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TRANSLOCATION OF ASSIMILATES IN THE TOMATO PLANT

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Summary The steps limiting the loading of assimilate in leaves and the unloading of imported sucrose in fruits have been examined in the tomato. The rate of leaf assimilate translocation was estimated as the difference between the change of carbon content and the CO_2 exchange in both leaves and fruits.

The rate of carbon export from a mature leaf is in proportion to the concurrent carbon fixation plus a constant supply from the mobile reserve and is therefore related to the concentration of labile sucrose. Three-quarters of the carbon being exported from a leaf, during its 100 day life, occurs during a period of 30 days when the leaf is 50-60 days old.

The supplies of leaf assimilate to individual trusses are similar. The possible causes of the reduction in final size of fruits from the base to the tip of a truss are discussed. It is proposed that the rate of fruit growth may be determined by the unloading process. The activity of one or more enzymes related to sucrose hydrolysis (e.g. acid invertase) may be a key factor regulating the rate of import.

INTRODUCTION

The ultimate aim of research on the translocation of leaf assimilate in fruiting crops is to utilize the knowledge of the partitioning of leaf assimilate in a whole plant to maximise fruit yield by manipulating assimilate distribution. Since structural features of the conducting tissue appear not to limit movement from source to sink (Milthorpe & Moorby, 1969; Wareing and Patrick, 1975), the driving force is located in either the source or the sink or both. Our work has therefore been concentrated on the identification of the limiting steps in the loading of labile assimilate in the leaves and the unloading of imported sucrose in the fruits of tomatoes.

Our measurement of the rate of translocation from leaves or into fruits was based on the carbon budget in these organs. This was obtained by measuring the change of carbon content and the CO_2 exchange of an organ over a given time. This approach provides an estimate of the rate of translocation more accurate than that based on dry matter accumulation; the latter underestimates the translocation by ignoring respiration. Moreover, since the rate of translocation is estimated in terms of carbon and is based on the measurements of carbon fixation or respiration and the carbon change of a given organ, the rate of carbon transport is an integral part of the carbon economy. Therefore, the rate of carbon transport can be directly related to all components of plant growth and the effect of the environment upon them. Furthermore, the rates of carbon transport from leaves or into fruits were measured concurrently with carbon metabolism in these organs. The causal relationship between the rate of carbon transport and these changes of carbon metabolites allows a further analysis of the limiting steps to carbon metabolism in regulating

the rate of carbon transport.

The following is a brief report of our findings and a statement of our understanding of the mechanism of leaf assimilate translocation in the tomato.

RATE OF CARBON EXPORT FROM LEAVES

The rate of carbon transport was measured using either the seventh or the ninth basal leaves in two-truss tomato plants. Export of leaf assimilate starts when the compound leaf is only 10 percent expanded and the capacity of export develops basipetally from the terminal leaflet to the basal-pair of leaflets (Ho & Shaw, 1977). The leaflets of a compound leaf become net exporters only 13 days after the leaf becomes visible (Ho & Shaw, 1978). A greater proportion of newly fixed carbon is exported with increasing leaf area during expansion. Since the rate of carbon fixation reaches a maximum just before the leaf is fully expanded (Ho, 1977), the amount of carbon being exported declines rapidly with time in a fully expanded leaf.

Based on this information, a tomato leaf with a final area of 6 dm^2, under a constant light flux density of 70 Wm^{-2} for 16 h daily, may export about 10 g of carbon over a period of 100 days with three-quarters of this carbon being exported during 30 days when the leaf is 30-60 days old. The amount of carbon being exported in the last 40 days amounts to only one-fifth of the total export. This is due to a combination of both low carbon fixation and a low proportion of newly fixed carbon being exported (Ho, 1976b). The continuing assimilate accumulation in a mature tomato leaf, results in an undesirable low export of assimilate.

The rate of carbon export from a mature tomato leaf in the light period is proportional to the rate of concurrent carbon fixation (Ho, 1976a). This relationship can be described satisfactorily by a linear regression (Ho, 1976b) indicating that the rate of carbon transport is proportional to the rate of concurrent carbon fixation plus a certain supply of carbon mobilized from reserves. Starch appears to be the principal source of reserve carbon hydrolysed for export (Charles-Edwards & Ho, 1976). The analysis of this relationship in plants grown at different radiations and CO_2 concentrations revealed that the proportion of newly fixed carbon being immediately exported is similar in leaves grown in different conditions, although it is slightly lower in plants grown in low radiation without CO_2 enrichment. On the other hand, the difference in the reserve supply is substantially affected by the growing condition, so that only a trivial amount of carbon is supplied by the reserve for carbon export in plants grown under low radiation without CO_2 enrichment (Ho, 1978).

Further analysis of the rate of carbon export by day and night revealed that the rate of day transport is more closely related to the sucrose concentration in the leaf than is that of night transport. Based on the relationship between the rates of night transport and of night respiration, the energy obtained from 1 g of respired carbon may facilitate the export of 4.55 g of carbon (Ho & Thornley, 1978).

RATE OF CARBON IMPORT INTO FRUITS

Factors affecting the import of leaf assimilate into fruits have been examined at crop, truss and fruit levels.

Based on dry matter partitioning, the harvest index for tomato is 0.5-0.6 (Tanaka et al., 1974). At crop level, the fruit yield is undoubtedly determined by the availability of leaf assimilates. Thus, the yield of an early crop with CO_2

enrichment can be over twice that of a non-enriched crop with the number and the size of the fruits increased (Calvert & Slack, 1975). In a multi-truss tomato plant, individual trusses appear to be supplied with assimilates primarily by a specific group of leaves (Bonnemain, 1966; Khan & Sagar, 1966). Since there are three leaves between successive trusses, the supply of leaf assimilate to individual trusses may be similar. The uniformity in the final yield of individual trusses in a 10-truss plant, all weigh about 400 $\pm$ 50 g and comprise 7-9 fruits each of 50 $\pm$12 g, supports such an idea (Slack, personal communication).

