in these "off" years by spray concentrations of ·01 and ·03 per cent. of NAA applied at full bloom. Such high concentrations caused considerable injury to the tree, and later concentrations of a tenth these values were shown to be effective. 2, 4-D has also the same action. From subsequent work on apples and peaches it has been shown that results are extremely variable. Some varieties are very sensitive, and almost complete thinning may be caused by applications even after petal fall (*e.g.* McIntosh and Baldwin). Other varieties are correspondingly resistant, and only moderate blossom-kill is obtained even at full bloom (*e.g.* Ontario Red Astrakan, Gravenstein). But even these wide differences may be masked by other factors, among which the general vigour of the tree is very important. Thus a much smaller response can be expected from vigorous than from weak fruiting spurs. The full details of species response and the effect of environmental and other conditions have still to be worked out. It is probable from the results so far reported that specific procedures may have to be determined for each species in order to obtain the best results.

It has been suggested that one way in which the hormones such as NAA could bring about ultimate thinning is by delaying the normal dropping of very young fruit (Struckmeyer and Roberts, 1950). Such a delay results subsequently in a more intensive competition between the fruits for available nutrients and a much heavier resultant June drop. This needs further investigation, but it is of interest in this connection that no thinning can be obtained if treatment is delayed until after June drop (Southwick and Weeks, 1950).

### BOLTING OF VEGETABLES

The ammonium salt of 2, 4-D, applied as a dilute solution spray at 20 p.p.m., has been used in America in an attempt to facilitate the release of the flowering stalk from certain lettuce varieties with tight compact hearts by causing outward curvature of the leaves. The loosening of the leaves of these normally non-bolting varieties is very important if we are to get unimpeded growth of the inflorescence and consequently adequate seed production (Franklin, 1948). Normally this is done by making a vertical cut in the head so that the floral axis can grow out from the heart. It was found that a solution spray of 20 p.p.m. 2, 4-D (ammonium salt) caused the necessary response but at the same time checked the growth of the floral axis. It is hoped that further work may disclose a compound that can induce the necessary curvature of the leaves without preventing the growth of the seed-stalk. Auxins have, however, been used successfully in preventing the bolting of celery, but this will be considered more appropriately in the next chapter.

### CROTCH-ANGLE STRENGTHENING IN FRUIT TREES

Fruit-growers over the centuries have observed that, as a general rule, lateral branches of trees that grow out at a small angle to the trunk are structurally much weaker than those growing at a large angle or perpendicularly to it. This is apparently due to the inclusion of a pocket of bark in the narrow angle of the crotch and the consequent failure of the contiguous wood tissue of the branch and trunk to unite and form a strong junction. Attempts have been made in America to influence the angle of development of branches in young apple trees by the application of hormones (Verner, 1938). Auxin (IBA) was applied in lanolin paste (5 to 25 mg. per 100 gm. lanolin) in a variety of ways to the young developing side branches, but the most effective method was found to be to cut the tree back to about 2 feet 6 inches and to apply the auxin paste to the cut apex. This was found to induce the developing lateral branches to grow at a much wider angle to the main stem (average of 65°) as compared with untreated trees (average 48°). The writer is unaware of any subsequent work along these lines.

CHAPTER 12

# HORMONES AND REPRODUCTION

## THE FLOWERING PLANT

### *Introduction*

ONE of the most refreshing and heartening sights after the cold dreariness of winter is the appearance of the spring flowers in our gardens and hedgerows. What a glory are the russets and golds of the autumnal chrysanthemums that grace our flower-beds long after the earlier summer blooms have withered and fallen. This never-failing rhythm of reproduction, each species producing its flowers and seeds in its proper season, is as commonplace to us as our very breathing. But the miraculous way in which plants succeed in keeping to the seasonal time-table, in spite of wide fluctuations in prevailing weather from year to year, has been, until relatively recent times, one of the greatest enigmas of plant physiology. Why should the crocus be one of the first of our spring flowers, while the meadow-saffron (*Colchicum*), with a remarkably similar flowering habit, produces its flowers in the autumn? Why should annual plants be able to mature their flowers and fruit in the first season of their growth, whereas biennials fail to do so until their second summer? Why should spring varieties of cereals in temperate regions be able to bear seed in the same season after spring sowing, whereas winter varieties need to spend the winter in the ground before ears can be formed? Why does such a predominating number of tropical plants flower all the year round with no perceptible rhythm in their fecundity? These are the sort of puzzling questions that we are constantly meeting in connection with these apparently inexorable cycles of sexual reproduction in plants, but gradually, over the last few decades, we have been getting at the answers.

As a mass of data accumulates concerning plant behaviour in a vast variety of plants, it has become clear that it is the regular alteration of certain environmental seasonal factors which imposes on the plant this developmental rhythm of repro-

duction. Environmental stimuli, the nature of which we shall consider later, set in train a series of plant responses which determine whether the appropriate meristems shall produce flowers or vegetative branches. Stimuli and reactions are both complex, and there is much that is highly speculative in the explanations of our experimental results, but an imposing amount of evidence is piling up to suggest that the vital co-ordinating factors between stimulus and ultimate flowering reactions are hormonic in nature. Although these hormones have not yet been satisfactorily isolated in crystalline form and therefore occupy the same position of doubt as did the auxins before the advent of Went's *Avena* assay, yet their interest and implications for the future are so great that this chapter on them has been written. Although they are not auxins, there is no lack of pointers to indicate that the auxins themselves may play some rôle in the physiology of flowering, and this aspect, too, will be discussed. This chapter therefore will contain much of plant physiology and little reference to the class of tangible growth substances that might be got out of a bottle, but it is hoped that the practical man will not find it too academic to be of interest to him.

### *Factors controlling Flowering*

The idea of a flower-inducing substance is by no means new. It figured prominently in the theoretical discussions of the nineteenth-century pioneer, Sachs (1865), long before the word hormone had been coined. But, as we have seen for other aspects of growth and development in plants, Sachs's theories were not very seriously considered by those immediately following him, since, at the close of that century and in the first decade or so of the present one, the rapid advance of our knowledge in the purely nutritive aspects of plant physiology dominated contemporary ideas on growth control. Such an approach to the problem culminated, in 1918, in a theory in which the initiation of flowering was attributed to the attainment of a certain balance between the carbon and nitrogen nutrition of the plant (Kraus and Kraybill, 1918). The theory was based on the result of experiments carried out on the nutrition of tomatoes. An abundance of nitrogenous manures greatly

lowered the fruitfulness of these plants and, when coupled with conditions favouring active manufacture of carbon compounds in the leaves, gave rise to lush vegetative growth. If the supply of nitrogen were lowered while maintaining a high level of carbon nutrition, there was plentiful fruiting and reduced vegetative growth, while low levels of both carbon and nitrogen nutrition, as would be expected, greatly reduced both vegetative and reproductive growth. This concept of fertility control by nutritional balance rapidly "caught on" and became the fashionable theory. A considerable amount of analytical data accumulated on carbon–nitrogen relations in the constitution of vegetative and reproductive plants, but, as the pile grew higher, it became increasingly more obvious that plants would flower over a very wide range of carbon–nitrogen ratios, by which measure this balance was distinguished. This will occasion no surprise to the present-day student of plant growth, since these crude gross ratios of total carbon compounds to total nitrogen compounds can give little insight into the true physiological condition of the plant, delicately controlled as it is by vanishingly small quantities of specific growth substances.

It was, however, during the hey-day of this theory that a discovery was made that revolutionized our ideas on the physiology of flowering and has led us straight back to the old Sachs concept of flowering substances. Two botanists in America, W. W. Garner and H. A. Allard (1920), were trying to carry out crossing experiments on tobacco in the state of Washington. In contrast to the usual behaviour of tobacco, a new variety, Maryland Mammoth, refused to flower in the open in the Washington growing season and did so only in the greenhouse in the autumn and winter. Crossings could not therefore be carried out between this new variety and other varieties. These two botanists tried every trick that they could think of to make the plant flower in the summer, but without avail. One seasonal factor after another was tried, and then, with little hope of success, the last possible variable, length of day, was investigated. By artificial shortening of the day-length during the summer the new variety was brought into regular and prolific flowering.

This startling discovery naturally led to an outburst of research on the new phenomenon of *photoperiodism*, as the growth reaction of plants to day-length was christened. Such investigations, which have steadily increased in volume in the intervening period, have established that the flowering of a great proportion of plants is controlled by the length of day. Three classes of plants have been recognized on a basis of their photoperiodic behaviour. Firstly, there are those which resemble the Maryland Mammoth tobacco, in that they will come into the flowering state only when the day-length is decreased to a certain level. These plants have been called short-day plants and include many of our spring and autumn flowers such as *Chrysanthemum*, *Salvia*, *Cosmos*, golden rod, some species of *Primula*, etc. The second class comprises long-day plants which will flower only if a certain minimum day-length is attained or exceeded. This embraces all the summer flowering plants of the temperate regions of the earth, such as most of the temperate grasses, the spring varieties of cereals, beet, radish, lettuce, potato, etc. Remaining plants fall into the last or day-neutral class that can flower in any day-length and where flowering is determined by other climatic factors. Examples of these plants are tomato, dandelion, buckwheat and the considerable variety of tropical plants that have no flowering rhythm, but produce their flowers, as they do their leaves, all the year round. This classification of plants is in no way paralleled by the natural classification into families, and there is no telling what the behaviour of any one species will be from a knowledge of its nearest relatives. Even varieties of the same species, as we have seen in the tobacco, can show widely differing responses to day-length. What is, however, of considerable importance to the plant geographer is that the world distribution of plant species is largely determined by their photoperiodic response. In the tropics, where the day-length varies little from twelve hours throughout the year, short-day or day-neutral plants only will be able to reproduce sexually and survive. Similarly, long-day plants will be restricted to the regions nearer the poles, where long summer days allow flowering: such plants in the tropics will remain non-flowering indefinitely, as the visitor to the tropics

learns to his chagrin if he is misguided enough to try and grow temperate summer flowers there.

There seems little doubt now that for long-day plants it is long exposure to light that is the sole stimulus to flower, and that a period of darkness is not at all necessary. This is proved by the fact that long-day plants flower in continuous light and do so with the minimum delay. Dark does, however, enter the picture, in that it in some way counteracts the effect of the light stimulus and greatly postponesthe onset of flowering. This was shown in experiments on spinach and beet (see Gregory, 1948), which were grown in different lengths of day above the minimum required, and as the night length increased relative to the day, the time taken to flower also increased. Finally, at the critical day-length (13 hours day : 11 hours night) flowering is never attained. At this point the antagonistic effect of the increasing dark period completely neutralizes the stimulus of the day. Whether a long-day plant will flower or not depends on the resultant of these two opposing tendencies. When, however, we consider the short-day plant, we find that an uninterrupted dark period of a definite minimum duration is essential for flowering. The interruption of this dark period by a very short interval of weak illumination will, in some of the more sensitive plants, completely prevent flowering. Thus, in *Kalanchoë Blossfeldiana*, an ornamental succulent plant much used in this work, a 9-hour day and a 15-hour night induces flowering. Only one minute of weak artificial light (700-lux *) given midway through the night will reduce the number of flowers formed to less than 10 per cent., while a similar period of intense light (12–15,000 lux, equivalent to sunlight) stops it completely (Harder and Bode, 1943). The importance of the uninterrupted dark period had given rise to the opinion that short-day plants, as it were the complements of long-day plants, required only darkness to induce flowers, but that since growth could not take place indefinitely without the provision of raw materials by photosynthesis, a certain minimum day-length became indirectly essential (Lysenko, 1931, 1932). It has been shown, however, that this is very much an over-simplification

* The lux is a unit in which the visible light energy falling on an object is measured.

and that a light period of a certain minimum intensity and duration preceding the long dark period is an integral step in flower induction, quite apart from any photosynthetic effect. It must be pointed out before we go any further that this photoperiodic effect does not work through variations of food supply brought about by different periods of photosynthesis. Very weak light is effective in inducing these phenomena, and in experimental work may often be of an intensity in which a net synthesis of food is not achieved.

These very remarkable reactions of plants are already being used by horticulturalists in flower culture. We have seen their potential use in facilitating the crossing of varieties with widely spaced flowering times. Low-intensity supplementary illumination during the winter nights is being used in greenhouses to maintain *Begonias* (short-day plants) in a vigorous vegetative condition so that cuttings can be taken and grown continuously throughout the winter. Short periods of artificial illumination at midnight in the autumn are being used in *Chrysanthemum* cultivation to prevent flower development in the long autumn nights, and thereby spread the supply of blooms over a much longer period.

One of the most remarkable features of these phenomena is that a very few cycles of alternating day and night of optimum length can, in many plants, trigger off the flowering process, which will then continue whatever the day-length. This was first discovered in the short-day varieties of the soya bean (Garner and Allard, 1923), where exposure to ten short days was all that was required to cause flowering, which took place thereafter in long days. In some plants, e.g. *Xanthium* (cocklebur), it had been shown that only one cycle * can induce flowering, although it may be very much delayed. As the number of cycles is increased, so the time taken to flower is reduced.

Day-length, however, is not the only climatic factor that may affect flowering. The temperature during one or more of the developmental phases of the growth of the individual plant may determine the future flowering behaviour. The best-known example of this is in the spring and winter varieties

* A cycle is a term used for one light plus one dark period.

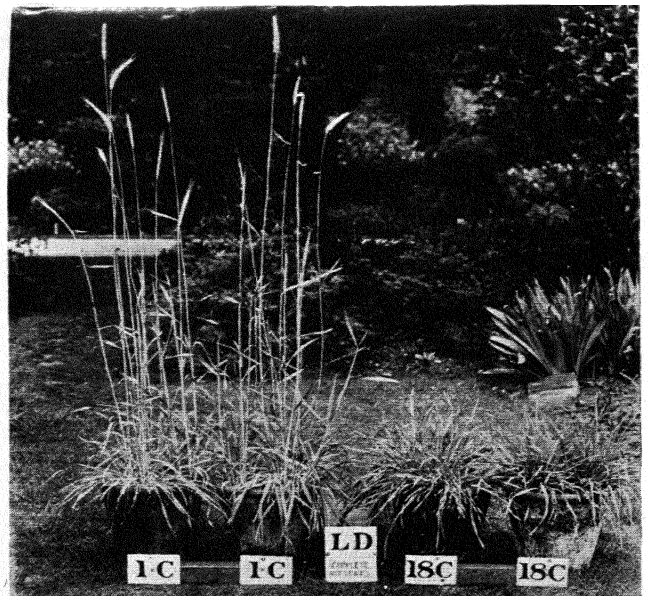



FIG. 42.—Petkus winter rye grown in long days with full nitrogen nutrition. The two plants on the left were grown from seed germinated at 1° C. for about 4 weeks, during which time they became vernalized. They have produced normal ears. The two plants on the right germinated at 18° C. (about 4 days) and were therefore not vernalized. They have produced no ears. Photographs were taken 11 weeks after planting.

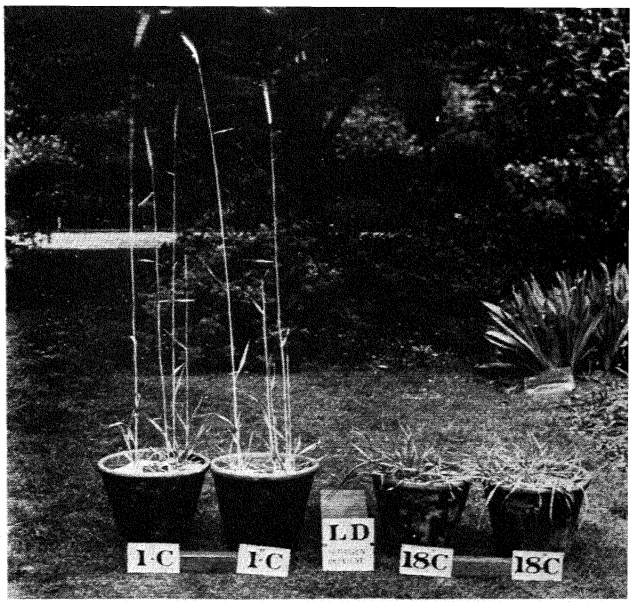



FIG. 43.—Petkus winter rye grown in long days on a low nitrogen diet. Other treatments as in Fig. 42. Note that the flowering behaviour is the same as in the plants with full nitrogen nutrition but that the general vigour of the plants is much lower.

*From Purvis, O. N.* (1934); *Ann. Bot. (Lond.)*, **48** (192), 919–955.




FIG. 44.—Petkus winter rye grown in short days with full nitrogen nutrition. Here, too, the left-hand plants were vernalized and the right-hand ones were not. Neither treatment has produced ears since short days has prevented shooting.

*From Purvis, O. N.* (1934); *Ann. Bot. (Lond.)*, **48 (192)**, 919–955.

of temperate region cereals such as wheat and rye. Spring varieties of these plants, if sown in the spring, will come into ear in the summer of the same year, while winter varieties will not. These latter need to be sown in the autumn and so spend the winter in the soil before ears will mature. If sown in the spring, they will not ear until the following season, *i.e.*, they behave as biennials. This phenomenon has been known for a very long time, and almost a hundred years ago an American agriculturalist described an empirical temperature treatment for winter grain that would make it behave as spring grain (Klippart, 1857). This consisted of allowing the wheat to germinate slightly in the autumn or winter, while keeping it from vegetating by a low temperature or freezing, until it could be sown in the spring. This new "trick" did not, for some reason, receive much attention from the scientific or practical agricultural world until the year 1928, when it was rediscovered in Russia by the now well-known agronomist Lysenko, who worked out a practical technique for the Russian farmer and called it *jarovizacija*, the English translation of which is vernalization. His technique for bestowing on winter wheat the flowering properties of spring wheat was virtually the same as that of Klippart. Thus the cereal was heaped on the concrete floor of the barn and soaked in water till it had absorbed about one and a half times its original weight of it. This is sufficient to cause the grain to germinate, and when roots just begin to form, the grain is cooled by opening the barn door and allowing cold air to enter. Thence for the period of treatment, the length of which is determined by the type of seed, the grain is periodically turned to ensure good aeration and prevent heating, and the water content is kept up to the optimum value above. If this treatment is long enough (*i.e.* about three months), then the grain can be dried and stored at a higher temperature without losing its acquired spring-flowering characteristics. This technique was widely adopted in the U.S.S.R., where it was used to ensure early crops in regions where growing periods were curtailed by early frosts or droughts, or even to obtain two crops per season on the same land. Needless to say, the phenomenon of vernalization has received much attention since, and other crops of agricultural

importance have been shown to have their flowering behaviour modified by temperature treatment in the early growing stages. Mustard grown in India will flower much earlier after cold treatment during germination. In rice, on the other hand, in the tropics, the same effect is brought about by high-temperature treatment. A more complicated state of affairs has been shown to exist in the temperature requirements for flower development in bulbous plants, mainly as the outcome of systematic research on tulips and hyacinths in Holland. One brief example will illustrate the complexity of the flower-forming process. The tulip at the time of lifting is ready to initiate the flower in the centre of the thick fleshy scale-leaves of the bulb. This initiation takes place most rapidly at 20° C. and is complete in about 2 to 3 weeks. For the development of this rudimentary flower to the state when it is ready to come above ground, temperatures of 8° to 9° C. are optimum, and this period lasts 13 to 14 weeks. When elongation of the flower-stalk actually begins, the optimum temperature slowly rises, reaching a maximum of about 23° C. at full bloom.

A close relationship between vernalization and photoperiodic phenomena is now reasonably well established. The most important work done on rye by Gregory and Purvis at Imperial College, London (see Gregory, 1948; Murneek and Whyte, 1948), has shown up the most complicated interactions between day-length and low-temperature vernalization in the initiation and maturation of the ears. These investigations have involved the painstaking dissection of the growing-points of many thousands of plants in every stage of development and have demonstrated unequivocally that the various discrete phases of ear development (*e.g.* initiation of young flowers, maturation and emergence from sheath) may each be separately determined by temperature or light treatment.

It was shown that the vernalization of winter rye by low temperatures was not an essential step for ear development, since such plants, grown through the winter in a warm greenhouse, matured normal ears in the following summer. This suggested that the internal changes brought about in the partly germinated seed under the cold winter soil could be induced also by the short days of winter in the growing plant. Un-




FIG. 45.—Interaction of day-length and low temperature vernalization in *Chrysanthemum rubellum*. *Left to right*: Unvernalized plant grown in short days, unvernalized plant grown in long days, vernalized plant grown in long days and vernalized plant grown in short days. Only the latter plant has flowered, showing that short days will not bring about flowering in this species unless flowering meristems have been induced by low temperatures.

*From Schwabe, W. W., J. exp. Bot.*, **1** (3), 329, 1950.

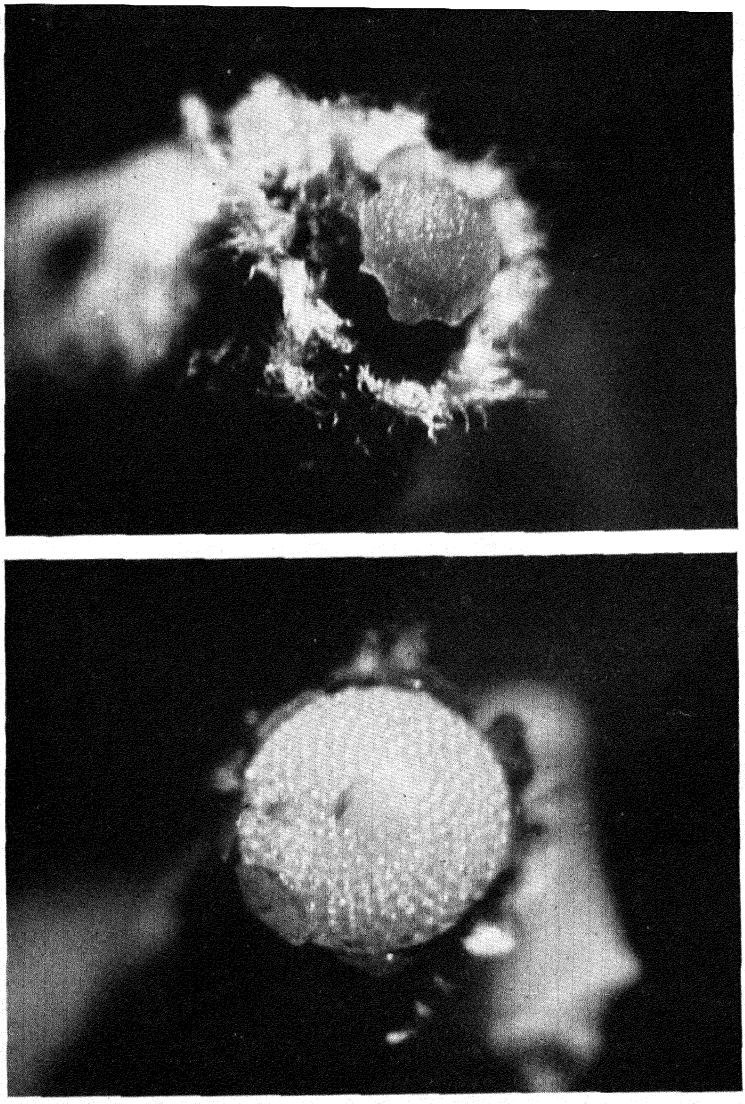


FIG. 46.—Inflorescence buds of *Chrysanthemum* dissected at a time when they first become visible to the naked eye.

*Top.* Bud from a vernalized plant in long days showing that, although the meristem is fully formed, the receptacle remains smooth and bare with no sign of flower initials.

*Bottom.* Bud from a comparable plant which has been vernalized and grown in short days where the initiation of the individual flowers on the receptacle is almost complete.

*From Schwabe, W. W., J. exp. Bot.*, 2 (5), 145, 1951.

vernalized winter rye sown in spring will, however, eventually initiate flowers, but the ears, which are produced late in the summer, never emerge but die within their sheaths. This failure to emerge is due to the effects of short days which stop "shooting" or elongation of the ear-stalk; this elongation is promoted by long days. In spring rye and low-temperature vernalized winter rye, in which the ears are initiated early in the season, the long summer days bring about emergence. A period of short days, however, will speed up the initiation of ears in non-vernalized winter varieties, and if long days are subsequently given, such varieties may be brought to ear in the first season. Some of the results of the early experimental work on rye can be seen in the photographs of Figs. 42 to 44.

This work, of which only the most sketchy outline has been given, indicates that both these external factors, low temperature and day-length, are exerting their influences on flowering via the same system in the plant. Furthermore, many of the characteristic features of the phenomena outlined in the preceding paragraphs suggest that this system is essentially hormonic in nature. In the first place, many unsuccessful attempts have been made to demonstrate consistent biochemical changes in the balance of protoplasmic components such as proteins, mobile food materials (sugars and amino acids), enzymes, etc. The induced changes must therefore be more subtle, *e.g.* the production of small quantities of a specific chemical substance not yet detectable by chemical means. Again, cold temperature vernalization can be reversed, *i.e.* its effects on flowering destroyed, by subsequent early exposure to high temperatures. This suggests that a substance formed in the cold and responsible for flower initiation is destroyed at the higher temperatures. Similarly, photoperiodic phenomena are best explained in terms of the production or non-production of flower-inducing substances under the various day-length conditions. A number of such hormone theories have been advanced to fit the known facts. The one with the widest application to the whole range of phenomena is due to Gregory (1948), who proposes that the ultimate flowering hormone is formed in the meristems from precursors carried

there from the leaves. In long-day plants the immediate precursor is a hypothetical substance A, formed only in the light in the leaves and converted in the night to some other inactive product, so stopping further export to the meristems. Only when sufficient length of day is given to such plants can enough A pass in the day to the meristems to be converted to the flowering hormone and induce flowering. In short-day plants the same precursor A is formed in the light but cannot be directly converted to the flowering hormone. Instead, an intermediate precursor B is first formed from A, but *only in the dark*. During the night period, then, this intermediate is carried to the meristems where it is converted to the flowering hormone. In short-day plants, therefore, a minimum period of darkness is necessary to allow sufficient B to pass from the leaves to the meristems to be changed into the flowering hormone, since B is immediately destroyed when the leaves are illuminated and its further transport thereby prevented. Needless to say, this is not the only scheme which has been put forward, but this is not the place for discussing the merits of conflicting hypotheses. The interested reader will find a further elementary discussion of these theories in a recent review by Heath (1949), while a full review of this aspect of the subject is given by Gregory himself (1948). What is of importance, however, is the experimental evidence we have to support the hormone theories.

The first basic fact is that the green leaves are definitely the organs which perceive the photoperiodic stimulus. One of the first to demonstrate this was the Russian Chailachjan (1936) using *Chrysanthemum* as experimental material. A number of similar plants with a terminal bushy habit was taken, and all the leaves were removed from the upper half of the plants. These plants were now divided into four sets. The first set was left to grow in the long summer days, with the result that no flowers were formed at the top of the upper leafless branches. In the second set the lower halves with the intact leaves were covered each afternoon with a light tight box so that that part of the plant received short days, and the upper leafless part long days. All these plants produced flowers at the top of the leafless branches. In the third set the tops were provided with

boxes and given short days, and the leafy lower part normal long days, and no flowering resulted. The fourth set, in which the whole plant had short days, flowered as did the second set. Obviously the stimulus of short days was received only by the leafy basal part of the plant and this stimulus was transmitted, presumably by means of a hormone, to the upper defoliated part where flowers were induced to form. The upper part, being robbed of its leaves, could not receive the stimulus. Chailachjan christened this theoretical hormone "florigen". This type of experiment, however, does not rule out the possibility that the "influence" transmitted from leaves to apical meristem might be the spread of some vital change in the living protoplasm in the intervening tissues. There is, indeed, much data indicating that the stimulus can only pass along living cells. Thus, if the intervening portion of stem between meristem and leaves is cooled, then no stimulus will pass through, owing, it is thought, to the greatly lowered activity of the living protoplasm in the cooled portion. It will also not pass through a portion of stem on which there are mature leaves subjected to a day-length unsuitable for flowering. This suggests that such leaves actually produce a substance which may destroy the hormone or prevent its movement. The stimulus will also pass a graft union, and this has been admirably shown in the tobacco varieties previously mentioned. Maryland Mammoth remains vegetative in long days or continuous illumination. If, however, such plants have their tops removed and a scion of the variety Sampson (a long-day variety) grafted upon it, then the Maryland Mammoth stock will produce lateral branches and flower not only in long-day but in continuous illumination (Moshkov, 1936). Since these first experiments, the phenomenon has been re-observed in a number of other plant species and this is a very strong indication that the flowering hormones of long- and short-day plants are identical. There is also considerable evidence that the hormone is non-specific, since it can be transmitted from a scion of one species to a stock of another, or even from stocks to scions of different genera, *e.g.* from tobacco to *Petunia* or henbane (Melchers, 1936, 1937). A most interesting observation in this respect is that the broom-rape (*Orobanche minor*), which is a root parasite

of red clover, will only flower when its host does (Holdsworth and Nutman, 1947), suggesting that the flowering hormones are identical in these two widely separated families of flowering plant.

A similar transmission of flowering stimulus has been noted in vernalization phenomena. In the henbane (*Hyoscyamus niger*) there are two varieties, an annual and a biennial one. The latter variety, if vernalized by exposure to 5° C. for six weeks, will behave as an annual and flower in the first year. The biennial variety will also flower in the first year without vernalization if there is grafted on it, near the growing-point, a shoot of the annual variety that is ready to flower, or a shoot of the biennial variety that has been vernalized (Melchers, 1936, 1937). This is a further indication that the ultimate hormone resulting from vernalization treatment may be identical with that brought into being by photoperiodic induction.

These grafting experiments, however, resulted in the actual organic union of tissue to a greater or lesser degree and therefore afford no positive proof of diffusive transport of a simple chemical substance. In one experiment on the American plant cockle-bur (*Xanthium pennsylvanicum*), lens paper * was inserted between scion and stock to prevent this union, and the usual transmission of flowering influence was demonstrated (Hamner and Bonner, 1938). In the absence of union such a transmission could have taken place only by diffusion of a soluble hormone through the water soaked up into the lens paper, but, unfortunately, it is not impossible that tissue union may be established through such paper (Withrow and Withrow, 1943). Some workers have gone a step further and explored the possibility of diffusive transmission through a water gap. This was done by fixing a leaf, which had received the correct day-length for flowering, to a debladed leaf-stalk on a plant in an unfavourable day-length. The two cut ends, separated by about a millimetre, were kept surrounded by water. In *Chrysanthemum* the passage of a stimulus through this gap has been

* Lens paper is very fine soft absorbent tissue paper made of pure cellulose and containing no weighting or dressing materials. It is used for cleaning the surfaces of very delicate lens systems such as are used in the compound microscope.

claimed (Moshkov, 1936), but these findings have very largely lost their significance since we now know that, although in this species the development of flowers from flower-buds may be brought about by short days, yet the *initiation* of those flower-buds is independent of day-length, although it is greatly hastened by low temperature vernalization of the vegetative plants. In fact in *Chrysanthemum*, flowering seems to take place in two stages. A first stage of initiation, in which the inflorescence meristems alone are formed with no flower initials, is induced by low-temperature vernalization. The second stage of flower development from these meristems is inhibited by long days, but promoted by short ones (Schwabe, 1950, 1951). Illustrations of these phenomena are shown in Figs. 45 and 46. Moshkov's experiments should obviously be repeated in the light of these recent findings, since we cannot tell whether or not his observations refer only to flower development. More recently, experiments on soya bean, similar to those of Moshkov, have met with no success (Galston, 1949). From all this indirect evidence there is still, therefore, no positive proof of the existence of a flowering hormone, and it would seem that such proof will come only when someone succeeds in preparing, from a flowering plant, an extract that will induce flowering when injected into a vegetative plant.

In 1936 a claim was made that such an extract had been prepared from the stigma of the crocus (Ulrich, 1939), but subsequent attempts at confirmation failed (Melchers and Lang, 1941). Kerosine-oil extracts of flowering cockle-bur (*Xanthium echinatum*) have yielded a water-soluble material of a crystalline nature with which the discoverer claims to have induced flowering in vegetative cockle-bur plants (Roberts, 1951). We await the confirmation of this new claim with interest.

### *The Rôle of Auxin in Flowering*

It has been pointed out that all flowering plants have, inherent in their hereditary make-up, the full potentiality to flower, and that failure to do so when the right development stage is reached must obviously be due to the action of some inhibiting factor. In other words, the problem of flowering may quite as properly

be considered as one of "failure to flower as of promoting flowering" (Gregory, 1948). It follows naturally from this new view-point that the stimulus of day-length or temperature might be explained by the destruction of a flowering inhibitor rather than the production of a flowering promotor.

In recent years a new approach to the solution of this problem has begun with the largely empirical study of the effect of external applications of various chemical compounds, that for some reason or other have been suspected of having physiological actions in the plant. The aim of such experiments has been to find a synthetic substance active either directly as a flower-promoting substance, or indirectly as a counteractant or antagonist, as the physiologist calls it, of the flowering inhibitor. The following substances have been so tested and have shown no action in stimulating flower initiation: Vitamins $B_1$, $B_2$, $B_6$, C, nicotinic acid, pantothenic acid, theelin, inositol (Hamner and Bonner, 1938) and dehydrofolliculin (Chouard, 1937).*

It is natural that the auxins should have received much attention in this connection, and here again, with one notable exception, no stimulating action was to be found. This exception is the pineapple, which is, of course, tropical and produces only one "fruit" in a lifetime. The flowering of this plant is apparently under environmental control, since initiation of the inflorescence † usually takes place in the autumn and

* Vitamin $B_1$ = Thiamin. Deficiencies of this vitamin cause neuritic diseases such as beriberi in man.

Vitamin $B_2$ = Riboflavin. Deficiencies cause mouth, eye and skin affectations in man.

Vitamin $B_6$ = Pyridoxine. Deficiencies cause skin diseases in animals. An essential growth-factor for many micro-organisms.

Vitamin C = Ascorbic acid. A deficiency of this vitamin is the main cause of scurvy in man.

