Rhubarb and buckwheat are the only economically important members of the Polygonaceae, to which Emex belongs. In a short survey undertaken by Hawaiian entomologists several insects were found attacking Emex in South Africa. One of these, a weevil Apion antiquum, has been liberated in Hawaii and is said to be causing a considerable reduction in Emex in certain areas. Another weevil, Alcidodes, was thought to be promising but the stock unfortunately died out in captivity (A.D. Thistle - personal communication).

Climatically conditions should favour the establishment of South African insects in Australia. All the main types of climate found in Australia (with the exception of the "warm temperate climate with rain likely in all months" found in coastal and subcoastal New South Wales) also occur in the Union of South Africa.

In addition to the major weed pests considered above, certain other Australian weeds have a southern African origin, and could be incorporated in the main investigation as opportunity offered. Such weeds include Asclepias fruticosa, Asclepias physocarpa (Balloon Cottons), Romulea rosea (Guildford Grass), Tripteris clandostina (Tripteris), and Berkheya rigida (Berkheya thistle). Cryptostegia grandiflora (Rubber vine), Zizyphus mauritiana (Chinee Apple), and Tribulus terrestris (Caltrop) are natives of tropical Africa.
INTRODUCTION

Since the enzymes, or organic catalysts, of biochemical reactions are of such importance in the functioning of living cells, physiologists and biochemists have turned their attention to possible effects of plant growth substances on such enzyme systems.

The evidence so far indicates that growth substances do not act as part of enzyme systems. The various chemical substances which have been shown to exert growth regulating effects in plants are, chemically speaking, of quite widely differing structure, and they do not seem to show the same specificity as exhibited, for example, by the vitamins in animal biochemistry, many of which are known to act as an essential part of an enzyme regulating an essential metabolic activity. Although many enzyme systems have been isolated from plants, no specific effect of plant growth had been reported, and there is no enzyme studied which has been reported to be affected in a specific manner by chemicals active as plant growth regulators.

While there is a lack of "in vitro" effects of plant growth substances on plant enzymes, there is a confusing multiplicity of effects "in vivo". By an "in vivo" effect is meant one which is exerted through, and depending on, the organization of the living cells. As is well known, "hormone herbicides" such as 2,4-D will cause a breakdown of carbohydrates in the leaves of beans and morning-glory plants, the net result being a depletion of the readily available carbohydrates in leaf tissues. This is accepted by some as a partial explanation of the herbicidal effects of this substance. One could multiply examples of the long term effects of 2,4-D and other growth substances on the enzyme

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activities of whole plants and tissues and get no closer to explaining the mechanism of action of these substances.

One difficulty in many of these studies was that there was no mechanism of action which could account for all the observed effects. Furthermore there was often no attempt made to localise the action of the growth substance, either in time or in space. Thus interpretation was, in most cases, difficult.

One of the confusing aspects of research into the mechanism of growth substance action is just this multiplicity of effect. Thus it may be profitable to examine in detail, the relationship between the plant growth substance and one enzyme system which is affected by it, rather than attempt to survey the whole spectrum of effects at once. In this way it might be possible to pick up effects of plant growth substances on the specific enzyme which might give an indication of the way that such substances could act on other systems.

Such investigations will naturally be guided by physiological studies. One of the most studied of the physiological effects of plant growth substances on cells is their ability to promote the cell extension of isolated tissues such as sections of the Avena coleoptile. This cell extension growth is accompanied by an uptake of water which is proportional to the increase in cell size. One of the explanations of this increased cell size is that the growth substance causes modifications in the cell walls which cause them to become more extensible. Thus Heyn (1931) was the first to show that the cell wall apparently became more extensible under mechanical loading when auxins such as 1AA were applied to cells of Avena coleoptiles. Later work supports his conclusions. There seems now no doubt that the application of plant growth substances of the auxin type does result in this increased plastic extensibility of the cells of some plant tissues. (Cleland & Bonner 1956, Cleland 1958, Adamson & Adamson 1958). Kerr (1951) suggested that the structure of the primary cell wall could be that of cellulose microfibrils as a discontinuous phase and pectic substances as the continuous phase. It was thought that many of the properties of the primary cell wall (i.e. the cell wall which is capable of growth) could be explained on this hypothesis, which could not be explained were it assumed that the cellulose alone controlled the cell wall properties. One approach to the study of auxin or growth substance action in plants has been to examine the effect of plant growth substances on the pectic enzymes and the pectin metabolism of the primary cell wall.
One way in which the properties of pectic substances could be controlled was pointed out by Bennett Clark (1956). It was pointed out that pectin consists of long chain molecules consisting of condensed galacturonic acid units. This means that there are fixed ionisable groups attached to these long chains. When these groups are methylated the attraction between them is minimal; when these groups are not methylated, consisting of the acid carboxyl group, the attraction is greater, especially in the presence of calcium ions, which could cross-link between these groups. Thus it was thought that the extent of methylation of the carboxyl groups in pectin could control the mechanical properties of the pectin of the primary cell wall, and thus, the properties of the cell wall itself.