The source-sink relationship between a specific group of leaves and an individual truss is dynamic. Slack and Calvert (1977) demonstrated that removing a truss increased the yield of its immediately adjacent trusses by up to 13%, the benefit deminishing with distance from the site of a removed truss. The redistribution of leaf assimilates from leaves originally supplying the removed truss, was most effecient when the fifth truss of a ten-truss plant was removed; in this situation the final yield of the remaining nine trusses was only 2% less than in an intact plant.

It is well known that the final tomato fruit size on a truss reduces from the base to the tip. Thus, the final size of the tip fruits may only be seven-tenths that of the basal ones. The recognition of the cause of this differential growth is important for improving productivity and for advancing our understanding of the regulation of carbon import. Three possible causes for the differential growth have been investigated.

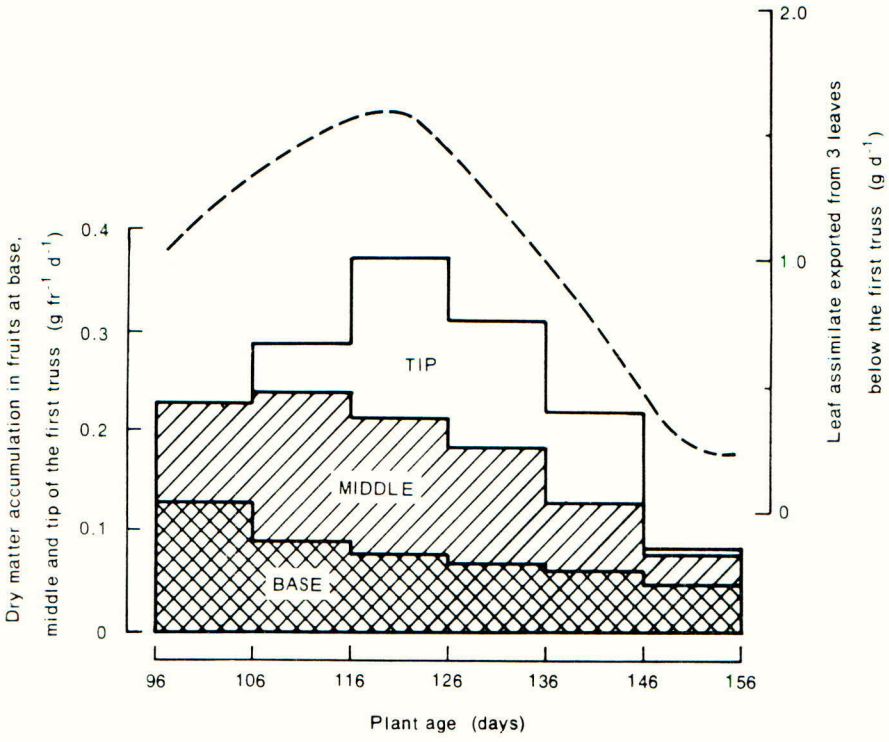
(1) Availability of leaf assimilate. Possibly, the smaller final size of the tip fruits is due to a shortage of leaf assimilate when these fruits developed.

In an early crop, the first flower of the first truss is pollinated about 60 days after sowing. If the average growing period of a fruit is 55 days and the tenth flower is pollinated about 10-15 days after the first one, then the total growing period of the truss is about 70 days. If the three leaves just below the first truss are the principal source of assimilates for the truss (Tanaka and Fujika, 1974), these leaves would be 28 $\pm$ 2 to 108 $\pm$ 2 days old over the same period. When the first fruit starts to enlarge, 10 days after pollination, all three leaves should be 80% expanded and just reaching their maximal rate of carbon fixation. The amount of carbon being exported from these leaves would amount to 75-80% of that fixed (Ho & Shaw, 1978). Soon after full leaf expansion, the rate of both carbon fixation and carbon transport drop, and by the end of fruit growing period the amount of carbon being exported from these three leaves would be less than a sixth of the maximum (Fig. 1).

The dry matter accumulation pattern of fruits at the base, middle and tip position of the first truss revealed that the period of the dry matter accumulation for the tip fruits is shorter than that of others. The decline of export from the source leaves during the growth of tip fruits could well be the main cause of the lower fruit growth rate. Since the contribution of assimilate from leaves other than the principal source leaves to the growth of the tip fruits is not accounted for, the evidence presented here is not conclusive.

If the smaller final size of the tip fruits is caused by inadequate supply of mobile leaf assimilates, removal of some fruits should result in all remaining fruits increasing to the same size regardless of their positions in the truss. To test this hypothesis an experiment was performed in which all fruits were removed, 35 days after the first flower was pollinated, leaving 1, 3 or 5 fruits at the base, middle and tip positions. The final size of all the remaining fruits were larger than those at the comparable positions on the control plants.

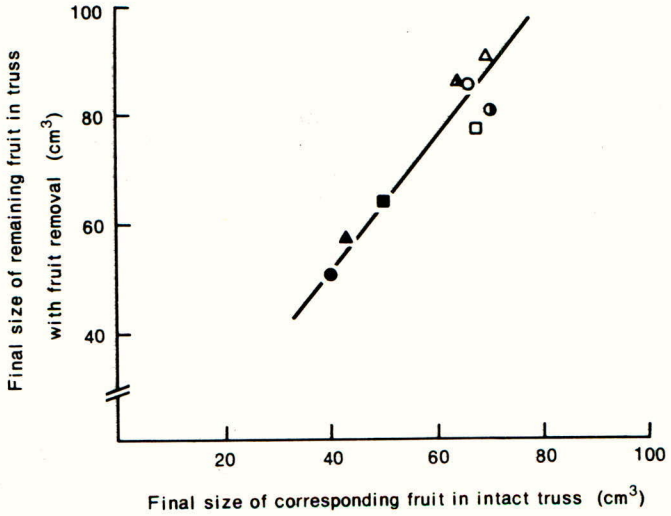
Fig. 1 Comparison between the dry matter accumulation in fruits of the first truss and the export of leaf assimilate from 3 leaves below the first truss over the same period.



In the control truss, the final size of individual fruits would be affected by both the availability of leaf assimilate to the truss as a whole and by the competition among the fruits for assimilate in the truss. Thus, the final size of a fruit in the control truss can be treated as its apparent sink strength. Because the final size of a fruit in the fruit-removal truss is achieved under conditions of non-limiting supply, it can be treated as a potential sink strength.