Nicotinic acid. Deficiencies of this vitamin cause pellagra, a skin and alimentary canal disease.

Pantothenic acid. A vitamin. A growth-factor for many micro-organisms.

Theelin. The female hormone produced by the ovaries.

Inositol. A vitamin. A growth-factor for certain micro-organisms.

Dehydrofolliculin = Œstradiol. Another female hormone.

† An inflorescence is a collection of flowers borne on one stem. In the pineapple the fruit comprises the whole inflorescence, the fleshy edible portion being made up of the true fruits, fleshy stalks and flower parts all fused together (see Fig. 21).

subsequent development and ripening continues throughout the winter. The several varieties differ in the length of time taken to reach a stage when the plants are ready to flower and this period ranges from one and a half to five years. It has been known for a long time that pineapple can be induced to flower outside this seasonal period and there was a practice of lighting smoky fires in the plantations to induce early flowering (van Overbeek, 1951). The active principle of the smoke was later discovered to be unsaturated hydrocarbon gases such as ethylene. The discovery that auxins (actually NAA) could similarly force the pineapple into flower was made as early as 1939 (Clark and Kerns, 1942). Since then the effect has been much studied, and it has been found possible, with some varieties, to induce flowering at any time of the year by spraying plants with dilute aqueous solutions of synthetic auxins. This easy and positive method of flowering control is now being used extensively by pineapple growers in South America and Hawaii, and allows for the first time a really planned cropping (van Overbeek, 1951). Thus, any particular area can be brought into flower at any time of the season, and a steady production of fruit throughout the year can be ensured from a series of such areas. In addition, the plants in any one uniformly treated area produce mature fruit at the same time and not spread over a period of a month or so as with natural uncontrolled fruiting. This enables a whole field to be harvested at once instead of in a series of operations. Fruiting can also be avoided at those seasons of the year when certain diseases are rife, *e.g.* black heart in Queensland (Groszmann, 1950). Naturally, there are certain drawbacks to this method of control (van Overbeek, 1951): one is that NAA causes rather thin stalks to the fruits. This makes them droop and therefore causes some loss. A cure can be effected by giving a second spray of NAA when the fruit is developing, and this has an added advantage of delaying fruit maturation and so increasing its final size at maturity. Only very low amounts of hormone are used, *i.e.* rates of application of the order of 25 gm. of sodium NAA per acre applied as a solution of 5 to 10 p.p.m.

In spite of this marked effect of applied auxins, virtually nothing is known of the function of the natural auxin IAA in

the normal flowering of the pineapple. This auxin seems to be present in plentiful amounts in the growing-point of the plant at all times, and is apparently produced by enzyme action from the precursor β-indolylacetaldehyde released from storage in the leaf bases. It seems, however, that auxin does play a part, since in one variety (*Caliezona*) flowering can be induced out of season by placing a plant on its side. This presumably disturbs the auxin distribution in the plant, which then resumes its normal growth position by geotropic response, and the altered hormone balance at the same time initiates an inflorescence (van Overbeek and Cruzado, 1948).

In all other plant species which have so far come under experiment there is no recorded instance of auxin application bringing about an initiation of flower primordia. There is, nevertheless, considerable evidence that high concentrations of added auxins will inhibit the production of such primordia under environmental conditions suitable for flowering and in plants otherwise ready to flower. This, indeed, happens in the pineapple if the applied concentrations are too high. This inhibition has been shown clearly in the cockle-bur (*Xanthium canadense*) (Bonner and Thurlow, 1949). This plant, a short-day plant, forms flower primordia when subjected to a dark period longer than the critical time of about eight and a half hours. In these experiments, flowering was induced by giving the experimental plants four consecutive short days of eight hours with corresponding sixteen-hour nights. These plants normally came into flower subsequently in long days. Plants kept continuously in long days did not, of course, flower. If, however, sprays of IAA or NAA (concentration 50 to 500 p.p.m.) were applied to the leaves of such plants during and for a few days before and after the short-day induction periods, then flower initiation was considerably delayed although it was not completely prevented. Similar cases of flower inhibition by applied auxins have been reported by other workers in enchanter's nightshade (*Circœa*) (Dostal and Hosek, 1937), *Kalanchoë* (Harder and van Senden, 1949; van Senden, 1951), balsam (*Impatiens balsamina*) and marigold (*Calendula officinalis*) (von Denffer, 1950). On the other hand, dilute sprays of the sodium salt of 2,4-D, 2,4,5-T and MCPA in the

field had no effect on the flowering of a range of crop plants, viz. winter oats, wheat, string beans, peas, beetroot, carrots and clover (Rice, 1950). A slight delay was observed in beans. That such inhibition is just another facet of the general inhibiting effect of high auxin concentrations on growth processes and not specifically an effect on flower primordia development is indicated by other work on flowering in two grasses, Chalco teosinte (*Euchlæna* sp.) (a short-day plant), and Wintex barley (a long-day plant) (Leopold and Thimann, 1949). Small quantities of solution of a range of concentrations of NAA (·01 to 400 p.p.m.) were applied in small phials to cut ends of leaves and were completely absorbed by them. In the short-day teosinte plant, brought to the point of flower formation by short days, all the auxin concentrations inhibited flowering. In the long-day barley, however, in a suitable long day, low concentrations of auxin (·01 to 1 p.p.m.) augmented the total number of flowers formed, but high concentrations greatly reduced that number. The vegetative growth of the plant, in addition, showed very closely correlated differences in relation to auxin concentration, indicating that the action of the hormone was a general one on the whole growth of the plant and not a specific one on flower production. In no case except the pineapple is there a recorded instance of low concentration of auxin causing flower initiation in plants that would otherwise have remained vegetative. This effect can probably be interpreted as a general effect of auxin on growth vigour, resulting not only in an augmented vegetative growth, but also in closely correlated effects on the growth of flower primordia already initiated by the flowering hormone.

Nevertheless, there are some botanists who still regard auxins as playing a contributory part in flower initiation, and support for their views is sought in the apparent correlations that exist between flowering and low auxin concentrations in the plant. Such correlative evidence is indirect but comes from many sources. Thus, both X-rays and ultra-violet light cause auxin destruction in the plant and at the same time tend to cause an accelerated flowering. The high ultra-violet content of light at high altitudes has been suggested as the reason why alpine plants flower early in the year (Bentley, 1950). Similarly,

removal of organs known to be a source of auxins have, in some instances, resulted in induction or acceleration of flowering. For example, in the mango, the removal of the terminal bud induced the lateral buds, which would otherwise have remained dormant or vegetative, to initiate flowers (Reece, Furr and Cooper, 1946). In *Chrysanthemum* the earliest stages of flower-head formation may begin even in long days, but normally flowers fail to develop fully unless subjected afterwards to short days. If, however, all side-shoots are removed from the plant, or if the shoot terminated by the flower-bud initials is cut off and re-rooted as a cutting, then flowering can be completed in long days. Both these latter treatments are such as might be expected to reduce the auxin content of the flower meristems. In a like manner, inflorescence buds formed in short days can be prevented from developing by placing the plant in long days. Similar effects are obtained in short days either by shading during the day (known to cause an increase in auxin content) or by the application of auxin in lanolin paste (Schwabe, 1951). It has already been seen in Chapter 4 that treatment of seeds with auxins may delay flowering. From such observations it seems that any conditions, natural or imposed, causing an overall lowering of the auxin content of plants, favours the initiation and development of flowers and any conditions increasing that content prevent their development.

In the last year or so, much attention has been paid to possible chemical means of counteracting auxin activity in the plant, and so cause production of flowers. Two chemicals have been claimed as such counteractants of auxin action, or "anti-auxins" as they have been called. They are 2, 3, 5-triiodobenzoic acid (TIBA) and 2, 4-dichloranisole. The flower-inducing activity of TIBA was first reported in 1942 (Zimmerman and Hitchcock), when it was shown that, after application of solutions to the soil around the roots (4 to 200 p.p.m.), or as a spray to the aerial parts of the plant, flower-clusters of tomatoes grew from axillary buds where shoots normally appear, and that terminal buds were replaced by flower-clusters (Zimmerman and Hitchcock, 1942): this phenomenon is illustrated in Fig. 47. These observations have since been verified by a number of independent investigators, and flower-

buds have actually been induced to form in the very earliest post-germination stages when plants were only an inch or two high and had produced three or so leaves (de Waard and Roodenberg, 1948). It has been pointed out (Galston, 1947) that the tomato is insensitive to day-length, and this effect of TIBA may be merely an acceleration of the development of flowers already differentiated, and not a true induction of flower primordia. This is now strengthened by the observation that in such day-length sensitive plants as soya bean (Galston, 1947) this compound will not induce flowers to form in vegetative plants (*i.e.* plants subjected to a day-length unfavourable for flowering), but will greatly augment the flowering response in a favourable day-length. A similar acceleration of flowering in cockle-bur plants had been claimed for 2, 4-dichloranisole (Bonner and Thurlow, 1949).

There is a certain amount of indirect evidence to suggest that this augmented flowering response caused by these chemicals is correlated with a lowered auxin activity in the plant. In the soya bean experiments cited above, TIBA caused certain growth responses, *i.e.* inhibition of stem (internode) growth, loss of apical dominance, premature abscission of apical leaves and buds, that are associated in normal plants with low auxin contents. Furthermore, TIBA will inhibit the action of auxin in the *Avena* test, if present with it in the agar blocks (Galston, 1947). The growth in length of root-hairs is also prevented, although cell-wall material (cellulose) still continues to be made, and forms an amorphous accretion at the tip of the inhibited cell (Gorter, 1949). Such evidence, coupled with the fact that the molecule of TIBA has a structure very similar to those of the auxins, points to some sort of competition between it and auxin in the plant, an action which has come to be known among physiologists as "antagonism". This type of phenomenon is of widespread occurrence in living organisms. Thus the action of the sulphonamide drugs, which prevent the growth of certain classes of bacteria, depends on competition with the chemically similar *para*aminobenzoic acid (PABA) in the system in which the latter is an essential factor. The drug, having entered the system, prevents the PABA from doing so: being itself ineffective as a growth-factor,

it brings about a partial or complete stoppage of that particular biochemical reaction sequence and hence prevents growth. As a crude analogy, this competitive inhibitor can be likened to that part of a broken key left in the lock when the break takes place in the key-shaft. The broken key has a shape which lets it fit exactly into the wards of the lock, just as the similar chemical structure of the inhibitor allows it to fit into the biochemical system it is inhibiting. The key has, however, lost the means by which it can be turned, and so it is useless; so, too, is the inhibitor which lacks the particular chemical structure necessary to allow the specific reaction to go on. Finally, the broken key prevents the insertion into the lock of an unbroken effective key, and in the same way, the competitive inhibitor blocks the entry of the normal essential reactant. Similarly, certain organic chemical compounds closely resembling particular vitamins in chemical structure, and therefore called vitamers, can compete with those vitamins in the particular biochemical systems of the animal in which they are essential components. Being themselves inactive in these processes, these competitive vitamers effectively clog the mechanism and, even though ample vitamins may be available, vitamin deficiency diseases inevitably result. For example, the action of vitamin $B_1$ (thiamin) can be blocked by the closely similar and purely synthetic pyrithiamin, nicotinic-acid action by IAA, etc. A number of such effects are discussed by Yudkin in a recent popular article to which the interested reader should refer.* Indeed, the search for specific biochemical competitors of this sort forms the basis of most modern chemotherapeutic research where selective inhibition of specific organisms or tissues (*e.g.* cancerous tissue) is the aim.

However, in the case of TIBA, the evidence so far available does not supply convincing proof that it is indeed an auxin antagonist. It is possible that its inhibition of auxin action in the *Avena* test is due to a general growth inhibition not specifically connected with auxins. In the author's laboratory, work on the growth of roots has been unable to demonstrate any significant antagonism of auxin inhibition by either TIBA or by 2, 4-dichloranisole (Audus and Shipton, 1952). Further-

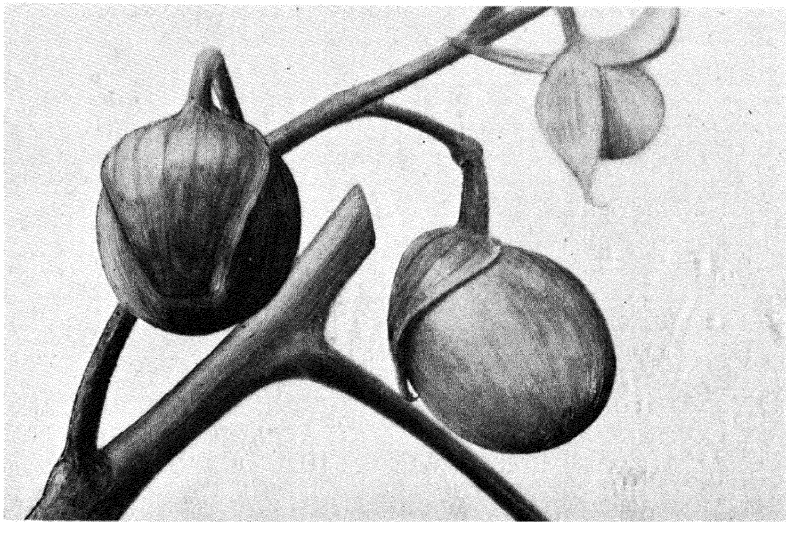
* Yudkin, *Science News*, 7.



FIG. 47.—A terminal cluster of flowers induced by treatment with 2, 3, 5-triiodobenzoic acid (TIBA) in a young tomato plant. In normal plants terminal shoots are always leafy.

*Photographs by permission of Professor R. L. Wain, Wye College, University of London.*

FIG. 48.—Complete fusion of the five sepals under the fruit of the tomato produced by spraying with solutions of $\alpha$-phenoxypropionic acid.



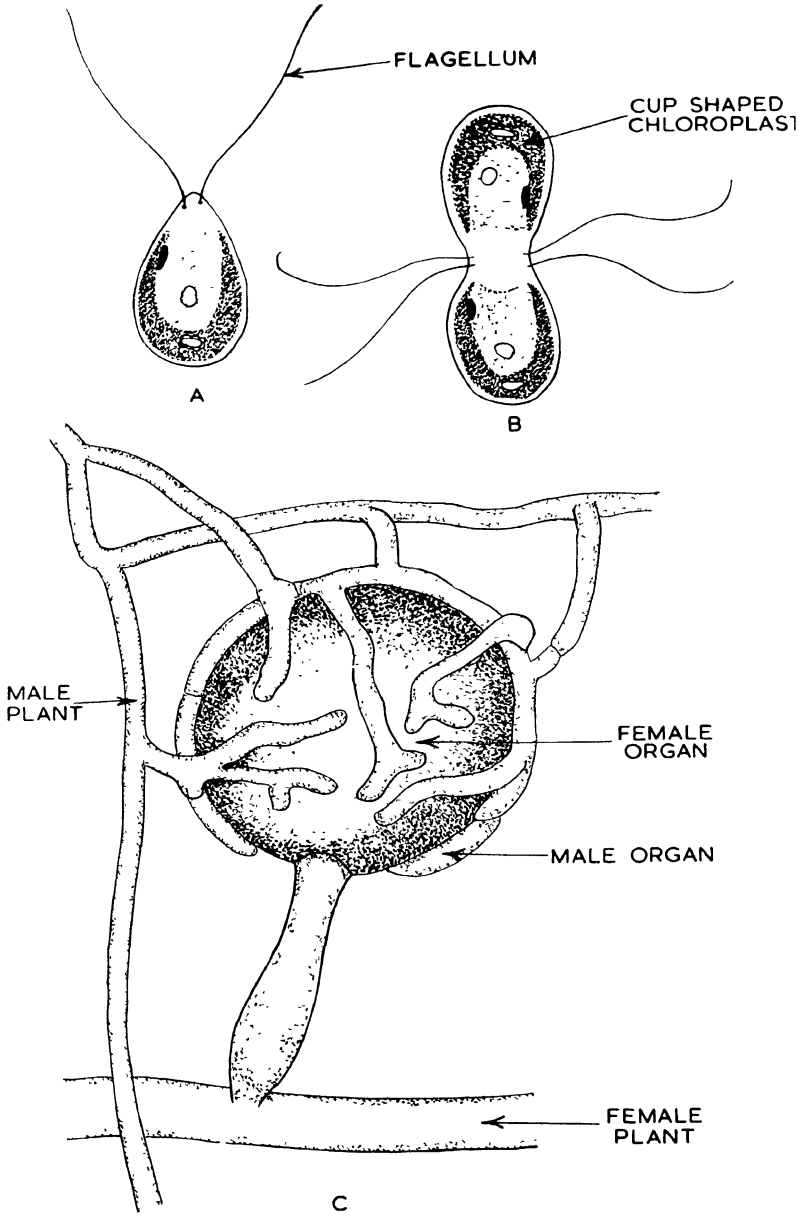



FIG. 49.—A. *Chlamydomonas* plant. B. Sexual fusion of two *Chlamydomonas* gametes. (×1500.) C. Envelopment of the young immature female organ of the water mould *Achlya ambisexualis* by numerous male hyphæ.

*After Raper, J. R., Amer. J. Bot.*, **26**, 619, 1939.

more, TIBA in addition to its growth-inhibiting effects can also stimulate growth in very low concentrations. This has been shown for the pea test, the *Avena* test, the *Avena* cylinder test (Thimann and Bonner, 1948), and the cress-root test of Moewus (unpublished work of Gorter, communicated verbally to the author). It will also induce the formation of parthenocarpic fruits in the tomato (Luckwill: personal communication). These latter two aspects of its action closely resemble that of auxin itself.

This conflicting evidence is not a little confusing, but there seems at the moment no justification for assuming that the so-called "anti-auxins" do indeed act on flowering by countering the action of the natural auxins. It seems more likely that they affect flowering directly as does auxin itself in the pineapple and Wintex barley.

However, the concept of growth control by auxin–anti-auxin balance appeals strongly to many physiologists and forms the basis of a recent theory of flowering (Resende, 1949). In this theory, flower initiation is regarded as determined, not by a specific "florigen" but by the attainment of a particular ratio of the natural auxin to an equally natural anti-auxin, identified with bound or inactive auxin. This critical balance varies markedly from species to species, and at least two groups of plants are recognized. Firstly, the "auxin plants" are those which, like the pineapple, require a high ratio of auxin to anti-auxin and which can therefore be brought into flower by the administration of auxin. Secondly, we have the "anti-auxin" plants, which require a low value of this ratio for flowering. These plants include the soya bean, the cocklebur, etc., where flowering can be induced by lowering the auxin content or by increasing the anti-auxin content. This theory is a valiant attempt to harmonize the conflicting flowering reactions of plants to auxins. It is, however, highly speculative and has little direct experimental evidence to support it. We need to have much more convincing proof of a specific antagonistic action of these chemical relatives of auxins in the auxin system before such a theory can be seriously considered.

The attention recently given in America to the synthetic

inhibitor maleic hydrazide has naturally included its possible effect on flowering behaviour. Its action here would be of particular relevance to the above theories, since there are strong indications from recent experiments that its inhibiting action may be very largely exerted by suppression of the activity of auxin (Leopold and Klein, 1952). Thus, spraying maize plants with ·025 per cent. solution causes the male flower tassels to become completely sterile but has no effect on the female flowers, which can produce normal viable seed when pollinated. It is suggested that this treatment may be a very useful tool to produce male sterile plants in hybridization work (Naylor, 1950). There are also indications that the day-length requirements for flowering of *Xanthium saccharatum* (cockle-bur) may be modified by maleic hydrazide treatment. Further study is obviously indicated.

As for the chemical control of flower initiation on a practical and economic scale, there is little promise as yet that the remarkable success with pineapples is likely to be repeated with other species of plants in the near future.

Before leaving the question of flowering, it is of interest to mention one case of the successful use of auxin in the *prevention* of flowering. Celery is normally a biennial and is cropped at the end of the first year before flower-stalks form. If, however, a spell of cool weather occurs after transplanting the young seedlings, then "bolting" or the formation of inflorescences may take place and the plant becomes an annual. This may result in a considerable loss to the grower. Similar losses result from the use of protective coverings or by late planting, and are therefore undesirable. Experiments in America, in which celery seedlings were subjected to low-temperature treatment at 41° F., demonstrated that the subsequent bolting response can be completely prevented by spraying with a solution of 100 p.p.m. α-(2-chlorophenoxy)-propionic acid (Wittwer, Coulter and Carolus, 1948). Similar results have been obtained for lettuce, using 2, 4-D (Clark and Wittwer, 1949). Whether this is yet another example of the inverse correlation between high auxin content of the plant and flower initiation has yet to be discovered.

*Growth Substances and Fertilization*

Pollination, the act of transfer of the male pollen grains from the ripe anthers of a flower to the female receptive surface of the stigma, is the first step leading to the fusion of male and female nuclei in the female organ (ovary) and the formation of the seed. If the pollen finds itself, as a result of pollination, on a compatible stigma, *e.g.* that of a flower of the same species, then it germinates and a long tube grows down into the female organ, penetrating to where the unfertilized seed initials (ovules) are situated, and finally passes into the female cell where it releases its nuclei to fuse with those of the female. This constitutes the act of fertilization. In the flowering plants many devices exist whereby pollen is prevented from fertilizing ovaries in the same flower or, in some cases, ovaries in different flowers on the same plant. The exact significance of this enforced cross-pollination is not, as yet, thoroughly understood, but it is thought that all flowering plants require at least the occasional stimulus of such a mating to maintain the vigour of the species. The well-known phenomenon of hybrid vigour * may be closely related to it.

Most of these devices are of a mechanical nature and depend on structural features of the flower; they need not concern us here. What are of interest, however, are those flowers which are self-sterile—that is, those in which pollen from the same flower fails to germinate on or grow down into the female organ. Such types of self-sterile flowers are of wide occurrence in the flowering plant kingdom, but it is only in the last year or so that definite proof has been obtained that their sterility is due to chemical growth inhibitors. This proof has come from observations on the beautiful early flowering shrub *Forsythia* (Moewus, 1950). In this shrub there are two types of plant, one bearing flowers with short pollen-bearing organs (stamens) and long female organs (pistils); the other has short pistils and long stamens. Furthermore, these two types are both self-sterile but interfertile. In other words, pollen from the short stamens will not germinate on the stigmas of long pistils but only on those of short pistils, and pollen from long stamens will germinate only on long pistils: in this way cross-

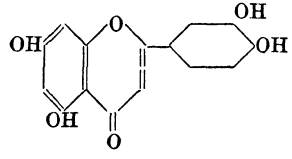
* See the excellent article by Ashby (*New Biology*, No. 4, 9–22, 1948) where this phenomenon is explained for the layman in simple terms.

pollination is assured. Now it has been found that pollen from long stamens contains an organic substance, rutin,* which is a germination inhibitor. The stigmas of the long pistils, however, contain an enzyme that destroys this inhibitor, allowing the pollen to germinate and subsequently fertilize the ovule. Stigmas of short pistils do not contain this enzyme and therefore such pollen will not germinate on these stigmas. On the other hand, short-stamen pollen contains a related inhibitor, quercitrin,* and short pistils an enzyme which will destroy it so that such pollen can germinate on the short but not on the long pistils, which do not contain the second enzyme. It is possible that such a system, whereby pollen is prevented from germination by its own natural inhibitor content until released from its inhibition by an enzyme or a counteracting growth substance from a compatible stigma, is the basis of many self-sterility phenomena in flowering plants. These results open up a new and promising field of research.

### *Hormones and Sex Determination in Flowers*

The majority of plant species have flowers which possess fully developed male and female organs in each flower, but in some groups of flowering plant the flowers may be unisexual and therefore of two kinds, one with only male and the other with only female organs. In these plants both male and female flowers may develop on the same individual parent or they may be confined to separate male and female plants. The distribution of the sexes between flowers in the former, and between individual plants in the latter, is usually determined by strict hereditary laws, but there are a number of well-founded observations that other factors, such as the state of the plant's nutrition, etc., may greatly influence sexual expression in those

* Rutin and quercitrin are compounds of sugars with the common yellow plant flavone pigment, quercitin, which has the following formula:—




Rutin = Rhamnose + glucose + quercitin.
Quercitrin = Rhamnose + quercitin.

plants (see Skene, 1932, pp. 329–338). One very interesting example of an induced change of this sort, reported in the literature of over forty years ago, concerns the red campion, *Melandrium dioicum*, which usually has male and female flowers on different plants. This plant is often attacked by a smut fungus (*Ustilago violacea*) which invades the male flowers, filling the pollen chambers with violet smut spores and preventing the formation of pollen. It was noticed that, if perchance a female plant were attacked, the fungus induced the formation of male organs in the flowers and formed its spores in the pollen chambers (Shull, 1910). This can only mean that the potentiality for maleness must be present in female as well as male plants, and that some stimulus from the invading smut fungus allowed this to express itself. In the present-day climate of botanical thought, the idea of a hormonic control of this sexual expression immediately comes to mind, and recent observations in Sweden support such a view (Löve and Löve, 1945). Thus the application of the mammalian female hormones œstrone * and œstradiol * in lanolin paste to the leaf axils of male plants of *Melandrium dioicum*, caused a partial suppression of the male organs (stamens) in the flowers which subsequently arose in those axils and promoted the formation of the female organ (pistil). The male hormone, testosterone,† has the opposite effect, suppressing the expression of femaleness in flowers on female plants and promoting the formation of stamens. Unfortunately these observations have yet to be confirmed, and a subsequent careful repetition of this work on two British races of the same species of plant failed to obtain any response except some slight stimulation of growth vigour (Harrison, 1948). There seems little doubt, however, that the Swedish results are real, and more work on this intriguing subject is certainly indicated.

Although we now know that the female mammalian sex hormones are widely distributed in plants and that their male counterpart also occurs, yet there has been little con-

* Œstrone and œstradiol are hormones elaborated by the ovary in mammals and are of fundamental importance in the control of the uterine cycle.

† Testosterone is the male mammalian hormone elaborated in the testes and is responsible for the control of male sexual characteristics.

clusive evidence that they play an important part in plant development. Many experiments on a considerable variety of plants and plant organs have demonstrated some stimulation of general growth vigour, and in one plant, *Callistephus sinensis*, in short days, the application of œstradiol greatly speeded up the onset of flowering (Chouard, 1937). Nevertheless, the above are the first claims of a positive effect of mammalian sex hormones on sexual expression in plants, but there is no evidence whatever as yet that the same or similar molecules have any such function in its control in normal plants.

## SIMPLE PLANTS

From what has been said before in this chapter, one might be led to regard reproduction in the flowering plants as being ultimately controlled by a relatively simple hormone system. Indeed, we have evidence that the ultimate hormone causing flower initiation is the same whether produced by vernalization or by long or short photoperiods. Furthermore, we have seen that some would identify this hormone with auxin at a specific concentration level. When we turn from these highly organized flowering plants, with their great complexity of structure and organ, to the simply organized plants such as the colourless moulds (fungi) and the microscopic green plants (algæ) growing in ponds, we find evidence of a very complex system of hormones controlling their reproductive processes. This state of affairs makes one wonder whether we are not grossly oversimplifying the whole hormone set-up in the higher plants.

The rapid growth and ease of culture of such simple organisms, under the carefully controlled environmental conditions of the laboratory, has enabled us to learn considerably more about the chemical regulation of their reproduction than that of the more highly organized flowering plants. Needless to say, many species and conditions have been investigated, but in a book such as this it will be possible only to outline some of the more important findings to illustrate this complexity.

Let us begin with the moulds. These are, for the most part, colourless plants devoid of the green colouring matter chlorophyll and therefore incapable of using the radiant energy of

the sun for their growth processes. Such growth energy must come, therefore, from organic materials such as starch, sugars, proteins, etc., which the mould is capable of decomposing. The body of the mould is composed of microscopic branched threads called hyphæ which ramify and spread over the organic medium serving it as food. Sexual reproduction, which occurs ultimately in the life of the majority of known moulds, takes place usually by the fusion of the contents of two separate parts of the hyphæ, which are often differentiated into distinct male and female organs. It is the differentiation of these organs and their behaviour during the processes leading up to actual sexual fusion that are apparently controlled by hormone complexes.

There is now much well-established evidence that nutrition, particularly the provision of certain specific chemical growth factors, exerts a marked influence on the sexual reproduction of moulds. Perhaps the best known is the work on *Phycomyces Blakesleeanus*, which is a close relative of the pin mould commonly found growing on old moist bread. This fungus requires vitamin $B_1$ (thiamin, aneurin) in its food in order to grow at all, since it is incapable of making this essential biochemical catalyst by itself (see review by Schopfer, 1943). Sexual reproduction is brought about by the close juxtaposition of two hyphæ, the subsequent fusion of their contents and the formation, at the point of fusion, of a large thick-walled spore, the so-called *zygospore*. For this sexual fusion to take place, at least two organic substances other than thiamin must be provided in minute traces. One of these factors is the purine base hypoxanthine (6-ketopurine), an important component of the proteins of plants (Robbins and Kavanagh, 1942). Some species of yeast, a group of microscopic unicellular fungi, which normally multiply solely by simple cell-division, show sexuality by the fusion of two cells. In one such yeast which has been intensively studied, viz. *Zygosaccharomyces* sp., sexual fusion is induced by vitamin $B_2$ (riboflavin) and the organic acid glutaric acid acting together (Nickerson and Thimann, 1943). There are indications that natural sexual fusion, which occurs most frequently in old cultures, may be brought about by the accumulation of these compounds produced by the ageing cells themselves.

Such sexuality-inducing substances produced by the organism itself and not provided in its food are essentially hormones, as we usually define them, and are the most interesting from our present point of view. The first demonstration of the production of such substances by fungi came from studies on *Mucor mucedo,* a white pin mould often found on damp bread. This mould exists in two distinct strains that are structurally indistinguishable under the microscope. Purely for the sake of convenience, they have been designated (+) and (−) strains respectively. The only difference between them is that sexual reproduction, which takes place, as in *Phycomyces Blakesleeanus,* by the fusion of two hyphal tips, will only do so between hyphæ of a (+) and a (−) strain. Two (+) hyphæ will not grow together and fuse, and neither will two (−) hyphæ. The participation of a diffusable hormone in such a process is indicated by the fact that considerably before the (+) and (−) hyphæ come into contact, the approaching tips begin to swell. A mutual stimulating influence must therefore have crossed the intervening space between one hyphal tip and the other, *i.e.,* either over the surface of or through the nutrient material on which the mould was growing; such a stimulating influence is most likely to be chemical. The matter was finally clinched when an agar block with (+) hyphæ was placed upon (−) hyphæ, also growing on agar, with a film of collodion between them. Although the hyphal tips could not pass the membrane and so fuse, yet they swelled up in the usual preparatory manner, which could only have been brought about by the diffusion of chemical substances from one strain to the other through the permeable membrane (Burgeff, 1924). It seems likely that at least two different substances are involved, one produced by the (+) strain and evoking a reaction in the (−) strain and *vice versa,* although, as far as the writer is aware, no two such compounds have yet been isolated.

The type of sexual reproduction so far discussed has been extremely simple. In other fungi we find a much more complicated sequence with the differentiation of male and female organs of markedly different structure. Such male and female organs may be borne on the same plant body, *i.e.* on the same hypha, which may be simple or highly branched. On the

other hand, as in *Mucor mucedo*, there may be two distinct strains, one bearing female organs only and the other male only: such a plant is said to be *heterothallic*. This is the case is some species of mould which grow in fresh water on higher plant debris such as twigs, berries, etc. It is in one of these genera of water moulds,* *Achlya* spp., that a most amazing complex of sexual hormones has recently been demonstrated. Here the reproductive sequence begins with the production by the female plant of a hormone (called for convenience hormone A) which diffuses to the male plant and there induces the formation of male sexual hyphæ, which are short lateral branches of the main hyphæ. The total number of such branches is determined by a variety of external physical factors such as temperature, salt concentration, etc., and also by the relative concentrations of a chemical activator from the female and a chemical inhibitor from the male plant. Soon after they have been formed, the male sexual hyphæ secrete another hormone (hormone B) which diffuses to the female plant and causes the female organ to be formed. These short, swollen female branches now produce a third hormone (hormone C), which also diffuses out into the medium and causes the male hyphæ to grow along the concentration gradient towards them. When the male branch finally reaches the female branch, the male organ is induced to form, under the joint action of contact and hormone C, as a short club-shaped terminal cell containing dense protoplasm and several nuclei. This stage of the process is illustrated in Fig. 49. As this male cell develops, it secretes a fourth hormone (hormone D) which in its turn brings about the maturation of the female organ and its contents. This ripe female organ consists of a large spherical cell at the end of a short female branch and contains a number of spherical masses of protoplasm, each with its own nucleus (the eggs). Fertilization takes place by the growth of a fine tube from the male into the female organ, the passage of male nuclei into that organ and the ultimate fusion of male and female nuclei. Such a fusion is followed by the formation from each fertilized egg of a thick-walled spore, which, on germination, gives rise to new plants.

* Water moulds are usually to be found growing on plant debris, *e.g.*, fleshy fruits of wild rose, hawthorn, etc., submerged in fresh-water streams and ponds.

So far these various hormones have not been identified with any known chemical substance, owing primarily to the incredibly small amounts of the compounds involved. For example, in one experiment nearly 1450 litres (320 gallons) of culture fluid, in which female plants had been growing, were concentrated until 0·0002 gm. of highly active substance was obtained: when this was dissolved in $10^{13}$ (ten billion) times its own weight of water, it could still induce the formation of male branches on male plants. The occurrence of these hormones in such extremely low concentrations makes the work of direct chemical identification wellnigh impossible. This over-simplified description has obviously omitted many important points of detail, and the interested reader should refer to the discoverer's own account in recent reviews, where reference to the original papers will be found (Raper, 1951 and 1952).

