Proceedings Joint BCPC and BPGRG Symposium - 'Opportunities for Chemical Plant Growth Regulation' (1978).

GROWTH REGULATORS AND THE CONTROL OF DEVELOPMENT IN FRUIT TREES

W.W. Schwabe

Wye College (University of London), Nr. Ashford, Kent, TN25 5AH

Summary Growth regulation by hormones in fruit trees may be used at several stages in the life history of the plant. In seedlings gibberellins and anti-gibberellins may be useful in overcoming juvenility. At maturity fruit set in apples, plums, cherries, etc. has been promoted by the "Wye 3 hormone mix". Seasonal variation in response to hormone sprays, due to flower 'quality', is now being studied. Hormone treatment also aids in fruit harvesting. Thus, deliberate manipulation of the tree by appropriate hormones at several stages of growth, would seem a likely method of normal tree and crop control in the future.

INTRODUCTION

Perhaps I may be permitted to start with a few platitudes. In evolutionary terms, we all accept that originating from wild ancestors, plants must have developed mechanisms to survive as individuals long enough to reproduce and leave the maximum number of offspring. Such mechanisms must comprise not only adaptations to survive the vagaries of the environment, pests and diseases, but also others to enable the plant to compete successfully with different species. Hence, much of the morphology of the plant represents an integration of successful forms and habits of growth. These adaptations are clearly not necessarily desirable from the agriculturalist's point of view, in inducing maximum yield of the particular crop product that is wanted. In fact, present technology has made many of these adaptive characteristics irrelevant, perhaps by eliminating competitors and/or by providing in abundance many of the things competed for, e.g. mineral fertiliser, water, etc. The morphological features aiding in survival may therefore now be regarded as limitations or brakes on the production of those plant parts wanted as crop products. The aim of hormone physiologists must therefore be to achieve a redistribution of growth activity and development in the plant, i.e. by the same means by which the plant normally integrates its morphogenetic functions. In other words, we must re-tailor the structure of the plant - something which may ultimately be done by the geneticist where the appropriate genes are available.

The application of hormones normally suffers from the fact that it is difficult to get the appropriate hormone to the target site in

adequate concentration, without swamping other tissues with unwanted hormones, bringing about undesirable responses. Nevertheless, it seems likely to me that, in the not too distant future, the regular application of natural and synthetic plant growth regulators to modify plant structure in a variety of ways, possibly by sequential application of different substances, will become normal and accepted practice in much the same way as mineral fertilisers have become accepted in the last 100 years or so. While the following discussion will be restricted to fruit trees in the temperate zone, I would like to view those more detailed studies in the light of these more general principles.

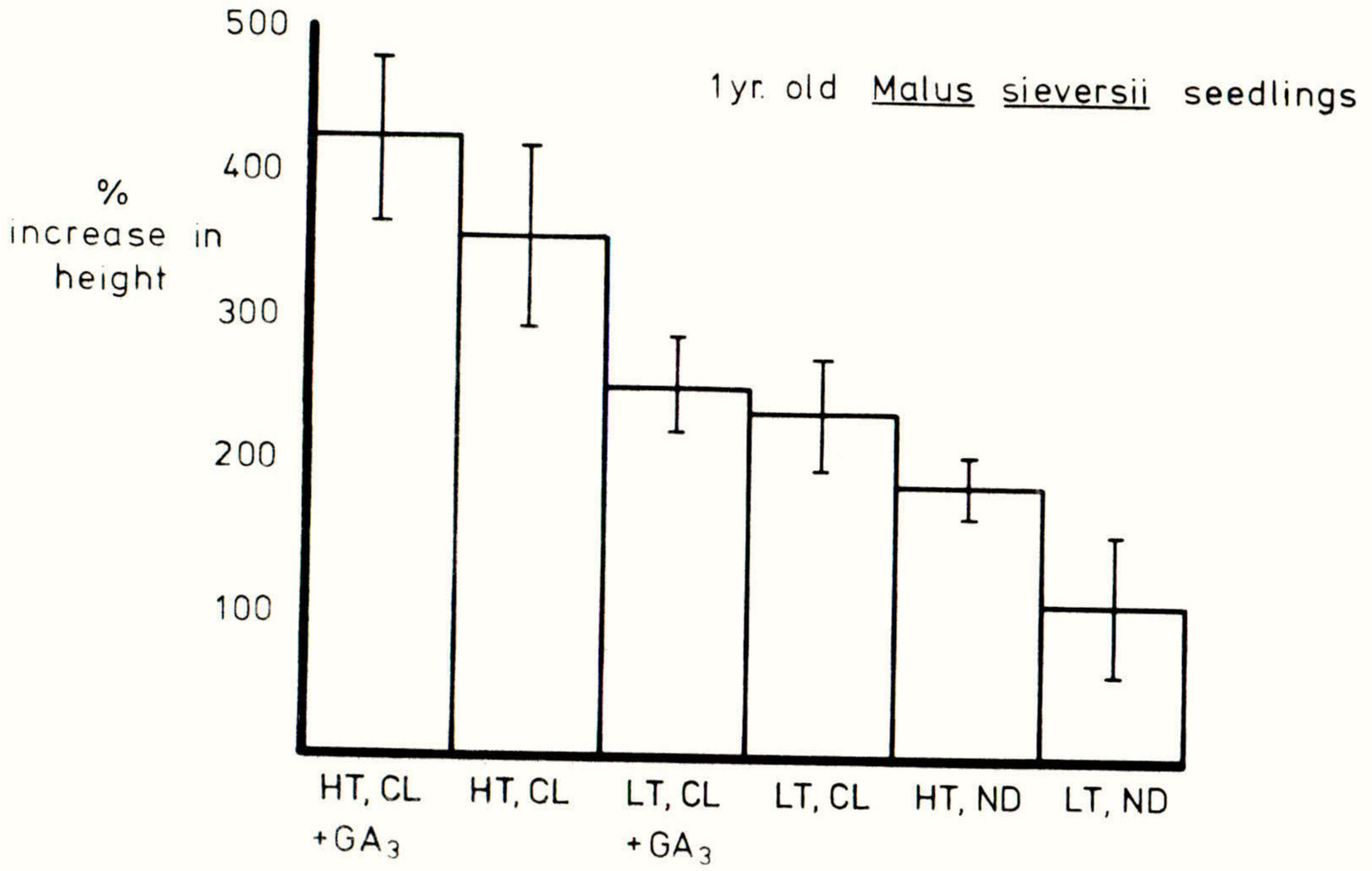
Also, since this is not meant to be a review lecture, the examples refer largely to recent work at Wye on fruit trees, fruit production, etc. Since this work was done by or in collaboration with a number of colleagues and research students, I wish especially to acknowledge the contributions by: Dr. A. Kotob, Dr. G. Goldwin, Dr. D. Smith, Mrs. D. Buszard, Mr. R. Branton, as well as Mr. A. Webster of East Malling Research Station.