Therefore the comparison of the fruit size in trusses with or without fruit removal treatment (Fig. 2) can be used to examine the relationship between the apparent sink strength and the potential sink strength. The linear correlation describing this relationship indicated that all fruits grow to only seven-tenths of their potential. This suggests that fruits at different positions have different potentials. In other words, as the competitive ability (Wareing and Patrick, 1975) of the tip fruits appears to be lower than that of the basal ones, it is a factor determining the final fruit size. Therefore, neither availability of leaf assimilate nor competition between fruits are sole causes of the observed differential growth of fruits within a truss.

Fig. 2 Correlation between the final size of fruits in comparable positions of a truss with and without fruit removal. In fruit removal treatments, different numbers of fruits were retained (1 = O ; 3 = Δ ; 5 = □) at different positions (tip = closed symbols; middle = half closed symbols; base = open symbols).



(2) Length of the transport pathway. The second possible cause for the smaller final size of the tip fruits is the higher resistance encountered by assimilate moved from leaves to the tip fruits.

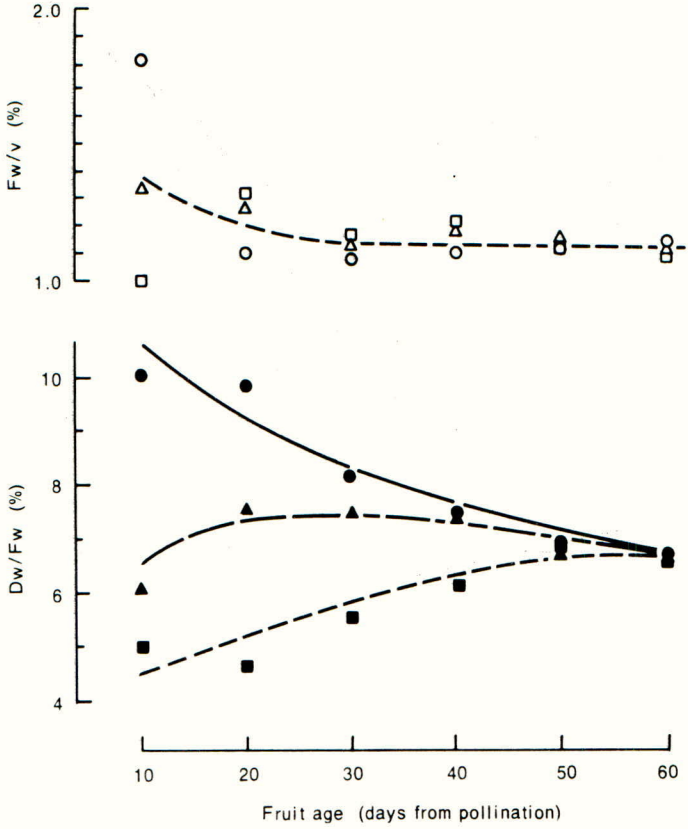
Analyses of the correlations between the leaf sucrose concentration and carbon export (Ho, 1976a, b; 1977) and between the fruit sucrose concentration and carbon import (Walker and Thornley, 1977) strongly suggest that leaf assimilates are translocated into tomato fruits along a decreasing gradient of sucrose. Thus, the rate of carbon import can be described as

$$F = \frac{D(S_1 - S_2)A}{X}$$

where, $S_1 - S_2$, is the sucrose concentration gradient between leaves and fruits, A, the cross sectional phloem area, D, the diffusion constant and X, the length of the pathway. Assuming that the sucrose concentration gradients between source leaves and individual fruits of the same truss are the same and that the phloem cross-sectional area of pedicels of individual fruits are comparable, the rate of carbon import will vary in inverse proportion to the distance between the source leaves and the fruit.

In cv. Kingley Cross, the average distance between the three leaves below the truss and the first fruit of the truss is about 23 cm and that between the first fruit and the tenth fruit is about 13 cm. In effect, the translocation distance for the first fruit would be 23 cm and that for the tenth fruit would be 36 cm. Thus, the rate of carbon import to the tenth fruit should be only two-thirds of that for the first fruit. The reduction of the carbon import caused by the resistance

Fig. 3 Changes in Fw/v (open symbol) and Dw/Fw (closed symbol) during fruit growth of fruits at tip (□,■), middle (△,▲) and base (○,●) positions of the first truss.



along the pathway is of the same magnitude as the reduction of the final fruit size.

Another possible effect of the pathway is that the assimilate may be withdrawn by fruits attached to the basal part of the pathway so that less would be available for the tip fruits. Although no measurement has been made of the concentration of phloem sap entering fruits at different positions of a truss, the dry weight/fresh weight ratio of individual fruits has been examined as an index of the concentration of imported assimilate sap.

Figure 3 shows that in early stage of fruit growth the DW/FW of the tip fruits is lower than that of other fruits. Since the fresh weight/volume ratio of all fruits are much less variable, the lower DW/FW of the tip fruits suggests that the concentration of assimilate sap reaching the tip fruits may be indeed lower than that reaching the basal fruits. However, such a dilution of assimilate sap along the pathway should not occur in the fruit removal truss. The observation of the

constant proportional increase in final fruit size induced by fruit removal suggests that the low DW/FW in the young tip fruits may result from the low potential sink strength of the tip fruits rather than the low concentration of the phloem sap.

(3) Potential sink strength of the fruit. The difference in DW/FW ratio in young fruits and the final size attained by ripe fruits at different positions on the truss suggests that there may be steps within the fruit which regulate carbon import.

It has been established that the rate of carbon import into tomato fruits changes with the stage of fruit development (McCollum & Skok, 1960; Walker and Ho, 1977a) and also with temperature (Walker and Ho, 1977b). The causal relationship between the rate of carbon import and the changes of carbon metabolites in fruits suggests that the rate of carbon import, apart from the availability of the labile leaf assimilate, is determined by the rate of unloading at the fruit. Hydrolysis of imported sucrose and the subsequent synthesis of starch appeared to limit the unloading process (Walker, Ho and Baker, 1978).