The fungi are not the only lower plants in which sexual processes are apparently controlled by diffusable hormones. In the algæ, a group which includes the microscopic plants constituting the green scums of stagnant ponds and slowly flowing streams, evidence has recently been obtained of a similar complex of hormones controlling sexual reproduction. Needless to say, this group of plants has received much less attention than the fungi, since they contain chlorophyll and so are capable of making all their organic food from the simple inorganic materials in the surrounding water, and this makes it much more difficult to check up on their exact nutritive requirements. There seems to be no indication therefore that reproduction in these organisms may at any time be limited, as in the fungi, by the lack of any specific nutrient such as a vitamin or an amino-acid.

Several species of alga have been studied from the point of view of the effect of external physical conditions of the environment (*e.g.* light intensity) on their sexual behaviour, and it appears that the effects of these environmental factors are mediated by specific hormones produced by the organisms under those conditions. The genus or organism most intensively studied is a microscopic unicellular form of freshwater alga called *Chlamydomonas*. The individuals of this plant genus are egg-shaped structures of about 1 to $1\frac{1}{2}$

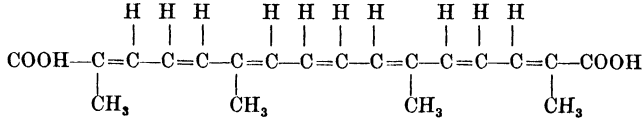
thousandths of an inch in diameter. Each contains a green protoplasmic body (chloroplast) for the synthesis of food in the light, and two thin whip-like protoplasmic structures (flagella) attached to the more pointed end of the cell. The rhythmic movement of these flagella propel the cell through the water in which it lives. If these motile organisms are transferred to the surface of a solid medium, *e.g.* agar-agar, the flagella are lost and the cells become embedded in a jelly-like matrix secreted by the cells.

Reproduction of *Chlamydomonas* may be sexual or asexual. When asexual reproduction takes place, individual cells simply divide into four or eight identical daughter cells which are exact miniature replicas of their mother. Under some conditions, however, such daughter cells fuse in pairs, this fusion constituting the sexual act: this is illustrated in Fig. 49. Even ordinary vegetative cells can fuse in some species without prior division to form sexual cells (called *gametes*). The most intensive investigations have been carried out in Germany (see Moewus, 1950) on a species, *Chlamydomonas eugametos*, which is characterized by producing two distinct strains of cell—a male and a female strain.* Such a phenomenon has already been noted in the fungal genera *Mucor* and *Achlya*. It was found that when non-motile individuals of this species growing on agar medium were flooded with water in the light, they grew new flagella and became motile; this did not happen if the flooding were done in the dark and, in addition, the cells became immobile again when returned to the dark, even though kept in water. The cells could, nevertheless, be made motile in the dark if certain sugars were provided in small concentrations (*e.g.* gentiobiose was the most active), or if some of the medium in which motile cells had been swimming was added. Thus, motile cells produce an active chemical substance which can induce motility in non-motile cells in the dark. Then it was discovered that

* *Chlamydomonas eugametos*, as *Mucor mucedo*, has male and female individuals that are indistinguishable in size and structure when seen under the microscope. But there are species (e.g. *Chlamydomonas Braunii*) which have female gametes that are larger than the male and therefore the sex of the equally-sized gametes of *Chlamydomonas eugametos* can be determined by mating experiments with *C. Braunii*.

this substance was probably identical with the orange-yellow carotinoid pigment, crocin, which was shown to have the same activity as the natural stimulant in dilutions of the order of one part in $2{\cdot}5 \times 10^{14}$ (250 billions). These latter claims have been viewed with some misgiving since this dilution would represent an availability of just over one molecule of the crocin per *Chlamydomonas* cell, and since it seems unlikely that a single molecule could so radically modify the behaviour of such a relatively vast structure as a whole organized cell. Cells, which had been made motile by crocin in the dark, remained incapable, however, of uniting with sexually functional cells of the opposite sex. But when the medium in which illuminated sexually functional cells had been growing was used to flood agar cultures in the dark, then the cells so flooded acquired both motility and the capacity to fuse with the opposite sex, provided that the flooding liquid came from a motile culture of the same sex; this shows that light induces the formation of two different chemical substances, one in motile potential male cells inducing male sexuality, and one in motile potential female cells inducing female sexuality. Experiments using different coloured light to illuminate the cells gave further information. Red light resulted in the production by the cells of an orange-yellow pigment which was later identified with *cis*-crocetin-dimethyl-ester,* a close relative of the motility hormone crocin; blue light caused this to be converted slowly in solution to the very closely related *trans*-crocetin-dimethyl-ester, and this transformation was complete in about one and a quarter hours. During the transformation of this substance, the cells flooded with the irradiated medium became sexually functional, first the female and then the male. It then became apparent that the male and female activating hormones were merely different mixtures of these two compounds, the female consisting of a high ratio of the *cis*- to the *trans*- compound, and the male of

* Crocetin has the formula:—



a much lower ratio: this was subsequently confirmed by direct application of the appropriate mixtures. Thus motility, a pre-requisite for sexual fusion, and the capacity to fuse are both induced by certain lighting conditions acting via the production of specific hormones.

Interestingly enough, there is a variety of this species of *Chlamydomonas* that is *homothallic*, *i.e.* there is no separation into male and female strains. When such cultures are illuminated, any cell may fuse with any other, showing that sex is not determined by inheritance from the mother-cell, as in the type variety, but a cell may become either male or female depending on conditions. Studies on this homothallic variety have indicated that here sex determination itself is also hormonic. For example, when these homothallic strains are illuminated, cells fuse in pairs. If they are first treated in the dark with a solution of the substance picrocrocin, another relative of the crocin discussed earlier, then subsequent illumination will not cause sexual union; but such cells will fuse sexually with male cells of the heterothallic strain but not with the female. This compound, picrocrocin, is therefore a female sex-determining hormone. In a similar way a related compound safranol was demonstrated to be the male determining hormone. Thus female cells of heterothallic varieties are female because they produce the compound picrocrocin, and male cells are male because they produce safranol. In homothallic varieties it is supposed that cells produce both compounds and that sexual behaviour of any individual cell is decided by the particular ratio of these two compounds produced in the metabolism of that individual. In this way a high ratio picrocrocin–safranol would make the cell containing it female, and a low ratio would make it male. It is very interesting to note that all these substances, motility-inducing crocin, sexual fusion activators *cis*- and *trans*-crocetin-dimethyl-esters and the latter sex-determining compounds, are all closely related chemically, and their ready interconversions in the cell are presumably under simple enzymatic control.

It is unfortunate that this complex story has not yet been independently confirmed, and it seems quite likely that in other species of *Chlamydomonas*, where the pattern of behaviour to

light and dark is by no means the same, that a very different hormone system may be in operation. As has been indicated above, there are features of the *Chlamydomonas eugametos* work that are difficult to accept and which require further critical investigation. In the words of one reviewer (Schopfer, 1943) concerning the results of this work, ". . . it would be comforting to see them confirmed". A balanced and fully critical account of all these studies to date has recently been written by Raper (1952).

When we consider the complexity of the hormone systems concerned with reproduction in these simply organized plants, one suspects that the state of affairs in the flowering plants may be even more complicated, and that simple theories of auxin level and auxin–anti-auxin balance are gross over-simplifications. If the development in proper order of such simple structures as the male and female organs of *Achlya* requires a series of hormones, how much more complicated a chemical organization might be necessary for the proper development of a flower.

CHAPTER 13

# SPECIFIC FACTORS FOR THE GROWTH OF ORGANS

## INTRODUCTION

WE have seen in the foregoing chapters how the single hormone β-indolylacetic acid can control the growth of many different organs of the higher plant. It has also been noted that a considerable body of current opinion tends to support the suggestions (Thimann, 1937) that this hormone may be the primary or even the sole factor concerned in the growth and differentiation of plant organs, the mechanism of differential control being based on the vastly differing sensitivity of various organs to the same compound. Changes in the concentration level would, by this means, favour the growth of one or other organ, depending on its sensitivity. When considering reproductive hormones in the algæ and fungi, we contrasted the complexity of the hormone control in these poorly organized plants with the simplicity of the postulated one-hormone system in the highly organized flowering plant, where one would have expected to have found instead even greater complexity. In being sceptical of this simplicity, we are in good company, since many students of growth phenomena have brought forward a considerable weight of data suggesting the collaboration of other natural hormones with auxin in the growth and development of plant organs. In Chapter 5 we have already devoted considerable space to sifting the evidence for rhizocaline, the specific root-forming hormone. Two other important theoretical organ-forming hormones (caulocaline and phyllocaline) have been postulated; let us now consider these calines in more detail.

## ORGAN GROWTH: INDIRECT APPROACH

### *The Calines*

When we were discussing the possible rôle of internal factors in the rooting response of cuttings to applied auxins,

it was concluded that some vital co-factor of auxin was contributed by the leaves and buds of the cutting. Unfortunately, it is still not possible to say with any degree of certainty whether such a factor is purely nutritive, consisting of the raw materials for growth (viz. sugars and organic nitrogenous compounds), or whether a specific root-forming hormone (rhizocaline) is needed to co-operate with the auxin: a number of well-known workers in the field adhere strongly to the idea of such a specific factor. In the hands of one of the originators, the theory has been elaborated so that three factors are now regarded as constituting the root initiation system (Bouillenne and Bouillenne-Walrand, 1948). Thus, in addition to auxin and the mobile rhizocaline coming from the leaves (see Chapter 5), there must be, according to this theory, a specific enzyme present in the cells to bring about the auxin-rhizocaline interaction, which results in root initiation. Such an enzyme, being confined to certain tissues, *e.g.* the pericycle, explains why it is only those tissues that can be induced to form roots under any conditions. Whether this rhizocaline plays any part in the subsequent growth of the root once it is formed has, so far, not been considered. We will return to this aspect of root growth later.

This specific rooting hormone has its counterpart in a similar theoretical stem-growth hormone which is supposed to be synthesized in roots. This hormone has been christened *caulocaline* (Went, 1938); its existence is based on rather slender indirect evidence which does not carry the conviction of that adduced for rhizocaline. Young etiolated seedlings of pea were taken, the top of the main shoot removed and the subsequent growth of the lateral shoots followed in darkness. The seed leaves (cotyledons), being the source of food and growth-factors, were removed, and in their place a solution of 2 per cent. sucrose was applied; this had little effect on the subsequent growth of the lateral shoots. When, however, the cotyledons were left intact and the roots removed instead, sugar solution being applied as before, there was virtually no subsequent growth of the lateral shoots. It was concluded from this that the roots were a source of an essential shoot growth-factor. This experimental restriction of growth might, however, be due entirely to serious interference with the water

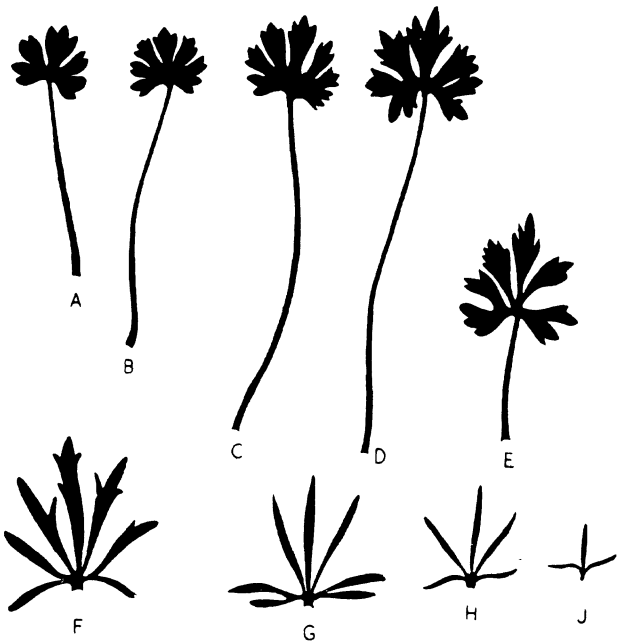
supply to the seedling, but these objections have been met by a modified experiment (Went, 1943), in which a tomato plant was grown with part of its root system in a nutrient solution and part in moist air. Such plants made much better shoot growth than control plants with the whole root system in a nutrient solution. Here conditions of good aeration of part of the root system had a marked stimulating effect on shoot growth, even although the total available water was restricted. Such improved aeration was supposed to act by causing more caulocaline to be made in the roots (Went, 1943).

Unfortunately, other conflicting evidence exists to show that isolated parts of stems and shoots can continue to grow indefinitely in the absence of any attached roots. This is so in *Asparagus*, where excised stem-tips will grow indefinitely in the light on a simple inorganic culture medium (Loo, 1945), and also in similar rootless tobacco shoots formed from callus tissue (Skoog, 1944). These results have been taken as disproving the necessity of caulocaline for stem growth, although the originator of the idea (Went, 1951) prefers to explain the discrepancies in these exceptional species in terms of the manufacture of this substance in the stem itself. Obviously only the isolation of this material can finally settle the question, but, so far, root extracts have failed to yield any active compounds (Went and Bonner, 1942). Substances active in stimulating the growth of rootless tomato shoots kept in water in the dark have, however, been demonstrated to be present in pea cotyledons and in coconut milk. These observations merely illustrate the complexity of growth-substance requirements in organs and do not help us to decide the caulocaline question. What some of these further growth-substance requirements may be we shall consider in the next section.

When we turn to the leaf, a further complication arises in that the growth of veins seems to be under the control of factors different from those controlling the growth of the intervein areas (mesophyll). It has been shown experimentally that auxins will stimulate or inhibit the growth of veins, depending on the concentrations applied, but it has no effect on the growth of mesophyll. This latter tissue does, however, require specific growth-factors which can be synthesized only

in the light. This can easily be demonstrated by growing pea seedlings in complete darkness, when the stems grow long and spindly while the leaves remain small and vestigial. This inability to grow is not due to lack of food manufactured in the light, but to the absence of a specific chemical factor produced photochemically (*i.e.* by a chemical reaction needing light energy) (Gregory, 1928). Such observations have recently been taken a step further by growing isolated discs of leaf mesophyll on extracts of pea cotyledons containing sugar (1 per cent. sucrose) (Bonner, Haagen Smit and Went, 1939). Although such discs grow in the sucrose solution alone, they showed a 40 per cent. greater increase in area in the presence of the extract. Whether we are dealing here with a complex of growth factors or a single growth substance we do not yet know, but the functional name *phyllocaline* has been given to this mesophyll stimulant (Went, 1951).

The concept of the dual control of leaf growth by two separate chemical systems, one regulating vein extension and the other the growth of intervein tissue, allows a relatively simple explanation of the intriguing variations in leaf form that can be observed on one and the same plant. Such variation is extremely common in flowering plants and is usually associated with age, the oldest and therefore the lowest leaf on the seedling often being the simplest in structure, the lobing or toothing becoming more pronounced in the younger, more recently formed leaves (see Fig. 50). On the other hand, a reversion to a simple form generally takes place when the plant goes into the reproductive state. This is very clearly seen in the ivy (*Hedera helix*), where leaves on the flowering shoots are usually relatively narrow and simple compared with the broad five-toothed leaves on the vegetative branches. Most broad-leaved plants with leafy flowering stems will show this phenomenon. On the other hand, in some species the leaves first formed in the season are often much more highly lobed than those formed later on. This occurs in the horse-radish (*Armoracia lapathifolia*) where the first rosette of leaves arising from the persistent underground root are usually pinnately lobed (feather-shaped), whereas the mature leaves are shaped like a spear-head. These sequences could be explained in terms



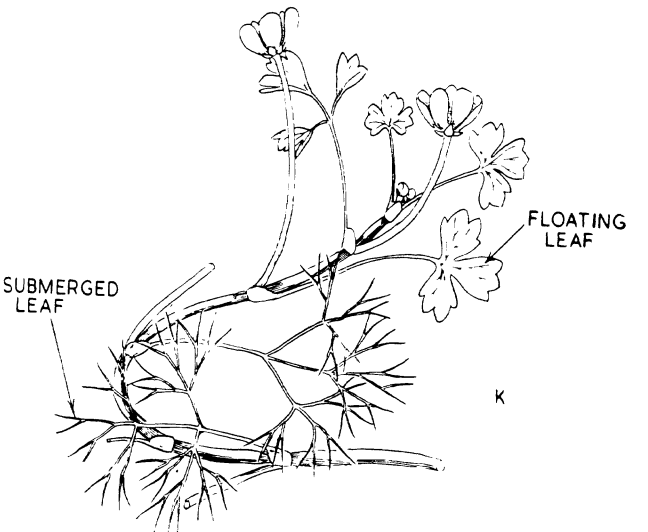




FIG. 50.

A–J. Silhouettes of successive leaves from base to apex of a plant of goldilocks (*Ranunculus auricomus*) showing the progressive increase in the depth of lobing and narrowing of the lobes as one passes from the oldest (A) to the youngest leaf (J). ($\times \frac{1}{3}$.)

K. Portion of a plant of water crowfoot (*Ranunculus baudotii*) showing the complete absence of inter-vein tissue in the submerged leaves and its full development in the typical buttercup-like floating leaves. ($\times \frac{1}{3}$.)

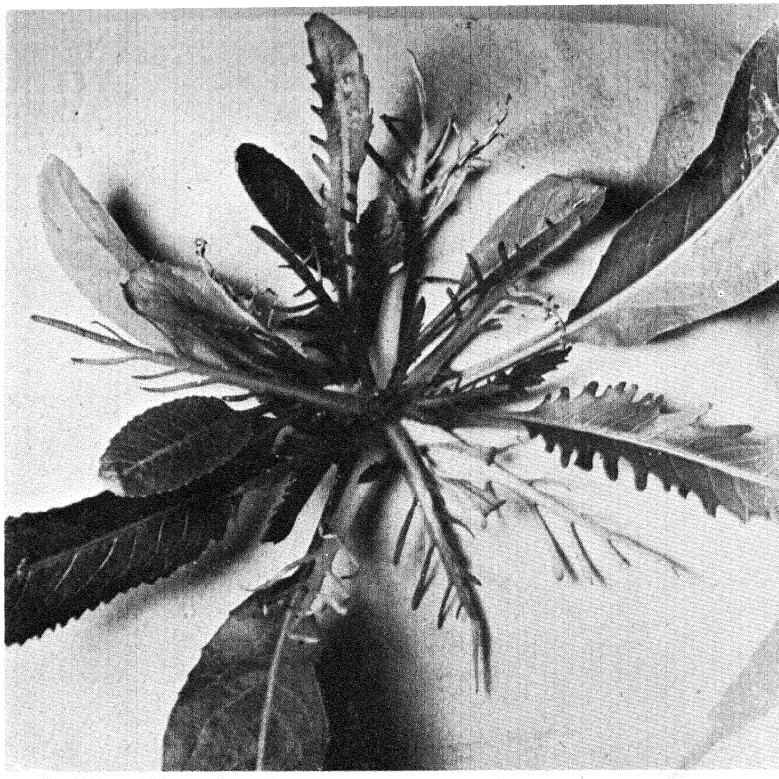


FIG. 51.—Photograph of horseradish plant (*Armoracea lapathifolia*) subjected to repeated removal of young root-tips causing a prolific production of deeply lobed leaves. The unlobed older leaves seen here are normal.

*Photograph by F. A. Barrett, by permission of Professor W. T. Williams, University College, Southampton.*

of changes in the ratio of vein to mesophyll growth-factors with age and position of leaf, a preponderance of the vein factors causing lobing when the mesophyll growth cannot keep pace with vein growth, while suitable proportions of the two factors produce balanced growth and a simple non-lobed leaf.

Another widespread phenomenon is the marked differences in leaf form associated in some plants with differences in environmental conditions under which the plant or organ may grow. This is most clearly seen in plants of inland waterways, where the earlier-formed leaves are usually completely submerged in the water but the later leaves project up into the air. The best example of such plants is the arrowhead (*Sagittaria sagittifolia*), where the submerged leaves are long and strap-shaped. Whenever the leaf reaches the surface, the tip expands and becomes oval; when it projects from the water surface it develops the typical arrowhead outline. In many of the species of water crowfoot (e.g. *Ranunculus bandotii*) there is a comparable limitation of mesophyll development in submerged leaves, which are finely divided and consist of little more than the forking veins, whereas the floating leaves have a considerable blade development as in the ordinary buttercups (see Fig. 50). The provocative suggestion has been advanced (Went, 1951) that the effect of submersion is to allow the "phyllocaline" to diffuse out into the surrounding water, where it is lost from the plant, and mesophyll growth is thereby prevented. This is supported by the observation that if arrowhead is grown in darkness, where presumably no phyllocaline could be produced, the leaves remain strap-shaped, whether they are submerged or not. That an "influence" affecting leaf shape can arise from other organs is demonstrable in the horse-radish (*Armoracea lapathifolia*), which we have seen may often produce lobed juvenile leaves before the mature spear-shaped ones. If a young plant is taken, and the root-tips cut off as fast as they are formed, then such highly lobed leaves are invariably induced to develop and continue to be produced for a very considerable time (Njoku, 1948): this phenomenon is illustrated in Fig. 51. If we apply to this phenomenon the reasoning of Went, we could postulate a site of phyllocaline synthesis in the root-tips.

That the growth of veins and mesophyll are separately controlled is supported by the many different leaf symptoms that arise from virus diseases and the application of certain growth-inhibiting chemicals. In some of these diseases mesophyll growth may be almost completely suppressed, giving a long whip-like leaf (*e.g.* the "shoe-string" virus of tobacco in America): similar blade suppression can also be produced by compounds allied to 2,4-D. If we accept the phyllocaline theory, then these widely different causes act presumably by reducing or destroying the supply of that growth-factor. Similarly, vein growth may be retarded more than mesophyll growth, and this tissue then bulges into large pockets between the main veins: this crisped distorted leaf is characteristic of other virus diseases of tobacco and of cotton, and can also be reproduced by treatment with chemical inhibitors, such as the substituted phenoxyacetic acids. One imagines that disease symptoms such as these can be produced by a reduction in the auxin supply or the availability of other stem and vein growth-factors (caulocalines). It is perhaps pertinent to note at this point that the halogen-substituted phenoxyacetic and benzoic acids, acting on plants at inhibitory levels, can produce a very wide variety of abnormal growth-forms in plant organs, particularly in leaves (Zimmerman, 1951). Those we have mentioned above arise as a result of the disturbance of the vein–mesophyll growth-factor ratio, but there are some that cannot be so explained. Thus, palmate leaves, *i.e.*, leaves composed of several leaflets arranged fan-wise on the top of a leaf-stalk (*e.g.* horse-chestnut), may show fusion of these leaflets, giving a lobed leaf without much reduction in total leaf area (Zimmerman, Hitchcock and Harvill, 1944). More striking still are the large cup-shaped organs which result from the fusion of the margins of several leaves. Fig. 48 shows the fusion of the five sepals at the base of the tomato fruit into one large unsymmetrical lobe as a result of spraying with α-phenoxypropionic acid. In the tomato itself (Zimmerman, 1951), TIBA application induces a ring-shaped fruit closely resembling the American doughnut in shape. Such ring fasciations, as they are called, are extremely rare in nature, and are the result of a much more fundamental action of the chemicals on the meristem from

which these organs are produced (Gorter, 1951). They require much more careful study. Formative effects, very similar to those produced by TIBA, have been claimed for the distantly related compound α-(2-naphthoxy)-phenylacetic acid (Osborne and Wain, 1950).

## ORGAN GROWTH: DIRECT APPROACH

### *The Effect of Growth Substances of Known Constitution*

All the indirect evidence of the foregoing section on the existence of substances specific for the growth of different plant organs has received strong confirmation from the results of a relatively new and revolutionary technique in the study of plant growth problems. In this technique, individual organs or tissues removed from the parent plant are grown for long periods of time on sterile nutrient media, usually in the dark, so that they are isolated from all nutrient or hormonic influences which would normally come to them from other organs and tissues. These "tissue culture" methods depend for their success on the maintenance of absolutely sterile conditions and the continued supply of fresh culture media of the correct constitution. Such conditions are usually obtained by transferring, at frequent intervals, the growing organ or tissue from one sterile culture vessel to another containing fresh medium: in this way indefinite growth can be obtained over long periods of time. This indefinite growth, in which the participating cells traverse the whole cycle from meristematic division to full maturation and differentiation, is therefore quite distinct from the limited extension growth of such portions of organs as oat coleoptile sections and pea-stem sections, etc., as discussed in previous chapters. The difference arises in that, in tissue cultures, some part of the tissue isolated is either already meristematic, or is induced to become so by the applied cultural conditions, whereas in the organ sections, used for the several auxin assay techniques, no such dividing cells are to be found. The perfection of this tissue-culture technique has enabled plant physiologists to study the precise requirements of a number of different plant organs and tissues,

and has enabled us to get one step nearer the delimitation and analysis of composite theoretical growth-factors like the calines.

Although the technique is new, the idea goes back to the famous German botanist, Haberlandt, who first considered the theoretical possibilities of it. Unfortunately, all his early attempts to realize such culture met with failure, due, as we now know, to the fact that mature differentiated tissues (*e.g.* the green cells from foliage leaves) were used and the nutrients employed were not suitable. After twenty years of unsuccessful experimentation, the secret was simultaneously hit upon by two independent workers, Kotte in Germany (1922) and Robbins in America (1922). They realized that the only tissues that might be induced to grow *in vitro* were those still capable of active cell-division at the time of separation from the parent plant (*i.e.* meristematic tissues) and that organic growth-factors, other than simple nutrients, might also be required. Applying these ideas, both workers succeeded in growing roots from isolated root-tip meristems in artificial nutrient media containing sugar, inorganic salts and yeast extract. The growth obtained was strictly limited, however, and it was not until another decade had passed that unlimited growth was attained. After a painstaking series of detailed experiments on the precise optimum concentrations of each of the components of the nutrient medium, a combination was eventually evolved which gave this unlimited growth when the medium was regularly and frequently renewed (White, 1934): this is usually done by removing the isolated root system from the culture medium under strictly sterile conditions and replacing it in fresh medium at regular intervals. Each transfer is called a "passage" (see Fig. 52). Tomato roots have been most consistently used in this work, and by allowing such isolated roots to form laterals and then preparing fresh isolates from these laterals, it has been possible to grow, over a period of years, very considerable quantities of root tissue all from one original root-tip.

Once the secret was out, the technique developed rapidly, and the growth-factor in yeast extract so necessary for continued root growth was soon identified as vitamin $B_1$ (thiamin

or aneurin) (White, 1937). Experiments were soon extended to stem-tips, and now a suitable combination of growth-factors has been worked out to allow indefinite growth of these organs in pure culture in a number of species.

Very much research has also been done on isolated tissue fragments and the growth of undifferentiated tissue masses, or callus as it is called. We have met such callus tissue before in our consideration of wound healing. The indefinite growth of this tissue in isolation was realized almost simultaneously by three independent workers, White (1938) in America, Gautheret (1939) in Paris, and Nobécourt in Grenoble (1939). Callus can be induced to form from a number of different plant tissues. White obtained his from the natural callus or tumour tissue which occasionally forms at a graft union in tobacco, and also from the tumour tissue caused in certain plants by the activities of the crown gall disease organism, *Phytomonas tumefaciens*.* Gautheret has obtained his from many sources, viz. the cambial tissue of many woody species such as willow (*Salix capræa*), the stems of herbaceous plants such as tobacco (*Nicotiana tabaccum*) and *Antirrhinum*, and the storage organs of certain vegetables such as carrot, chicory, *Scorzonera*, etc.; Nobécourt obtained his from carrot. The tissue formed is usually compact and made up of regular undifferentiated cells in which occasional isolated conducting elements are found. Under some growth conditions it may develop into a loose, friable, cancerous mass, in which the cells are either swollen and rounded or grown out into filaments like those of a mould fungus.

In all these successful growth media, not only were sugars and mineral salts necessary as normal growth nutrients, but certain organic growth-factors had also to be present in very small amounts. The accessory factors most commonly required are vitamin $B_1$ (as used in isolated root-growth), vitamin $B_6$, nicotinic acid and glycine. One fundamental deduction that we can make from these facts is that such tissues

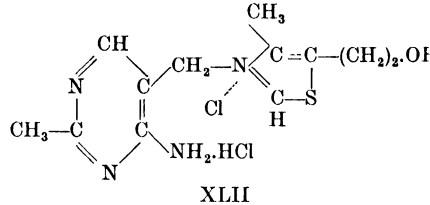
* This tumorous tissue was grown free of the causative organism and shown to be capable of more rapid growth than normal tissue, thus demonstrating for the first time the possibility of true plant cancers. See review by Gautheret (*Endeavour*, 1950).

and organs are incapable of making their accessory growth-factors for themselves from the simple sugars and mineral salt nutrients provided. This is carried out in the normally growing plant in organs possessing the appropriate enzyme mechanisms, *i.e.* in most cases in the leaves. It is not surprising that tissues from different organs have both qualitatively and quantitatively differing growth-substance requirements. To quote only one example, Gautheret (1942 *a*) has shown that for some tissues addition of IAA is necessary for continued growth. This applies to tissues from the tuber of the Jerusalem artichoke (*Helianthus tuberosus*) where a concentration of about 1 part in 10 million produces optimum growth. Cultures of isolated roots of any species, however, do not require the supply of IAA and show optimal growth in its complete absence. What is even more interesting is that, after protracted growth of artichoke tuber tissue in the presence of auxin, it eventually acquires the capacity to make its own auxin and thereby to continue indefinite growth without an external supply (Gautheret, 1947).

It will be appreciated that this new approach to tissue growth offers great opportunities for the study of specific growth-factors, and the intriguing results so far obtained indicate the complexity of requirement that may exist even between one tissue and another in the same species. There is little point in further elaboration here, and interested readers should refer to the two manuals on tissue culture by White (1943) and by Gautheret (1942), and to some of the many recent short reviews that have appeared (Gautheret, 1947, 1950; White, 1951). We must now return to our consideration of the specific growth requirements of individual plant organs.

(a) *Growth Requirements of Roots.*—Since the first demonstration that vitamin $B_1$ was essential for the continued growth of excised tomato roots, a very considerable number of species of plant have been similarly studied. For many the exact requirements in growth substances have not yet been worked out, but for those fully investigated it would seem that one or two of the following three substances, all vitamins, are necessary (Robbins, 1951).

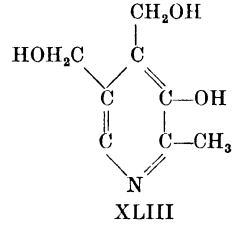
The first is vitamin $B_1$, also called thiamin and aneurin, and it has the following formula (XLII):



XLII

It was the first vitamin discovered, and is now known to act in both plant and animal as an essential component in an enzyme system (decarboxylase) responsible for one of the steps in the breakdown of carbohydrate, etc., and therefore in the provision of growth energy. It is widely distributed in nature in all actively growing tissue; lack of it in mammals (including man) gives rise to a wide range of disease symptoms, known collectively as beriberi, of which the most serious is nerve degeneration. It is very prevalent in the East, where polished rice poor in this vitamin is eaten, and was the scourge of Japanese prisoner-of-war camps in the second World War.

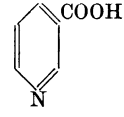
The second substance necessary is vitamin $B_6$, or pyridoxine, with the following formula (XLIII):



XLIII

It is a member of the "vitamin $B_2$ complex"; *i.e.* it is an essential growth-factor for some micro-organisms; for instance, the lactic-acid bacterium (see Schopfer, 1943).

The third factor is nicotinic acid, or niacin, which has the formula (XLIV):



XLIV

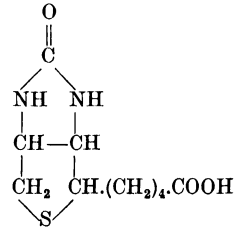
A deficiency of this vitamin causes the disease pellagra in man: this was, at one time, serious among the black population of the southern states of America. It is an essential growth-factor for many micro-organisms. Its biochemical function in the organism is as a component in yet another enzyme system taking part in carbohydrate oxidation and, therefore, the provision of growth energy.

The requirements of the roots of different plant species for these three vitamins vary considerably. Some species will not grow in culture unless all three are provided. The majority need thiamin but may be able to dispense with nicotinic acid and/or pyridoxine. White clover, on the other hand, can grow in the absence of thiamin but needs nicotinic acid, while flax can grow slowly without any of the three, although growth is greatly enhanced by them. As a rule, the growth of all roots is stimulated by suitable concentrations of these compounds. These phenomena are well illustrated in tomato varieties in Fig. 52. It seems that roots of different species possess, in varying degrees, the capacity to build up these growth-factors from simple nutrient materials. It should, however, be borne in mind that any one isolated root system will not continue to grow through an indefinite number of passages, but that the apical meristem eventually loses its capacity to produce new tissue, and growth in length stops. The exact reason for this is not yet known, but it is not impossible that it may be due to deficiency of yet another unidentified growth-factor, although obviously there are other explanations.

In most roots the power to synthesize thiamin seems to be very slight or lacking altogether, so that in normal growth the supply of this factor must come from the aerial parts of the plant: the seat of synthesis there is most probably the mature leaves, from whence it is transported in the cortical bark tissue. This has been shown clearly by studies on the distribution of thiamin in the tomato plant, and the effect on that distribution of killing a small ring of bark tissue by steaming it ("ringing" or "girdling") at several points on the plant. Such a girdle of dead bark cells between roots and mature leaves causes an accumulation of thiamin above the point of ringing, showing that the treatment has made a blockage in the downward flow

of the vitamin from leaves to roots (Bonner, 1942); this demonstrates thiamin to be a "hormone" in the original sense of the definition (Thimann, 1947). With pyrodoxine, it is at present not possible to say whether those roots, which do not require an external supply for growth, can actually synthesize it themselves, or whether indeed they can dispense with it altogether in their growth processes. In any case, in the tomato, the vitamin seems to be synthesized in the mature leaves, whence it is transported in bark tissue to both the roots and the stem apex (Bonner and Dorlund, 1943). The varying requirements of roots of different species for nicotinic acid is undoubtedly a reflection of their several capacities for its manufacture, as it is most improbable that any growing cell could dispense with such an essential biochemical factor altogether. Again, in those roots in which growth in isolated culture is stimulated by an external supply, the full growth requirements for this compound are presumably provided from the organs above ground.