An enzymatic approach to this problem is possible. The enzyme which is capable of controlling, in part, the extent of the methylation of pectin in plants, is pectin methyl-esterase. This enzyme converts the methylated carboxyl groups of pectin to the unmethylated form - it hydrolyses the ester linkage, and thus by its action would be capable of maintaining the pectin with the minimum of methoxyl content, and thus in a condition of minimum plastic extensibility, according to the hypothesis of Bennett Clark.

Pectin methyl-esterase could control the extensibility by an entirely different mechanism. According to one theory of pectin breakdown, the long chains of pectin molecules cannot be broken up enzymatically until the pectin molecule is first de-methylated by the enzyme PME. After this has been accomplished, other enzymes can act to breakdown the pectin molecules to shorter chain lengths. This also would lead eventually to increased plastic extensibility of the ground substance of the cell wall.

Bryan and Newcomb (1954) found that IAA would increase the PME activity of tobacco pith tissue expanding under the influence of IAA. Their interpretation of the results was that, for pectin breakdown to occur, the pectin had to be first de-methylated. Since IAA promoted PME activity, it would promote pectin breakdown, and thus the extensibility of the walls would be increased.

Glasziou (1957a, 1957b, 1958) adopted a different approach. Reasoning that plant growth substances could control the extent of methylation of pectin by "tying up" the enzyme PME, he studied the effect of plant growth substances on the state of PME in the cell. It was found that, at certain concentrations of IAA and 2,4-D, these substances promoted the binding of PME to both whole tissue slices and
to "cell wall fractions" of tobacco pith tissue. Similar results were obtained for Jerusalem artichoke tuber tissue as for tobacco pith tissue. Glasziou, from these studies, thought that IAA or other growth substances might control pectin metabolism in the cell wall by controlling the availability of the enzyme, pectin methylesterase. It was assumed that the enzyme was active when free in the cell wall, and inactive when bound. Since auxins (p.g.s.) had been found to bind the enzyme to cell wall fractions and whole cells, it was said that plant growth substances could cause the enzyme to become inactive. This would result in the build-up of methyl-ester groups in the pectin of the cell wall. This would result in the cell wall becoming more extensible, and so growth would occur with increased water uptake.

PRESENT WORK - RESULTS & DISCUSSION

The present work was initially undertaken to investigate the PME effect in whole tissue. After some preliminary studies, artichoke tuber tissue was chosen for this. This tissue has been much used in studies of plant growth substance action. Providing conditions are suitable, it will respond well to additions of 2,4-D and other plant growth substances, and at the so-called physiological concentrations, its water uptake over about 120 hrs. can be such as to increase the fresh weight by nearly 100%.