The reasoning for the suggestion that hydrolysis of sucrose limits the rate of carbon import is simple: (1) 90% of the imported assimilate is sucrose but the sucrose concentration in growing tomato fruits is only 0.1 - 0.5% of fresh weight. Imported sucrose is the principal source of carbon for all carbon metabolities. (2) The rate of carbon import is inversely proportional to the fruit sucrose concentration, implying that the rate of carbon import is closely related to the rate of hydrolysis of imported sucrose.

Further support for this hypothesis can be found from differences between modern cultivars (Lycopersicon esculentum) and wild species (e.g. L. hirsutum & L. peruvianum), of tomato. The fruits of wild species are small but the fruit sucrose concentration is 10-30 times higher than that in the modern cultivar (Davies, 1966). More important is that the activity of acid invertase in the fruits of these wild species is only a tenth to a fifth of that of modern cultivars (Manning and Maw, 1975). If sucrose concentration in tomato fruit is an accurate index of sucrose hydrolysis, the activity of acid invertase appears to be the key factor in controlling the rate of carbon import (Walker, Ho and Baker, 1978).

CONCLUSION

A tomato leaf is an efficient carbon exporter for only a short period (about 30 days) just prior to and after the leaf is fully expanded. During the last 40 days of its life, a tomato leaf exports little but accumulates dry matter continuously. The efficiency of carbon export from a young mature leaf can be improved by high radiation and high CO_2. To improve the efficiency of an ageing leaf it would be necessary to reduce the carbon flow destined for storage and increase that for labile sucrose synthesis. The factor controlling the partitioning of labile and storage sucrose is as yet unknown.

Although the availability of mobile leaf assimilate is the primary limiting factor in the partitioning of assimilates, the carbon metabolism within the fruit may constitute a second limitation to the import of assimilate in individual fruits.

Investigations of the role of growth regulators and enzymes on steps limiting carbon metabolism should shed some light on mechanisms regulating carbon transport.

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SOME ALTERNATIVE SCREENING TECHNIQUES FOR PLANT GROWTH REGULATOR ACTIVITY BASED ON PHYSIOLOGICAL PROCESSES

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Summary Certain physiological processes such as photosynthesis, assimilate partitioning and leaf senescence have been suggested as being limiting factors in the production of major food crops. The possibility of developing screening techniques for plant growth regulators based on our present knowledge of these processes is discussed. It is concluded that the most effective method would be by choosing target crops and employing screens involving a combination of whole plant and bioassay systems to monitor specific, fundamental plant processes.

INTRODUCTION

Methods of increasing food production have been sought for as long as civilized man has cultivated the soil. In this respect, the plant breeder has made considerable impact, such that many of the major food crops bear little resemblance to their original, wild ancestors either in terms of appearance or yield. The introduction of new varieties and improved agronomic practices has contributed in no small measure to improving uniformity and yield thus partially satisfying the world demand for more and better food. Of equal importance has been the discovery and application of a wide range of chemicals to control pests and diseases, both during production and storage of crops. These various factors have resulted in an increase on average of 35% in world food production over the decade up to 1975 (F.A.O. Statistics). Nevertheless, improved food production is likely to remain one of the most important future requirements and Hardy et al (1977) have predicted that "the space-time productivity of the economic component of world crops must be increased 3% per year". Thus any additional methods such as those offered by growth regulator technology may be of great importance in future agronomic practice.

The discovery that certain plant constituents possess growth regulatory properties has stimulated intense research over the last 20 years directed towards understanding the hormonal mechanisms which control certain plant processes. Parallel, but sometimes unrelated attempts, have been made to discover effective synthetic plant growth regulators which could be used on a commercial scale. The interest now being directed towards finding such compounds has accelerated for a number of reasons. Not least of these has been the undoubted success of certain compounds such as daminozide, chlormequat chloride, ethephon and the gibberellins which have found various useful if somewhat limited horticultural outlets. In the past the major emphasis in the Agrochemicals Industry has been placed on the production of herbicides, insecticides and more recently fungicides. For obvious reasons, the screening procedures adopted have required a less sophisticated approach than those demanded for plant growth regulator screening. Because of this, and since there has until recently always been a large demand for new and better herbicides and pesticides, growth regulator tests have tended to be of secondary importance to those for other agrochemicals.

Indeed it is probably true to say that with some notable exceptions, many active compounds have been produced as by-products of an overall general pesticide screening procedure rather than through tests designed for assaying specific growth regulatory properties. There has tended to be an emphasis on screening for retardant-type activity since these compounds were known to have important uses through suppressing growth, altering plant shape, affecting fruit production and alleviating plant stress. Such primary screens have been simple to operate since the visual effects produced could be assessed quickly and large numbers of chemicals could be examined using a range of test species. Thus, retardant-type compounds could be easily detected in a conventional herbicide screening programme.

There is considerable literature on the effects of growth retardants and other growth regulators on physiological and biochemical processes involved in plant development but it is becoming more apparent that our fundamental knowledge of these processes, particularly in crop species, is very limited. Even so, within these limitations, the likelihood of developing new commercial PGRs would be improved considerably by increased co-operation between all scientists interested in the control of plant growth. It is with this in mind, that various groups such as the BPGRG have become established over the last few years.

In this paper, an attempt will be made to illustrate how our knowledge of the factors affecting various physiological processes may be used to devise specific screening tests for plant growth regulator activity. However, it should be pointed out that many of the possibilities discussed are probably already being investigated by some commercial researchers. Because of the competitive nature of the industry, it is difficult to obtain detailed information about current screening operations and this point must be borne in mind in relation to this presentation.

DEVELOPMENT OF SCREENING PROGRAMMES

When considering the development of new screening techniques it is soon apparent that the main problem is to produce tests which will provide the answers to specific questions but which will allow a throughput of many chemicals each year. This is not possible with some of the standard primary screens using whole plants of indicator species. Problems associated with uptake and metabolism of components or with growing the plants under artificial conditions can result in incorrect conclusions. Thus, some potentially-active compounds might appear to possess little or no activity whereas others which are effective in conditions of stress may have no commercial value when applied on a field scale. In addition, the responses of various indicator species may differ considerably from those of the crop species. It is suggested that a more useful approach is that of defining targets for research in that specific crops of high economic value are selected for intensive physiological studies and chemical screening programmes. A good example of this type of approach is illustrated with sugar cane where careful examination of the limitations to increased sugar production has resulted in the development of regulators for preventing flowering, enhancing ripening and increasing the sucrose storage capacity. Other crops have been studied in similar detail and it is apparent that most major Agrochemicals companies adopt this approach and have developed or are developing sophisticated secondary screening programmes in addition to the more simple primary tests.