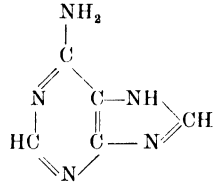
It is appropriate to mention here the observations that the initiation of roots by auxins in pea-stem sections is greatly augmented by other growth substances. Firstly, the effect of the vitamin biotin, which is apparently a co-factor in this process, has already been discussed in Chapter 5. Biotin (sometimes called vitamin H), which is another member of the vitamin $B_2$ complex, is also an essential growth-factor for yeasts and other micro-organisms. Its formula (XLV) is:




XLV

There seems little doubt that this vitamin is synthesized in young leaves, particularly in the spring. If it is an essential co-factor of auxin in root initiation and is translocated into the root systems from the leaves, then it has some of the properties of

the "rhizocaline" mentioned in the first section of this chapter, although root-forming activity is certainly not likely to be its only function in the plant. A similar argument applies to the substance adenine and its derivative adenosine, both of which can augment the root-forming activity of auxins in stem sections (Galston and Hand, 1949). Adenine, which has the following formula (XLVI), is actively synthesized in leaves: it is an essential component of a number of enzyme systems, which are fundamentally concerned with the provision and



XLVI

utilization of chemical energy in growth processes. This could easily be another component of "rhizocaline".

(b) *Growth Requirements of Stems.*—The growth in isolation of the apical meristems of stems is complicated by the frequency with which such tips develop roots: since, when this happens, one is dealing with a complete plant, the whole point of the investigation is lost. There are, however, plants that are relatively free from this complication, and stem-tips of *Asparagus* (Loo, 1945) and also of the parasitic dodder (Loo, 1946) have been grown successfully for long periods on simple nutrient media. Cultures of these can be kept growing in diffuse light for very long periods, and these results have led to a denial that a "caulocaline" supplied by the roots is necessary for stem growth. Some essential factor is, however, synthesized in the light, since these isolated tips cannot maintain indefinite growth in the dark, even when supplied with yeast extracts. Such a factor, which still remains unidentified, could be called "caulocaline", although, of course, it may have nothing to do with the substance supposed to come from roots.

(c) *Growth Requirements of Leaves.*—The growth of veins

seems to be under the control of auxins, whereas the intervein portions of the blade are controlled by another growth-factor. Attempts have been made to identify this latter factor by studying the growth of small discs cut from the lamina of radish or tobacco leaves floated on various nutrient solutions (Bonner and Haagen Smit, 1939). A range of vitamins, including thiamin, were found ineffective, but growth was promoted up to a maximum of 20 per cent. increase in surface area by a number of amino-acids of which arginine proved to be the most effective. The most active substance tried was the purine base adenine (XLVI), which we have already seen to be active in root initiation. Activity was claimed for dilutions as low as 2 parts in 100 million. An extension of these experiments, in which whole plants of *Cosmos* sp. were grown in sand watered with dilute solutions of this compound (1 part in 10 million), showed a considerable increase (about three times normal) in the leaf growth. Unfortunately, a repetition of these latter experiments in Holland (Kruyt and Veldstra, 1947 *a*) yielded only small increases of the order of 15 per cent., which, furthermore, could be obtained by other synthetic chemicals resembling adenine in structure, *i.e.*, $\alpha$- and $\beta$-naphthylamine. It is very unlikely that these naphthalene derivatives can substitute for adenine in the enzyme systems of which it is a component, so that this small non-specific action is not due to an effect on such systems. Clearly, then, adenine is not yet finally established as a leaf growth-factor of the "phyllocaline" type.

That adenine does, however, play a part in the growth of buds has been demonstrated in two independent researches. In tobacco stem and in carrot and radish root segments in sterile nutrient solutions, the formation and growth of buds can be very greatly augmented by the addition of adenine at concentrations of 10 to 40 p.p.m. (Skoog and Tsui, 1951). Auxins exert their usual inhibiting action in bud development, but this inhibition can be completely overcome by high concentrations of adenine. Similar effects are produced in lateral buds growing on cut pea-stems when the cut ends of the stem are supplied with sucrose and adenine at the rate of 10 p.p.m. Even apical buds, detached and floated on the solution, show a marked

growth stimulation by the same compound (Galston and Hand, 1949).

The fact that adenine, and possibly some of the vitamins, *e.g.*, thiamin, play a part in the growth of *several* organs, *i.e.*, root, leaf and bud, and thereby fulfil the rôles of the postulated calines, is beginning once more to throw doubt on the existence of specific growth substances for each particular organ. Opinion is now tending towards the concept of a complex of factors, each organ requiring different specific proportions of the various components of this complex for optimum growth.

(d) *Growth Requirements of Plant Embryos.*—The growth and development, from a single fertilized egg-cell, of the young plant embryo in the seed, raises many intriguing problems for the plant physiologist. As in the elaborately organized mature plant, both nutritional and hormonal factors may play equally vital rôles. Apart from such fundamental questions as why one end of the egg should develop into root and the other into shoot, botanists would like to know why development and growth, once started, should not continue and the seed germinate on the parent plant without a dormant period. Another puzzle is what determines the tremendous growth of the seed leaves in some plants, while the embryo proper (root and shoot) remains small and undeveloped. In recent years, now that the failure of many attempted crossings between species and varieties has been traced to the abortion of the young embryo after successful fusion of male and female nuclei, geneticists and physiologists alike have been concerned with discovering the reasons for this inability, on the part of the hybrid embryo, to grow and develop in the tissue of one of its parents.

The study of excised embryo growth promises to solve these questions, although the subject is still in its infancy. It has been known for a long time that once embryos have reached a certain size they are capable of developing into mature plants when supplied with mineral salts and sugars. This means that, long before they reach the full size characteristic of the ripe seed, they have already acquired the enzyme systems needed

to synthesize all the complex proteins, hormones, etc., for normal growth. On the other hand, embryos that have not reached this minimum size must be supplied with the whole range of amino-acids and vitamins. Even so, if the embryo is young enough, it will show no growth on *any* such artificial media. In the embryo of the thornapple (*Datura*) this lower limit of size is about half a millimetre, but smaller embryos ($\frac{1}{10}$ to $\frac{1}{2}$ mm.) can be successfully grown *in vitro* if fresh coconut milk is added to the culture medium. Coconut milk, which is the natural fluid bathing the young coconut embryo, therefore contains an unknown growth-factor, apparently not purely a nutrient, which is essential for these very early stages of embryo growth (van Overbeek, Conklin and Blakeslee, 1942). But this is by no means the whole story, since in other species coconut milk is ineffective. Apparently a whole array of these early growth-factors may exist, and one forms, therefore, a picture of the embryo acquiring progressively, as it develops, the capacity to synthesize the many components of this complex of growth-factors and becoming less and less dependent on the supply from its parent.

As for the cessation of the growth after the attainment of a certain stage of development, this would seem to be induced by the onset of inhibiting conditions such as high concentrations of soluble materials in the neighbourhood of the embryo, high acidities or the accumulation of chemical inhibitors. The importance of natural growth inhibitors has become increasingly more apparent in recent years, and it may easily be that all growth is controlled by the maintenance of a suitable balance between growth-factor stimulant and natural inhibitor. The abortion of embryos in the incompatible crosses mentioned above may be due to the production of some such specific inhibitor (Blakeslee, 1951) by the parent tissue. There is a wide and almost unexplored field for research in this fascinating aspect of plant development where discoveries of great practical value may be made.

Naturally occurring plant growth inhibitors form the subject of the next chapter.

CHAPTER 14

# NATURAL PLANT GROWTH INHIBITORS

## INTRODUCTION

So far, in our consideration of the normal rôle of plant growth substances, we have been concerned mainly with direct growth promotion. We have, nevertheless, noted some inhibition phenomena associated with auxin action, namely the growth dominance of apical buds, root-growth inhibition and possibly the inhibition of flowering, but it is not yet established by unequivocal evidence that direct auxin inhibitions are an integral part of normal growth control in the plant. Whatever the future may decide on this point, there is no doubt that growth-inhibiting substances are of very wide occurrence in plants, and it is plausible, indeed logical, to assume that they play their part in regulating the initiation and growth of plant organs. Such an idea of a dual balanced control is by no means new, since it had already been formulated at the turn of the century by Czapek (1903), the famous Czech botanist, who gave the name of "negative catalysts" or "anti-enzymes" to these hypothetical inhibiting substances. We have already met some of these specific inhibitors. That responsible for the natural dormancy of potato tubers is one (see p. 166). A theoretical inhibitor, not auxin, supposed by some workers to be the effective agent in apical dominance phenomena, is another (see Thimann, 1939).

The presence of growth inhibitors in plant tissues, and the fact that they are extracted by the same techniques as those employed for the auxins, may account for the inconsistencies which are apparent in the literature concerning the concentrations, distribution and nature of the naturally occurring auxins (see Chapter 3). It also indicates the need for greatly improved techniques to separate inhibitors from auxins before absolute reliance can be placed upon the *Avena* test. Chemical separation is difficult to apply, since the exact constitutions of

the inhibitors are very largely unknown. Physical methods have been employed using different rates of diffusion of inhibitor and auxin into agar, and there is distinct promise that the application of chromatographic techniques may be of great help. Preliminary experiments using alcohol solutions with aluminium-oxide columns, and petrol-ether solutions with chalk or sugar columns, have enabled extracts of leaves of Brussels sprouts and elder (*Sambucus nigra*) to be separated into a number of distinct growth-substance components. These components were each separately assayed using the *Avena* test, the growth-promoting auxins giving a negative curvature (away from applied growth substance), while the growth inhibitors gave a positive curvature. Sprouts yielded two auxins, one of which was IAA; a growth inhibitor was also present. From elder extracts, in addition to the auxins, two distinct growth-inhibiting components were separated (Linser, 1951).

One test recently devised has been claimed to allow the estimation of auxins and inhibitors at one and the same time in the same extract (Moewus, 1948). By using a very wide range of dilutions of the extract containing both types of growth substance, the effect of concentration on the growth of the roots of selected cress seedlings can be followed. Such growth concentration curves show significant growth stimulations in very high dilutions, and the position of this growth peak can be used to calculate auxin concentrations in the mixture (see Chapter 2). Having fixed this point, the relationships of growth inhibition to concentration in the low dilution ranges is compared with that of a standard curve for IAA; any departure from this curve (usually a greater inhibition than given solely by comparable auxin concentrations) is held to be due to non-auxin inhibitors, the concentration of which can be calculated from the degree of this departure. This inhibitor assay is of doubtful validity, since it depends apparently on the separate inhibitions (*i.e.* by auxin and non-auxin inhibitor) being additive. Even though this appears to be true for some of the compounds tested (*i.e.* coumarin; see later) it need not necessarily hold for all of the many inhibitors which undoubtedly occur in plants.

The difficulties attendant on the separation of stimulant and

inhibitor are considerable, especially since recent work indicates a close chemical relationship between some of those inhibitors and IAA. As early as 1939, an inhibitor was isolated from cotyledons and leaves of radish, and was shown to release auxin on hydrolysis with alkalies (Stewart, 1939). Workers in Germany have also isolated from seedlings of *Zea Mays* and *Avena sativa* and from potato tubers, a similar inhibitor which liberates auxin when left in contact with coleoptile sections (Funke and Söding, 1948). A further complication is that the inhibitor seems to be formed by the combination of the auxin with a second inhibitor of unknown constitution (Tegethoff, 1951). The first inhibitor, which has been unfortunately named "anti-auxin" by its discoverers (Funke and Söding, 1948) and also "inhibitor-auxin complex" (Larsen, 1951), has therefore the essential properties of an auxin precursor. It is resistant to both hot alkali and hydrogen peroxide, and is therefore a much more stable molecule than the auxin to which it gives rise. The splitting of this auxin-precursor, presumably by an enzyme in the living tissue, makes biological testing of its inhibiting activity very unreliable, even if complete separation from all growth-promoting substances has been achieved, since one can never be quite sure that growth-promoting auxins may not have been formed from it during the course of the test.

There is, however, a class of natural inhibitor, which has, apparently, little direct chemical relationship to the auxins and which plays a very important part in the biology of the seed. These compounds, which have received considerable attention in recent years, are natural seed-germination inhibitors, or "blastocholines" * as they have been termed by their discoverer (Köckemann, 1934). They are simple molecules of diverse chemical affinities, but all possessing the property of preventing germination in relatively low concentrations. In nature they occur in fruit or seed-coats and may be the most important single factors in the control of germination of many seeds. We shall return to a more detailed consideration of these important compounds later in the chapter.

All the above types of inhibitor are concerned with the

* Blastocholine was coined by Köckemann from the Greek words *blastanĕin* = germination and *chōlyĕin* = prevent.

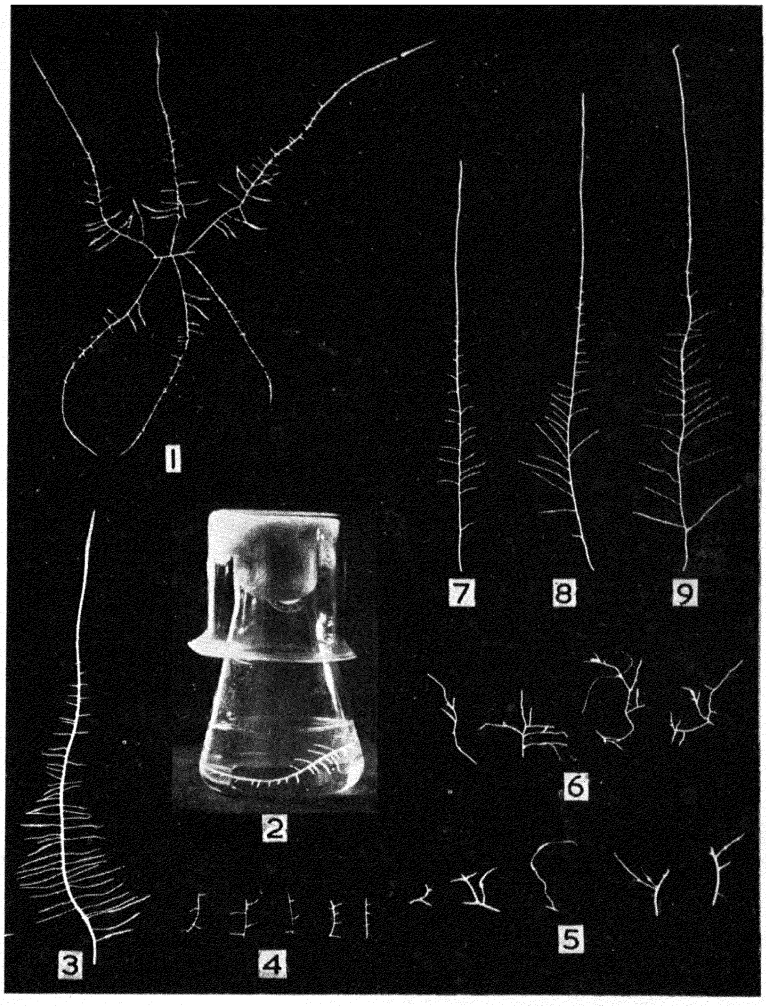



FIG. 52.—Culture of excised tomato roots (*Lycopersicum esculentum*).

1–3. Roots of *Lycopersicum esculentum* (var. Best-of-All) grown in a modified White's medium.

1. A 7-day-old culture from a sector of mature root. 2. Culture flask containing a tip culture. 3. A 7-day-old tip culture.

4–9. Roots of *Lycopersicum esculentum* (var. Pritchard) grown in a medium containing mineral salts and sucrose, together with various combinations of the growth-factors thiamin (Vitamin $B_1$), pyridoxine (Vitamin $B_6$), niacin (nicotinic acid) and glycine. Growth-factors present are as follows:—

4. Pyridoxine, niacin and glycine (2nd passage). 5. Thiamin (4th passage). 6. Thiamin and niacin (5th passage). 7. Thiamin and pyridoxine (7th passage). 8. Thiamin, pyridoxine and niacin (7th passage). 9. Thiamin, pyridoxine, niacin and glycine (7th passage). Note that the maximum growth is obtained when all four growth-factors are present.

*Photographs by permission of Drs. H. E. Street and W. G. Boll, Manchester University.*

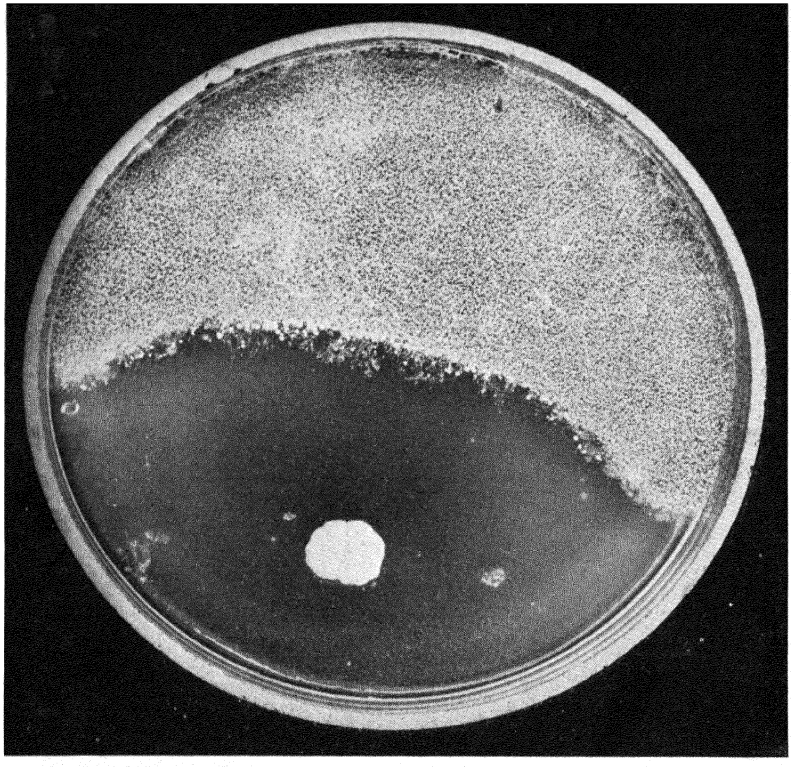


FIG. 53.—The phenomenon of antagonism in the growth of micro-organisms.

The photograph shows a petri dish of nutrient agar on which two micro-organisms are growing. The large crescent-shaped colony is of the rapidly growing onion disease fungus, *Botrytis allii*. The smaller circular colony is the slower-growing soil organism, *Streptomyces* sp. In the absence of the latter the plant pathogen would have covered the dish, but all growth has been prevented here in an area surrounding the *Streptomyces*. This is due to the diffusion of a fungistatic substance from the cells of the latter into the agar forming a wide circular area of growth inhibition around the colony.

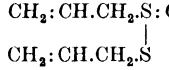
*Photograph by permission of Dr. P. W. Brian, Imperial Chemical Industries.*

growth of the particular plant organ with which they are associated, and one could regard them as normal components in the hormonic growth-control system of the plant cell. There is, however, a very large class of growth inhibitors that cannot be so categorized. These are substances which are produced by one organism and which bring about growth inhibitions or even death in another, sometimes quite unrelated, organism. This phenomenon, although familiar to botanists for a long time, came recently into great prominence during the second World War, when it was shown that a chemical substance, produced by the mould *Penicillium notatum*, completely inhibited the growth of a large number of bacteria causing disease in man. The fact that this compound, later christened penicillin, was harmless to man himself, enabled it to be injected into infected individuals, with the startling curative results that the general public have long since come to accept as commonplace. Thus, a vast new branch of biological science sprung up almost overnight, and, in the intervening ten years or so, a multitude of moulds and other micro-organisms have been studied and their chemical products tested for inhibiting action on pathogenic bacteria. Many such inhibiting compounds or "antibiotics" have been isolated, but only a few have proved to be of practical use, since the majority are toxic to man. The most important of these new drugs are penicillin from *Penicillium notatum* and streptomycin from the soil micro-organism *Streptomyces griseus*.* The chemical structures of some of these compounds have been worked out, and some have even been synthesized, *e.g.* penicillin and chloromycetin. What is striking about them is that they are all of diverse chemical structure,† and it is not surprising, therefore, that some are quite specific in their action on different classes of organism and this may make them of great value medicinally if they will kill disease organisms but not harm the diseased animal. Most of these antibiotics are, however, general poisons and are toxic to a greater or lesser degree to all organisms.

* *Streptomyces griseus* belongs to that group of organisms known as Actinomycetes. These are very simple organisms very closely related to certain groups of bacteria. Their natural habitat is the soil.

† The interested reader will find the subject treated in detail in Waksman (1948) and Brian (1951).

"Antibiotic" action is not, moreover, confined to the interaction between micro-organisms. Not only do bacteria and fungi produce inhibitors which prevent the growth of other micro-organisms, but higher plants may also have in their cells specific chemical substances which will prevent the growth of certain classes of micro-organism. For example, garlic (*Allium sativum*) produces a substance called allicin to which the formula (XLVII)



XLVII

has been given and which is inhibiting to a very wide range of bacteria. Many families of higher plants contain species which possess similar antibiotic substances. Furthermore, a very considerable body of evidence is accumulating to show that specific chemical compounds of this sort may also inhibit the growth of other higher plants of quite unrelated families or genera. The liberation of such substances into the soil on the death of the plant producing them, or their diffusion out of the roots of living plants, may result in their accumulation in the soil and a resulting toxic action on the roots of sensitive plants in the neighbourhood.

Now these antagonistic inhibitors are in somewhat of a different class from those described at the beginning of the chapter, *i.e.* those associated with auxins in the growth control of the flowering plant, the blastocholines, the dormancy inducers, etc. Here the inhibitors seem to be by-products of metabolism to which the generating organism is often very largely or sometimes completely indifferent; such indifference is not shared by other plants with a different biochemical constitution. In these latter sensitive organisms the by-product, owing to its molecular shape, clogs up certain essential enzyme systems, and so prevents the normal growth processes from going forward. Such clogging may happen in the organism producing the antibiotic but not until much higher concentrations are reached. The reason for the insensitivity of the generating organism to its own metabolic by-product is by no means clear and probably rests on quite different causes in different plants.

It is possible, for example, that the particular sensitive enzyme system is not essential for its own growth or that it possesses an alternative non-sensitive enzyme system not inhibited by the "antibiotic". This is a fascinating field for biochemical study as yet almost unexplored. Whatever the underlying causes, the final effect is clearly apparent. The organism, which by some happy evolutionary chance has acquired the power of synthesizing and, at the same time, growing in the presence of such growth inhibitors, will gain a great advantage over its competitors in the acquisition of nutrients. In the soil, for example, in the absence of antibiotic production, the efficiency with which it can utilize the limited nutrient supply will determine the success of any one species of organism. If, on the other hand, it suppresses the growth of any of its competitors by means of a chemical inhibitor, it gains an overwhelming advantage in the struggle for existence. The significance of such chemical factors in the continuous competition of soil organisms and/or higher plants has been very largely overlooked in the past. We shall be considering it again in the next chapter.

## GERMINATION INHIBITORS IN SEEDS AND FRUITS

A phenomenon of almost universal occurrence in the plant kingdom is the inability of reproductive structures, such as spores and seeds, to germinate and grow into new plants while still associated with the organs on the mother plant in which they are borne. With the exception of a few species of higher plant that are confined to the salt swamps (mangroves) of tropical estuaries and seacoasts and which produce "viviparous" seedlings, the seeds of higher plants will not generally germinate while still within the fruit or pod, even though they may be fully mature and capable of immediate germination when shed. In many plants this state of affairs is induced by a germination inhibitor present in some part of the fruit. In the pear, apple and fig, for instance, it is present in the fleshy part of the fruit. Indeed, blastocholines in fruit flesh are of very wide occurrence, a recent survey revealing that they have so far been demonstrated in 33 genera of 16 families from 16 widely spaced orders of plant (Moewus, Moewus and Schader, 1951). In the

majority they can be found also in the juice of the fruit as in the tomato, the orange, the berry of mountain ash, etc. On the other hand, in very many seeds an inhibitor is resident in the seed-coat itself, as in cabbage, lettuce, clover, coffee, etc. In many plants, where the "seed" of commerce is really a fruit, *e.g.* wheat, buckwheat, sunflower and beet, the inhibitor is in the fruit "coat". A comprehensive survey of the occurrence of such inhibitors in higher plants is given in a recent review by Evenari (1949). In the lower plants the same phenomenon is widely observed. Spores of ferns and mosses will not germinate when still within their containers (sporangia, capsules, etc.). In the small, green, ribbon-like liverwort *Marchantia*, which grows on the surface of damp soil in shady places, reproduction may take place by the development of minute, detachable green "buds" (gemmæ), formed inside special cup-like containers on the upper surface of the ribbon. These, too, will not develop into new liverwort plants until they have been detached and separated from the parent plant. Presumably in all these plants the controlling agent may be a specific inhibitor produced by the parent tissue. In the green water plant *Chlamydomonas* (see p. 312) the resting body produced after sexual fusion also contains a germination inhibitor. In one variety this can be washed away by flowing water, but in another it cannot escape through the thick coat until the spore has been subjected to a certain minimum temperature. Subsequently the washing away of the inhibitor allows the spore to germinate when more favourable temperature conditions return (Moewus, 1950 *a*).

In seed plants the main function of these inhibitors, when present in fruit tissue, would seem to be the prevention of premature germination before adequate dispersal is attained. A correlated effect in some plants is the spreading out of germination over a long period, amounting to years in some cases. Thus in charlock (*Sinapis arvensis*) some seeds may be retained in the pod when it falls. Those that are freed at once germinate immediately, while those retained in this way do not; these latter seeds will germinate only when the pod has rotted or the inhibitor has been washed out by prolonged exposure to rain. In this way germination may be spread over at least two years

(Evenari, 1949). This phenomenon may be of widespread occurrence. Mechanically cracking, scratching or otherwise rupturing the seed-coat—a necessary prelude to germination in many seeds—may act purely by allowing the inhibitor to escape, and this may be just as important for the germination process as facilitating the entry of water.

Inhibitors present in the seeds themselves may play an all-important rôle in those species that require special external conditions for their germination. This is particularly apparent in seeds that are sensitive to light. Lettuce is an example of the type of plant which, under certain conditions, shows a much higher percentage germination in light than in darkness. The most likely explanation of this phenomenon is that light destroys a germination inhibitor, present in lettuce seed, which is maintaining the seed in a dormant state in spite of otherwise favourable conditions. This theory is supported by a number of experimental facts. Firstly, the seed does contain a blastocholine; secondly, non-dormant seeds in the dark can be made dormant by treatment with a number of known blastocholines (Nutile, 1945; Weintraub, 1948) including the widely occurring coumarin: lastly, induced dormancy can be very largely removed by exposure to light (Nutile, 1945; Weintraub, 1948). In the other type of seed, in which germination is inhibited by light, we would need to postulate either the production of an inhibitor in the light or the photo-sensitization of one already present. That the latter might be the true state of affairs is suggested by several experiments in which blastocholines extracted from beet seed, from the seeds of the dark-germinating *Phacelia tanacetifolia* and from red kidney bean (*Phaseolus vulgaris*) seeds (Siegel, 1950) are much more effective in the light (see Evenari, 1949, pp. 178–9). We must not lose sight of the fact, however, that these natural inhibitors, in spite of their obvious importance, are not the only factors controlling seed dormancy. The necessity for caution in our approach to the subject has been stressed by recent work on apple seeds, which require an exposure to a 60-day "after-ripening" period under cold moist conditions before they will germinate. One of the contributing causes of the subsequent breaking of dormancy seems to be the disappearance of a natural inhibitor from the

embryo itself and the leaching of a similar inhibitor from the seed-coat (Luckwill, 1952). But excised embryos from seeds not exposed to "after-ripening" conditions will not germinate, even though they contain no inhibitor. This germination is apparently governed by the production of a growth-stimulating auxin, triggered off by the cold temperature treatment. We are only now just beginning to get a glimpse of the complex of chemical factors underlying seed germination.

It is appropriate to recall at this point the natural inhibitor obtained from the tuber of the dormant potato and mentioned in Chapter 8. Although the constitution of this inhibitor is still unknown, there is no doubt that it performs the same function as the naturally occurring seed-germination inhibitors, namely the maintenance of the reproductive structure in a dormant state until conditions are optimal for the growth of the plant arising from it.

The rôle of inhibitors, present in seeds, in the struggle for space in natural plant communities has already been hinted at. The precise importance of this factor is very difficult to assess, and very little systematic work has been done on that aspect of ecology.* A number of clear demonstrations of this chemical antagonism between seeds are known from laboratory experiments. Thus, seeds of beet (*Beta*) will inhibit the growth of rye grass and campion (*Melandrium* sp.) when placed in the same pot of soil. Similarly, wheat and rye grass will suppress the germination of certain weed seeds, *e.g.* corn camomile (*Anthemis arvensis*) and scented mayweed (*Matricaria inodora*). Red kidney bean seeds will inhibit the germination and growth of flax and wheat, and seeds of *Viola* will also inhibit the germination of wheat. These few examples stress the complexity of possible interaction in a natural plant community.

### GROWTH AND GERMINATION INHIBITORS FROM PLANT ORGANS OTHER THAN SEEDS AND FRUITS

The seed interactions mentioned above lead naturally to the wider question whether a similar chemical antagonism exists between plant species in the later stages of their development

* Ecology is the scientific study of the interrelationships of the organism and its natural environment and their mutual interactions.

and at maturity. Is it possible that plants may excrete from their roots toxic chemicals that retard the growth of other susceptible plants growing with them? Here, indeed, we find, accumulated over the last century, a considerable amount of evidence of marked antagonisms between crop plants and their associated weeds. The decline in vigour and productivity of crops grown continuously on the same soil, in spite of adequate cultivation and fertilization of that soil and the depressing effect of some crops (*e.g.* maize, rye and buckwheat) on certain succeeding crops (*e.g.* fruit trees, onions, tomatoes), suggest the possibility of the accumulation in the soil of a growth-inhibiting factor from the roots of the offending plants.* As early as 1832 the famous French botanist, De Candolle, pointed out the apparent specific inhibition of flax by spurge (*Euphorbia* sp.) and of oats by thistles (*Cnicus* sp.) and postulated the production of specific toxic substances by these weeds. However, his ideas were not accepted at the time, and for the remainder of the century competition for essential inorganic nutrients was held to underlie all such antagonistic effects. Interest in the possibility of excreted toxic substances was aroused in America at the beginning of the present century by the loss of fertility in certain soils as the result of continued cropping of one plant species for a series of generations. Workers in the U.S. Department of Agriculture took such soils, made water extracts of them and showed that these extracts prevented the growth of wheat seedlings (Livingstone, 1907; Schreiner and Reed, 1907). Some of the toxic substances were isolated and identified with known organic compounds, *e.g.* picolonic acid, salicaldehyde, vanillin and dihydroxystearic acid, and shown to be sufficiently toxic to plant growth to exert similar inhibitions at the concentrations found in the soil (Schreiner and Reed, 1908). Unfortunately, this work produced no evidence that the inhibitors had their origins in the plants grown previously in those soils. The part played by exhaustion of nutrients in the loss of soil fertility was also

* This aspect of plant growth inhibitors has been comprehensively reviewed by Loehwing (1937) and more recently by Bonner (1950), from whose articles this section has been very largely taken. The reader should consult these papers for the details of references to original work.

ignored and, as a result, the implications of this work were not seriously considered at the time. In spite of this, observations of the toxic effects of plant products in the soil continue to accumulate in recent years. Thus the old dead roots of brome grass (*Bromus inermis*) liberate into the soil a toxic substance that has a marked deleterious effect on the growth of seedlings of the same species, and this was held to account very largely for the gradual loss of vigour of pure stands of this grass growing in the same place for a number of years (Benedict, 1941). Again, it has been observed that young peach trees are very difficult to establish in ground that has previously been a peach orchard, and this fits in with the observation that old peach roots contain a substance inhibitory to the growth of young peach trees (Proebsting and Gilmore, 1940). A similar phenomenon in old *Citrus* orchards has been much more intensively studied within the last few years (Martin, 1950). Soils from these orchards are quite inhibitory to the growth of young *Citrus* seedlings, 50 per cent. to 175 per cent. more growth being made on similar soils from outside the orchard area. Tomato seedlings were not affected in this way and this suggested the action of a toxic agent specific to *Citrus* itself. Other possible causes were fully investigated. Fumigation to destroy harmful soil organisms gave only a slight alleviation of the toxic action. Deterioration of soil structure was ruled out, since the same effects were observed on light sandy soil as well as on medium and heavy soils. Continued water leaching for six weeks gave only slight responses, and extraction with alcohol and ammonium chloride solution had little better effects. Finally, treatment of the soil with 2 per cent. sulphuric acid or 2 per cent. caustic potash, followed by saturation with calcium, was found to restore these orchard soils to the full fertility of the normal soil. The most reasonable explanation of these results is that toxicity of these soils is due to the accumulation of a growth inhibitor specific for *Citrus* itself and excreted by its roots. Presumably this inhibitor is normally adsorbed into soil colloids so that it is not washed out by rain, and it can be removed or destroyed only by treatment with relatively strong acids or alkalies.

Just before the second World War the commercial implica-

tions of such antagonisms in rubber and coffee estates in the Far East led to a comprehensive series of experiments with the different plant species used for "cover crops". In the tropics, where jungle has been cleared and young plantations established, the removal of natural vegetation cover may have very serious effects on the structure of the soil so exposed. The very violent rainfall will, in the absence of suitable precautions, result in serious erosion of the soil, and, in any event, a rapid removal of humus components accumulated slowly in the natural jungle. To combat these effects it is customary, among other things, for the planter to grow, on the soil between and under his young tree seedlings, certain rapidly growing herbaceous plants that form a continuous cover to the bare soil. These so-called "cover crops" bind the soil with their roots, preventing erosion and also allowing the accumulation of humus. The use of suitable species of leguminous plant, e.g. *Centrosema pubescens*, which bring about the enrichment of the soil in nitrogen compounds (see Chapter 15), is an added advantage. The deleterious effect of such cover crops on the growth of young trees had been known since the introduction of the practice. The effect had always been regarded as due almost entirely to competition for nutrients, but a series of widespread experiments in young coffee plantations in Java indicated strongly that toxic root exudates were the basis of this competition (van der Veen, 1935). In a series of laboratory experiments with one of the worst offenders, *Salvia occidentalis*, drainage water from soil in which the plant was growing was shown to have a marked inhibitory and toxic effect on the growth of coffee seedlings when applied to their roots. These results are very similar to those obtained nearly twenty years earlier on the toxicity of grass to apple trees in orchards (Pickering, 1917). All cover crops are fortunately not similarly toxic, and rubber reacts differently from coffee. Some of the worst offenders are *Paspalum conjugatum*, *Cynodon dactylon*, *Salvia occidentalis*, *Passiflora fœtida* and *Amarantus spinosus*.