OVERCOMING JUVENILITY

Starting with aspects of propagation, there is a serious problem in fruit breeding, i.e. the relatively long persistence of the juvenile condition which may last for three or often several more years and thus delays the assessment of possible varieties as far as any character associated with reproduction and fruit quality are concerned, often for a long time. There is evidence (13, 11, 4) that in some species at least, juvenility is associated with gibberellins produced probably by the roots, and maturation as a phase change may result partly from increasing the distance of the top of the plant from its own roots. This certainly seems to be operative in the case of flower induction in the blackcurrant (12). In the case of fruit trees, we are now attempting to see whether the transition to becoming capable of flowering can be artificially accelerated. Zimmerman (16, 17) has already shown that Malus hupehensis can be induced to grow faster and longer under glass, leading to flowering in 9.5 months. While Aldwinckle (1) using M. pumila (Miller) obtained flowering by this method of growth in 16 months. We are now trying to combine high temperature with continuous illumination and gibberellin treatment. Some data on height growth with seedling trees of M. sieversii are shown in Figure 1. We are greatly indebted to Dr. Watkins of East Malling Research Station for the gift of the one-year old seedlings. They were 35 cm long when subjected to differential treatment, i.e. from the time of bud break in March 1977 until measurement 7 months later when the tallest were some 1.5 m in height. Clearly the greatest amount of growth in length resulted from a combination of gibberellin with high temperature and continuous light. It remains to be seen whether this will also result in a faster transition to flowering. In this respect one might suggest that once a tree has reached the 'appropriate' size (and this is still unknown), the opposite treatment by substances acting as anti-gibberellins may be effective in inducing flower bud formation, in the same way as this has been done with young grafted trees (15), though Jonkers' (5) results with seedlings were less encouraging.

In experiments at Wye, Dr. Smith subjected oak seedlings to such a 'forcing treatment' that heights over 2 m were attained in about 18 months growth and one such tree produced catkins within 2 years, something which may take oak trees some 80 years under natural conditions.

Figure 1 Acceleration of height growth by: glasshouse temperature (HT) v. outdoor temperature (LT), continuous light, i.e. natural daylength supplemented by low intensity light (CL), v. natural daylength (ND), and gibberellic acid treatment (GA_3).



FRUIT SETTING

Once the tree is reproductive, the question of fruit set becomes important, and the need for regular cropping in spite of the vagaries of the weather especially at blossom time can hardly be overstressed. In this respect we have worked for a good many years now on fruit setting hormones. In the early work (6), it was found that the production of full-sized Cox's Orange Pippin apples was only achieved from emasculated and effeminated (decapitated) flowers by simultaneous application of three hormones, an auxin (NOXA 40 ppm), gibberellic acid (600 ppm) and a cytokinin (SD 8339, 6-(benzylamino)-9-(tetrahydro-2-pyranyl)-(9H-purine) at 300 ppm. Whereas in pears the use of gibberellin alone (following the pioneering work of Luckwill (7) and Modlibowska (8)) is now common practice, the triple mixture was needed for Cox. It should be made clear that there are three categories of result:

(i) setting of emasculated flowers and seedless setting of fruit due to failure of pollination - giving rise to truly parthenocarpic fruit;

(ii) setting and development of fruit which was pollinated, but where pollen tube growth has failed (due to selfing - or other incompatability) or where embryo death has occurred giving seedless fruit, and

(iii) retention of seeded fruit in competition with other seeded fruit.

Full size fruit has been obtained in all three types of situation, and under orchard conditions all may occur and be important. Since then, the 'Wye mixture' has been modified and we have more or less standardised on 200 ppm GA_3, 10 ppm NAA or 50 ppm NOXA, and 300 ppm DPU (diphenylurea).

This mixture has proved effective in three field trials in Wye College Orchards, as will be seen from Table 1.

Table 1

Percentage increase in fruit yield due to triple hormone spraying at 560 l/ha.