Unfortunately, unless the primary screening techniques are designed to determine the effects of new chemicals on specific physiological and biochemical mechanisms, many potentially useful growth regulators may be discarded during this initial operation. The commercial researcher is faced with the dilemma of obtaining meaningful information on the mode of action of new compounds whilst keeping the screening techniques simple enough to ensure a through-put of all his chemicals. However, once a target crop has been selected for improvement after careful economic consideration, the development of suitable growth regulators can be envisaged as a simple three-step procedure:

1. Definition of the physiological limitations to improved production of the specific crop.
2. Decision on which physiological aspects to study and modify with growth regulators.
3. Development of screening techniques for suitable growth regulators.

It seems rational to suppose that the successful development of screening techniques must be based on the previous experience of academic and commercial researchers. It is envisaged that many important pointers to the action of new compounds could be obtained by selecting tests based on a wide variety of techniques. Thus a combination of whole plant testing, bioassay, tissue or explant culture and specific biochemical tests could perhaps be developed with a particular physiological goal in mind. In order to decide which kinds of tests should be employed, it is first necessary to have a detailed knowledge of the physiology of the crop.

PHYSIOLOGICAL LIMITATIONS TO INCREASED YIELD

It has already been pointed out that considerable research is still necessary on many major crop plants before the physiological limitations to yield can be defined in detail. However, as a generalization there appear to be certain major areas of study which would be rewarding in determining the type of new plant growth regulators for commercial use. Thus the following three major physiological aspects appear to be of particular relevance.

1. Increased photosynthetic capacity.
2. Alteration of the pattern of assimilate distribution.
3. Delayed senescence to extend leaf area duration.

INCREASED PHOTOSYNTHETIC CAPACITY

It is well known that CO_2 supplementation will enhance the growth of plants resulting in increased crop production and this practice is used widely in the glasshouse crops industry. The difficulties of such treatment on a field scale are obvious though Hardy et al., (1977), demonstrated yield increases of about 50% in four field-grown legumes by enrichment to 1000-1500 ppm CO_2 around the canopy from anthesis to senescence. These results and others indicate the potential for increased yield through photosynthetic modification and there have been various reports of the stimulatory effects of growth regulators on photosynthesis (see report by Treharne at this Symposium).

Two possible methods of increasing the photosynthetic capacity of the sugar beet crop have been defined by Lenton and Milford (1977), these being the establishment of leaf cover early in the season and increasing photosynthetic rate of the established canopy. Both these factors can impose limitations to increased yield of many major crops, particularly those grown in temperate climates.

i. Improving leaf development: A good example of the failure to produce a sound economic yield because of the limitation of leaf development by low temperature is afforded by the Navy bean crop in the UK. Intensive research at NVRS, Wellesbourne has been aimed towards selecting new lines with increased tolerance to low temperature. Chemical stimulation of leaf development and photosynthesis at low temperature would be a valuable alternative treatment. It is clear that conventional screening under normal temperatures might indicate certain chemicals which would enhance growth, but only by testing at low temperatures would one ensure that commercially useful chemicals were available. The difficulties of such tests, because of the slow growth rates at low temperature, are obvious but there are certain plants such as Lemna and Arabidopsis (Lawrence, 1968) which have rapid growth rates and short life cycles which could perhaps be used as ancillary screening material. With Lemna, or leaf

discs of other species, the measurement of leaf area could be determined very simply by automatic scanning with a TV monitoring device.

The damaging effects of low temperature on leaf development in certain susceptible species operates partly through affecting membrane integrity so causing the cells to "leak". Thus a standard test for the effects of chemicals on cold tolerance of plants could be developed by removing treated leaves, placing them in water and measuring the conductivity of the solution after a predetermined time.

ii. Increasing photosynthesis: Methods of screening chemicals for beneficial effects on the rate of synthesis of assimilates must depend on detecting increases in photosynthetic rate or decreases in rate of photorespiration. In view of the extensive research on photosynthesis, as illustrated by the number of papers given at the recent 4th International Congress on Photosynthesis (1977), one would anticipate the development of a number of simple tests to detect such effects. In the case of photorespiration, biochemical pathways can be identified and through characterisation of some of the important enzymes it may be possible to produce inhibitors to suppress the process.

The measurement of photosynthetic rates seems to be an obvious parameter to include in a routine screening programme. However, the experience of plant breeders in attempting to select cultivars for higher photosynthetic rate and more effective canopies should not be ignored. The relationship between these characteristics and final yield has been inconsistent for a number of species including sugar cane (Irvine, 1975) and soybean (Curtis et al., 1969). Nevertheless, since plant growth is determined to a large extent by photosynthetic capacity, the development of informative tests based on these processes should be a primary aim. Hardy et al., (1977) suggested that a study of the balance of photosynthesis and photorespiration could be used to evaluate yield potential. This introduces the possibility of developing an in vitro system, using leaf tissue or lower plant organisms, based on measuring enzyme activity. Measurement of the carboxylase/oxygenase ratio of RuDP carboxylase might provide a suitable basis for such a screen. Ogren (1977) has suggested that increasing net photosynthesis by reducing the oxygen sensitivity of this enzyme is a feasible objective. However, no chemical has yet been found which will act in this way. Effects of GA and chlormequat chloride on the synthesis of other enzymes associated with photorespiration have been reported recently (Oben and Marcelle,1977).

More laborious techniques for measuring photosynthesis such as the use of Warburg apparatus or measurements involving $^{14}CO_2$ utilization are unlikely to find use in primary screens but can be incorporated into detailed secondary studies. However, isolated chloroplast systems might well provide suitable primary material particularly in relation to the measurement of the Hill reaction where CO_2 and O_2 measurements can be made. The effect of chemicals on the alteration of O_2 evolution and CO_2 uptake by isolated soybean chloroplasts has been reported by Stutte and Blem (1976). Chloroplast systems have been used extensively for studying biochemical pathways of photosynthesis but have been little used for testing the modifying effects of chemicals on these processes.