In Sweden, recent observations have confirmed the excretion of toxic compounds from various grass roots. The investigations were initiated by the observation that rape (*Brassica napus* var. *oleifera* and *B. rapa* var. *oleifera*) germinated and developed

very poorly in fields where couch grass (*Agropyrum repens*) was growing well. Watery or dilute ammonia extracts of dried powdered roots and stolons * showed that these organs contained a toxic substance which prevented the germination of rape seed and allowed it to be attacked and destroyed by moulds (Osvald, 1947 *a*). This suggested that the well-known success of grasses in competition with other species in natural grassland communities might be due to a similar toxin production and led to the study of soil extracts from such grassland and from agricultural land. A particular meadow, dominated by an almost pure stand of red fescue grass (*Festuca rubra*), was chosen, and extracts of the soil made with alcohol and with dilute ammonia: these extracts stopped the rape seeds from germinating, whereas similar extracts from cultivated soils had no effect. The toxic principle was destroyed by heating to 80–90° C. (Osvald, 1949). Some evidence has also been obtained that certain crop plants may be mutually inhibitory to each other's growth when grown in close association in the same plot. Spinach and radish are such a pair, and it has been suggested that the saponins of the spinach and the mustard oils of the radish may be the inhibitory compounds involved (Schuphan, 1948).

All these results so far are of an indirect nature and do not afford direct proof, either that the toxic substances of such soils actually come from the underground portions of the plants concerned or, on the other hand, that the inhibitors extracted from those organs do actually exude into the soil. One recent piece of work leaves very little doubt that such an exudation can take place. In the early days of the second World War, experiments were started in America on the production of rubber by the guayule plant (*Parthenium argentatum*).† In plantations of this species mutual antagonisms were obvious, since individuals in the centre of a block of plants showed much feebler growth than those at the edges. This was not due to competition for water or nutrients but presumably to toxic root exudates, since roots

* Stolon is the botanical name for the long underground stems or runners by which this grass spreads so effectively and rapidly.

† Guayule is a Central American plant of the family Compositæ, which has been used for rubber production for many years. (For a survey of rubber-forming plants, see Bonner and Galston, 1947.)

of adjacent plants did not intermingle but grew out into areas unoccupied by other guayule roots. An elaborate series of pot experiments confirmed this antagonism and the presence of toxic compounds in the soil solution. 1·6 grammes of a highly toxic crystalline material were obtained by soaking 20,000 roots of normal living plants in distilled water. This material was subsequently identified as *trans*-cinnamic acid (XXXIII, p. 69), a normal constituent of the guayule plant. This strongly supported the view that the toxicity of the soil-water under normal conditions was due to an exudation from living roots and not a release due to the death of the old roots. It is interesting to note that the related *cis*-cinnamic acid (XXXIV, p. 69) has the properties of an auxin (see Chapter 3). The toxic action of this exudate seems to be very selective, since tomato is only a hundredth as sensitive to it as guayule itself.

There can be little doubt from this accumulated evidence that roots and underground stems of a very wide range of plant species produce specific chemical compounds that are active inhibitors of seed germination and root growth: it seems highly probable that these compounds are normally excreted into the soil where they may play a very important rôle in the selective suppression of the growth of competing seedlings. There is, however, another source of growth inhibitor in the soil, namely the normal decomposition of plant organs when the plant dies. Such inhibitors may also play an important part in determining the nature of the vegetation growing on that soil. This has been clearly shown recently in America for the desert shrub, *Encelia farinosa*. This plant, in contrast to most other desert shrubs, does not shelter a small colony of annual plants under its branches. This is due to a chemical inhibitor, contained in its leaves, which passes out from the leaves into the surrounding soil after the leaves have been shed and covered by the sandy soil under the bush. Analysis of leaf extracts showed that the active inhibitor was a compound new to the organic chemist * and one which could bring about inhibition of growth in such plants as tomato in concentrations as low as 0·002 per cent. (Gray and Bonner, 1948). In one plant that has been studied, *Artemisia absinthium*, the wormwood, inhibitors present in the

* Actually 3-acetyl-6-methoxybenzaldehyde.

leaves may even be washed out by rain and severely stunt plants of certain species growing under it (Bode, 1939).

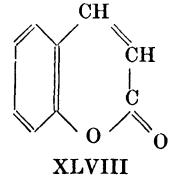
How long these organic substances from living roots or decomposing plant organs can remain in the soil is at present unknown. It is possible that they may disappear quickly under the action of micro-organisms, except perhaps under certain unfavourable conditions (*e.g.* low aeration in water-logged or heavy clay soils). However, when active production is going on, concentrations may be maintained at equilibrium levels high enough to exercise a considerable inhibiting influence on surrounding plants, even under conditions favouring rapid decomposition. There is no doubt that this large field of natural growth inhibitors still remains to be explored, and may yield a host of new compounds useful to man in the selective control of plant growth.

## THE CHEMICAL NATURE OF THE NATURALLY OCCURRING GROWTH AND GERMINATION INHIBITORS

The natural growth inhibitors in plants are exceedingly numerous and correspondingly varied in their chemical structure. This aspect has been comprehensively reviewed in an easily available article by Evenari (1949), but here we can merely indicate the main groups to which they can be allocated.

The vast majority of these substances are simple organic compounds, although in a number of plants inorganic products liberated by enzyme action from plant constituents can be effective. Two important examples of the latter are ammonia, produced from organic nitrogenous materials, and prussic acid from the cyanogenetic glucosides reserves in seeds of certain groups of plants, particularly those to which the plum and apple belong.

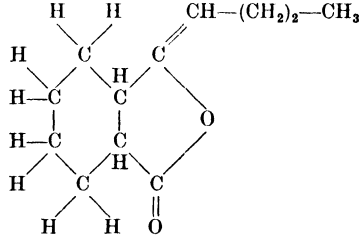
Of the organic compounds, the unsaturated lactones are among the most important, since the original "blastocholine" isolated from the fruit of mountain ash (*Sorbus aucuparia*) belongs to this group. Coumarin (XLVIII), a widely dis-




XLVIII

tributed substance responsible for the scent of woodruff (*Asperula odorata*) and new-mown hay, also belongs here and is highly effective as a germination inhibitor. It has recently received much attention from plant biochemists, since it is the internal lactone, and therefore a close relative, of *ortho*-hydroxy-*cis*-cinnamic acid. We have already seen that *cis*-cinnamic acid has the property of an auxin and that *trans*-cinnamic acid can be a strong growth inhibitor. It has therefore been thought that a study of the biochemical behaviour of these closely related compounds might lead the way to the secrets of the mechanisms of auxin action and of the action of the blastocholines. It has been suggested that *trans*-cinnamic acid itself is the blastocholine of the resting spore of the green alga *Chlamydomonas* (Moewus and Banerjee, 1951). Another point of contact of the inhibitors with the auxins is through the substance auxin A lactone, which is supposed to change in the light, by internal rearrangement of atoms, into an inhibiting substance, lumi-auxon, the proposed formula of which has an unsaturated lactone structure. It has been suggested that the formation of this inhibitor might play an important part in causing the plant curvatures in response to unilateral illumination (Veldstra and Havinga, 1943) (see footnote, p. 51). These are, however, at the moment merely theoretical speculations.

A closely related group of compounds, apparently the germination inhibitors of many seeds of the family Umbelliferæ,* are the phthalides, the most active compound so far isolated being *n*-butylidene-hexahydro-phthalide (Moewus and Schader, 1951 *a*). It has the formula (XLIX):




XLIX

* Umbelliferæ is the family to which carrot, celery, parsnip, parsley and fennel, each with their characteristic aromatic oils, belong.

A group of compounds specific to one family of plants (Cruciferæ) are the mustard oils. These are volatile substances present not only in seeds (*e.g.* the dormancy-inducing substances of *Sinapis arvensis*), but also in the roots of such species as radish, horse-radish, etc. Essential oils from a range of other plant families can also be effective germination inhibitors, *e.g.* those from the skin of the orange and lemon, the flower of the clove, leaves of peppermint, thyme, rosemary and *Eucalyptus* and fruits of fennel. These compounds are very heterogeneous from a chemical standpoint. Some of their vapours are effective in very small quantities. For example, the emanations from certain types of resinous wood (*e.g.* spruce, poplar, etc.) can inhibit seed germination (Weintraub and Price, 1948). Wood is obviously a material to be used with care in germination tests in seed-testing institutions. Certain organic acids are also very effective germination inhibitors, and are usually the active agents in tissues of fleshy fruits. As an example may be quoted malic and citric acids in the apple, or caffeic acid (3, 4-dihydroxy-cinnamic acid) and ferulic acid (3-methoxy-4-hydroxycinnamic acid) in the tomato (Akkerman and Veldstra, 1947). The activity of *trans*-cinnamic acid from guayule has already been noted. The last important group of compounds are the alkaloids, many of which are of value pharmaceutically. Not all alkaloids have these properties, and only a few are strong inhibitors: the most important are cocaine, physostigmin, caffein and quinine.

One important point which emerges from this survey of the chemical nature of inhibitors is the large variety of chemical structure which these inhibitors exhibit. It is obvious, therefore, that the mechanisms of their several actions are correspondingly varied and that the germinative capacity of the seed or the growth of the plant may be attacked through one or more of its complex of essential enzyme systems.

So far, few attempts have been made to find practical use for these naturally occurring inhibitors. In one recent series of tests, coumarin, a substance which can be easily obtained synthetically, showed considerable promise as a dormancy promoter in potato tubers in storage. Dipping of the tubers before storage in a 0·1 per cent. aqueous solution resulted in a

48 per cent. inhibition of sprouting, while treatment with a solution of similar concentration in lanolin prevented sprouting completely (Moewus and Schader, 1951). Whether this can be modified into a practicable and effective storage treatment method remains to be seen.

## SYNTHETIC PLANT GROWTH INHIBITORS

It should perhaps be noted at this point that a considerable amount of empirical biological testing of various unusual chemical compounds has led to the discovery of many active plant inhibitors. Maleic hydrazide has already been discussed in connection with several practical aspects of plant inhibition. Another group of compounds are derivatives of pteroylglutamic acid, *e.g.* 4-amino-9-methylpteroylglutamic acid. These are even more effective than IAA in inhibiting root-growth and, like IAA, are much less effective on stem tissue (de Ropp, 1950). Both maleic hydrazide and these latter compounds seem to act by inhibiting cell-division. A third class of compounds are the substituted benzene derivatives of quaternary ammonium compounds * which are very effective in inhibiting stem-growth in bean seedlings (Wirwille and Mitchell, 1950). Another group of highly toxic organic compounds are certain imidazoline derivatives with long chain aliphatic substitutions in the 2-position, already mentioned in Chapter 9. These compounds are very strong respiratory inhibitors. The necessary word of caution should here be reiterated, that it would be most unwise to adopt these substances for use in practical control of food-crop growth until they have been conclusively proved to be harmless to man.

## ANTIBIOTICS IN PLANT PATHOLOGY

Plant diseases are caused by many different agents, by bacteria, by viruses, by parasitic animals and by fungi. Now more than ever before, when a rapidly increasing world population is outrunning the earth's food-bearing capacity, it is vitally necessary to reduce to the minimum all losses that might result from such disease attack in food-bearing plants.

* The most active compound described in this class is (4-hydroxy-5-isopropyl-2-methylphenyl) trimethylammonium chloride, 1-piperidene carboxylate.

Diseases produced by fungi constitute the majority that the plant pathologist has to combat. They include the rusts, the smuts, the mildews, the blights, and last but not least, the soil-borne wilts that are of particular importance in the tropics. So far, the practical means employed to prevent epidemics of these diseases have been the breeding and growing of strains resistant to the pathogen and the treatment of the surface of the growing plant with a chemical solution toxic to the fungus concerned. The gardener will be familiar with such fungicides as Bordeaux mixture, in which copper salts are the toxic agents. He will also realize the limitations of such sprays, which will merely prevent the infection of the host plant by the spores of the fungus, but can have no effect on the fungus parasite that is established within the tissues of the host plant. To attack such deeply seated fungi, botanists have tried injecting a number of synthetic organic compounds, inhibitory to their growth *in vitro*, in the hope that they might similarly inhibit the growth of the parasite without harming the host. This was tried, for example, in an attempt to stop the ravages of the notorious Dutch elm disease by injecting the chemical solution into the tree trunks, but there was little permanent success.

With the advent of the antibiotic and its remarkable success in the treatment of human diseases, new hope has come to the plant pathologist and there is distinct promise that specific antibiotics, with which susceptible crop plants may be treated and thereby rendered immune from disease attack, may soon be discovered and developed. Naturally some of the first substances to be tried were those which had been so successful in combating bacterial diseases in man, *i.e.* penicillin and streptomycin. These substances have been applied to disease tissues caused by plant pathogenic bacteria, with some considerable success. Streptomycin is much more effective than penicillin and is particularly active against the soft rots of root crops (caused by *Bacterium carotovorum*) and crown gall disease (caused by *Bacterium tumifaciens*). It has been used with success for dressing tomato seed as a preventative against bacterial canker (caused by *Corynebacterium michiganense*) (see Grossbard, 1951). As far as the writer is aware, no such antagonistic effects have ever been observed on fungal diseases.

It should be noted, however, that streptomycin is not without its effect on the higher plant since a number of independent workers have shown that it prevents the development of the green colouring matter, chlorophyll.

In recent years, due largely to the activities of Dr. P. W. Brian and his collaborators in England, a large number of the fungal products that are specific antagonists of fungal growth have been isolated, and in many the chemical structures have been worked out. The most important of these "fungistatic" * compounds are:—

*Alternaric Acid.*—This is produced by the fungus *Alternaria solani*, which causes a foliage disease in the tomato family. It is highly toxic to plants, and the killing of the host by the fungal parasite may be brought about largely by means of this substance. Although it has a highly specific antifungal action on certain pathogenic fungi, its poisonous action on higher plants precludes its use in the control of the diseases caused by these fungi. It is not antibacterial.

*Frequentin.*—Produced by strains of *Penicillium frequentatum.* It is strongly antifungal but only moderately antibacterial.

*Gladiolic Acid.*—This is produced by *Penicillium gladioli*, a parasite of *Gladiolus* corms. It is also moderately antibacterial.

*Gliotoxin.*—This is produced by a number of fungi including *Trichoderma viride* and *Aspergillus fumigatus.* It is also strongly antibacterial.

*Griseofulvin.*—This is produced by *Penicillium griseofulvum* and others and is very strongly antifungal, causing a characteristic curling growth of the young hyphæ of susceptible fungi. This peculiar growth reaction is very reminiscent of the effect of IAA on the growth of root-hairs in higher plants, and suggests that this compound might be performing a function in the growth of fungal cellulose cell-walls similar to that of IAA in the growth of the cellulose walls of the higher plant, a possibility still to be followed up (Brian, 1947).

* "Fungistatic" is a word which has been coined to describe this class of inhibitor and means "stopping fungal growth". Similarly, the antibiotics penicillin and streptomycin and the synthetic sulphonamides are called "bacteriostatic", since they stop bacterial growth.

*Viridin.*—This is produced by the green mould *Trichoderma viride* and is very specific in its action, being highly active against the fungus causing neck rot of onions (*Botrytis allii*) but much less so to others, including of course itself. In addition, it is not antibacterial.

All these are crystalline organic compounds of relatively small molecular weight and are not proteins. This means, of course, that they can easily pass out from the fungal cells, in which they are synthesized, into the surrounding medium, where they exert their antagonistic effects. The structural formulæ of griseofulvin and gliotoxin only are known at present. A complete list of such fungistatics, giving their chemical properties and structural formulæ as far as known, has recently been completed by Brian (1951).

The fungistatic properties of these compounds have been established by growth experiments with susceptible fungi on nutrient agar. The vital question now is whether such an action can be extended into the tissues of the higher plants, so that the growth of pathogenic fungi (or bacteria) can be prevented. In this respect, great promise is shown by a very recent report from Brian and his colleagues (Brian, Wright, Stubbs and Way, 1951) on the results of experiments with griseofulvin. This compound was chosen on account of its low toxicity to higher plants compared with that of the other known fungistatics. Firstly, it was established that the compound could be taken up by cut shoots of a variety of plants, if they were placed with their cut ends in solutions, and was distributed throughout the whole of the shoot without any obvious harmful effects. Subsequently it was shown that it could be taken up by the roots of oat seedlings and distributed thence to the leaves. Then it was demonstrated that plants of lettuce and tomato, when grown with their roots supplied with a griseofulvin solution, took up the compound and acquired therefrom a considerable resistance to fungal attack. With lettuce, which was subsequently sprayed with a suspension of spores of the virulent rot fungus, *Botrytis cinerea*, all the untreated plants succumbed, whereas at least 60 per cent. of the treated plants remained free from attack. In tomato, similarly treated and tested with *Alternaria solani*, three-quarters of

the treated plants resisted attack, whereas all the untreated ones became infected.

These are splendid beginnings, but it must be noted that as yet the majority of the antibiotic compounds that have been tested are more active in inhibiting the germination of seeds in crop plants (*i.e.* wheat, mustard and clover) than the blastocholine coumarin itself (Wright, 1951). Of the three least toxic compounds (penicillin, streptomycin and griseofulvin) griseofulvin may inhibit germination markedly at 25 p.p.m., while streptomycin, which has no detectable action on such seedlings at that concentration, is markedly toxic at concentrations above about 0·1 per cent., when the development of the green pigment chlorophyll may be partially or even completely prevented (van Euler *et al.*, 1948; Wright, 1951).

However, a new chapter is beginning in man's struggle to grow more food, and we may expect, in the not too distant future, that a whole range of fungistatic compounds may become available for inducing resistance to specific diseases in plants of economic importance. As with penicillin, etc., initial production costs will be very high, but no doubt the organic chemist will develop methods for the synthesis of these compounds, or prepare other related active molecules, which will make their use a practical proposition.

It is an interesting fact that most of the antibiotic compounds so far discovered are produced by micro-organisms that are normal inhabitants of the soil. One wonders, therefore, whether antibiotics are produced by these organisms when growing in the soil and, if so, what part they play in the intense competition which must be going on in the soil at all times between the myriads of organisms which make up its living population. This aspect of plant growth substances will be dealt with in our next and final chapter.

CHAPTER 15

# GROWTH SUBSTANCES IN SOIL

## INTRODUCTION

In the last chapter we saw that the soil may acquire, from many sources, specific growth-inhibiting substances that may exert a marked effect on the growth and development of plants rooted in it. We must now explore this subject in somewhat more detail and consider how far growth substances such as hormones and vitamins may also be present, and whether they exert any effect on the nature and vigour of plant growth. We cannot embark here on the closely relevant subject of the mineral nutrition of the plant and the supply and function of the many inorganic salts necessary for normal plant development. While the majority of plant physiologists would hold that provision of a properly balanced mixture of such inorganic nutrients is all that is required by plants to give maximum growth, and while this may very well be true, yet in the past many claims have been put forward for beneficial effects of organic compounds present in natural manures, and recent evidence suggests that these claims cannot be dismissed as lightly as has been the previous custom. This uncertainty will certainly not be dispelled in this chapter, but it is hoped herein to draw a rough picture of the complexity of what we may call "soil biochemistry" and to indicate what part organic growth regulators of various types and specificities may play in it and in the growth of the organisms concerned.

## THE BIOCHEMISTRY OF SOIL

The ultimate source of all organic matter in the soil is, of course, the dead plants and animals that accumulate in it. The complex proteins and other structural compounds that make up the body of the dead organism do not stay long as such, but are rapidly decomposed by one component of the

soil microbial population liberating a vast complex of simpler organic (and inorganic) products into the soil: these, in turn, may be attacked by other organisms and further broken down into simple inorganic salts, the gas carbon dioxide and water. During this complicated sequence of events, populations of many different organisms may wax and wane as the availability of the particular substances serving them as a source of raw materials and chemical growth energy similarly rises and falls. Micro-organisms themselves die and their proteins enter into the cycle and serve as food for their successors.

Thus, the structure of the soil microbial population will be extremely complex and determined very largely by the nature and amount of organic food available. Many different organisms will have the same food requirements and this will naturally result in intense competition for it. Here other soil factors such as acidity and accessibility of oxygen from the atmosphere will exert a controlling influence and determine the success or failure of any one class of organism. Since the chemical processes employed to obtain energy and raw materials from any given organic source will differ from one type of organism to another, it will be seen that these soil factors will affect the balance of the breakdown products and hence the whole subsequent sequence of soil populations: in this way the final end-products of the breakdown process may differ very widely. To quote an extreme example, in the water-logged soil of marshes, where oxygen is lacking, the gas methane is formed from the decomposition of organic carbon compounds, instead of carbon dioxide and water which are normally formed. There may be present in the decomposing matter, substances that resist breakdown, and these may accumulate temporarily in the soil until suitable organisms capable of attacking them have grown in sufficient numbers. The inhibitor from *Encelia farinosa* leaves is a case in point (see previous Chapter, p. 347). During the growth of soil micro-organisms, specific by-products of their particular metabolism will arise. We have already seen that many soil organisms, such as fungi and actinomycetes, can synthesize large amounts of compounds which are specific growth-inhibitors of other micro-organisms (antibiotics). In Chapter 3 it was noted that IAA was similarly produced by

certain moulds. It is possible that these by-products may also be formed in soil and will diffuse out from the cells into the soil solution and may there exert a regulating effect on the growth of other soil organisms (Brian, 1949).

Micro-organisms are not the only living things that can contribute simple soluble organic molecules to the soil solution. A large variety of such compounds can diffuse out from the roots of higher plants. Amino-acids, the building bricks of proteins, have been demonstrated by many independent experimenters to pass out into the soil in this way. It has long been a practice to grow leguminous plants like clover side by side with non-leguminous crops, since the latter grow much better as a result of the association. In the nodules which are caused to form on the roots of these leguminous plants after invasion by specific associated bacteria, atmospheric nitrogen is converted into organic nitrogen compounds and there seems little doubt that the benefit derived by the associated non-leguminous crop is due to augmented nitrogen nutrition provided by material (presumably amino-acids) diffusing from the leguminous nodules (Nicol, 1934). Pea roots have also been shown to release nucleotides and flavanones * into the external solution (Lundegårdh and Stenlid, 1944). The vitamins, biotin and thiamin can be released by flax roots (West, 1939). Sugars may also be excreted and, in some members of the grass family, such excretions may have very important biological effects. In the tropics of the Old World, many grasses are parasitized by a genus of plants closely related to the familiar yellow rattle (*Rhinanthus*) of our own pastures. This genus, which is called *Striga*, sends small suckers into the roots of the host grass and so considerably supplements its food supply. One striking thing is that the seeds of this semiparasite† will germinate only in the presence of the living roots of a suitable grass host; this has been shown to be due to the presence, in the diffusate

* Nucleotides are complex organic molecules containing phosphorus. They are the units from which the nucleo-proteins of the cell nucleus are very largely constructed. The flavanones are yellow-plant pigments very closely related to the flavones (see footnote on p. 306).

† A semiparasite is a parasite which is only partly dependent on its host for food supply, being able itself to synthesize carbohydrates, etc. since it possesses green aerial organs which can manufacture some of its food.

from the host roots, of a specific germination stimulant (Brown and Edwards, 1944) which has been identified as a pentose sugar closely related to, if not identical with, *d*-keto-xylose (Brown *et al.*, 1949). Organic root exudates, specific to certain species, may be of considerable practical value. For example, the seedling roots of Italian rye grass exude a substance showing a bluish fluorescence in ultra-violet light: this fact allows the seed tester to distinguish the seeds of this from other rye grass species. We have already discussed the positive evidence for toxic exudates from the roots of guayule and the strong indication of similar phenomena in a large variety of other plants.

There is no doubt, therefore, that the soil solution, in addition to inorganic mineral salts, may contain a very considerable variety of simple organic molecules arising in the metabolism of the living organisms which inhabit it, and it is very likely that many of these compounds may play a vital rôle in the intense competition that goes on there. Experience shows, however, that organic compounds, no matter how toxic, will eventually disappear from the soil, and this disappearance seems to be due to the activity of soil micro-organisms that are insensitive to their toxic actions. What evidence there is suggests that, in response to the appearance of any particular organic compound in the soil, a new population of a specific micro-organism, capable of attacking it, will arise from the vast and infinitely varied pool of the normal population, will proliferate to a peak and then decline to normal levels when the source of its energy, the particular organic molecule, is used up. Thus an appreciable accumulation of these simple organic molecules, be they hormones, vitamins, amino-acids, sugars or specific growth-inhibitors, will not be usual in ordinary unsterilized soils. In such soils the most that can be expected is the maintenance of a low level of concentration, the outcome of the establishment of a balance between production and breakdown, although these low concentration levels may still be high enough to have a decided effect on plant growth. Only in soils where conditions (*e.g.* water-logging) do not favour the growth of the particular micro-organism may concentrations reach a high level.

## BIOLOGICAL EQUILIBRIA IN SOIL

From what has been so briefly outlined above, it may have become apparent that the various populations of soil micro-organisms are in a condition of very delicate equilibrium, maintained by a variety of factors. The most obvious is their relative success in the competition for the available common nutrients. Secondly, their susceptibility to or tolerance of inhibiting metabolic by-products of other organisms may determine whether or not an otherwise successful organism may thrive. Again, the availability of specific growth-factors may discriminate between one class of organism and another. Thus it has been recently shown (Lochhead and Thexton, 1951) that a considerable proportion of soil bacteria require vitamin $B_{12}$* as an accessory growth-factor, since they are incapable of synthesizing it themselves, at least in amounts adequate for optimum growth. It seems possible that these organisms may receive this factor from other soil organisms which do synthesize it. In this way the growth of one species of organism might assist the growth of another and not antagonize it. This equilibrium is also very much under the influence of conditions such as soil acidity and degree of aeration, factors which the soil organisms themselves may partially control.

It is also well established that the roots of plants will affect and be affected by this microbiological equilibrium in the soil. Quantitative and qualitative studies on the microscopic flora of soils have demonstrated that the distribution of this microbial population is by no means uniform. In particular, there is a manifold increase in numbers as we pass into the immediate vicinity of the roots, and the greatest numbers are found actually on the surface of the root itself. Furthermore, it has been shown that the predominant organisms in this thin, highly populated soil-sheath surrounding the root, are those which have relatively complex food requirements, especially for certain amino-acids and vitamins such as thiamin. Presumably this accounts for their presence in the neighbourhood

* Vitamin $B_{12}$ is the anti-pernicious anæmia factor present in liver and recently found to be synthesized by numerous soil micro-organisms. It is a complex organic compound of as yet undetermined constitution containing the metal cobalt.

of the roots, which may supply these requirements in their exudates. On the other hand, there is also an increase in less exacting organisms, and so obviously other influences cannot be ruled out, and the underlying causes of the whole disturbed equilibrium in the soil population in the neighbourhood of the root is still far from being solved.* The extreme case of this augmented microbial population on and near root surfaces is seen in the widely occurring *mycorrhizas.* These structures arise in a very great variety of plants as small branched lateral rootlets that have become covered with a thick layer of interwoven threads of a fungus, which is usually specific to the particular plant affected. In some instances the fungus may even penetrate the tissue of the host root where it apparently behaves as a weak parasite. This close association of fungus and plant root has been extensively studied for many years, especially by foresters, since it is in forest trees that this phenomenon is most clearly seen. The exact significance of this association is still obscure. Foresters claim that the plant definitely gains, in that more vigorous growth is shown when mycorrhizas are present, and it has been severally suggested that the plant receives from its fungal associate nutrients and growth-factors including hormones (auxins) and vitamins. It is probable that the fungus also derives some similar benefit from the host root.

The effect of root exudates on the soil micro-flora may result in both advantages and disadvantages to the plants concerned. There is an increasing volume of evidence to support the view that the resistance or susceptibility to invasion by pathogenic soil organisms of the roots of certain crop plants may be directly correlated with their effects on the micro-flora of the soil immediately surrounding them. Thus, in two varieties of flax, Bison, which is resistant, and Novelty, which is susceptible to the soil-borne *Fusarium* wilt disease, it has been shown that root exudations from the susceptible variety Novelty stimulate the growth of the responsible fungus, whereas exudations from the resistant variety, Bison, do not. On the other hand,

* The reader is referred to the following authors for an exhaustive survey: Katznelson, Lochhead and Timonin, 1948; Harley, 1948; Garrett, 1950. (See List of Citations.)

exudates from Bison do stimulate the growth of the soil fungus *Trichoderma viride*, which, as we have seen previously, is an active producer of fungistatic compounds (Timonin, 1941). It is very probable that these exudates play a fundamental part in determining the resistance of flax plants to wilt disease.

Many other factors will, however, disturb the balance of pathogenic and non-pathogenic organisms in the soil, and thereby determine the incidence of disease in plants. In East Anglia the root rot of pines caused by *Fomes annosus* spreads much more rapidly on alkaline than on acid soils (Rishbeth, 1949). This is due to the fact that acid conditions greatly stimulate the growth of *Trichoderma viride*, which antagonizes the growth of the pathogenic *Fomes* and greatly reduces its spread and the correlated disease incidence. There are now many examples in the literature of antagonistic action of saprophytic fungi such as *Trichoderma viride* on plant pathogenic fungi in the soil, resulting in a great reduction in disease incidence. The most important have been reviewed by Brian (1949) and Garrett (1950). These considerations underlie the practice of seed "bacterization", which has received considerable attention in Russia. By incubation treatment of moist seed, the natural microbial flora of the seed-coat is increased. The antagonistic action of these non-pathogenic organisms on the pathogens in the soil is expected thereby to reduce attack when the seed is sown. Again, in certain soils heavily infected with potato-scab disease, "take-all" disease of wheat or root rot of cotton, the incorporation of green manures has been used with considerable success to disturb the microbiological equilibrium in the soil and encourage the growth of harmless antagonistic organisms. A marked effect of this type has been demonstrated in soils in an afforestation area on Wareham Heath in Dorset, where pine seedlings usually fail to become established. This has been investigated over a period of years by Rayner and by Neilson-Jones (see Rayner and Neilson-Jones, 1946), who found that not only did roots and shoots become stunted, but that the normal association of the roots with their specific mycorrhizal fungi was greatly disturbed. It was shown that this poor development of the plant and its associated fungus was due to the presence of a toxic compound

produced by a soil micro-organism, probably a species of *Penicillium* (see Brian, 1949). Sterilization of the soil by heat or by disinfectants killed this fungus, and subsequent inoculation of the soil with a suitable mycorrhizal fungus allowed the vigorous growth of pine seedlings. This same effect could be obtained, without sterilizing, by treating the soil with certain composts which presumably acted by so altering the microbiological equilibrium in the soil that the growth of the toxin-producing *Penicillia* was suppressed.

The above rather disjointed account of the interaction of micro-organisms and plant roots serves to illustrate the importance of excreted metabolic products in controlling soil equilibria, and has pointed out the important indirect effects on the plant that may result from the disturbance of these equilibria (*e.g.* an increase in pathogenic fungi, increase in toxin-producing organisms, etc.). It now remains to consider the occurrence in the soil of growth-factors, such as hormones and vitamins, which may have a direct effect on the growth of higher plants.

## ORGANIC GROWTH-FACTORS FOR HIGHER PLANTS

The question of the relative merits of organic farmyard manure and artificial fertilizers has long been a bone of contention between farmers and scientists alike. Those who favour the use of farmyard manure would have us believe that, quite apart from its beneficial effect on the physical properties of the soil, it provides specific organic growth substances essential for the best growth of the plant. On the other hand, their opponents, who are still very much in the majority, hold that optimal growth of a plant can take place if inorganic salts only are provided in a balanced mixture of optimal concentrations, and they point for proof to the success of the new "soilless culture" of market-garden produce, or "hydroponics", as it is unnecessarily called.

The adherents to the former view are not without experimental support. Nearly fifty years ago, Bottomley, one of the earliest proponents of the theory, showed that "bacterized peat", prepared by subjecting raw peat to the action of certain unspecified aerobic "humating" bacteria, gave watery extracts which he claimed markedly stimulated the growth of wheat

and other crop plants (Bottomley, 1914): this, he suggested, was due to the presence of specific organic growth-promoting substances to which he gave the name "auximones"; later, he showed them to be present in other organic sources, such as well-rotted stable manure, leaf-mould, yeasts and germinating seeds. Subsequently (Bottomley, 1917), experiments were extended to the small duckweed (*Lemna minor*), the small floating green fronds of which are characteristic of stagnant fresh-water ponds. From the results it was concluded that "auximones" were essential for the sustained growth and reproduction of this plant, and some evidence was obtained that the effective compounds might be derived from the breakdown of nucleic acids (Bottomley, 1920). Although much of the stimulating effects noted by Bottomley may have been due to the provision in his extracts of small traces of inorganic nutrients (*e.g.* iron salts, etc.) otherwise present only in suboptimal concentrations in his untreated culture medium (Bonner, 1946), and although it is now clear that *Lemna* can grow for long periods on a purely inorganic culture medium, yet a number of subsequent workers (see Chesters and Street, 1948) have demonstrated the marked growth-stimulating effects of small traces of organic matter, *e.g.* sterilized extracts of fresh horse-dung, on the growth of *Lemna* and the closely allied genus *Spirodela*.