1973	9.8%
1976	20.6%
1977	8.0%

Since much of the earlier work has been published (6, 2, 3, 10), I need not go into details. Unfortunately, the possibility of mammalian toxicity of DPU in the long term, has not yet been completely tested, and this means that the DPU component of the mixture has been omitted from some commercial trials in the last few years. Nevertheless, increases in yield have been obtained ranging from 8 to 38%. Thus, while very worthwhile increases have been obtained with only a two-component mixture, the additional presence of DPU has led to increases in yield over the two-component spray, i.e. mean set of fruit for such trials were:

	With DPU	Without DPU
1975	14.7%	12.7%
1976	9.4%	8.3%
1977	21% more than without	

The amounts of hormone effective per fruit are very small indeed. Dr. Goldwin (in press) has estimated that each flower may on average receive some 50-60 µg of DPU and proportional quantities of GA_3 and NOXA, when sprayed to run-off (2250 l/ha), a quantity which becomes reduced to about 10% of the original after 6 weeks, and disappears below detectable levels at harvest. Of the original amount deposited on the flower approximately 80% lies on the petals. In a preliminary trial with C^{14} labelled GA_3, he found that very little of this, if any, moves to the fruitlet before the petals are dropped.

It seems that GA_{4+7} mixtures are more effective than GA_3, but from the practical point of view, it is much more economical to increase GA_3 concentration, if required, than to use these other gibberellins.

Fruit setting by the 'Wye mix' hormones is highly effective also with stone fruit, and experiments on plums and cherries conducted on a collaborative basis at Wye and East Malling Research Station by Dr. Goldwin and Mr. Webster have shown remarkable increases in setting and fruit formation (Tables 2 and 3).

Table 2

Spray experiment on Victoria plum, using a mixture of 150 ppm GA_3, 300 ppm DPU and 50 ppm NOXA. (East Malling Research Station Report 1975)

Time of applications	Fruits harvested/ 100 fruit buds	Mean fruit wt. (g)
1 + 2	0.11	33
1 + 3	7.42	29
2 + 3	10.39	31
1 + 2 + 3	14.65	23
Control	1.23	46

1=50% petal-fall, 2=50% petal-fall+2 weeks, 3=50% petal-fall+4 weeks

Table 3

Effect of GA_3 concentration in the 'Wye mix' on yield of Early Rivers Cherries sprayed at 50% petal fall. (East Malling Research Station Report 1976)

Concentration of GA_3 in ppm	Fruits harvested/ 100 fruit buds	Mean fruit wt. (g)	Percentage seedless fruit
500	55	3.6	79
200	53	3.5	73
100	37	3.8	54
50	29	3.7	58
Control	11	4.2	8

The timing of the spray is of considerable importance and varies with species and probably variety. Thus in Cox's Orange Pippin, it has been found that generally the best time of application is between full bloom and 50% petal fall. In earlier experiments in cool wet Springs, a somewhat later stage was optimal. In the cherry, again 50% petal fall has been found to be very effective, while in the plum later applications, i.e. 4 - 6 weeks after petal fall were best. In fact, more than one spray may be much more effective (Table 2). In Cox too, a second spray at a later stage may prove valuable to increase retention at the time of 'June drop', if containing an auxin or to increase fruit size by additional kinin supply (6). This aspect has been the subject of detailed studies by Wertheim (14).

FLOWER 'QUALITY'

In most of our experiments with Cox apples, seasonal comparisons indicate marked differences between years. This suggested that differences in flower quality may be important, and this is confirmed by comparison in the effectiveness of the spray on trees cropped heavily in the previous year and trees which had either cropped very lightly or had been prevented from being pollinated or were de-blossomed. This aspect is now under study by Mrs. Buszard, who finds the fruit bud diameters in autumn on the two types of trees were significantly different. Classified into large and small buds, the mean diameters (mm) are shown below together with the total numbers of flowers per tree and the final numbers of fruit produced for these flowers:

(A) Uncropped 3.25 (small), 3.50 (large) 3780 fls/tree 213 fruits/tree

(B) Heavily cropped 2.75 (small), 3.00 (large) 1440 fls/tree 111 fruits/tree

When the level of fruit set of such trees (expressed as a percentage of all flowers) was compared (averaging over spur buds, one year axillary shoots and five treatments) this was reduced substantially by the previous year's cropping - especially for the initial set - and also by bud size.

Thus:	Initial set	Final set
A	28.5	9.3
B	12.0	8.6
Large buds	46.2	22.0
Small buds	34.6	13.8

These data on flower quality taken in conjunction with the detailed work by D.L. Abbott and colleagues at Long Ashton suggest that we can at least recognise what may be a good or bad flower from their subsequent setting response, but it is also clear that there are interactions of all sorts in the final response, e.g. a much lower first set on previously heavily cropped trees may be pulled up almost to the same percentage as on a tree in its 'on' year, but that greater retention of first set flowers cannot make up for the overall reduced number of flower buds. Clearly, a lot more work needs to be done here, to give some meaning to 'good' or 'bad' in this context, but the setting spray may prove a good tool in such studies.

AFTER-EFFECTS ON FRUIT AND TREES

Other aspects to be studied are secondary effects on fruit and after-effects of sprays on the tree in the following season. Here we have done a detailed investigation with Cox. Thus, the distortion (elongation) of fruit so noticeable with GA_3-sprayed pears, is also seen initially with Cox but by harvest the change in shape is so small as to be unnoticeable. Neither are taste and storage qualities affected in any detectable degree. The extra fruit is usually of rather smaller, but fully marketable size.