ALTERATION OF THE PATTERN OF ASSIMILATE DISTRIBUTION

Increased total plant growth does not necessarily result in larger economic yields. For example, increased nitrogen fertiliser can stimulate leaf development and increase plant dry matter production of sugar beet with no corresponding increase in sugar yield since root/shoot ratios are affected adversely (Draycott & Webb,1971). Narrower plant spacing tends to produce higher total yields, until plant competition becomes limiting but I.E.Currah (Personal Communication) has shown that carrot plants retain a fixed allometric pattern throughout development regardless of the spacing and time of sowing. However, other environmental factors such as photoperiod and chemical treatments (Thomas et al., 1973) can induce major changes in the ratio of root to shoot weights.

There are a number of potential agronomic uses for chemicals which might modify plant allometry, particularly those which would enhance root development without decreasing total plant weight. Conventional primary screens are ideal for detecting dwarfing chemicals but are not suitable for assaying any related increases in root growth. The use of plants grown in nutrient solution is not attractive because of the increased labour requirements but does have the advantage that the effects of foliar sprays on root development can be assessed visually. Alternatively, root culture might afford a suitable vehicle for determining the direct effects of growth regulators particularly if it is possible to culture roots of the target crop. The effects of abscisic acid on root development have been extensively studied using this technique (Gaither et al., 1975; Weston, 1974). An advantage of using root culture is that effects on root morphology and anatomy can be determined fairly easily. This is of particular relevance to sugar beet since Milford (1973) has demonstrated that young, small cells within the storage root are more efficient at accumulating sugar than mature, large cells. Thus, chemicals which increase cell number as well as root size could be detected using such a screening method.

Although plant tissue and cell cultures have been in existence for many years, they have not been widely used to assess growth regulator effects. They have many potential uses in the study of growth regulatory compounds and could be used as rapid microtest for specific hormonal properties. The effects of a number of herbicides and growth regulators on seedlings and tissue cultures of various weed species have been reported by Zilkah and Gressel (1977 a, b) and Zilkah et al.,(1977), who discussed the relative merits of whole plant and cell culture systems. The use of tissue cultures as screening materials would eliminate many of the problems caused by the inherent variation of certain crop plants and could also act as a source of uniform plantlets for whole plant tests.

The partitioning of assimilates in cereal plants has received wide attention particularly in relation to the development of the flag leaf and the photosynthetic capacity of the plant. However, recent research has indicated two important physiological features during the development of the grain.

First, it has been shown by Brocklehurst (1977) that the rate of increase in grain dry weight is closely related to sink size as determined by the number of endosperm cells formed. The period of cell division occurs during the 14 days following anthesis in wheat and this process could provide one suitable measure of the potential yield of the crop. A test based on the measurement of dry weight or cell number 14 days after anthesis could be used to assess the potential effects of growth regulators on grain development and yield. The feasibility of using whole plants or excised inflorescences for such a test deserves examination.

Secondly, Radley (1976) demonstrated that starch formation in wheat grain ceases shortly after a period of rapid water loss from the grain and that this is preceded by an increase in abscisic acid content. Presumably, if this water loss is delayed and the ABA content decreased, starch synthesis would continue since it has been shown that decreased synthesis is not due to reduced sucrose supply to the grain. It is possible that these physiological features could also be used as a basis of a growth regulator screen using whole plants or excised inflorescences in sucrose solution, measuring permiability of the seed or using conductivity measurements to determine leakage of solutes from the seed.

DELAYED SENESCENCE TO EXTEND LEAF AREA DURATION

The contribution of older, senescing leaves to the final yield of root, legume and cereal crops is not clear. The continued production of foliage may even compete with the final, filling processes of the economically important crop parts. However, extension or delay in leaf senescence can be simply and easily measured by the conventional bioassays employed for determining cytokinin and gibberellin activity.

Such tests could be modified, using excised leaves or leaf discs of the target species, providing a rapid screen for chemicals which affect leaf senescence. However, until more is known about the role of leaves during the later growth stages of crop plants, the value of such tests is questionable.

CONCLUSIONS

In this paper it has been possible to discuss only some of the physiological and biochemical processes which could be monitored in screening for growth regulatory chemicals. However, other processes known to be affected by hormones might provide more useful parameters for including in a screening programme. The significance of certain hormonal effects such as the control of ion excretion through membranes, is not clear but could provide the key to the discovery of even more powerful and specific growth regulators than those available at present.

It has become very apparent that the use of whole plant tests only is not satisfactory, particularly if the crop plant has a long growth period. Shorter, ancillary screens involving specific growth mechanisms are essential to obtain meaningful results but much depends on further progress in plant physiology and biochemistry. The possibility of using explants and cultures could be explored more thoroughly. Powell and Edgerton (1974) have shown that apple bud explants respond to several chemicals in a similar manner to intact trees and can be used successfully as a screen for fruit regulation chemicals. However it seems likely that the best information will be obtained by following a programme involving whole plant and bioassay systems. In this way specific effects, whole plant correlative effects and crop responses, such as those caused by changes in canopy structure, can all be assessed.

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SOME NEW CYTOKININS: ACTIVITY OF METHYL-BENZYLOXYPURINES

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Summary The effects of methyl substitution on the cytokinin activity of 6-benzyloxypurine, as measured in the tobacco pith test, is discussed in terms of the molecular shape of the side chain. The 2- and 3-methyl substituents cause little change whereas the 4-methyl reduces the activity markedly. The effects of transferring the tobacco explants from the culture medium containing nutrients and the cytokinins to one containing nutrients only are discussed in terms of hormonal effects on tissue growth and differentiation.

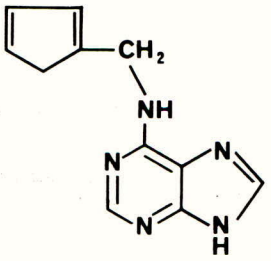
INTRODUCTION

Since the discovery of kinetin (I) in autoclaved deoxyribonucleic acid (Miller et al., 1955a, b, 1956) a large number of synthetic 6-substituted aminopurines have been prepared and examined as cytokinins (see review by Leonard, 1974 and references therein). Also most cytokinin-active substances isolated from plants are 6-N-substituted adenines (Leonard, 1974).