Although the "auximone" interpretation of the stimulation effects observed by Bottomley on higher plants have never been accepted by the majority of plant physiologists, yet there has been, in the interval, a steady trickle of experimental results, suggesting that under certain conditions organic factors in manures may produce significant stimulations of plant growth. Some "enthusiasts" have even claimed that organic manures produce crops that have a higher nutritive value to man than those receiving artificial fertilizers only, this being due to their higher vitamin content. Unfortunately, many of these positive results can be explained by the stimulating effect of small traces of mineral nutrients in the organic manures and the extracts used. On the other hand, as has been pointed out in a recent review (Chesters and Street, 1948), in all water-culture experiments so far carried out, where plants have been

grown supposedly in the complete absence of any external supply of organic compounds, no adequate precautions have, in actual fact, been taken to exclude such sources as dust and micro-organisms from the air. Recent experiments have shown that very appreciable quantities of nitrogen and phosphorus compounds are present in submicroscopic dust particles (natural aerosols) even in the cleanest country air, and this is returned to the soil in rain (Ingham, 1950). Chesters and Street carried out some of the most careful experiments in sand cultures in which lettuce and radish were grown from seed in solutions containing optimal amounts of all the known mineral nutrients, including trace elements. The effects of adding aqueous extracts of leaf-mould, yeast and the products of hydrolysis of casein were compared with controls in which extra nitrogen, supplied as inorganic nitrate, was added in amounts equivalent to that in the organic extracts (Chesters and Street, 1948; Street, 1950). The addition of the leaf-mould extract significantly increased the growth of these plants. Although these extracts contained small traces (about one part in $10^{10}$) of IAA, this was not responsible for the effects noted, and the absence of response to the casein hydrolysate and yeast suggested that neither vitamins, nucleic acids nor amino-acids were involved in the stimulation. It is, however, possible that these effects may not be the direct result of the action of a specific organic compound present in the extract, but may be indirectly due to some interaction with the microflora of the sand culture. This is suggested by other results (Swaby, 1942), in which organic materials were shown to have no effect on plant growth in sterile sand culture, but did so only under conditions favouring the development of micro-organisms.

There is now ample evidence that both auxins and vitamins are present in appreciable quantities in many different types of soil and organic manures. The vitamins, as we have seen, arise mainly from the metabolism of soil micro-organisms, and the auxins, presumably by the decomposition of dead plants. Naturally the highest concentrations of those compounds occur in the surface layers of the soil, where organic detritus has accumulated and the activity of associated micro-organisms is highest: there even seems to be a positive correlation

between the concentrations of these growth substances in the soil and its fertility. Details of authors and results are given in a recent review by Schmidt (1951). There is, however, very little evidence that these growth substances can, in any direct way, stimulate the growth of crop plants. In Chapter 4 the position with regard to auxins has been reviewed and no consistent effects could be observed in the welter of conflicting results. With the vitamins, very little work has been done. A few claims have been put forward that an external supply of thiamin, ascorbic acid, etc. increased root and shoot growth in a number of plant species when given in solution to the roots, but they have remained unconfirmed by subsequent investigators. Indeed, some of the early claims of positive effects have now been withdrawn (see Chesters and Street, 1948; Schmidt, 1951).

Both on experimental evidence and from theoretical considerations, it seems unlikely, therefore, that vitamins and auxins present in the soil produce any stimulation of the vigour of the plant growing in that soil. Any normal plant in receipt of optimal nutrient supply is able to fulfil, by synthesis, its full requirements of these particular growth-factors, and any additional external supply is unlikely to exert anything but the harmful effects of supra-optimal concentrations. Such effects may account, at least partially, for the "sickness" of over-manured soils, where growth-factors may be present in high concentrations together with specific organic inhibitors. The stimulating effects of the organic content of such material as leaf-mould, etc., if they do prove to be well founded, must be due, then, either to some, as yet unidentified, growth stimulant, or to an indirect action on the associated microflora of the plant roots, which themselves supply growth-factors or otherwise favourably modify the environment of the root. We are only now beginning to appreciate the complexity of these root micro-organism interrelationships and their importance in plant growth.

# GLOSSARY

*AEROSOL.* The name given to the very fine mist of particles suspended in a gas. Aerosols of growth substances for application to plants are produced by allowing a dilute solution of the compound in a very volatile solvent (usually a gas liquefied by pressure) to escape from its container through a fine nozzle. The solvent evaporates leaving the growth substance to settle on the plant as a fine mist.

*AGAR-AGAR* (=*AGAR*). A carbohydrate prepared from seaweed. It dissolves in water to give, even in low concentrations, an inert jelly which is in universal use as a medium on which to grow bacteria or fungi. For this purpose various nutrients have to be supplied in the agar, which itself is not attacked in any way by the organisms.

*AMINO-ACID.* The building bricks of proteins. They contain, in addition to the elements carbon, hydrogen and oxygen (which make up the carbohydrates), the element nitrogen and, in some cases, sulphur.

*ANNUAL RINGS.* The alternating rings of close-grained dark and open-grained light wood seen when the trunk or branch of a tree is cut across. The open-grained ring is formed in the spring and early summer, and is composed largely of wide water-conducting tubes, whereas the close-grained wood is formed in the late summer and is mainly thick-walled fibrous cells of great strength.

*ANTI-AUXIN.* A theoretical chemical compound that competes with auxin for its place in the growth system of the plant cell and is itself inactive in promoting growth. It therefore prevents the growth action of auxin to a degree determined by the success of that competition.

*APICAL DOMINANCE.* The phenomenon in which the growth of side buds on a shoot is inhibited by the presence of a terminal (apical) bud.

*BIOCHEMISTRY.* The chemistry of living processes.

*CALIBRATION.* The process whereby the arbitrary readings obtained from a measuring instrument or material are related to the absolute measure of the system under observation. In an auxin assay, calibration would consist of constructing a graph relating the growth response of the organ (curvature, increase in length, etc.) to the concentration or amount of a known auxin applied. The graph then forms a scale from which auxin amount can be read from a knowledge of the relevant response to the material assayed.

*CALLUS.* Soft undifferentiated tissue which is produced at the edge of a wound in living plant tissue and which tends, under favourable conditions, to seal off the wound and to prevent infection by disease organisms.

*CAMBIUM.* A sheath of cells which possesses the power of active growth and division, and which is responsible for the production of new tissues in mature organs. The two most important cambia are (*a*) the cylinder which is situated between wood and bark and which causes growth in girth in stem and root, and (*b*) the sheet in the outer regions of the bark in mature stems and roots which gives rise to the protective cork tissue.

*CARBOHYDRATE.* Organic substances composed of the elements carbon, hydrogen and oxygen. They form the dead structural elements of the plant (cellulose in the cell-wall) and the storage units for chemical energy and raw material for growth synthesis (starch, sugars).

*CARBON DIOXIDE.* A gas present in the air to the extent of 3 parts in 10,000 and composed of the elements carbon and oxygen. It is the sole source of these elements for the synthesis of the whole complex or organic materials making up the living plant.

*CELL-SAP.* The watery solution of substances filling the vesicles found in the protoplasm of almost all mature living plant cells. In the majority of such cells, there is only one large vesicle which is called the vacuole.

*CELLULOSE.* The complex carbohydrate which constitutes the main structural material of the cell-wall. Cotton is almost pure cellulose.

*CHLOROPHYLL.* The green colouring matter of leaves, etc. Only in the presence of chlorophyll can the radiant energy of the sunlight be converted to molecular or chemical energy, necessary for the growth and vital activities of all living organisms.

*CHLOROPLAST.* Small microscopic protoplasmic structures which contain the green chlorophyll and in which the energy conversion mentioned above is brought about. In green leaf cells the chloroplasts are discoid and about $10\mu$ ($\frac{1}{2500}$ inch) in diameter, a large number being present in most cells.

*CHROMOSOME.* Short, thread-like, protoplasmic bodies, characteristic of the nuclei of cells. Their number, form and constitution are constant for the cells of any one variety or species, and they are the bearers of the hereditary characters of the organism.

*CHROMATOGRAPHY.* A sensitive technique for the complete separation of closely related chemical compounds by their selective removal from solution by various inert powders, or by special absorptive paper (see footnote, p. 34).

*COLEOPTILE.* The tubular first leaf produced by a cereal or a grass seedling. The second leaf, with a flattened blade, grows up inside the coleoptile and eventually bursts through it at the tip.

*CONTROL EXPERIMENT.* In all research in which the effect on a system of a condition or a particular treatment is being investigated it is vital that a check is kept on the behaviour of the system in the absence of such treatment with which the behaviour under the treatment itself can be accurately compared. The experiment designed to follow this normal behaviour is called a control experiment.

*2-CPA.* 2-chlorophenoxyacetic acid.
= o-chlorophenoxyacetic acid.

*4-CPA.* 4-chlorophenoxyacetic acid.
= p-chlorophenoxyacetic acid.

*CYTOPLASM.* That part of the living cell protoplasm which lies outside the nucleus. The protoplasm of the nucleus is generally termed nucleoplasm.

*2, 4-D.* 2, 4-dichlorophenoxyacetic acid.

*DECAPITATION.* A term used in plant physiology to denote the removal of the apex of a plant organ, *e.g.* coleoptile tip in the *Avena* assay for auxins (see Chapter 2).

*DICOTYLEDON.* A plant belonging to that great division of flowering plants which have two seed-leaves. The other division consists of all the remaining flowering plants; these have one seed-leaf and are called monocotyledons.

*DIFFERENTIATION.* The process whereby the cell, after reaching its full size, becomes structurally modified to play its specific rôle in the working of the organ or tissue of which it forms a part.

*ECOLOGY.* The scientific study of the interrelationships of the organism and its natural environment and their mutual interactions.

*EMBRYO-SAC.* The large cell which is found in the centre of the potential seed (ovule) in the female organ of a flower, and which contains the female nuclei destined to fuse with the male nuclei from the pollen grain. After the fusion, the embryo starts its development within the embryo-sac.

*ENDOCARP.* The innermost layer of the fruit-wall.

*ENDOSPERM.* The main reserve-food tissue of many seeds (*e.g.* cereal, castor oil, ash, etc.); it forms a layer between the young embryo and the seed-coat. Seeds that have no endosperm (*e.g.* pea, bean, etc.) have their reserve food in their seed-leaves.

*ENZYME.* An organic catalyst of the living cell. A catalyst is a substance which, when present in a chemical system in very small amounts, will greatly speed up the chemical reaction taking place there, without itself being changed in the process.

*EPINASTY.* The phenomenon of plant growth movements in relation to an *internal* stimulus, *e.g.* unequal distribution of a growth substance arising from a local external application. In the epinastic response of leaves, the upper side of the stalk grows faster than the lower side, so that the leaf appears to sink under its own weight.

*ETHYLENE.* An explosive gas having the formula $C_2H_4$; it has a very marked physiological action on plants even in very low concentrations, causing distorted growth in seedlings, greatly accelerated rates of fruit ripening, breaking of dormancy in potatoes, etc.

*ETIOLATION.* A condition produced in plants by continued growth in very weak light or in darkness. The plant grows long and spindly, has very small undeveloped leaves and forms no green chlorophyll.

*GENE.* The name given to the individual units of structure, making up the chromosome, which are associated each with the expression of an hereditary character or group of characters.

*GENETIC* (adjective). Having to do with genes and their action in heredity.

*GENETICS.* The study of the genes and of heredity.

*HEAVY METALS.* Metallic elements of large molecular weight. The salts of such metals are usually very toxic to living tissue, since they have the power, in very low concentrations, to put many vital enzyme systems out of action. Among the worst offenders are copper and lead.

*HUMUS.* The non-living organic material of the soil, produced by the decay of dead plants and animals. A very important constituent for the maintenance of desirable physical properties such as lightness of texture, etc.

*HYPHA.* The botanical name for the microscopic thread which is the basic structural unit of all fungi. In the moulds, etc., these threads are separate and clearly visible under the high-powered lens. In the large fruiting bodies, such as we find in the mushroom, these threads are twisted together almost indistinguishably into a compact "tissue".

*IAA.* $\beta$-indolylacetic acid.
= Indole-3-acetic acid.

*IBA.* $\gamma$-(indole-3)-butyric acid.

*IPA.* $\alpha$-(indole-3)-propionic acid.

*LANOLIN.* The natural fat of wool; of great value as a carrier for growth substances to be applied to plants, since it is itself without action on the plant.

*LIGNIN.* A chemical substance of as yet unknown constitution which, in combination with cellulose, is responsible for the remarkable mechanical strength of wood.

*MCPA.* 2-methyl-4-chlorophenoxyacetic acid.

*MeNA.* The methyl ester of α-naphthylacetic acid.

*MERISTEM.* A plant tissue in which the cells are all capable of and, under favourable growth conditions usually in process of, active division to produce new daughter cells.

*METABOLISM.* The sum-total of the chemical processes taking place in all their complexity in the normal life and growth of the organism.

*MICROGRAMME.* (Symbol :—γ (Gamma))
=0·001 milligramme = 0·000001 gramme.

*MOLECULE.* The smallest unit into which a chemical compound can be divided and still retain its characteristic properties.

*MONOCOTYLEDON* (see Dicotyledon).

*MORPHOLOGY.* The study of the form or shape of the living organism. Often used (rather loosely) to mean the shape or the form itself.

*MULTICELLULAR.* Composed of many cells as contrasted with unicellular, which described those organisms whose complete body is made up of only one cell.

*NAA.* α-naphthylacetic acid.

*NAAm.* α-naphthylacetamide.

*NoXA.* β-naphthoxyacetic acid.

*NUCLEUS.* The spherical or ovoid protoplasmic body which is present in all cells and which controls their vital activities in some as yet unexplained way. It contains the chromosomes, the bearers of the hereditary characters. It contains a high proportion of a special protein material (nucleoprotein) which is characterized by a great affinity for certain basic stains.

*OVARY.* In the flower, that portion of the female organ which contains the potential seeds (ovules). After fertilization it grows into the true fruit.

*OVULE.* The potential seed before fertilization.

*PAA.* Phenylacetic acid.

*PARTHENOCARPY.* The development of a fruit from an unfertilized ovary. Such fruits are necessarily seedless.

*PETRI DISH.* A circular glass dish with a loosely fitting glass lid, the whole the shape of a flat pill-box of about three and a half inches diameter. The standard vessel used for the cultivation of bacteria and fungi on a thin layer of nutrient agar with which the experimenter lines the bottom.

*PHLOEM.* That tissue which is concerned with the transport of organic food materials from one plant organ to another. In woody dicotyledonous plants it forms the innermost layer of the bark.

*PHOTOSYNTHESIS.* Synthesis of organic compounds from carbon dioxide and water, using the radiant energy of sunlight. This radiant energy is absorbed by the green colouring matter of plants (chlorophyll) and is there converted into the molecular energy essential for the synthesis.

*PHYTOHORMONE.* A plant hormone.

*PISTIL.* The female organ of a flower (see illustration and description, p. 134).

*POLAR MOVEMENT.* Movement restricted to a particular direction in a plant organ. When applied to auxins, for example in the coleoptile, the movement is strictly basipetal, *i.e.* always from the apex to the base and never in the reverse direction no matter what the orientation of the organ in space.

*PRECURSOR.* A forerunner. The chemical compound from which the substance under consideration (*e.g.* hormone) is formed in the organism.

*PROTOPLASM.* The living matter of the organism.

*RESPIRATION.* Chemical breakdown processes taking place in the living organism and resulting in the release of large quantities of chemical energy necessary for the maintenance of life and the continuance of growth. In the majority of organisms under normal conditions these processes also involve the release of the gas carbon dioxide.

*SPHAGNUM.* A genus of moss characteristic of acid bogs. Its special spongy nature, due to the high proportion of large, empty, perforated cells in its leaves, makes it an ideal medium for the maintenance, round plant organs, of moist but yet well-aerated conditions, necessary in some horticultural operations, *e.g.* the rooting of cuttings.

*STAMEN.* The male organ of the flower (see p. 134).

*STIGMA.* The apical portion of the female organ on which the pollen is deposited and germinates.

*STOMA* (Pl. stomata). The microscopic pores in the external skin (epidermis) of leaves and similar organs. Almost all the necessary interchange of gases between environment and interior of the leaf takes place by diffusion through these pores.

*2, 4, 5-T.* 2, 4, 5-trichlorophenoxyacetic acid.

*THIAMIN.* = Aneurin = Vitamin $B_1$ (see footnote, p. 296).

*TIBA.* 2, 3, 5-triiodobenzoic acid.

*TRANSPIRATION.* The loss of water by evaporation from the aerial parts of plants.

*TRAVELLING MICROSCOPE.* A microscope mounted on a special stage enabling it to be moved in a direction perpendicular to its optical axis by means of a graduated micrometer screw. Its use is to measure with great accuracy distances between objects or points on an object observed through the microscope.

*UNICELLULAR.* Describes an organism composed of one cell only. A bacterium is a unicellular organism.

*VACUOLE.* The central vesicle of a mature plant cell containing a watery solution of various substances—the cell-sap.

*VITAMINS* $B_1$, $B_2$, $B_6$, *etc.* (See footnote, p. 296.)

# APPENDIX A

## RESPONSES OF CUTTINGS TO AUXIN TREATMENT

The following list of plants showing their rooting reactions to auxin treatment has been condensed from Thimann and Behnke, 1947; Avery and Johnson, 1947; Pearse, 1948; and Satiadiredja, 1949. No attempts have been made to record the type of treatment for which these results are recorded, although they were mainly obtained with the dilute solution soak method. In a broad classification of this kind no details of variations between varieties and species or any experimental details can be given. These lists merely serve to indicate the general aspects of cutting behaviour both with and without auxin treatment for the more important genera and species of cultivated plant, as far as they appear in the above detailed lists. For detailed information reference should be made to those lists, where complete citations of the original investigations are recorded.

### GOOD ROOTERS

(60 to 100 per cent. of untreated cuttings root under optimal conditions)

*Significant Stimulation*

| *Botanical Name* | *Common Name* | *Effective Compound* * |
|---|---|---|
| Acalypha spp. | Red-hot Cat-tail | IAA |
| Alternanthera amœna | | IAA |
| Calluna vulgaris | Scotch Heather | IBA |
| Caryopteris incana | Bluebeard | IBA |
| Enkianthus spp. | | IBA |
| Epigæa repens | Mayflower | NAA |
| Iresine spp. | Bloodleaf | IAA, IBA |
| Ixora amboinica | | IAA, NAA |
| Melastoma spp. | | NAA, NAAm |
| Saintpaulia spp. | African Violet | IAA |

* For *Effective Compound*, pp. 377–385, see p. 385.

| *Botanical Name* | *Common Name* | *Effective Compound* * |
|---|---|---|
| Salix spp. | Willow | IAA |
| Sambucus spp. | Elder | IBA, NAA |
| | *Slight or No Stimulation* | |
| Bambusa spp. | Bamboo | IAA, IBA, 2, 4-D |
| Campsis spp. | Common Trumpet Creeper | IAA |
| Cephalanthus occidentalis | Buttonbush | IAA |
| Cladrastis lutea | Yellowwood | IAA |
| Enkianthus perulatus | White Enkianthus | IAA |
| Euonymus furtunei colorata | Purpleleaf Wintercreeper Euonymus | IAA |
| Fothergilla spp. | Dwarf Alder | IAA, IBA |
| Ginkgo biloba | Maidenhair Tree | IBA |
| Gypsophila paniculata | | IAA |
| Leucothoë catesbæi | | IBA |
| Lonicera nitida | Box Honeysuckle | IAA |
| Physocarpus opulifolius | Ninebark | IBA |
| Prunus laurocerasus | Cherry Laurel | IBA |
| Rhododendron dauricum sempervirens | Everygreen Dahurian Rhododendron | IAA |
| Rhododendron japonicum aureum | Golden Japanese Azalea | IAA |
| Rhododendron mucronatum | Snow Azalea | IAA |
| Sauropus androgynus | | IAA |

## FAIR ROOTERS

(30 to 60 per cent. of untreated cuttings root under optimal conditions)

| | *Excellent Stimulation* | |
|---|---|---|
| Chorizema varium | Flame Pea | IAA |
| Chrysanthemum spp. | | IAA, IBA |
| Codiæum variegatum | Croton | IBA |
| Pelargonium spp. | Pelargonium, Geranium | IBA |

| *Botanical Name* | *Common Name* | *Effective Compound** |
|---|---|---|
| | *Fair to Good Stimulation* | |
| Abelia spp. | | IBA |
| Achras sapota | Sapodilla | IBA |
| Ageratum houstonianum | | IBA |
| Antirrhinum majus | | IBA |
| Aucuba japonica | Japanese Laurel | IAA |
| Buxus spp. | Box Tree | IBA |
| Callicarpa spp. | Beautyberry | IBA |
| Camellia japonica | Camellia | IAA, IBA |
| Caragana spp. | Pea Tree | IAA |
| Chamæcyparis spp. | Lawson's Cypress | IBA |
| Citrus spp. | {Orange, Lemon, Lime, Grapefruit, etc.} | IAA, IBA |
| Clematis spp. | Clematis | IBA |
| Clerodendron spp. | Glorybower | IAA, IBA |
| Cordyline stricta | Dracena Palm | IAA |
| Coriaria spp. | | NAA |
| Cyrilla racemiflora | Leatherwood | NAA, NAAm |
| Datura cornucopia | Thornapple | IAA |
| Derris spp. | | NAA, IAA |
| Dianthus caryophyllea | Carnation | IBA |
| Euonymus spp. | Spindle Tree | IBA |
| Eupatorium spp. | Mist-flower | IBA |
| Euphorbia pulcherrima | Poinsettia | IBA |
| Euphorbia splendens | Crown of Thorns | IBA |
| Exochorda racemosa | Common Pearlbush | IAA, IBA |
| Ficus spp. | Figs | IAA |
| Forsythia spp. | | IAA, IBA |
| Genista spp. | Needle-Gorse | IAA, NAA |
| Hedera helix | Ivy | IAA, IBA, NAA |
| Helichrysum spp. | Everlasting Flower | IAA |
| Hydrangea spp. | | IAA, IBA |
| Hypericum spp. | St. John's Wort, Tutson, etc. | IAA |
| Idesia polycarpa | | IBA |
| Iberis sempervirens | Candytuft | IAA |
| Ixora pulcherrima | | IAA |
| Jasminum spp. | Jasmine | IAA |
| Leycesteria formosa | | IAAm |

| *Botanical Name* | *Common Name* | *Effective Compound* * |
|---|---|---|
| Libocedrus decurrens | White Cedar | IBA |
| Maclura aurantiaca | Bow Wood | IAA |
| Metrosideros robusta | Bottle-brush | IBA |
| Morus spp. | Mulberry | IAA |
| Myrica gale | Sweet Gale | IAA |
| Osmanthus spp. | | IAA |
| Pachysandra terminalis | | IBA |
| Parthenium argentatum | Guayule | IBA, NAAm |
| Passiflora vitifolia | Passion Flower | IAA |
| Petunia hybrida | | IBA |
| Phleum pratense | Timothy Grass | NAA |
| Phlox paniculata | Summer Perennial Phlox | IBA |
| Pieris japonica | | IBA |
| Piper geniculata | Pepper | IAA |
| Piqueria trinervis | | IBA |
| Prunus cerasifera | Cherry Plum | IAA |
| Prunus padus | Bird Cherry | IBA |
| Pyracantha spp. | Firethorn | IBA |
| Ribes grossularia | Gooseberry | NAA |
| Ribes alpinum | Alpine Currant | IBA |
| Rosa spp. | Rose | IAA, IBA |
| Senecio spp. | Groundsel | IBA |
| Skimmia spp. | | IAA, IAAm |
| Symphoricarpus albus | Snowberry | IAA |
| Taxus spp. | Yew | IAA, IBA |
| Viburnum spp. | Guelder rose, Laurestinus, etc. | IBA |

*Slight or No Stimulation*

| | | |
|---|---|---|
| Cotoneaster spp. | | IBA |
| Deutzia spp. | | IAA, IBA |
| Erica spp. | Heath | IAA, IBA, NAA |
| Fortunella hindsii | Hong Kong Kumquat | IAA |
| Gardenia jasminioides | | IAA |
| Pilea microphylla | Artillery Plant | IBA |
| Podocarpus macrophyllus | | IAA, NAA |
| Rosa canina | Senff's Dog Rose | IAA |

| *Botanical Name* | *Common Name* | *Effective Compound* * |
|---|---|---|

## POOR ROOTERS

(Less than 30 per cent. of untreated cuttings rooting under optimal conditions)

*Excellent Stimulation*

| | | |
|---|---|---|
| Abies spp. | Fir | IAA |
| Boronia elatior | | IBA |
| Bougainvillea spp. | | IAA, IBA |
| Capparis Heyneana | Caper-bush | IAA |
| Cerastium tomentosum | Snow-in-summer | IBA |
| Cissus rhombifolia | Treebine | IBA |
| Derris elliptica | Derris | IAA, NAA |
| Fuchsia spp. | | IBA |
| Pentstemon barbatus | | IBA |
| Podocarpus spp. | | IAA |
| Prunus persica | Peach | IBA |
| Vinca minor | Periwinkle | IBA |
| Wistaria spp. | | IAA |

*Fair to Good Stimulation*

| | | |
|---|---|---|
| Amorpha sp. | False Indigo | IAA |
| Begonia semperflorens | Perpetual Begonia | IBA |
| Berberis spp. | Barberry | IBA, IPA |
| Bignonia spp. | | NAA |
| Buddleia spp. | | IAA, IBA, NAA |
| Carica papaya | Papaya, Paw-paw | IAA, IBA |
| Centaurea gymnocarpa | Velvet Cornflower | IBA |
| Coffea spp. | Coffee | IAA |
| Combretum intermedium | | NAA |
| Cydonia oblonga | Quince | IAA |
| Cytisus spp. | Broom | IAA, IBA |
| Daphne spp. | | IAA |
| Diervilla spp. | Weigela | IBA |
| Eleagnus spp. | Oleaster | IBA |
| Eriostemon myoporoides | | IAA, IBA |
| Flacourtia ramontchi | Governor's Plum | IAA |
| Hamamelis spp. | Witch Hazel | IAA, IBA |
| Hevea braziliensis | Rubber | NAA |

| *Botanical Name* | *Common Name* | *Effective Compound* * |
|---|---|---|
| Hibiscus spp. | Hibiscus | IAA, IBA |
| Ilex spp. | Holly | IBA |
| Jasminum grandiflorum | Jasmine | IAA |
| Juniperus spp. | Juniper | IBA |
| Kalmia spp. | American Laurel | IAA |
| Lagerstrœmia indica | Crape Myrtle | IBA |
| Lantana spp. | | IBA |
| Larix sibirica | Larch | IAA |
| Laurus nobilis | Grecian Laurel | IAA |
| Ligustrum vulgare | Privet | IAA, IBA |
| Liriodendron tulipfera | Tulip Tree | IAA |
| Lithospermum prostratum | Gentian-blue Gromwell | IAA, IBA, NAA |
| Lonicera spp. | Honeysuckle | IBA |
| Magnolia soulangeana | | IAA |
| Nereum oleander | Oleander | IBA |
| Olearia haastii | Tree Aster | IAA |
| Philadelphus spp. | Syringa | IBA |
| Photina spp. | | IAA |
| Picea spp. | Spruce | IAA, IBA |
| Populus spp. | Poplar, Aspen | IBA |
| Potentilla fruticosa | Shrubby Cinquefoil | IAA, IBA |
| Prunus spp. | Plum | IBA |
| Prunus triloba | Flowering Almond | IBA |
| Pterostyrax hispida | Fragrant Epaulette Tree | IAA |
| Quercus spp. | Oak | IAA |
| Sansevieria guiniensis | Bowstring Hemp | IAA |
| Spiræa spp. | | IAA, IBA |
| Syringa spp. | Lilac | IBA |
| Thuya spp. | Arbor vitæ | IBA |
| Ugni molinæ | | IAA |
| Ulmus spp. | Elm | IAA, IBA |
| Vaccinium corymbosum | Blueberry | IBA |
| Verbena spp. | | IBA |
| Veronica longifolia | | IBA |
| | *Slight or No Stimulation* | |
| Agathis australis | Kauri Pine | IBA |
| Carya pecan | Pecan Nut | IBA |

| *Botanical Name* | *Common Name* | *Effective Compound* * |
|---|---|---|
| Ceanothus spp. | | IAA, IBA |
| Chænomeles spp. | | IAA |
| Cinnamomum | Camphor | IAA |
| Cornus spp. | Dogwood | IAA, IBA |
| Licania rigida | | IAA |
| Linum flavum | Golden Flax | IBA |
| Magnolia spp. | | IAA |
| Pinus spp. | Pine | IAA, IBA |
| Stewartia spp. | | IAA, IBA |
| Thea spp. | Tea | |

## NON-ROOTERS

(No rooting of untreated cuttings under optimal conditions)

*Excellent Stimulation*

| | | |
|---|---|---|
| Acer spp. | Maple | IAA, IBA, NAA |
| Aleurites fordii | Tung Tree | IAA |
| Allamandra cathartica | | IBA |
| Artocarpus communis | Breadfruit | IBA |
| Celastrus scandens | False Bittersweet | IBA |
| Chodanthus splendens | | IAA |
| Cinchona spp. | Quinine | IBA |
| Corylus spp. | Hazel Nut | IBA |
| Erythrina coralloden-dron | Coral Tree | IAA |
| Frankenia alatamalia | | IBA |
| Hibiscus syriacus | Rose of Sharon | IAA |
| Impatiens spp. | Touch-me-not, Balsam | IAA |
| Ixora incarnata | | IAA |
| Oxydendrum arboreum | Sorrel Tree | IBA |
| Psidium guajava | Guava | IBA |
| Pseudotsuga taxifolia | Douglas Fir | IBA |
| Pyrus communis | Pear | IBA, IAA, NAA |
| Pyrus malus | Apple | IBA |
| Sciadopitys verticil-lata | Umbrella Pine | IBA |
| Teucrium chamædrys | Wall Germander | IBA |

| *Botanical Name* | *Common Name* | *Effective Compound* * |
|---|---|---|
| | *Fair to Good Stimulation* | |
| Aristolochia sipho | Dutchman's Pipe | IAA |
| Betula spp. | Birch | IAA |
| Brunfelsia uniflora | | IAA |
| Davidia involucrata | Dove Tree | IAA |
| Eurya japonica | | IAA |
| Fagus sylvatica | Beech | IAA |
| Garrya elliptica | | IAA |
| Gleditschia triacanthos | Honey Locust | IBA |
| Hedysarum multijugatum | Mongolian Sweet Vetch | IAA |
| Heuchera tiarelloides | | IBA |
| Ixora chinensis | | IAA |
| Lespedeza Thunbergii | Bush Clover | IAA |
| Litchi chinensis | | IAA |
| Mussænda spp. | | IAA, IBA |
| Myrciaria cauliflora | Jaboticaba | IAA |
| Myrtus pubescens | Myrtle | IAA |
| Olea spp. | Olive | IBA |
| Pterocarya stenoptera | | IAA |
| Punica granatum | Pomegranate | IAA |
| Quercus robur | English Oak | IAA |
| Rhodotypos kerrioides | | IBA |
| Rondeletia odorata | | IAA |
| Sequoia sempervirens | Californian Redwood | IAA |
| Tsuga canadensis | Canadian Hemlock | IAA, IBA |
| | *Slight or No Stimulation* | |
| Acer spp. | Maple | |
| Aleurites moluccana | Candlenut | IAA |
| Alnus spp. | Alder | |
| Arctostaphylos manzanita | Common Manzanita | IAA, IBA, NAA |
| Calycanthus spp. | Sweetshrub | IAA, IBA |
| Cratægus spp. | Hawthorn | IAA |
| Fraxinus americana | Ash (American) | IAA |
| Larix decidua | European Larch | IBA |
| Pimelia officinalis | Rice Flower | IAA |
| Pittosporum spp. | | IAA |

| *Botanical Name* | *Common Name* | *Effective Compound* * |
|---|---|---|
| Podocarpus Nogi | | IAA, NAA |
| Polygonum paniculatum | Knotweed | NAA |
| Rosa willmottiæ | Willmott Rose | IAA |
| Stifftia chrysantha | | IAA |
| Uroskinnera spectabilis | | IAA |
| Warszewiczia coccinea | | IAA |

## VARIABLE ROOTERS

(Genera in which there is a wide range of behaviour in untreated cuttings of the many species and varieties studied. For details consult above lists. Both genera, with few exceptions (see *Rhododendron* under "Good Rooters"), show fair to good stimulation).

| | | |
|---|---|---|
| Rhododendron spp. | Rhododendron, Azaleas | IAA, IBA |
| Vitis spp. | Grapes | IAA, IBA<br>NoXA, 2-CPA |

* IAA = β-indolylacetic acid.
IAAm = β-indolylacetamide.
IBA = γ-(indole-3)-butyric acid.
IPA = α-(indole-3)-propionic acid.
NAA = α-naphthylacetic acid.
NAAm = α-naphthylacetamide.
NoXA = β-naphthoxyacetic acid.
2-CPA = 2-chlorophenoxyacetic acid.
2, 4-D = 2, 4-dichlorophenoxyacetic acid.