In cherries and plums, seedless fruit is smaller and there are, moreover, quite marked effects on blossoming in the following year - in contrast to apples where no such effects will be detected. In the stone fruit with the very high yield increases induced (up to 25 times) flower numbers can be markedly reduced. This may be remedied by using more dilute sprays - or alternatively, one might deliberately accept a biennial situation of bearing, which might reduce picking costs, e.g. one might spray every second tree in alternate years, or even half trees (one side only).

As regards other species, we are now extending these studies but no firm results are yet at hand. In a small trial with olives (in the Wye glasshouses, using cuttings) we found a significant effect (Table 4) but whether this will be repeated in the field is as yet uncertain. Other species are now being tried in various parts of the world.

Table 4

Effect of 'Wye mix' spray on glasshouse grown miniature olive trees. Sprayed at full bloom and 4 weeks later.

	Control	Treated	LSD
1976			
Mean no. fruits/ sprayed plant	3.4	7.6	-
1977			
Mean no. fruits/ sprayed plant	14.4	22.2	5.5
Mean weight/fruit	0.94	0.86	N.S.

The final stage in this story from seedlings to crop is that of harvesting. Here we have not contributed anything at Wye, but reference must be made to the series of investigations in the use of ethylene producers to cause synchronized ripening and fruit drop recently summarised by Proebsting (9) and here too there is clear evidence that manipulation of the plant's activities by hormones is possible.

So one might perhaps list the possible sequence of changing the initial hormone balance given at different stages like this:

1. (seedling to overcome juvenility): GA_3 Alar
2. (mature tree fruit set): triple mix
3. (ripening & harvesting): Ethrel

References

1. ALDWINCKLE, J.S. (1976) Early flowering of cultivated apple seedlings forced in the greenhouse. Acta Horticulturae, 56, Symposium on juvenility in woody perennials, 201-203.

2. GOLDWIN, G.K. & SCHWABE, W.W. (1974) Use of a plant growth regulator mixture for parthenocarpic fruit setting in apple. Proceedings of the 12th British Weed Control Conference, 131-135.

3. GOLDWIN, G.K. and SCHWABE, W.W. (1975) Parthenocarpic fruit in Cox's Orange Pippin apples, obtained without hormones. Journal of Horticultural Science, 50, 175-178.

4. HACKETT, W.P. (1976) Control of phase change in woody plants. Acta Horticulturae, 56, Symposium on juvenility in woody perennials, 143-154.

5. JONKERS, H. (1973) Effect of SADH on juvenility of apple seedlings. Acta Horticulturae, 34, Symposium on growth regulators in fruit production, 143-150.

6. KOTOB, M.A. & SCHWABE, W.W. (1971) Induction of parthenocarpic fruit in Cox's Orange Pippin apples. Journal of Horticultural Science, 46, 89-93.

7. LUCKWILL, L.C. (1960) The effect of gibberellic acid on fruit set in apples and pears. Report Long Ashton Research Station for 1959, 59-64.

8. MODLIBOWSKA, I. (1961) Stimulation of fruit development in frost damaged pears. Report East Malling Research Station for 1960, 46-48.

9. PROEBSTING, E.L. Jr. (1973) Value of growth regulators as aids to mechanical harvesting of apples, cherries and grapes. Acta Horticulturae, 34. Symposium on growth regulators in fruit production, 363-371.

10. SCHWABE, W.W. (1973) The induction of parthenocarpy in Cox's Orange Pippin. Acta Horticulturae, 34. Symposium on growth regulators in fruit production, 311-316.

11. SCHWABE, W.W. (1976) Applied aspects of juvenility and some theoretical considerations. Acta Horticulturae, 56. Symposium on juvenility in woody perennials, 45-56.

12. SCHWABE, W.W. & AL-DOORI, A.H. (1973) Analysis of a juvenile-like condition affecting flowering in blackcurrant (Ribes nigrum). Journal of Experimental Botany, 24, 969-981.

13. WAREING, P.F. & FRYDMAN, V.M. (1976) General aspects of phase change, with special reference to Hedera helix L. Acta Horticulturae, 56, Symposium on juvenility in woody perennials, 57-69.

14. WERTHEIM, S.J. (1971) The drop of flowers and fruit in apple, with special reference to the June drop of Cox's Orange Pippin and its control with growth regulators. Mededelingen Landbouwhogeschool, Wageningen, The Netherlands, 71, 1-73.

15. WILLIAMS, M.W. (1972) Induction of spur and flower bud formation in young apple trees with chemical growth retardants. Journal of the American Society for Horticultural Science, 97, 210-212.

16. ZIMMERMAN, R.H. (1971) Flowering in Crabapple seedlings: methods of shortening the juvenile phase. Journal of the American Society for Horticultural Science, 96, 404-411.

17. ———————— (1973) Juvenility and flowering of fruit trees. Acta Horticulturae, 34, Symposium on growth regulators in fruit production, 139-142.

ACKNOWLEDGEMENT

The author is much endebted to the Agricultural Research Council for a support grant.

NOTES

Proceedings Joint BCPC and BPGRG Symposium -
'Opportunities for Chemical Plant Growth Regulation' (1978)

PHOTOSYNTHESIS AND ITS HORMONAL CONTROL

Kenneth J. Treharne

East Malling Research Station, Maidstone, Kent

Summary Plant growth substances have been shown to modulate photosynthetic rate. Effects are recognized on both stomatal conductance of CO_2 and on chloroplast activity but the mode of action of hormones remains obscure. Several investigations suggest cytokinins affect protein synthesis whereas gibberellins enhance enzyme activity seemingly unrelated to synthesis but which may be ascribed to changes in membrane permeability. Abscisic acid regulates stomata whilst its metabolite phaseic acid is thought to inhibit chloroplast light reactions. Aspects of hormone function are discussed in relation to chloroplast development and senescence.