It has recently been shown that a nitrogen atom in the bridge between the 6-substituent and the purine ring is not a prerequisite for cytokinin activity as oxygen (IIa) (Wilcox & Wain, 1976), sulphur (IIb) (Kuraishi, 1959) and carbon (IIc) (Henderson et al., 1975) bridging groups all give rise to compounds that possess cytokinin activity.

Figure 1

I



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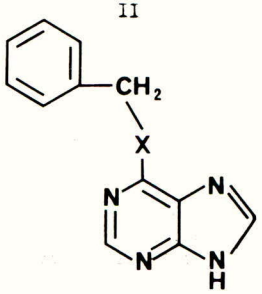
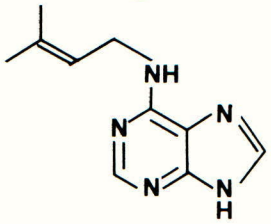


X = a, O; b, S; c, CH_2

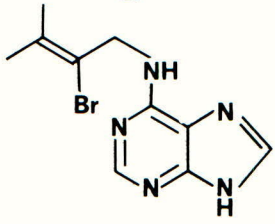
It has been shown that for adenine derivatives large changes in cytokinin activity can be caused by minor modifications to the 6-N-substituent, for example when a hydrogen atom in 6-(3-methyl-2-butenyl)-aminopurine (III) is replaced by a bromine atom to give 6-(2-bromo-3-methyl-2-butenyl)-aminopurine (IV) the change results in a 99% loss of activity (Hecht et al., 1970). In the present work the effect of substitution of the benzyl ring of 6-benzyloxypurine (IIa) was investigated.

Figure 2

III



IV



MATERIALS AND METHODS

Chemical

The alkoxypurines were prepared by reaction of the sodium salt of the corresponding benzylalcohol with 6-chloropurine (prepared by the method of Bendick et al., 1954). In order to obtain maximum yields of the required compounds it was essential that water was rigorously excluded from the reaction. Consequently the benzyl alcohols were dried by reaction with a small amount of sodium and then distilled under vacuum from the resultant salts. The 6-chloropurine was dried over phosphorous pentoxide and the required sodium phenylmethoxides were prepared either by reacting an excess of the benzyl alcohol with sodium, or by reacting a solution of the benzyl alcohol in dioxane with sodium hydride (50% oil dispersion). The solution of the sodium phenyl methoxide and the 6-chloropurine was then heated at 100 to 120°C for 2 to 10 h.

The products were purified either by thin layer or column chromatography followed by recrystallisation from ethanol/water. All compounds gave infrared, ultraviolet and nuclear magnetic resonance (proton) spectra consistent with their proposed structures.

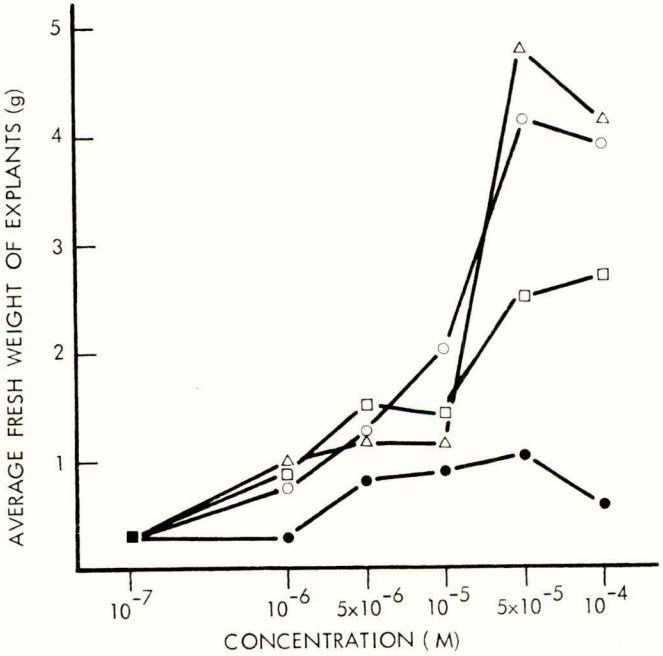
Biological

Tobacco pith test. The procedure was as described by Murashige & Skoog (1962) using their basal medium containing 2 mg l^{-1} of indole-3-acetic acid. The pith tissue was obtained from tobacco plants grown to c. 1 m high in the greenhouse.

The agar medium was prepared as described by Murashige & Skoog (1962) and the compounds were added to the cooling media as solutions in dimethyl sulphoxide (DMSO). The DMSO concentration was never more than 0.05%, a concentration that has no effect on tobacco tissue growth (Schmitz & Skoog, 1970).

Five replicate flasks were used for each concentration with 3 pieces of tissue to a flask. The cultures were maintained under diffuse light for 4 weeks when the explants were removed and weighed under sterile conditions; the results are shown in Fig. 3. The explants were replaced on the culture medium and the tissue differentiation was assessed after a further 2 weeks growth (Fig. 4).

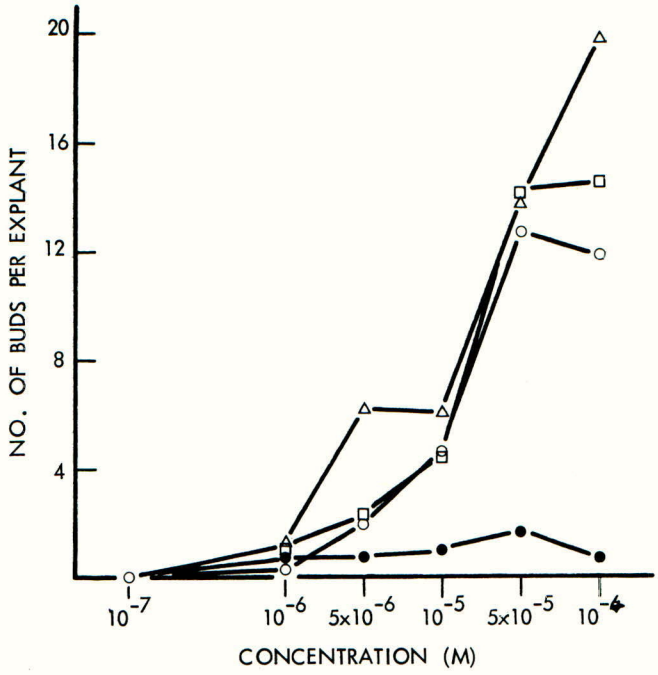
Figure 3. Tissue growth promoted by methyl substituted benzyloxypurines after four weeks growth



o—— o Unsubstituted; △—△ 2-methyl; □—□ 3-methyl; ●—● 4-methyl.
The average fresh weight of 15 explants is recorded for each concentration.