In preliminary studies it was found that 2,4-D induced water uptake of the sliced tuber tissue was much influenced by the external conditions. Calcium ions in very low concentrations during pretreatment were found to increase the subsequent water uptake in solutions of 2,4-D at the low (physiological) concentrations where this substance promotes the water uptake of responsive tissue. The procedure for demonstrating water uptake by discs of tissue is usually to cut into slices and thoroughly wash the tissue in distilled water, and then to aerate for a varying period in order to let the tissue recover from the effect of the cutting process. After this, the discs are placed in a solution of plant growth substance at an appropriate concentration, and water uptake is measured by fresh weight increases. In the present work it was found advantageous to include calcium at 20 ppm in the solution used for pretreatment, as well as the dilute phosphate buffer usually employed, as the response to 2,4-D was then greater.
The effect of 2,4-D (in particular) on the PME activity of artichoke tuber tissue was then studied. The method used to assay the PME (pectin methyl-esterase) activity was as follows, and is, in essentials, the technique used by Glasziou (1958), and Bryan and Newcomb (1954), adapted for use with artichoke tuber tissue. Finely chopped tissue was thoroughly washed in distilled water and then placed in a continuously aerated solution of dilute phosphate buffer, pH 5.0, molarity 0.005. Calcium at the low concentration of 20 ppm was included in the case of the experiments where an effect of 2,4-D was observed. This treatment in aerated solution was called the pretreatment. It was necessary in order to obtain reasonably consistent responses. After the pretreatment, batches of 2 gm fresh weight of tissue were weighed out, the tissue being carefully blotted through gauze after straining off from the solution. The tissue was then transferred to an assay solution consisting of 0.5% citrus pectin and made 0.2M with sodium chloride. The assay solution was adjusted to pH 7.0 with sodium hydroxide. The enzyme in the tissue is made active by the sodium ions and partly desorbed from the tissue. It acts on the pectin, converting methoxyl groups to hydroxyl groups, thus decreasing the pH of the solution. The decrease in pH was followed with a pH meter, and enough sodium hydroxide was then titrated to keep the pH at a value of 7.0 - 0.2. The amount of sodium hydroxide (0.005N) used in a certain time to maintain a constant pH was proportional to the PME activity of the tissue. The substrate, pectin, was non limiting at 0.5%. The technique used to measure general esterase (GE) activity was essentially similar, but in the GE studies no pretreatment was given, the pH used was 7.5 and the substrate naphthyl acetate instead of pectin. In all cases vigorous stirring with a magnetic stirrer was employed to thoroughly mix the tissue with the assay solution.

When 2,4-D was added to finely cut tissue at physiological concentration, the PME activity of the tissue increased by about 14-15%. It was shown that this response occurred consistently only when calcium had been included in the pretreatment solution. This requirement for calcium, given previously, was similar to the apparent requirement needed for maximum response to 2,4-D in the growth studies mentioned above. Fig (1) shows the response of PME activity to 2,4-D added to a final concentration of 6.0x 10⁻⁵M, using tissue which had been previously treated with small quantities of calcium.

The interaction with calcium ions was consistent with the idea that 2,4-D was interacting with a cell structure which is stabilised in some manner by calcium ions.
Such a structure could be formed by a phospholipid–water interface. It is well known that small quantities of calcium will stabilise plant tissues in the sense of preventing leakiness which develops when such tissues are placed in distilled water. This effect is very likely to be the result of the stabilisation of the cell membranes.

It should be emphasised that the results obtained by Glasziou (1958) and in the present work, differed from most other studies of plant growth substances in relation to enzymes in one important respect. While the effect of plant growth substances on enzymes is widespread when tissues are treated for long periods with 2,4-D doses, or when plants are sprayed with 2,4-D and then analysed, short term effects on enzymes are less well known. In Glasziou's (1958) and the present work, the effect on the enzyme PME could be measured within five to ten minutes of adding 2,4-D to the assay solution. There were differences of detail concerning the nature of the response in the two cases, but this was a common feature, this immediate response of the measured enzyme activity to the addition of 2,4-D (and 1AA in the case of Glasziou's work). In the present work a binding of PME to the tissue could not be demonstrated.

Certain features of the response of PME activity to added 2,4-D in the present work led the author to consider the possibility that other enzymes might be involved, as well as PME. One of these features was that high concentrations of 2,4-D of $10^{-2}$M and above, had no effect on PME activity, and also that the high concentration of 2,4-D did, in some experiments at least, reverse the effect of the lower concentration. The effect of high concentrations of 2,4-D were then investigated using not pectin methyl-esterase activity, but rather general esterase activity of the tissue as a guide.