INTRODUCTION

The past twenty years of research on photosynthesis have seen major advances in our understanding of the process and in the quantification of its component parts. In the first decade, physiologists on the one hand capitalised on the new technology of infra red gas analysis for measurement of carbon dioxide exchange between leaf and atmosphere, with stomates occupying a central role as the major rate-determining step in CO_2 assimilation and with the unexplained "residual resistance" to CO_2 flow occasionally acknowledged. Studies moved from single leaves to field measurements of canopies, linked with growth rates, into the era of "photosynthesis limiting productivity".

On the other hand, biochemists focussed on the chloroplast, established the photosystems converting light energy into ATP and NADPH and the intermediates and enzymes involved in the passage of CO_2 to carbohydrates, "survived the crisis" of the C_4 adjunct-pathway in certain species and turned more toward researching the complexities of regulation of chloroplast activities.

During this same period, new substances were isolated from higher plants which are present only in small amounts, but which produce marked effects on certain aspects of growth and differentiation. These substances, added to the already known auxins, include gibberellins, cytokinins, abscisic acid, ethylene and, most recently, phaseic acid. Their physiological role in plant growth regulation and in control mechanisms of metabolic processes continues to be an active research area. The wide range of effects of these growth substances, their production, movement and role in integrating plant growth has recently been reviewed (Wareing, 1977). Although information on their role in integrating metabolism is scarce, several observations suggest some, if not all, of these growth substances are involved in regulation of the processes associated with photosynthesis.

RESPONSE TO HORMONES

The major approach has been to apply growth substances, or their inhibitors and estimate the in vivo photosynthetic response. Thus auxins, gibberellins and cytokinins have been shown to promote photosynthesis in many species (Coulombe and Paquin, 1959; Lester et al., 1972; Marcelle and Oben, 1973; Poskuta et al., 1975; Treharne and Stoddart, 1968; Wareing et al., 1968) whilst abscisic acid and gibberellin inhibitors such as (2-chloroethyl) trimethylammonium chloride (CCC) reduce photosynthetic rate (Boyer, 1971; Cummins et al., 1971; Kriedemann et al., 1972; Kriedemann and Loveys, 1975; Marcelle and Oben, 1973). The majority of these studies, however, have not demonstrated degree of uptake of applied chemical, or change in concentration in target tissues or alteration in hormone balance. Other investigations have indicated relationships between extractable levels of endogenous hormones and photosynthetic activity (Treharne and Stoddart, 1968), particularly in situations where environmental factors such as water status produce stresses on the plant or where manipulation, such as partial defoliation, influence endogenous levels of the growth substances with correlated changes in photosynthesis (Loveys and Kriedemann, 1974; Neales et al., 1971; Treharne et al., 1970).

Questions arise as to the manner in which these plant hormones affect the photosynthetic capacity of leaves; is the response direct or secondary, are stomates or chloroplasts limiting the rate and are they selectively affected, do hormones affect the development of the photosynthetic apparatus, synthesis, activity or rate of breakdown of enzymes, and do the different classes of growth substances act in a similar or completely different way?

HORMONAL ACTIVITY IN THE CHLOROPLAST

Since stomatal physiology and response to stress are dealt with in session 2 of this symposium, I shall confine myself to consideration of the chloroplast and known or speculative interactions of cytokinins, gibberellins and abscisic and phaseic acids. From various studies it is clear that chloroplast activity and particularly the initial carboxylation reaction can control the rate of leaf photosynthesis (Björkman, 1966; Treharne, 1972; Wareing et al., 1968). Full consideration of how the activity of the enzyme ribulose-15-bisphosphate carboxylase (RuBPc), responsible for catalysing the carboxylation of ribulose-15-bisphosphate to 3-phosphoglyceric acid, is controlled is beyond the scope of this paper. Many alternative models have been offered in recent years to explain the regulatory properties of RuBPc (see reviews by Jensen and Bahr and Kluge). A brief consideration of the enzyme properties and speculative in vivo control of the chloroplast environment may, however, suggest possible roles for the different growth regulators. The lamellar membranes, or grana, of the chloroplast in producing ATP and NADPH, in light, take up protons in exchange for Mg^{2+} resulting in a light-mediated alkalization of the stroma from pH 7 in the dark to pH 8 (Heldt et al., 1973) and an increase in Mg^{2+} concentration (Lin and Nobel, 1971) consistent with "ideal" in vivo conditions for RuBPc activity. The enzyme RuBPc has allosteric properties and can be regulated in a complex manner (Chu and Bassham, 1975), is activated by Mg^{2+} and CO_2 (Lorrimer et al., 1977; Walker, 1973) and can be modified by sugar phosphates and is thus subject to feedback control (Buchanan and Schurmann, 1973). Selective transport of compounds, particularly sugar phosphates, across the inner membrane of the chloroplast envelope (Heber, 1974) serves as a further type of regulation of activity of the reductive pentose phosphate cycle. The degree of dependence of chloroplasts on cytoplasmic components or other cell organelles is not understood, but it may be envisaged that growth substances, particularly gibberellins, may well exert influence on movement of metabolites through the membranes by changing their permeability characteristics, and thereby modulate photosynthesis. It is reasonable to expect some relationship between gibberellins and photosynthesis since up to 60% of total leaf content of GA is associated with the chloroplast (Stoddart, 1968).