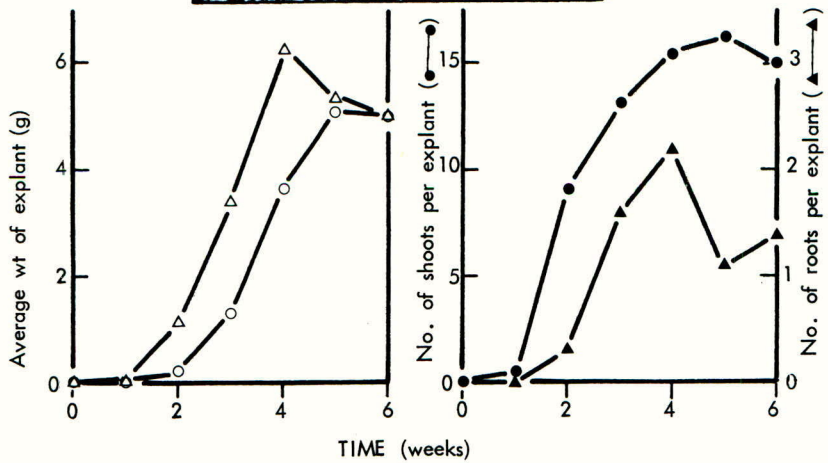
The effect of transferring the explants to a basal medium containing all the nutrients but no hormones was investigated. The explants were grown on the culture medium for a period of from 1 to 6 weeks, and then transferred to the basal medium and grown for a further 5 to 0 weeks. All explants were grown for a total of 6 weeks. At 6 weeks the explants were weighed and the number of shoots and roots was assessed (Fig. 5).

Figure 4. Tissue differentiation initiated by methyl substituted benzyloxypurines



o——o Unsubstituted; △——△ 2-methyl; □——□ 3-methyl; ●——● 4-methyl.
The number of buds per explant is recorded for a growth period of six weeks.

Figure 5. Effects of transferring explants from the culture medium containing IAA (2 mg l^{-1}) and 2-methylbenzyloxypurine (12 mg l^{-1}) to a basal medium with no growth hormones after 0 to 6 weeks



o——o weight of explant at time of transfer to basal medium; △—△ weight of explant at 6 weeks; number of shoots ●——● and roots ▲——▲ per explant at 6 weeks. All results are for a total growth period of 6 weeks.

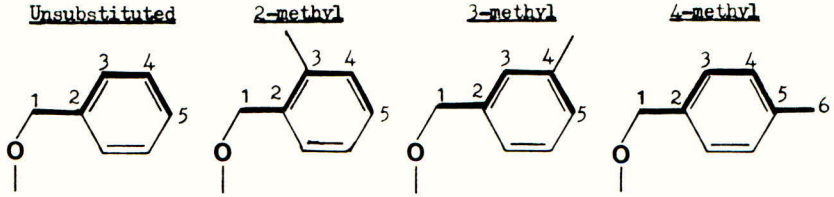
DISCUSSION

It can be seen from Fig. 3 that the introduction of a 4-methyl substituent on the benzyl group resulted in a lowering of the cytokinin activity. A 2- or 3-methyl substituent only caused a small change in activity, the 2-methyl being slightly more active and the 3-methyl slightly less. The results for the 2- and 4-methyl compounds are similar to those found by Kuraishi (1959) for 2- and 4-methylbenzyladenine.

As both the 2- and 4-methyl groups would give rise to very similar electronic peturbation in the molecule the effect is almost certainly caused by a steric effect.

The unsubstituted and the 2- and 3-methyloxypurines can be considered to have a substituent chain length of 5 carbon atoms whilst the 4-methyl is equivalent to a 6 carbon chain.

Figure 6



It is known that for simple 6-N-n-alkyladenines the maximum activity is associated with a 5 carbon atom chain (N-pentylaminopurine) (Skoog et al., 1967). The size is very critical as both the butyl and hexyl compounds are much less active. It has also been suggested that planarity of the 6-substituent on the purine ring is necessary for optimum activity (Hecht et al., 1970). The presence of a 4-methyl substituent may prevent the side chain attaining planarity at its active site.

The 2- and 3-methyl substituents caused little change suggesting that the spacial requirements for optimum activity at these points were not very critical.

The effects on tissue differentiation followed a very similar pattern to that observed for the tissue growth and is shown in Fig. 4.

The growth of the tissue was investigated further by transferring the explants from the culture medium to one containing all the nutrients but no hormones. From the results in Fig. 5, it can be seen that on the culture medium little growth occurred during the first 2 weeks, steady growth for the next 3 weeks and little in the last week. The explants continued to grow after transference to the basal medium: the growth of those transferred after 4 and 5 weeks exceeded those left on the culture medium for the full 6 weeks. This was almost certainly due to the explants suffering a nutrient deficiency on the culture medium after 4 weeks growth.

The effect on tissue differentiation was interesting; the greatest differentiation occurred after 4 or 5 weeks, which was consistent with the maximum growth. When the explant was transferred after 2 or 3 weeks there was little growth on the

basal medium, but the tissue gave rise to a large number of shoots. Thus the hormone balance in the explant suited differentiation but not growth. Using 2,4-D as auxin rather than IAA in the culture medium led to the formation of a soft callus which grew well but little or no differentiation occurred (Selby & Wilcox, unpublished data). As the cells required a definite balance of auxin and cytokinin to differentiate, the stability of the exogenously applied hormone may have been important in controlling the growth and differentiation of the explant. A number of different auxins and cytokinins are now being investigated in the laboratory.

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