# APPENDIX B

## EFFECTIVE CONCENTRATIONS OF AUXINS IN THE FRUIT-SET OF TOMATOES

| Auxin | Effective Concentration Range in parts per million (Optimum concentrations in brackets) | | | |
|---|---|---|---|---|
| | Lanolin Paste | Water Spray | Aerosol | Vapour |
| β-indolylacetic acid (IAA) .. .. | 5000 | 3000 | | |
| γ-(indole-3)-butyric acid (IBA) .. .. | 100–50,000 (5000) | 2000–3000* | 250 | |
| α-naphthylacetic acid (NAA) .. .. | 100–50,000 | 50–30,000 | | |
| β-naphthoxyacetic acid (NoXA) .. .. | 2500 | 10–300* (50) | 100–500 | |
| Do. as ethyl or methyl ester .. .. | | | | 10–50 mg. per cu. ft. |
| α-(1-naphthoxy)-propionic acid .. .. | | 10–250 (50) | | |
| α-(2-naphthoxy)-propionic acid .. | 100 | 10–150 | | |
| α-(2-naphthoxy)-phenylacetic acid .. | | 10–50 | | |
| Phenylacetic acid (PA) .. .. .. | | 400–1000 | | |
| α-phenoxypropionic acid .. .. .. | | 10–250 | | |
| α-phenoxy-n-butyric acid .. .. .. | | 10–250 | | |
| 2-chlorophenoxyacetic acid (2CPA) .. | | 50–100* (50) | | |
| 4-chlorophenoxyacetic acid (4CPA) .. | | 25–300* (50) | | |
| α-(2-chlorophenoxy)-propionic acid .. | | 10–50 | | |
| α-(2-chlorophenoxy)-n-butyric acid .. | | 25–50 | | |
| 2, 4-dichlorophenoxyacetic acid (2, 4–D) | | 2·6–100* (10) | | 1–10 mg. per 1000 cu. ft. |
| α-(2, 4-dichlorophenoxy)-propionic acid .. | | 7·5 | | |
| α-(2, 4-dichlorophenoxy)-n-butyric acid .. | | 10–100 | | |
| 2-methyl-4-chlorophenoxyacetic acid (MCPA) .. .. .. .. | | 1–5 | | |
| α-(2-methyl-4-chlorophenoxy)-propionic acid .. .. .. .. .. | | 1–50 (50) | | |
| 2, 3-dimethylphenoxyacetic acid .. | | 100 | | |
| 3, 5-dimethyl-4-chlorophenoxyacetic acid | | 20–250 | | |
| 2, 4, 5-trichlorophenoxyacetic acid (2, 4, 5–T) .. .. .. .. .. | | 10–50 | | |
| α-(2, 4, 5-trichlorophenoxy)-propionic acid | | 1–50 (50) | | |
| α-(2, 4, 5-trichlorophenoxy)-n-butyric acid | | 10–50 (50) | | |
| 2, 3, 5-trimethylphenoxyacetic acid .. | | 100 | | |
| 2, 5-dichlorobenzoic acid .. .. .. | | 100 | | |
| *Mixtures* | | | | |
| NoXA + 4CPA .. .. .. | | 40 + 10 | 2500 + 1000 | |
| NoXA + IBA .. .. .. | | | 125 + 125 | |
| NoXA + 4CPA + IBA .. .. | | | 125 + 10 + 125 | |

# APPENDIX C

## THE EFFECTIVENESS OF AUXINS IN THE PRODUCTION OF PARTHENOCARPIC FRUIT IN SPECIES OTHER THAN TOMATO

** = Good response; * = fair to doubtful response; o = no response; D = damage.

| Plant Species | | Effective Compound | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Botanical Name | Common Name | β-indolylacetic acid | γ-(indole-3)-butyric acid | α-naphthylacetic acid | β-naphthoxyacetic acid | 2, 4-dichlorophenoxy-acetic acid | 4-chlorophenoxyacetic acid | 2-chlorophenoxyacetic acid | α-naphthylacetamide | α-(indole-3)-propionic acid | Phenylacetic acid | 2, 4, 5-trichlorophenoxy-acetic acid | α-(2-naphthoxy)-propionic acid |
| Agapanthus sp. | | * | | | | | | | | | | | |
| Ananas sativa | Pineapple | | | ** | | | | | | | | | |
| Antirrhinum sp. | Snapdragon | * | | * | | | | | | * | * | | |
| Begonia sp. | | ** | ** | | | | | | | | | | |
| Capsicum spp. | Pepper, Paprika | ** | ** | ** | | | | | | | | | |
| Carya pecan | Pecan Nut | o | o | | | | | | | | | | |
| Citrullus vulgaris | Water Melon | * | o | * | | o | | | | | | | |
| Citrus aurantium | Orange | o | o | | o | | | | | | | | |
| Citrus decumana | Grapefruit | o | o | | o | | | | | | | | |
| Crinum sp. | | | | ** | | | | | | | | | |
| Cucumis Melo | Musk Melon | * | | o | | | | | | | | | |
| Cucumis sativus | Cucumber | ** | | ** | | | | | | | | | |
| Cucurbita maxima | Squash | * | * | ** | | | | | | | | | |
| Cucurbita pepo | Pumpkin | * | | * | | o | | | | | | | |
| Cymbidium sp. | | | | ** | | | | | | | | | |
| Datura stramonium | Thornapple | | ** | ** | | | | | | | | | |
| Digitalis purpurea | Foxglove | ** | | | | | | | | | | | |
| Ficus carica | Fig | | ** | ** | o | * | ** | | | | | ** | |
| Fragaria spp. | Strawberry | ** | ** | * | ** | | | | | | | | |
| Fuchsia sp. | | ** | | ** | | | | | | | | | |
| Gladiolus sp. | | ** | | ** | | | | | | | | | |
| Godetia sp. | | * | | | | | | | | | | | |
| Ilex opaca | American Holly | ** | ** | ** | | | | | ** | ** | | | |
| Lantana sellowiana | | ** | ** | ** | | | | | | | | | |
| Luffa cylindrica | Loofah | ** | | | | | | | | | | | |
| Matthiola sp. | Stock | * | | | | | | | | | | | |
| Melandrium dioicum | Red Campion | * | ** | ** | | | | | | | | | |
| Nicotiana sp. | Tobacco | ** | | | | | | | | | | | |
| Œnothera organensis | | | | | | | | | ** | | | | |
| Oncidium longipes | | | | ** | | | | | | | | | |
| Orchis sp. | Orchid | * | | | | | | | | | | | |
| Petunia sp. | | ** | ** | ** | | | | | | | | | |
| Phaseolus sp. | Snap Beans | | | | * | | ** | | * | | | | |
| Phœnix dactylifera | Date | o | o | o | | | | | | | | | |
| Prunus armeniaca | Apricot | ** | | | | | | | | | | | |
| Prunus avium | Cherry | | | | | | | | o | | | | |
| Prunus domestica | Plum | | | | | | | | o | | | | |
| Prunus persica | Peach | | | * | | | | | | | | | |
| Pyrus communis | Pear | * | | | | | | | o | | o | | ** |
| Pyrus malus | Apple | o | o | o | | | | | | o | | | |
| Rubus cæsius | Dewberry | o | | | | | | | | | | | |
| Rubus fruticosus | Blackberry | ** | ** | | ** | ** | D** | * | | | | | |
| Rubus idæus | Raspberry | o | | | | | | | | | | | |
| Salpiglossis variabilis | | ** | ** | | | | | | | ** | | | |
| Solanum melongena | Aubergine | ** | ** | ** | | ** | | | | | | | |
| Solanum tuberosum | Potato | | ** | ** | | | | | | | | | |
| Vaccinium sp. | Blueberry | o | | | | | | | | | | | |
| Vitis vinifera | Grape | * | | ** | | | | | | | | | |
| Zephyranthes canina | | * | | | | | | | | | | | |

# APPENDIX D

## SENSITIVITIES OF WEED, CROP AND ORNAMENTAL PLANTS TO TREATMENT WITH HORMONE HERBICIDES

This list of plant species and their sensitivities to hormones applied at toxic concentration levels has been compiled from the following sources:—


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A few additional species have been included from individual observations in a sprinkling of original research papers. It represents the results of very extensive experiments carried out mainly in Great Britain and the Commonwealth, in Europe and America.

The following categories of response have been distinguished:—

S = Highly susceptible, plants being killed at all stages in their development by the usually recommended rates of hormone application.

s = Moderately susceptible, but plants showing some degree of resistance to the hormone at normal application rates at some stage in their development.

r = Resistant, although normal rates of application can bring about control or serious damage if treatment is carried out under optimal conditions for action and when the plant is at its most sensitive developmental stage.

R = Highly resistant, plants being unaffected by all but impracticably high concentrations and at all developmental stages, or, if affected, then quickly recovering from all toxic symptoms.

Var. = Response very variable (experimentors category).

In some species reported sensitivities from different sources are at variance and this is undoubtedly due to treatments under different conditions and/or at different stages of the plant's development. Such reactions are indicated S–r, s–R, etc.

The majority of these records are for 2, 4-D, although much of the Swedish and British records include corresponding data for MCPA. Some of the reactions of woody species are to 2, 4, 5-T. Although the reactions of any one species to these compounds and their derivatives are seldom identical, yet they tend to fall together into one of the above four categories and no attempt has been made separately to record these different reactions.

* w = Weed plant or wild species.
c = Crop plant.
o = Ornamental plant.

** A = United States of America.
As = Austria.
Au = Australia.
B = Great Britain.
Ea = East Africa.
Eu = Europe (including Great Britain).
F = France.
H = Holland.
M = Mauritius.
Ml = Malaya.
P = Palestine.
Sa = South America.
Sw = Sweden.
WI = West Indies.
Z = New Zealand.

| *Botanical Name* | *Common Name* | *Class of Plant* * | *Region of Observation* ** | *Sensitivity* |
|---|---|---|---|---|
| Abutilon theophrasti | Velvetleaf | w | A | S |
| Acacia spp. | Wattle | w | A | r–R |
| Acæna ovina | Sheep's Burr | w | Au | R |
| Acæna sanguisorbæ | Bidi-bidi | w | Z | R |
| Acanthospermum hispidum | Star Burr | w | Au | S |
| Acer negundo | Manitoba Maple | o | A | S |
| Acer rubrum | Red Maple | o | A | r |
| Acer saccharum | Sugar Maple | c | A | r |
| Achillea millefolium | Yarrow | w | B, Eu, A, Z | r |
| Actinea odorata | Bitterweed | w | A | r–s |
| Ægopodium podagraria | Ground Elder / Bishop's Weed | w | Eu | R |
| Æschynomene virginica | Curly Indigo | o | A | r–s |
| Ageratum conyzoides | Bluetop | w | Au | S |
| Agropyron repens | Couch or Quack Grass | w | Eu, A | R |
| Agrostemma githago | Corn Cockle | w | A, Sw | r–s |
| Agrostis stolonifera | Black Bent Grass | w | B | R |
| Agrostis tenuis | Browntop | w | Au, B | R–r |
| Ailanthus glandulosus | Tree of Heaven | o | Au | S |
| Ajuga reptans | Bugle | w | B | R |
| Alchemilla arvensis | Parsley Piert | w | B | r–s |
| Alectorolophus hirsutus | Yellow Rattle | w | As | r–s |
| Alhagi camelorum | Camel Thorn | w | Au | S |
| Allium cepa | Onion | c | A, Eu | s–S |
| Allium cernuum | Wild Onion | w | A | s |
| Allium schœnoprasum | Chives | c | Eu | S |
| Allium vineale | Crow Garlic | w | B, Sw, Z | r–s |
| Alnus sp. | Alder | w | Sw | S |
| Alnus incana | Speckled Alder | w | A | S–s |
| Alternanthera denticulata | Lesser Joyweed | w | Au | s–S |
| Alternanthera philoxeroides | Alligator Weed | w | A | S |
| Alternanthera repens | Joyweed | w | Ea | r–s |
| Althæa spp. | Hollyhock | o | A, Eu | S |
| Althæa officinalis | Marshmallow | w | Z | R |
| Amaranthus spp. | Pigweed | w | Ea, A | S |
| Amaranthus blitoides | | w | A | S |
| Amaranthus caudatus | | w | A | S |
| Amaranthus paniculatus | Panicled Amaranth | w | Au | s |

| *Botanical Name* | *Common Name* | *Class of Plant* * | *Region of Observation* ** | *Sensitivity* |
|---|---|---|---|---|
| Amaranthus retroflexus | Red-root | w | A, Au, Eu | S |
| Amaranthus spinosus | Spiny Pigweed | w | A, Au | s–S |
| Amaranthus viridis | Green Amaranth | w | Au | r–s |
| Ambrosia spp. | Ragweed | w | A | S |
| Ambrosia artemisiifolia | „ | w | A, M | S |
| Amelanchier oblongifolia | Shadbush | w | A | S |
| Ampelopsis quinquefolia | Virginia Creeper | o | Au, Eu | S |
| Ampelopsis veitchi | | o | | S |
| Anagalis arvensis | Scarlet Pimpernel | w | Au, Z, Eu | S |
| Ananas sativa | Pineapple | c | A | S |
| Anaphalis margaritacea | Pearly Everlasting | w | A | S |
| Anthemis arvensis | Corn Camomile | w | Eu | r–s |
| Anthemis cotula | Stinking Mayweed | w | A, Eu | r–s |
| Anthemis tinctoria | Yellow Camomile | w | Eu | s |
| Anthriscus sylvestris | Keck, Wild Chervil | w | Eu | S–s |
| Antirrhinum majus | Snapdragon | o | | S |
| Apocynum androsæmifolium | Spreading Dogbane | w | A | r |
| Apocynum cannabinum | Dog-bane | w | A | S |
| Aquilegia canadensis | Columbine | o | A | r |
| Arabis arenosa | | w | Sw | S |
| Arctium lappa | Great Burdock | w | B, Sw, Z | r–s |
| Arctium minus | Small Burdock | w | A, B | S |
| Arctostaphylos spp. | Manzanita | w | A | S |
| Argemone mexicana | Prickly Poppy | w | Au | s |
| Arisæma triphyllum | Jack-in-the-pulpit | w | A | r |
| Armeria maritima | Thrift, Sea Pink | w, o | B | S |
| Artemisia spp. | Wormwood | w | A | S |
| Artemisia californica | Californian Sage | w | A | S |
| Artemisia campestris | Wormwood | w | As | s |
| Artemisia filifolia | Sand Sagebrush | w | A | r–s |
| Artemisia ludovicianum | Californian Mugwort | w | A | S |
| Artemisia montana | Silver King | o | A | S |
| Artemisia tridentata | Sagebrush | w | A | s |
| Artemisia vulgaris | Mugwort | w | Eu, M | S |
| Asclepias spp. | Milkweeds | w | A, Au | R |
| Asclepias fruticosa | Narrow-leaved Cotton Bush | w | Au | r |

| *Botanical Name* | *Common Name* | *Class of Plant* * | *Region of Observation* ** | *Sensitivity* |
|---|---|---|---|---|
| Asclepias syriaca | Milkweed | w | A | S |
| Asparagus officinalis | Asparagus | c | Eu, Au | r–S |
| Asperula conferta | Woodruff | w | Au | r–s |
| Asphodelus fistulosus | Onion Weed | w | Au | R–s |
| Aspidium filix-fœmina | Lady Fern | w | A | r–s |
| Aspidium novæboracense | New York Fern | w | A | r–s |
| Aster spp. | Aster | o | A | r–s |
| Astralagus spp. | Loco Weeds | w | A | r |
| Asystasia gangetica | Herbe Pistache | w | M | S |
| Atriplex hastata | Orache | w | Eu | r–s |
| Atriplex patula | ,, | w | B | r–s |
| Avena fatua | Wild Oat | w | A, Sw | R |
| Avena sativa | Oat | c | A, Eu | R |
| Axonopus compressus | Savannah Grass | w | Ml, A | r–s |
| Baccharis halmifolia | Groundsel Bush | w | Au | r–s |
| Baccharis pilularis | Cayote Brush | w | A | S |
| Baptisia tinctoria | Indigo | c | A | S |
| Barbarea spp. | Land Cress | w | Z | s |
| Barbarea vulgaris | Winter Cress | w | A, Sw | S |
| Bassia birchii | Galvanized Burr | w | Au | s |
| Bassia quinquecuspis | Black Roly-poly | w | Au | S |
| Bassia tetracuspis | Brigalow Burr | w | Au | S |
| Begonia semperflorens | Elephant Ear | w | A | s |
| Bellis perennis | Daisy | w | Eu, Z | r–s |
| Benzoin æstivale | Spicebush | w | A | S |
| Berberis spp. | Barberry | o | A, Eu | R–r |
| Berteroa incana | Hoary Alyssum | w | A | R |
| Beta spp. | Beets | c | A, Eu | S |
| Betula alba | Birch | w | A, Eu | S |
| Betula lenta | Black Birch | w | A | S |
| Betula lutea | Yellow Birch | w | A | S |
| Betula papyrifera | Paper Birch | w | A | S |
| Betula populifolia | Gray Birch | w | A | S |
| Biddens frondosa | Beggars' Ticks | w | A | S |
| Bidens pilosa | Cobbler's Pegs<br>Spanish Needle<br>Blackjack | w | Au<br>Wi<br>Ea | r–s |
| Boerhaavia diffusa | Hogmeat, Spiderling | w | A | r–s |
| Brassica spp. | | w | Ea | S |
| Brassica adpressa | Buchan Weed | w | Au | S |

| *Botanical Name* | *Common Name* | *Class of Plant* * | *Region of Observation* ** | *Sensitivity* |
|---|---|---|---|---|
| Brassica alba | White Mustard | w | B | S |
| Brassica campestris | Field Cabbage | w | Eu, P, Z | S |
| Brassica juncea | | w | A | S |
| Brassica napus | Rape | c | A, Eu | S |
| Brassica nigra | Mustard | c | Eu, A | S |
| Brassica oleracea | Cabbage | c | A, Eu | s–S |
| Brassica tournefortii | Wild Turnip | w | Au | S |
| Bromus hordeaceus | Soft Brome Grass | w | A, Au | R |
| Bromus rigidus | Ripgut | w | A | R |
| Bromus secalinus | Rye Brome Grass | w | Sw | R |
| Bryophyllum calycinum | | | Au | S |
| Bulbine bulbosa | Bulbine Lily | w | Au | R |
| Bunias orientalis | | w | Sw | S |
| Buxus sempervirens | Box Tree | w, o | A | R |
| Caladium spp. | | o | Eu | R–r |
| Calendula officinalis | Marigold | o | A, Eu | S |
| Calystegia sepium | Greater Bindweed | w | A, Eu, Z | s |
| Camelina spp. | False Flax | w | A | S |
| Campanula spp. | Bellflower | w | A | r |
| Cannabis sativa | Hemp | c | A | s |
| Caperonia castaneafolia | Birds' Eyes | w | A | S |
| Capsella bursa-pastoris | Shepherd's Purse | w | A, Au, Eu, Z | s–S |
| Cardaria draba | Hoary Cress | w | A, Au, Eu, Z | r–s |
| Carduus nutans | Nodding Thistle | w | B, Z | S |
| Carduus tenuiflorus | Winged Thistle | w | A, Au, B, Z | S |
| Carex spp. | Sedges | w | Z | R |
| Carex flava | Yellow Sedge | w | A | S |
| Carrichtera annuua | Ward's Weed | w | Au | S |
| Carthamnus lunatus | Saffron Thistle | w | Au | S–s |
| Carya ovata | Shagbark Hickory | w | A | r |
| Cassia occidentalis | Bladder Senna | w | A | S |
| Castanea dentata | Chestnut | c | A | s |
| Catalpa bignonioides | | | A | r |
| Ceanothus cuneatus | | w | A | r–s |
| Celastrus scandens | | o | | s |
| Celosia argentea | Quail-grass | w | A | S |
| Celtis occidentalis | Common Hackberry | w | A | r–s |
| Cenchrus pauciflorus | | w | A | R |
| Centaurea calcitrappa | Star Thistle | w | Au | r–S |
| Centaurea cyanus | Cornflower | w, o | Eu | s–S |
| Centaurea melitensis | Maltese Cockspur | w | Au | S |

| *Botanical Name* | *Common Name* | *Class of Plant* * | *Region of Observation* ** | *Sensitivity* |
|---|---|---|---|---|
| Centaurea nigra | Hardheads | w | B | s |
| Centaurea repens | Russian Knapweed | w | A, Au | R–r |
| Centaurea solstitialis | Yellow Star Thistle | w | A, Au | s–S |
| Cephalanthus occidentalis | Button Bush | w | A | R |
| Cerastium cæspitosum | | w | Sw | r–s |
| Cerastium glomeratum | Mouse-eared Chickweed | w | Z | R |
| Cerastium vulgatum | ,, | w | A, B | r–S |
| Cestrum parqui | Green Cestrum | w | A | r |
| Chæræfolium sylvestre | | w | As | R |
| Chætochloa spp. | | w | A | R |
| Chætochloa triangulare | Fishweed | w | Au | s |
| Chamæcrista fasciculata | Partridge Pea | w | A | r |
| Chamæcyparis spp. | White Cedar | o | | R |
| Chenopodium spp. | Goosefoot | w | A | s |
| Chenopodium album | Fat-hen, Lambs' Quarters | w | A, Au, B, Z | s |
| Chenopodium botrys | Stinkweed | w | Ea, A | S |
| Chenopodium carinatum | Keeled Goosefoot | w | Au | S |
| Chenopodium glaucum | | w | A | r–s |
| Chenopodium multifidum | Scented Goosefoot | w | Au | S |
| Chicorium Intybus | Chicory | c | A, Au<br>Z | S<br>r |
| Choisia ternata | Choisy | | A | S |
| Chondrilla juncea | Skeleton Weed | w | A, Au | r–s |
| Chrysanthemum spp. | | o | Eu | S |
| Chrysanthemum leucanthemum | Ox-eye Daisy | w | A, B, Eu | r–s |
| Chrysanthemum segetum | Corn Marigold | w | A, Eu | R |
| Cicuta maculata | Water Hemlock | w | A | S |
| Cirsium arvense | Field Thistle | w | A, Eu<br>Au, Z | s<br>r |
| Cirsium lanceolatum | Scotch Thistle | w | A, Au, Eu, Z | s–S |
| Cirsium plattense | Prairie Thistle | w | A | s |
| Cirsium pumilum | Bull Thistle | w | A | s |
| Cirsium undulatum | | w | A | s |
| Cirsium vulgare | Spear Thistle | w | B | S |

| *Botanical Name* | *Common Name* | *Class of Plant* * | *Region of Observation* ** | *Sensitivity* |
|---|---|---|---|---|
| Cissus arborea | Peppervine | w | A | S |
| Citrullus vulgaris | Water-melon | c | A | S |
| Citrus aurantium | Orange | c | A | r–s |
| Citrus decumana | Grapefruit | c | A | r–s |
| Citrus medica var. limonum | Lemon | c | A | S |
| Claytonia perfoliata | | w | B | R |
| Clematis virginiana | Virgin's Bower | w | A | s |
| Cleome spp. | | w | A | S |
| Clerodendron spp. | | w | Sa | S |
| Coffea arabica | Coffee | c | Sa | R–s |
| Colchicum autumnale | Autumn Crocus | w | As | r–s |
| Commelina communis | Dayflower | w | A | S |
| Commelina cyanea | Wandering Jew | w | Au | S |
| Commelina longicaulis | Water Grass, Listening Willie | w | A, Ea | S |
| Commelina nudiflora | | w | Ml | s |
| Comocladia dentata | Guao (Cuba) | w | Wi | r–s |
| Comptonia peregrina | Sweet Fern | w | A | S |
| Conium maculatum | Hemlock | w | Au, B, Z | s–S |
| Conringia orientalis | | w | A | S |
| Convolvulus arvensis | Field Bindweed | w | A, Au, Eu, Z | r–S |
| Convolvulus erubescens | Australian Bindweed | w | Au | S |
| Corchorus trilocularis | Jew's Mallow | w | A | S |
| Cordia macrostachya | Black Sage, Herbe Condé | w | M | R |
| Coreopsis spp. | | o | | S |
| Cornus alternifolia | Alternate-leaved Dogwood | w | A | r |
| Cornus sanguinea | Dogwood | w | Sw | R |
| Cornus stolonifera | | o | A | s |
| Coronopus didymus | Hog-cress | w | Au, B, Z | s–S |
| Corylus avellana | Hazel Nut | c | Sw | S |
| Corylus rostrata | Beaked Hazel | w | A | S |
| Cotula australis | Common Cotula | w | Au | R |
| Crassula sieberiana | | w | Au | S |
| Cratægus spp. | Hawthorn | w | A | S |
| Crepis biennis | Rough Hawkweed | w | B | R |
| Crepis capillaris | Hawkweed | w | Z | S |
| Crepis virens | Smooth Hawks-beard | w | As | r |
| Crotolaria striata | Streaked Rattlepod | w | Au | S |

| *Botanical Name* | *Common Name* | *Class of Plant* * | *Region of Observation* ** | *Sensitivity* |
|---|---|---|---|---|
| Croton texensis | Texas Croton | w | A | S |
| Cryptostemma calendulaceum | Cape Weed | w | Au, Z | r–S |
| Cucumis Melo | Cantaloupe | c | A | S |
| Cucumis myriocarpus | Paddy Melon | w | Au | R |
| Cucumis sativus | Cucumber | c | A | S |
| Cucurbita pepo | Pumpkin | c | A | S |
| Cuscuta spp. | Dodder | w | A, Sw | s |
| Cuscuta australis | ,, | w | Au | S |
| Cydonia japonica | | o | | S |
| Cymbonotus lawsonianus | Bear's Ear | w | Au | S |
| Cynodon dactylon | Bermuda Grass | w | A, F | R |
| Cyperus esculentus | | w | Ea | r–s |
| Cyperus rotundus | Nut Grass | w | A, Au, M, Ea | r–s |
| Cytisus scoparius | Broom | w | Au, B, Z | r |
| Dactylis glomerata | Cocksfoot | w | B | R |
| Danthonia spp. | Wallaby Grass | w | Au | R |
| Daphne spp. | | o | | r–R |
| Datura ferox | Long-spined Thornapple | w | Au | R |
| Datura stramonium | Common Thornapple | w | Au, Eu, Ea | S |
| | Jimson Weed | | A | S |
| Daucus carota | Carrot | c | A, Eu | S |
| Daucus glochidiatus | Carrot Weed | w | Au | S |
| Delphinium spp. | | o | A | r–s |
| Dennstædtia punctilobula | Hay-scented Fern | w | A | r |
| Derris elliptica | | c | Sa | r |
| Descantaria elongata var. diuretica | | c | Sa | S |
| Deschampsia cæspitosa | Tufted Hair-grass | w | Sw | R |
| Desmodium triflorum | Tick Trefoil | w | Au | Var. |
| Deutzia gracilis | | o | A | R |
| Dianthus spp. | Pink | o | A | S |
| Dianthus barbatus | Sweet William | o | | S |
| Dicentra sp. | Bleeding Heart | o | | S |
| Dichondra repens | | w | A | S |
| Dichrostachys nutans | Aroma Marabu (Cuba) | w | Wi | S |
| Diervilla spp. | Weigelia | o | A | S |

| *Botanical Name* | *Common Name* | *Class of Plant* * | *Region of Observation* ** | *Sensitivity* |
|---|---|---|---|---|
| Digitalis purpurea | Foxglove | w | Z | s |
| Digitaria spp. | | w | Ml | r–s |
| Digitaria adscendens | | w | A | R |
| Digitaria ischæmum | | w | A | R |
| Digitaria sanguinalis | Crab Grass | w | A | R |
| Diplotaxis tenuifolia | Sand Rocket | w | Au, Eu | S–s |
| Dipsacus fullonium | Teazel | w | B | r |
| Doronicum spp. | Leopard's Bane | o | A | r–R |
| Draba verna | Whitlow Grass | w | B | R |
| Duchesnea indica | Indian Strawberry | w | A | S |
| Echinochloa crus-galli | Barnyard Grass | w | A | R |
| Echium plantagineum | Patterson's Curse / Salvation Jane | w | Au | S |
| Echium vulgare | Viper's Bugloss / Blue Weed | w | Eu / A | r–s |
| Eichornia crassipes | Water Hyacinth | w | A, Au, Ml | S |
| Elephantopus scaber | | w | A | r–s |
| Eleusine indica | Goose Grass | w | A, Ea, Ml | R–s |
| Emex australis | Double Gee, Three-cornered Jack | w | Au | s |
| Enhydrias angustipetala | | w | Ml | R |
| Epilobium spp. | Willow Weed | w | Z | r |
| Epilobium angustifolium | Fire Weed | w | A | S |
| Epilobium glabellum | Smooth Willow-herb | w | Au | r–S |
| Equisetum arvense | Field Horsetail | w | Eu | r–s |
| Equisetum hyemale | Scouring Rush | w | A | S |
| Equisetum palustre | Marsh Horsetail | w | H | S |
| Erechtites quadridentata | Cotton Fireweed | w | Au | S |
| Erica lusitanica | Spanish Heath | w | Z | s |
| Erigeron sp. | | w | Ea | S |
| Erigeron annuus | Fleabane Whitetop | w | A | s |
| Erigeron canadensis | Canadian Fleabane | w | A, Au, B | s–S |
| Erigeron linifolius | Flax-leaved Fleabane | w | Au | S |
| Eriogonum annuum | | w | A | r–s |
| Erodium spp. | Crowfoot | w | Z, A | S |
| Erodium cicutarium | Stork's Bill | w | B, Au | S |

| *Botanical Name* | *Common Name* | *Class of Plant* * | *Region of Observation* ** | *Sensitivity* |
|---|---|---|---|---|
| Erodium cygnorum | Crowfoot | w | Au | S |
| Erophila vulgaris | Whitlow Grass | w | B | S |
| Erysmium cheiranthoides | Treacle Mustard | w | Eu | S |
| Erythræa australis | Australian Centaury | w | Au | S |
| Erythrina sp. | | o | Sa | R–r |
| Eupatorium capillifolium | Dog Fennel | w | A | S |
| Eupatorium riparium | Mist Flower | w | Au | Var. |
| Euphorbia drummondii | Caustic Weed | w | Au | Var. |
| Euphorbia esula | Leafy Spurge | w | A | R |
| Euphorbia helioscopia | Sun Spurge | w | Au | S |
| Euphorbia heterophylla | Poinsettia | w | Au | S |
| Euphorbia hirta | Asthma Plant | w | Au | S |
| Euphorbia maculata | | w | A | r–s |
| Euphorbia peplus | Petty Spurge | w | Au, Eu | r–S |
| Euphorbia prostrata | Red Caustic Creeper | w | Au | S |
| Euphorbia pulcherrima | Poinsettia | o | A | S |
| Euphorbia terracina | False Caper | w | Au | S |
| Fagopyrum esculentum | Buckwheat | w | A | S |
| Fagus grandifolia | Beech | | A | r |
| Festuca rubra | Red Fescue | w | B | R |
| Filipendula ulmaria | Meadowsweet | w | B | r |
| Fœniculum vulgare | Fennel | w | A, Au, B, Z | s–S |
| Forsythia spp. | | o | | S |
| Fragaria spp. | Strawberry (Wild) | w | A | r |
| Fragaria vesca | Strawberry | c | | r–R |
| Fraxinus americana | White Ash | c | A | r |
| Fraxinus excelsior | Ash | c | Sw | R |
| Fumaria muralis | Fumitory | w | Z | r |
| Fumaria officinalis | ,, | w | A, Au, Eu | r |
| Fumaria parviflora | ,, | w | Au | s |
| Fumaria vaillantii | ,, | w | Sw | r |
| Gaillardia spp. | | o | | S |
| Galega officinalis | Goat's-rue | w | Z | r |
| Galeopsis ladanum | Red Hamp Nettle | w | Eu | r |
| Galeopsis speciosa | Large-flowered Hemp Nettle | w | Sw | r |

| *Botanical Name* | *Common Name* | *Class of Plant* * | *Region of Observation* ** | *Sensitivity* |
|---|---|---|---|---|
| Galeopsis tetrahit | Common Hemp Nettle | w | A, Eu | r–s |
| Galinsoga ciliata | | w | A | S |
| Galinsoga parviflora | Yellow Weed | w | Ea, Eu<br>Au | r–s<br>S |
| Galium aparine | Cleavers | w | Au, B, Sw, Z | R |
| Galium molugo | Great Hedge Bedstraw | w | Sw | R |
| Galium vaillantii | | w | Sw | R |
| Galium verum | Lady's Bedstraw | w | B | R |
| Gaultheria procumbens | Wintergreen | | A | S |
| Gaura sp. | | | A | S |
| Gaylussacia baccata | Huckleberry | w | A | S |
| Geranium spp. | Crane's Bill | w | B, Z | r–s |
| Glaux maritima | Sea Milkwort | w | B | r–s |
| Glechoma hederacea | Ground Ivy | w | A, Eu | s–S |
| Gleditschia triacanthos | Black Locust | | A | S |
| Glyceria aquatica | Reed Mead Grass | w | Z | R |
| Gnaphalium luteo-album | Cudweed | w | Z | R |
| Gomphrena celosoides | Gomphrena Weed | w | Au | S |
| Goodenia pinnatifida | | w | Au | S |
| Grindelia squarrosa | Gumweed | w | A | S |
| Gypsophila spp. | | o | | S |
| Hamamelis virginiana | Witch Hazel | w | A | S |
| Helenium hoopesi | Orange Sneeze-weed | w | A | S |
| Heleocharis spp. | Club Rushes | w | Ml | r–s |
| Helianthus anuus | Sunflower | o | A | S |
| Helianthus tuberosus | Jerusalem Artichoke | c | A | S |
| Heliopsis patula | | o | A | S |
| Heliotropium amplexicaule | Wild Verbena | w | Au | r–s |
| Helxine soleiroli | | w | A | R |
| Hemerocallis fulva | Day Lily | o | A | r |
| Heracleum sphondylium | Hog-weed, Cow Parsnip | w | As | R |
| Hibiscus spp. | Hibiscus | o | A | S |
| Hieracium spp. | Hawkweeds | w | B | R |
| Hieracium canadense | ,, | | | s |