The bulk of Fraction I protein synthesis occurs during the early phase of leaf expansion and only a low turnover can be detected thereafter (Smillie et al., 1967; Treharne et al., 1970). Although synthesis of cytoplasmic RNA continues throughout the life of a leaf, synthesis of plastid ribosomal RNA occurs mainly in the early stages of leaf development (Ingle, 1968). Fraction I protein, containing RuBPc activity, consists of a major sub-unit, known from inhibitor studies to be synthesised on 70 S ribosomes within the plastid, and a small sub-unit which is synthesised outside the plastid on 80 S ribosomes, as are the bulk of the other enzymes of the Calvin cycle (Ellis and Hartley, 1971; Graham et al., 1970). The possibility exists that hormones enhancing photosynthetic activity may promote synthesis of RuBPc, either the component in the plastid or in the cytoplasm, or may reduce synthesis of a repressor molecule or inhibitory protein. Hormones may, alternatively, alter the permeability of the chloroplast membrane and promote entry of the extra-plastid synthesised small sub-unit.

In our earlier work (Treharne and Stoddart, 1968; Wareing et al., 1968), treatments with GA and CK increased RuBPc activity and photosynthesis of fully expanded leaves, but during the phase of active leaf growth and Fraction I protein synthesis, endogenous levels of hormones were high and GA_3 depressed synthesis of plastid enzymes. During the active phase of plastid-RNA synthesis, no significant effects of either GA_3 or kinetin were detected. However, RuBPc activity was enhanced by both GA_3 and kinetin when both plastid-RNA and Fraction I protein synthesis were very low. Thus, it would seem that stimulation in enzyme activity after hormone applications was due to activation rather than synthesis of new enzyme in the plastid. The experiments did not determine whether synthesis of messenger or transfer RNA was affected by hormone treatment, nor did they preclude the possibility of enzyme stimulated synthesis in the cytoplasm, subsequently transferred to the plastid (Treharne et al., 1970).

Feierabend reported stimulated rates of synthesis of RuBPc after applications of kinetin during greening of etiolate leaves exposed to light. Effects of applications of GA3 were examined during the rapid phase of development of the photosynthetic apparatus when etiolate monocotyledon seedlings were illuminated (Treharne et al., 1971). Known inhibitors of GA biosynthesis (Kende et al., 1963; Zeevart, 1966) were used to attempt to reduce endogenous hormone levels and thus allow study of the mechanism of GA response. Activity of RuBPc was enhanced by GA_3 in etiolate seedlings, but after greening, activity was the same as that of the untreated control. Thus, GA apparently substituted for light which could be explained by activation rather than increased synthesis of RuBPc. The inhibitors CCC and AMO 1618 however, also increased RuBPc in the etiolate seedlings and produced responses on a spectrum of enzymes which were difficult to explain solely in terms of inhibited GA synthesis. In the case of CCC it is feasible that only low concentrations actually increased GA content (Reid and Crozier, 1970).

Chloroplast differentiation is accelerated by the cytokinin benzyladenine (BA) and photosynthetic enzyme activity in cucumber cotyledons is stimulated (Harvey et al., 1974). In the etiolate system BA reduced the lag phase for production of grana and inhibitor studies suggested BA enhanced synthesis of RuBPc. Similarly, BA stimulates production of Ɣ-aminolaevulinic acid, a chlorophyll precursor, in cucumber cotyledons (Fletcher and McCullagh, 1971). Chloroplast division in tobacco is stimulated by cytokinins (Laetsch and Boasson, 1972), but inhibited by auxin (Laudi, 1967). Ultrastructural morphogenesis of plastids of greening cereals was enhanced by GA_3, whilst CCC and abscisic acid (ABA) showed the reverse effect (Wellburn et al., 1973). With isolated etio-chloroplasts, although GA_3 did not enhance development, it was able to neutralise ABA-induced retardation of development. In monocotyledons RNA metabolism is stimulated by GA and reduced by ABA (Poulson and Beevers, 1970) and is attributable to the RNA polymerase component (Pearson and Wareing, 1969) and thus, Wellburn et al. suggested ABA depressed the availability of m RNA responsible for synthesis of lamellar proteins

in the chloroplast. The situation was less clear for GA which presumably functioned at the same site as ABA, but was also likely to be affecting cytoplasmic RNA metabolism. The parallel nature of the overall structural responses and RuBPc activity suggested a relationship between the two parameters, but relative contributions of cytoplasm and plastid toward regulation of RuBPc are unknown.

From previous work on hormone concentration effects (Wareing et al., 1968) response is clearly dependent on endogenous status or balance of regulators in a system. The etiolate situation seems to have a more readily discernible response than young normally grown leaf tissue, but it should be borne in mind that, although providing a useful biochemical test system, it need not reflect the real pattern of chloroplast development or natural hormonal role. Exposure of etiolate leaves to red light releases large amounts of gibberellins which are attributed to plastids (Cooke and Saunders, 1975) and related to a phytochrome response (Evans and Smith, 1976). This could act by regulating chloroplast permeability to gibberellins allowing access to other target sites (Stoddart, 1976). These observations afford an explanation for a demonstrable effect of adding GA to etiolate tissue whereas young green tissue has a high endogenous GA level possibly related to light-stimulated phytochrome release from chloroplasts with a consequently GA-saturated system and no response to increase through application.