It was found that artichoke tissue did have considerable general esterase activity, which was followed by using, as a substrate, α-naphthyl acetate. The enzyme acted on this substance to produce naphthol and acetic acid. Both naphthol and the acid produced were estimated over short time periods and found to be equivalent. The studies of general esterase activity were conducted using acid production as a measure of enzyme activity.

At high concentrations, 2,4-D was found to decrease the general esterase activity of artichoke tissue by about 40-50%. The effect was shown by IAA as well as all chlorinated phenoxyacetic acids which were tried, but not by the unchlorinated phenoxyacetic acid. Fig. 2 shows the effect
of high 2,4-D concentration on the general esterase activity of artichoke tuber tissue, and in addition illustrates the lack of effect of high concentration of 2,4-D on PME activity. It also shows the lack of effect of 2,4-D at the same concentration on an enzyme extract from artichoke tuber tissue. The substances so far used in this investigation have been 2,4-D, 2,6-D, 2,4,6-T, POA and IAA. All these substances were added as the sodium salt and the only one which had no effect on general esterase activity was phenoxyacetic acid, the parent substance of the substituted phenoxyacetic acids used. POA is generally regarded as a very weak "auxin" and has, of course, no herbicidal properties. However, no general correlation with herbicidal properties was indicated by the results.

The conditions under which these results were obtained were different from those of the PME experiments, and the two sets of results are best regarded as distinct phenomena. Certain features of the general esterase (GE) experiments suggested that the chlorinated phenoxyacetic acids and IAA were acting to partially prevent the approach of the substrate to a surface responsible for its hydrolysis in the absence of growth substance. In both PME and GE experiments, the effect of 2,4-D was immediate and seemed to depend on a physical effect. In the case of the general esterase it was shown that the enzyme activity was due to enzyme which was still attached to the tissue at the time of the 2,4-D effect on it.

In summary the main findings of the present work have been:

1. Pectin methyl-esterase activity of tissue was increased by 2,4-D under conditions which had previously been found to favour a physiological response to 2,4-D. The interaction of the effect with calcium ions suggested that a certain stabilised surface was involved in these experiments.

2. General esterase activity of tissue was suppressed by high concentrations of a variety of growth substances, including IAA, but not by POA.

3. The effects on pectin methyl-esterase and on general esterase activity were immediate, and measured within minutes of adding the substances to the tissue.

4. The growth substances had no effect on the activity of crude enzyme extracts of either enzyme.
It is hoped that further work will do more to elucidate the nature of these immediate effects of growth substances at the enzyme level in plant cells.

**ABBREVIATIONS**

**Compounds**
- 2,4-D: 2,4 dichlorophenoxyacetic acid.
- 2,6-D: 2,6 " " "
- 2,4,6-T: 2,4,6 trichlorophenoxyacetic acid.
- POA: phenoxyacetic acid.
- IAA: indole - 3 acetic acid.

**Enzymes**
- PME: pectin methyl-esterase.
- GE: general esterase.

**REFERENCES**


- (1957b). - " " " 10,4,426-434.

Showing the response of PME activity to additions of 2,4-D made during assay of enzyme activity of tissue slices. 2,4-D at a concentration of $6 \times 10^{-5}$M was added at 35 mins. Note that where Ca pre-treatment was given, a response occurred; where no Ca pre-treatment was given, no response occurred.
Effect of 2,4D on enzymatic est. activ. in vitro.

- + 2,4D
- - 
- spontaneous hydrolysis

2,4D
O.001

1.0

2.0

3.0

1.0

2.0

3.0

0.005 N

MLN NA0H

MINS.

A: PME activity, 2,4D 1.0 \times 10^{-3} M.

\# " " " ZERO

O: GEN. ESTERASE = 2,6-D

\# " " " 1.0 \times 10^{-3} M.

\#: ENDOGENOUS ACID PROD.

Showing the general esterase (GE) activity and PME activity with and without 2,4-D at 10^{-3} M. No pre-treatment was given. Note the lack of effect of 2,4-D on PME activity, under these conditions which contrasts with the strong suppression of GE activity. This response was produced with 2,6-D, 2,4,6-T, and IAA also, but not with POA.