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|---|---|---|---|---|
| Hieracium pilosella | Mouse-ear Hawkweed | w | Sw | s–S |
| Hieracium pratense | King Devil | w | A | S |
| Holcus halepensis | Johnson Grass | w | A | R |
| Holcus mollis | Soft Grass | w | B | R |
| Homeria spp. | Cape Tulip | w | Au | r |
| Hordeum jubatum | Squirrel-tail Grass | w | A | R |
| Hordeum leporinum | Barley Grass | w | Au | R |
| Hordeum vulgare | Barley | c | A, Eu | r–R |
| Hydrangea spp. | | o | | S |
| Hydrocotyle spp. | Pennywort, Waxweed | w | A, Au, Z | s–S |
| Hydrocotyle bonariensis | Herbe Bol | w | M | s |
| Hymenachne myuros | | w | Ml | R |
| Hymenopappus sulphureus | Woollywhite | w | A | S |
| Hypericum androsæmum | Tutsan | w | Z | R |
| Hypericum perforatum | St. John's Wort | w | A, Au, B, Z | s–S |
| Hypochæris radicata | Cat's Ear | w | A, Au, B, Z | s–S |
| Ilex verticillata | Winterberry | | A | r–s |
| Ilex vomitoria | Yaupon | w | A | S |
| Imperata cylindrica | Lalang | w | A, Ml | R |
| Indigofera spp. | Indigo | w | A | r |
| Inula graveolens | Stinkwort | w | Au | S |
| Ipomœa aquatica | Kankun | w, c | Fiji | S |
| Ipomœa batatas | Sweet Potato | c | A | S |
| Ipomœa cairica | Liane Lastique | w | M | S |
| Ipomœa calobra | Weir Vine | w | Au | S |
| Ipomœa plebeia | Bell Vine | w | Au | S |
| Ipomœa purpurea | Morning Glory | o, w | A | R |
| Ipomœa quamoclit | Star of Bethlehem | w | Au | S |
| Iris germanica | Iris | o | A | r–s |
| Iris kæmpferi | | o | A | R |
| Iva axillaris | Poverty Weed | w | A | r–s |
| Iva xanthifolia | False Ragweed | w | A | S |
| Jasminum spp. | Jasmine | o | | s–S |
| Juncus spp. | Rushes | w | B, Z | r |
| Juniperus communis | Juniper | w | A | R |
| Juniperus virginiana | Red Cedar | w | A | R–r |
| Jussieua spp. | Water Primrose | w | Ml, A | S |

| *Botanical Name* | *Common Name* | *Class of Plant* * | *Region of Observation* ** | *Sensitivity* |
|---|---|---|---|---|
| Jussieua diffusa | | w | A | S |
| Jussieua californica | | w | A | S |
| Kalmia angustifolia | Sheep Laurel | w | A | s |
| Kalmia latifolia | Mountain Laurel | w | A | r |
| Kochia scoparia | Kochia | w | A | S |
| Kœleria phleoides | | w | F | R |
| Kyllinga monocephala | Mullumbumby Couch | w | Au | S |
| Lactuca pulchella | Blue Lettuce | w | A | r–s |
| Lactuca sativa | Cultivated Lettuce | c | | S |
| Lactuca scariola | Prickly Lettuce | w | A, Au, Sw | s–S |
| Lactuca taraxacifolia | Wild Lettuce | w | Ea | r |
| Lactuca virosa | ,, | w | As | s |
| Lagenaria vulgaris | Wild Gourd | w | A | S |
| Lamium amplexicaule | Henbit, Dead-nettle | w | A, Au | s–S |
| Lamium purpureum | Purple Deadnettle | w | Sw, Z | s |
| Lantana spp. | Lantana | w | Au, A | S |
| Lappula echinata | Blue Burr | w | A | S |
| Lapsana communis | Nipplewort | w | Sw | s |
| Larix spp. | Larch | c | A | r–s |
| Lathyrus aphaca | Yellow Vetchling | w | Eu | r–s |
| Lathyrus odorata | Sweet Pea | o | | S |
| Lathyrus pratensis | Yellow Meadow Vetchling | w | As | s–r |
| Lemna minor | Lesser Duckweed | w | A | R |
| Lemna oligorrhiza | Duckweed | w | Au | S |
| Leontodon spp. | Hawkbit | w | B, Z | s |
| Lepidium apetalum | | w | A | S |
| Lepidium hyssopifolium | Pepperwort | w | Au | S |
| Lepidium virginicum | Peppergrass | w | A | S |
| Leptospermum scoparium | Manuka | w | Z | R |
| Lespedeza spp. | Bush Clover | w | A | S |
| Ligustrum vulgare | Privet | o | | R |
| Lilium philadelphicum | Wood Lily | o | A | s |
| Linaria cymbalaria | Ivy-leaved Toad-flax, Wander-ing Jew | w | A | S |
| Linaria vulgaris | Toadflax, Butter and Eggs | w | A, Eu | R–r |

| *Botanical Name* | *Common Name* | *Class of Plant* * | *Region of Observation* ** | *Sensitivity* |
|---|---|---|---|---|
| Linum marginale | Australian Flax | w | Z | r |
| Linum perenne | Flax | o | | S |
| Linum usitatissimum | ,, | c | | r–s |
| Lippia spp. | | | A | S |
| Liquidambar styracifolia | Sweet Gum | w | A | S |
| Lithospermum arvense | Corn Gromwell | w | Au, Eu | S |
| Lolium multiflorum | | w | A | R |
| Lolium perenne | Perennial Rye-grass | c | A, Au, B | R |
| Lolium rigidum | Wimmera Rye-grass | c | Au | R |
| Lomandra spp. | | w | Au | R |
| Lonas inodora | African Daisy | | A | s |
| Lonicera japonica | Japanese Honey-suckle | w | A | S |
| Lonicera periclymenum | Honeysuckle | o | | S |
| Lonicera xylosteum | | w | A, Sw | r–s |
| Lupinus spp. | Garden Lupin | o | Z, A | S |
| Lupinus arborescens | Tree Lupin | | | S |
| Luzula spp. | Woodrush | w | B | R |
| Lychnis alba | White Campion | w | B | R |
| Lychnis dioica | Red Campion | w | Eu | R–s |
| Lycium ferocissimum | African Box Thorn | w | Au | r–s |
| Lycopersicum esculentum | Tomato | c | Au | S |
| Lycopodium obscurum | Ground Pine | w | A | R |
| Lyonia ligustrina | Maleberry | w | A | s |
| Lysimachia coliata | Loosestrife | w | A | r |
| Lysimachia nummularia | Moneywort | w | A | R |
| Maianthemum canadense | False Lily-of-the Valley | w | A | s |
| Malva parviflora | Small-flowered Mallow | w | A, Au | S |
| Malva rotundifolia | Dwarf Mallow | w | A, B | S |
| Malvastrum coromandelinum | False Mallow | w | Au | r–s |
| Manihot utilissima | Tapioca | c | A | S |
| Marrubium vulgare | Horehound | w | Au, B, Z | r–S |
| Martynia louisiana | Devil's Claw | w | Au | S |
| Matricaria chamomilla | Chamomile | w | B, Sw | r–s |

| *Botanical Name* | *Common Name* | *Class of Plant* * | *Region of Observation* ** | *Sensitivity* |
|---|---|---|---|---|
| Matricaria inodora | Scentless Mayweed | w | B, Sw, Z | r–s |
| Mazus sp. | | | A | s |
| Medicago arabica | Spotted Medick | w | Au, B | S |
| Medicago denticulata | Burr Medick | w | Au | S |
| Medicago hispida | Burr Clover | w | A, Eu | S |
| Medicago lupulina | Black Medick | w | A, Au, B | S |
| Medicago minima | Small Woolly Burr Medick | w | Au | S |
| Medicago sativa | Lucern | c | Au, B | S |
| Melastoma malabathricum | | w | Ml | R–r |
| Melilotus spp. | Sweet Clovers | w | A, B | S |
| Melilotus indica | Hexham Scent | w | Au | S |
| Mentha arvensis | Field Mint | w | Sw, Z | s |
| Mentha pulegium | Pennyroyal | w | Z | s |
| Mentha satureioides | ,, | w | Au | S |
| Mentzelia stricta | Stick leaf | w | A | S |
| Mercurialis annua | Annual Mercury | w | As | s |
| Mikania scandens | Mile-a-minute | w | M | R |
| Mimosa spp. | | w | Ml | r |
| Mimosa invisa | | w | A | r–s |
| Mimosa pudica | Sensitive Plant | w | Au | Var. |
| Mimulus guttatus | Monkey Musk | w | Z | s |
| Mirabilis jalapa | Marvel of Peru, False Jalap | c | Sa | S |
| Modiola caroliniana | Red-flowered Mallow | w | Au | r–s |
| Mollugo verticillata | Carpet Weed | w | A | S |
| Momordica charantia | | c | A | r–s |
| Monardia pectinata | Tall Mint | w | A | s |
| Monochoria spp. | | w | Ml | S |
| Muhlenbergia schreberi | Nimblewill | w | A | S |
| Myrica cerifera | Wax Myrtle | w | A | s |
| Nasturtium officinale | Water-cress | w | Z | S |
| Nasturtium palustre | Marsh Water-cress | w | | r–s |
| Nelumbo lutea | Lotus Lily | | A | S |
| Nelumbo nucifera | ,, | | A | s |
| Neslia paniculata | | w | A | S |
| Nicandra physaloides | Wild Hop | w | Au | S |
| Nicotiana tabaccum | Tobacco | c | A | S |
| Nuphar adrena | | | A | S |

| *Botanical Name* | *Common Name* | *Class of Plant* * | *Region of Observation* ** | *Sensitivity* |
|---|---|---|---|---|
| Nuttalia decapatala | Prairie Lily | w | A | s |
| Nymphæa flava | Yellow Water Lily | w | A | S |
| Nyssanthes diffusa | Barbwire Weed | w | Au | S |
| Oakesia sessilifolia | Bellwort | w | A | S |
| Obolaria virginica | Pennywort | w | A | S |
| Ocimum canum | Hoary Basil | w | A | S |
| Œnothera biennis | Evening Primrose | w | A, Au | r–s |
| Onoclea sensibilis | Sensitive Fern | w | A | r–s |
| Onopordon acanthium | Cotton Thistle | w | B | S |
| Opuntia spp. | Prickly Pear | w | A | r–s |
| Oryza sativa | Rice | c | | r–R |
| Osmunda cinnamomea | Cinnamon Fern | w | A | r–s |
| Osmunda claytoniana | Interrupted Fern | w | A | r–s |
| Ostrya virginiana | Hop Hornbeam | | A | S |
| Oxalis sp. | Wood-sorrel | w | Ea | R |
| Oxalis acetocella | ,, | w | A, B | r |
| Oxalis cernua | Soursob, Yellow Sorrel | w | Au, A | S |
| Oxalis corniculata | Yellow Wood-sorrel | w | A | r–s |
| | Ladies Sorrel | | Au | R |
| Oxalis corymbosa | Lilac-flowered Wood-sorrel | w | Au | R |
| Oxygonium atriplicifolium | Devil Thorn | w | Ea | R |
| Oxytropis spp. | Loco Weeds | w | A | r |
| Pæderia fœtida | Liane Lingue | w | M | r |
| Pæsia scaberula | Hard Fern | w | Z | R |
| Panicum maximum | | w | A | R |
| Papaver hybridum | Rough Poppy | w | Au, B | S |
| Papaver rhœas | Corn Poppy | w | Eu | s–S |
| Papaver somniferum | Opium Poppy | c | Fr | R |
| Parthenocissus dumetorum | Virginia Creeper | o | A | s |
| Paspalum conjugatim | | w | A | R |
| Pastinaca sativa | Parsnip | c | Eu, Z | s–S |
| Petalostemon villosus | Silky Prairie Clover | w | A | r |
| Phalaris tuberosa | Canary Grass | w | Au, A | r |
| Phaseolus spp. | Beans | c | | S |
| Phaseolus lunatus | Lima Bean | c | A | S |

| *Botanical Name* | *Common Name* | *Class of Plant* * | *Region of Observation* ** | *Sensitivity* |
|---|---|---|---|---|
| Philadelphus spp. | Mock Orange | o | A | r–s |
| Phleum pratense | Timothy Grass | c | | R |
| Phlox spp. | | o | A | r–s |
| Phyllanthus urinaria | Leaf-flower | w | A | S |
| Physalis spp. | Ground Cherry | w | A | R |
| | Cape Gooseberry | | Au | S |
| Phytolacca decandra | Pokeweed | w | A | S |
| Phytolacca octandra | Inkweed | w | Au | S |
| Picea abies | Norway Spruce | c | A | s |
| Pinus strobus | White Pine | c | A | r–s |
| Pinus virginianus | Virginian Pine | c | A | R |
| Pisum sativum | Garden Pea | c | | S |
| Plantago coronopus | Buckhorn Plantain | w | Au, B | S |
| Plantago lanceolata | Ribwort Plantain | w | A, Au, B, Z | s–S |
| Plantago maritima | Sea Plantain | w | B | r–s |
| Plantago major | Great Plantain | w | A, Eu, Z | s–S |
| Plantago media | Hoary Plantain | w | Sw | S |
| Plantago rugelii | | w | A | r–s |
| Plantago varia | Small Plantain | w | Au | S |
| Pluchea lanceolata | | w | India | r–s |
| Poa annuua | Annual Meadow-grass | w | Sw, B | R |
| Poa pratensis | Smooth Meadow-grass / Kentucky Blue-grass | w | A, Au | R |
| Polycarpon tetraphyllum | Four-leaved Allseed | w | Au | S |
| Polygonum aviculare | Knot-grass, Wire-weed | w | A, Au, Eu, Z | r–R |
| Polygonum coccineum | Smart Weed | w | A | S |
| Polygonum convolvulus | Black Bindweed | w | A, Eu | r–s |
| | Wild Buckwheat | | Au, Z | R |
| Polygonum hydropiper | Waterpepper | w | Au | Var. |
| Polygonum pennsylvanicum | Smartweed | w | A | S |
| Polygonum persicaria | Spotted Persicaria / Lady's Thumb | w | A, B, Z | r |
| Polygonum prostratum | Creeping Knot-weed | w | Au | r–s |
| Polygonum tartaricum | | w | A | R |
| Polygonum tomentosum | | w | Sw | r–s |

| *Botanical Name* | *Common Name* | *Class of Plant* * | *Region of Observation* ** | *Sensitivity* |
|---|---|---|---|---|
| Polystichum acrostichoides | Xmas Fern | w | A | R |
| Pontederia cordata | | | A | S |
| Populus alba | White Poplar | | Au | S |
| Populus deltoides | Cottonwood | w | A | r–s |
| Populus grandidentata | Large-toothed Aspen | | A | s |
| Populus tremula | Aspen | | Sw | S |
| Populus tremuloides | Trembling American Aspen | w | A | S |
| Portulacca oleracea | Purslane | w | A, Au, Z | s–S |
| Potamogeton americanus | Pondweed | w | A | R |
| Potamogeton diversifolius | ,, | w | A | R |
| Potentilla anserina | Silverweed | w | Eu | S |
| Potentilla arguta | Tall Cinquefoil | w | A | S |
| Potentilla canadensis | Fivefingers | | A | S |
| Potentilla fruticosa | Shrubby Cinquefoil | w | A | s |
| Potentilla reptans | Creeping Cinquefoil | w | Eu | s |
| Poterium sanguisorba | Salad Burnett | w | B, Au | R |
| Prosopsis juliflora var. glandulosa | Mesquite | w | A | s |
| Prunella vulgaris | Self-heal | w | A, Eu, Z | r–S |
| Prunus amygdalus | Almond | o | | S |
| Prunus angustifolia | Sand Plum | w | A | r–s |
| Prunus cerasus | Cherry | c | Sw | s–S |
| Prunus domesticus | Plum | c | Sw | S |
| Prunus glandulosa | | o | A | S |
| Prunus pennsylvanica | Fire Cherry | w | A | S |
| Prunus persica | Peach | c | | r–s |
| Prunus serotina | Black Cherry | c | A | s |
| Prunus virginiana | Choke Cherry | c | A | s |
| Psedera quinquefolia | Woodbine | | A | S |
| Psidium guajava | Guava | c | Au | S |
| Psoralea sp. | Scurf Pea | w | A | r |
| Pteridium aquilinum | Bracken Fern | w | A, Eu, Au | R |
| Pteridium esculentum | ,, | w | Z | R |
| Pyrethrum spp. | | o | | S |
| Pyrrhopappus carolinianus | False Dandelion | w | A | S |

| *Botanical Name* | *Common Name* | *Class of Plant* * | *Region of Observation* ** | *Sensitivity* |
|---|---|---|---|---|
| Pyrus communis | Pear | o | A | r |
| Pyrus malus | Apple | c | A | s |
| Quercus dumosa | Scrub Oak | w | A | r–s |
| Quescus pavardi | Shinnery Oak | w | A | s |
| Quercus rubra | Northern Red Oak | | A | r |
| Ranunculus spp. | Buttercup | w | Z | S |
| Ranunculus acris | ,, | w | Eu | s–S |
| Ranunculus arvensis | Corn Buttercup | w | Eu | S |
| Ranunculus bulbosus | Bulbous Buttercup | w | B | r |
| Ranunculus repens | Creeping Buttercup | w | Eu | s |
| Raphanus raphanistrum | Wild Radish | w | Au, Eu | S |
| Raphanus sativus | Radish | c | A | S |
| Raphistrum rugosum | Turnip Weed | w | Au | S |
| Rhagodia hastata | Spear-head Fishweed | w | Au | S |
| Rheum officinale | Rhubarb | c | | S |
| Rhinanthus spp. | Yellow Rattle | w | B | s |
| Rhododendron spp. | Rhododendron | o | | r |
| Rhus spp. | Sumach | w | A | S |
| Rhus diversiloba | Poison Oak | w | A | R–S |
| Rhus toxicodendron | Poison Ivy | w | A | s |
| Rhus typhina | Staghorn Sumach | w | A | s |
| Ribes grossularia | Gooseberry | c | A | S |
| Ribes vulgare | Wild Red Currant | w | A | S |
| Richardia brasiliensis | Mexican Clover | w | Au | S |
| Ricinus communis | Castor Oil Plant | w | Au, Sa | S |
| Robinia pseudacacia | False Acacia | c | A, Sw | r–s |
| Rosa spp. | Wild Rose | w | Sw | R–r |
| Rosa spp. | Cultivated Rose | o | | s–S |
| Rosa bracteata | Chickasaw Rose | w | A | r–s |
| Rosa californica | Wild Rose | w | A | S |
| Rosa eglanteria | Sweet Briar | w | Z | r |
| Rosa rubiginosa | ,, | w | Au | r–s |
| Rotala ramosior | Toothcup | w | A | S |
| Rubus fruticosus | Blackberry | w | Au, B | r–R |
| Rubus idœus | Raspberry | c | A | r |
| Rubus procerus | | w | A | R |
| Rubus vitifolius | California Blackberry | w | A | S |

| *Botanical Name* | *Common Name* | *Class of Plant* * | *Region of Observation* ** | *Sensitivity* |
|---|---|---|---|---|
| Rudbeckia sp. | Prairie Cornflower | w | A | S |
| Rudbeckia hirta | Black-eyed Susan | w | A | S |
| Rumex spp. | Docks | w | Z | s |
| Rumex acetosa | Sorrel | w | Eu | r–s |
| Rumex acetosella | Sheep's Sorrel | w | A, Au, B, Z | R–s |
| Rumex brownii | Swamp Dock | w | Au | r–S |
| Rumex crispus | Curled Dock | w | Au, Eu, A | s–S |
| Rumex domesticus | | w | Sw | r–s |
| Rumex obtusifolius | Broad-leaved Dock | w | A, Eu | r–s |
| Sabal spp. | Palmetto, Thatch Palm | | A | r |
| Saccharum officinarum | Sugar Cane | c | A, Sa | R |
| Sagina procumbens | Pearlwort | w | B | R |
| Sagittaria guayamensis | Arrowhead | w | Ml | S |
| Sagittaria latifolia | ,, | w | A | S |
| Salix spp. | Willows | w | Eu, A | s |
| Salix caprea | Goat Sallow | w | Sw | s |
| Salsola pestifera | Russian Thistle | w | A | r–s |
| Salvia coccinea | Texas Sage | w | Au | S |
| Salvia leucophylla | Purple Sage | w | A | S |
| Salvia officinalis | Sage | c | | S |
| Salvia reflexa | Mint Weed | w | Au | S |
| Salvia verbenaca | Wild Sage | w | Au, Eu | S |
| Sambucus canadensis | Elderberry | w | A | S |
| Sambucus nigra | ,, | w | B | S |
| | | | Z | R |
| Sapindus drumondii | Soapberry | w | A | S |
| Sapium sebiferum | Chinese Tallow Tree | w | A | S |
| Saponaria vaccaria | | w | A | R |
| Sassafras spp. | | w | A | s |
| Saxifraga spp. | Saxifrages | w | As, A | s |
| Scandix pecten-veneris | Venus Comb, Shepherd's Needle | w | Eu | r–s |
| Scirpus spp. | Club Rushes | w | Ml | r–s |
| Scirpus spp. | Tule | w | A | S |
| Scirpus acutus | | w | | S |
| Scirpus validus | | w | A | s |
| Scleranthus annuus | Knawel | w | B | S |
| Sedum acre | Biting Stonecrop | w | A | S |

| *Botanical Name* | *Common Name* | *Class of Plant* * | *Region of Observation* ** | *Sensitivity* |
|---|---|---|---|---|
| Sedum purpureum | Livelong | w | A | r |
| Senecio jacobea | Ragwort | w | Au, B, Sw, Z | r–s |
| Senecio viscosus | Stinking Groundsel | w | B | s |
| Senecio vulgaris | Groundsel | w | Eu, Z | r–S |
| Sesbania macrocarpa | Indigo Weed | w | A | s |
| Sesuvium portulacastrum | Sea-side Purslane | w | A | r–s |
| Setaria spp. | Fox-tails | w | | R |
| Setaria glauca | Pigeon Grass | w | A | R |
| Setaria lutescens | | w | A | R |
| Setaria viridis | | w | A | R |
| Sida cordifolia | Flannel Weed | w | Au | S |
| Sida hederacea | | w | A | R |
| Sida retusa | | w | Au | r–s |
| Sida rhombifolia | | w | Au | r–s |
| Siegsbeckia orientalis | Indian Weed | w | Au | S |
| Silene cucubalis | Bladder Campion | w | Au, Z | r |
| Silene dichotoma | | w | Sw | r–s |
| Silene gallica | French Catchfly | w | Au, Z | r–S |
| Silene inflata | | w | Eu | r |
| Silene latifolia | Campion | w | Eu | R |
| Silene noctiflora | Night-flowering Catchfly | w | A | r–s |
| Silene vulgaris | Catchfly | w | B | r–s |
| Silybium marianum | Milk Thistle | w | A, Au, Z | s–S |
| Sinapis arvensis | Yellow Charlock | w | Au, Eu | S |
| Sisymbrium altissimum | Tumble Mustard | w | A | S |
| Sisymbrium officinale | Hedge Mustard | w | A, Au, Z | S |
| Sisymbrium orientale | Indian Hedge Mustard | w | Au | S |
| Sisymbrium thalliana | Thale-cress | w | B | R |
| Solanum auriculatum | Wild Tobacco | w | Au | r–s |
| Solanum caroliniense | Horse Nettle | w | A | s |
| Solanum dulcamara | Bitter-sweet | w | A, Sw | r–s |
| Solanum melongena | {Egg Plant, Aubergine} | c | A | S |
| Solanum nigrum | Nightshade | w | A, Au, Sw, Z | r–s |
| Solanum opacum | Black-berried Nightshade | w | Au | S |
| Solanum rostratum | Buffalo Burr | w | A | r–S |
| Solanum sodomaeum | Devil's Apple | w | Au | S |
| Solanum torvum | Devil's Fig | w | Au | r–s |

| *Botanical Name* | *Common Name* | *Class of Plant* * | *Region of Observation* ** | *Sensitivity* |
|---|---|---|---|---|
| Solanum tuberosum | Potato | c | | R |
| Solidago spp. | Golden-rod | w | A | s |
| Soliva sessilis | Jojo-weed | w | Au | S |
| Sonchus arvensis | Corn Sowthistle | w | A, Eu | s–S |
| Sonchus asper | | w | A | S |
| Sonchus oleraceus | Common Sow-thistle | w | Au, Eu, Z | s |
| Sorbus aucuparia | Mountain Ash | w | A | R |
| Sorghum vulgare | Millet | c | | r–s |
| Sparganium spp. | Burr Reed | w | A | r–s |
| Spergula arvensis | Spurrey | w | A, Eu, Z | r–s |
| Spergula rubra | Sand Spurrey | w | Au, B | r–s |
| Spinacia oleracea | Spinach | c | | s–S |
| Spiræa latifolia | Meadow-sweet | w | A | S |
| Spiræa salicifolia | | w | Sw | R |
| Spiræa tomentosa | Hard-hack | w | A | s |
| Spirodela polyrhiza | | w | A | S |
| Stachys arvensis | Field Woundwort | w | Sw, Au | s–S |
| | Stagger Weed | | Z | R |
| Stachys palustris | Marsh Woundwort | w | A, Sw | r–s |
| Steironema ciliata | | | A | r–s |
| Stellaria media | Chickweed | w | A, Au, Eu, Z | r–S |
| Stillingia sylvatica | Queenroot | w | A | r–s |
| Stizolobium pruritum | Pica-pica | w | Sa, A | S |
| Striga spp. | Witchweed | w | India, A | s |
| Symphoricarpus orbiculatus | Coral Berry | w | A | s |
| Symphoricarpus racemosus | Snow Berry | o | | S |
| Synedrella nodiflora | | | A | S |
| Syntherisma sp. | Crabgrass | w | A | R |
| Tagetes minuta | Mexican Marigold | w | Ea | r |
| | Stinking Roger | | Au | S |
| Tamarix africana | Tamarisk | o | | s |
| Tamarix gallica | ,, | o | | r–s |
| Tanacetum vulgare | Tansy | w | A | S |
| Taraxacum officinale | Dandelion | w | A, Au, Eu, Z | r–S |
| Taxus spp. | Yew | o | | R |
| Tecoma radicans | Trumpet Creeper | w | A | s |
| Tephrosia virginiana | Sand Pea | w | A | r |

| *Botanical Name* | *Common Name* | *Class of Plant* * | *Region of Observation* ** | *Sensitivity* |
|---|---|---|---|---|
| Tetragonia expansa | New Zealand Spinach | w | Au | S |
| Thlaspi arvense | Penny-cress | w | A, Eu | S |
| Thrinax spp. | {Thatch Palms / Palmettos} | | Sa | r |
| Thunbergia fragrans | Liane blanc | w | M | r |
| Thymus serpyllum | Thyme | c | A | S |
| Tilia americana | Basswood | | A | r |
| Tithymalus esula | Leafy Spurge | w | A | S |
| Tragapogon pratensis | Goatsbeard | w | A | S |
| Tragapogon porrifolius | Salsify | w | Au | R |
| Trianthema portulacastrum | Black Pigweed | w | Au | Var. |
| Tribulis terrestris | Caltrop | w | A, Au, Ea | S |
| Tricoryne elatior | | w | Au | R |
| Tridax procumbens | | w | A | S |
| Trifolium arvense | Hare's Foot Trefoil | w | Au; B | R |
| Trifolium dubium | Small Yellow Trefoil | w | A, Au, B | r–s |
| Trifolium glomeratum | Small Round-headed Trefoil | w | Au | R |
| Trifolium procumbens | Hop Trefoil | w | A, Au | r |
| Trifolium repens | Dutch Clover | c | Au, B, A | r–R |
| Trifolium subterraneum | Subterranean Clover | | Au | R |
| Triticum vulgare | Wheat | c | | R |
| Tropæolum spp. | Nasturtium | o | | S |
| Tsuga canadensis | Hemlock | c | A | R |
| Tussilago farfara | Colt's-foot | w | Eu | r–R |
| Typha angustifolia | Lesser Bullrush | w | Au, B | R |
| Typha latifolia | {Greater Bullrush / Cat-tail} | w | A | s |
| Ulex europæus | Gorse | w | B, Z | r–R |
| Ulmus americana | White Elm | | A | s |
| Ulmus glabra | | | Sw | s |
| Urena lobata | Pink-flowered Chinese Burr | w | Au | S |
| Urtica dioica | Stinging Nettle | w | A, Au, Eu | S |
| Urtica urens | Small Nettle | w | Au, Eu, Z | S |
| Utricularia flexuosa | Bladderwort | w | Ml | R |
| Uvularia sessilifolia | Bellwort | w | A | r |

| *Botanical Name* | *Common Name* | *Class of Plant* * | *Region of Observation* ** | *Sensitivity* |
|---|---|---|---|---|
| Vaccinium spp. | Blueberry | w, c | A | s |
| Veratrum viride | False Hellebore | w | A | s |
| Verbascum thapsus | Great Mullein | w | A, Au, B | s–r |
| Verbascum virgatum | Twiggy Mullein | w | Au | S |
| Verbena spp. | | w, o | A | s |
| Verbena bonariensis | Purple Top | w | Au | s |
| Verbena officinalis | | w | Au | S |
| Verbena rigida | Veined Verbena | w | Au | S |
| Verbena tenera | Mayne's Pest | w | Au | S |
| Verbesina occidentalis | Stinkweed | w | A | r–s |
| Veronica spp. | Speedwell | w | Z | R |
| Veronica agrestis | Field Speedwell | w | Sw | r–R |
| Veronica arvensis | Wall Speedwell | w | Eu | r–s |
| Veronica chamædrys | Germander Speedwell | w | B | r |
| Veronica hederæfolia | Ivy-leaved Speedwell | w | Eu | r–s |
| Veronica serpyllifolia | Thyme-leaved Speedwell | w | Sw | r–s |
| Veronica spicata | | o | A | r |
| Veronica tournefortii | Large Field Speedwell | w | | S |
| Viburnum alnifolium | Hobblebush | w | A | r–s |
| Viburnum cassinoides | Wild Raisin | w | A | s |
| Viburnum dentatum | Arrowwood | w | A | r |
| Viburnum lentago | Nannyberry | w | A | s |
| Viburnum opulus | Guelder Rose / Rough-leaved Marshholder | w | A | S |
| Vicia spp. | Vetches, Tares | w | Z, Eu | r–s |
| Vicia sativa | Vetch | w | Eu, Au | r–s |
| Vigna vexillata | Wild Cow-pea | w | Ea | S |
| Vinca major | Periwinkle | w, o | Au, Eu, Z | R |
| Viola arvensis | Field Pansy | w | Z | R |
| Viola odorata | Violet | o | A | R |
| Viola tricolor | Heartsease | w | Eu | r–R |
| Vitis spp. | Wild Grapes | w | A | S |
| | Cultivated Grapes | c | | S |
| Vittadenia triloba | New Holland Daisy | w | Au | R |
| Wyethia sp. | Mule-ear | w | A | S |

| *Botanical Name* | *Common Name* | *Class of Plant* * | *Region of Observation* ** | *Sensitivity* |
|---|---|---|---|---|
| Xanthium californicum | Californian Burr | w | Au | S |
| Xanthium canadense | Cocklebur | w | A | S |
| Xanthium orientale | ,, | w | A | S |
| Xanthium pungens | Noogoora Burr | w | Au | S |
| Xanthium spinosum | Bathurst Burr | w | A, Au | S |
| Yucca glauca | Small Soapweed | w | A | r–s |
| Zea mais | Indian Corn | c | A | R |
| Zinnia spp. | | o | A | S |

# APPENDIX E

## HORMONES AND THE PRE-HARVEST DROP OF APPLES

The following table shows the responses of a wide range of apple varieties tested in Europe and America to pre-harvest drop-sprays of α-naphthylacetic acid (NAA). (Modified from a comprehensive set of tables given by Vyvyan, 1946.)

* Normal Dropping Tendency
- Heavy Dropper = Normal percentage accumulated drop in season 60 to 100 per cent.
- Medium Dropper = Ditto 30 to 60 per cent.
- Light Dropper = Ditto 0 to 30 per cent.

** Effect of Hormone Treatment — Percentage reduction in percentage accumulated drop:—
- 75–100 per cent. = Very Good
- 50–75 per cent. = Good
- 30–50 per cent. = Fair
- 0–30 per cent. = Poor
- 0 per cent. = Nil

| Variety | Country | Normal Picking Period | Normal Dropping Tendency * | Effect of Hormone Treatment ** |
|---|---|---|---|---|
| Allington | England | End Sept. | Light | Fair |
| Baldwin | U.S.A. | Early Oct. | Medium–Heavy | Good |
| Beauty of Bath | England | July–Aug. | Heavy | Very Good |
| Bramley Seedling | England | Sept.–Oct. | Light | Poor–Nil |
| Colville Lesans | Netherlands | — | Light | Poor |
| Cox's Orange Pippin | England | Sept. | Light | Fair |
| Delicious | U.S.A. | Sept. | Medium | Good |
| Duchess of Oldenberg | U.S.A. | — | Medium | Good |
| Early McIntosh | U.S.A. | — | Medium–Heavy | Very Good |
| Edward VII | England | — | Medium–Light | Poor |
| Framboosappel | Netherlands | Early Oct. | Medium | Poor |
| Goudreinette | Netherlands | Early Oct. | Light | Poor–Nil |
| Grime's Golden | U.S.A. | — | Light | Poor |
| Gronsvalder Klumpke | Netherlands | Early Oct. | Heavy | Good |
| Jonathan | U.S.A. | — | Light | Poor–Fair |
| King David | U.S.A. | — | Medium | Very Good |
| Lemoenappel | Netherlands | End Sept. | Light | Good |
| McIntosh | U.S.A. | — | Medium | Good |
| Miller's Seedling | England | End Aug. | Medium | Fair–Good |
| Northern Spy | U.S.A. | — | Medium–Heavy | Good |
| Red Gravenstein | U.S.A. | — | Medium–Heavy | Good |
| Ribston Pippin | Netherlands | — | Heavy | Good |
| Rome Beauty | U.S.A. | — | Medium | Good |
| Stayman's Winesap | U.S.A. | — | Medium | Fair–Good |
| Sterappel | Netherlands | End Sept. | Medium | Good |
| Wealthy | U.S.A. | — | Medium | Good–Fair |
| William's Early Red | U.S.A. | — | Medium | Very Good |
| Winesap | U.S.A. | — | Medium | Good |
| Worcester | England | Early Sept. | Medium | Good |
| Yellow Transparent | Netherlands | — | Medium | Good |
| York Imperial | U.S.A. | — | Medium | Good |

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