ABSCISIC ACID

Chloroplasts from normal leaves are able to synthesise ABA (Milborrow, 1974) and virtually all the leaf ABA is located in the chloroplast fraction (Loveys, 1977). It seems that in response to water stress, ABA is synthesised in the mesophyll chloroplasts and migrates to the epidermis and there exerts its well established stomatal control. Isolated plastids are permeable to ABA and ther seems to be no barrier to its movement across chloroplast membranes (Wellburn and Hampp, 1976). Alleviation of water stress results in a rapid fall in ABA with a rise in its metabolites phaseic and dihydrophaseic acids (Harrison and Walton, 1975; Kriedemann and Loveys, 1975; Kriedemann et al., 1975). Rates of both synthesis and metabolism of ABA were increased and ABA was thought not to be released from an inactive conjugate, although this is questioned by Rasmussen. During the post-stress phase photosynthetic inhibition is observed and attributed to phaseic acid acting as a chloroplast inhibitor, rather than to stomatal conductance of CO_2. This phaseic acid inhibition is thought to be on a photochemical locus rather than enzyme activity (Kriedemann and Loveys, 1975) and it has been demonstrated that water stress does not affect protein synthesis via ABA (Dhindsa and Cleland, 1975). The role of ABA in non-stressed tissue is not understood, although seemingly well-defined in relation to water stress. It may be speculated that, like GA, ABA release from chloroplasts may also be related to phytochrome in a "normal" system.

SENESCENCE

Apart from the possible effects on chloroplast development the various growth substances have long been established as retardants of leaf senescence. The loss in activity of RuBPc during early leaf senescence is known to constitute the limiting "resistance" to CO_2 assimilation (Woolhouse, 1968; Neales et al., 1971). Senescence is a selective sequential process which, in many species, can be delayed by application of cytokinins (Letham, 1967; Skoog and Armstrong, 1970; Stobart et al., 1972). The early degradation of chloroplast proteins, including RuBPc, was delayed by kinetin treatment, and was prevented by inhibiting proteolytic activity developing on cytoplasmic 80 S ribosomes (Peterson and Huffaker, 1975). In dark grown Rumex, both GA and cytokinin inhibit senescence, whilst ABA accelerates the process (Manos and Goldthwaite, 1975). It was suggested that neither interacted directly with ABA in this system, but that ABA induced stomatal closure, reduced xylem flux, thus reducing cytokinin supply and consequently speeding senescence, and that intrinsic fluctuations in senescence,

according to season, may be related to more GA or CK or to less ABA in the system. It is proposed that the interplay of cytokinin and ABA levels regulate senescence (Even-Chen and Itai, 1975) related to changes in bound ABA with high cytokinins reducing the level of free ABA.

This paper has focussed on the chloroplast as a reasonable well-defined sub-system where metabolic processes are fairly well characterised, to attempt to examine the biochemical nature of hormone control. Although plastids contain gibberellins and abscisic acid their mode of action in modulating chloroplast activity and resultant photosynthesis of leaves is clearly an open question. Although some evidence is presented on the apparent ability of hormones to promote the rate of membrane organisation in chloroplast development and to stimulate some enzyme activities, it is no way certain that these effects may be ascribed directly to protein synthesis in the chloroplast *per se*. The establishment of the mode of action of growth regulators on the important process of photosynthesis is clearly a priority area for future study.

References

BJÖRKMANN, O. (1966) Physiologia Plantarum, 21, 1-8.

BOYER, J. S. (1971) Plant Physiology, 48, 532-536.

BUCHANAN, B. B. and SCHURMANN, P. (1973) Journal of Biological Chemistry, 248, 4956-4964.

CHU, D. K. and BASSHAM, J. A. (1975) Plant Physiology, 55, 720-726.

COOKE, R. J. and SAUNDERS, P. F. (1975) Planta, Berlin, 123, 299.

COULOMBE, L. J. and PAQUIN, R. (1959) Canadian Journal of Botany, 37, 897-902.

CUMMINS, W. R., KENDE, H. and RASCHE, K. (1971). Planta, Berlin, 99, 347-351.

DHINDSA, R. S. and CLELAND, R. E. (1975) Plant Physiology, 55, 782-785.

ELLIS, R. J. and HARTLEY, M. R. (1971) Nature, London, 233, 193-196.

EVANS, A. and SMITH, H. (1976) Proceedings of the National Academy of Sciences of the United States of America, 73, 138-142.

EVEN-CHEN, Z. and ITAI, C. (1975) Physiologia Plantarum, 34, 97-100.

FEIERABEND, J. (1966) Planta, Berlin, 84, 11-15.

FLETCHER, R. A. and McCULLAGH, D. (1971) Canadian Journal of Botany, 49, 2197-2201.

GRAHAM, D., HATCH, M. D., SLACK, C. R. and SMILLIE, R. N. (1970) Phytochemistry, 9, 521-532.

HARRISON, M. A. and WALTON, D. C. (1975) Plant Physiology, 56, 250-254.

HARVEY, B. M. R., LU, B. C. and FLETCHER, R. A. (1974) Canadian Journal of Botany, 52, 2581-2586.

HEBER, U. (1974) Annual Review of Plant Physiology, 25, 393-421.

HELDT, H. W., WERDAN, K. MILOVANEER, M. and GELLER, G. (1973) Biochimica et Biophysica Acta, 314, 224-241.

INGLE, J. (1968) Plant Physiology, 43, 1448.

JENSEN, R. G. and BAHR, J. T. (1976) In 'CO_2 metabolism and plant productivity' (Eds R. H. Burris and C. C. Black), pp 3-18, University Park Press.

KENDE, H. and NINNEMAN, H. and LANG, A. (1963). Naturwissenschaften Berlin, 50, 599-604.

KLUGE, M. (1977) In 'Integration of activity in the higher plant' (Ed. D. H. Jennings), pp 155-175, Cambridge University Press.

KRIEDEMANN, P. E. and LOVEYS, B. R. (1975) In 'Environmental and biological control of photosynthesis' (Ed. R. Marcelle), pp 227-236, Dr. W. Junk bv Publ.

KRIEDEMANN, P. E., LOVEYS, B. R. and DOWNTON, W. J. (1975) Australian Journal of Plant Physiology, 2, 553-567.

KRIEDEMANN, P. E., LOVEYS, B. R., FULLER, G. C. and LEOPOLD, A. C. (1972) Plant Physiology, 49, 842-847.

LAETSCH, W. M. and BOASSON, R. (1972) In 'Hormonal regulation of plant growth and development', Proceedings of the Advanced Study Institute Izmir 1971, pp 453-65 Weinheim: Verlag Chemie.

LAUDI, G. (1967) Giornale Botanico Italiano, 101, 97-109
LESTER, D. C., CARTER, O. G., KELLEHER, F. M. and LAING, D. R. (1972) Australian Journal of Agricultural Research, 23, 205-13.
LETHAM, D. S. (1967) Annual Review of Plant Physiology, 18, 349-364.
LIN, D. C. and NOBEL, P. S. (1971) Archives of Biochemistry and Biophysics, 145, 622-632.
LORRIMER, G. H., BADGER, M. R. and ANDREWS, T. J. (1977) Analytical Biochemistry, 78, 66-75.
LOVEYS, B. R. (1977) Physiologia Plantarum, 40, 6-10.
LOVEYS, B. R. and KRIEDEMANN, P. E. (1974) Australian Journal of Plant Physiology, 1, 407-415.
MANOS, P. J. and GOLDTHWAITE, J. (1975) Plant Physiology, 55, 192-198.
MARCELLE, R. and OBEN, G. (1973) Acta Horticulturae, 34, 55-58.
MILBORROW, B. V. (1974) Phytochemistry, 13, 131-136.
NEALES, T. F., TREHARNE, K. J. and WAREING, P. F. (1971) In 'Photosynthesis and phororespiration' (Eds M. D. Hatch, C. B. Osmond and R. O. Slatyer), pp 89-96. Wiley Interscience New York.
PEARSON, J. A. and WAREING, P. F. (1969) Nature, London, 221, 672.
PETERSON, L. W. and HUFFAKER, R. C. (1975) Plant Physiology, 55, 1009-1015.
POSKUTA, J., PARYS, E., OSTROWSKA, E. and WOLKOWA, E. (1975) In 'Environmental and biological control of photosynthesis' (Ed. R. Marcelle), pp 201-209. Dr. W. Junk bv Publ.
POULSON, R. and BEEVERS, L. (1970) Plant Physiology, 46, 782-785.
RASMUSSEN, O. S. (1976) Plant Physiology, 36, 208-212.
REID, D. M. and CROZIER, A. (1970) Planta (Berl.), 100, 309.
SKOOG, F. and ARMSTRONG, D. J. (1970) Annual Review of Plant Physiology, 21, 359-384
SMILLIE, R. M., GRAHAM, D., DWYER, M. R., GRIEVE, A. and TOBIN, N. F. (1967) Biochemical and Biophysical Research Communications, 28, 604.
STOBART, A. K., SHREWRY, R. R. and THOMAS, D. R. (1972) Phytochemistry, 11, 571-577.
STODDART, J. L. (1968) Planta Berlin, 81-106.
STODDART, J. L. (1976) Nature, London, 261, 454-455.
TREHARNE, K. J. (1972) In 'Crop processes in controlled environments' (Eds A. R. Rees, K. E. Cockshull, D. W. Hand and R. G. Hurd), pp 285-303. Academic Press.
TREHARNE, K. J. and STODDART, J. L. (1968) Nature, London, 220, 457-458.
TREHARNE, K. J., STODDART, J. L. and HEDLEY, C. L. (1971) In 'Proceedings IInd International Congress on Photosynthesis' pp 2497-2509.
TREHARNE, K. J., STODDART, J. L., PUGHE, J., PARANJOTHY, K. and WAREING, P. F. (1970) Nature, London, 228, 129-131.
WALKER, D. A. (1973) New Phytologist, 72, 209-235.
WAREING, P. F. (1977) In 'Integration of activity in the higher plant' (Ed. D. H. Jennings), pp 337-366. Cambridge University Press.
WAREING, P. F., KHALIFA, M. M. and TREHARNE, K. J. (1968) Nature, London, 220, 453-457.
WELLBURN, A. R. and HAMPP, R. (1976) Ibid., 131, 95-96.
WELLBURN, F. A. M., WELLBURN, A. R., STODDART, J. L. and TREHARNE, K. J. (1973) Planta, Berlin, 111, 337-346.
WOOLHOUSE, H. W. (1968) Hilger J., 11, 7-12.
ZEEVART, J. A. D. (1966) Plant Physiology, 51, 856-860.