CHANGES IN INHIBITOR LEVELS, STOMATAL APERTURE, AND GROWTH OF *PISUM SATIVUM* L. SUBJECTED TO WILTING STRESS CYCLES DURING DIFFERENT DEVELOPMENTAL STAGES.

A thesis submitted in partial fulfilment of the requirements for the degree of Master of Horticultural Science at Massey University

IAN DAVID IVEY

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Plants of Pisum sativum L., grown under controlled environmental conditions, were subjected to wilting stress cycles at different developmental stages and analyzed for changes in inhibitor levels, stomatal aperture, water status, and effects on final yield.

As leaf water potential decreased past a critical level, stomatal aperture decreased markedly and, at the same time, inhibitor levels increased rapidly. The maximum inhibitor levels attained, as determined by several different methods of assessment, approximately halved with each later wilting cycle, whilst the degree of stomatal closure was approximately the same for each cycle. During the recovery phase, plant water status recovered to normal 24 hours after rewatering. At this time inhibitor levels had decreased markedly and, in the later cycles, had apparently declined to normal levels. However stomatal aperture had only recovered slightly at this point in all cycles and by 4 days after rewatering stomata had generally regained normal apertures.

Results of the final yield analysis were confounded somewhat by the shooting of basal buds, particularly on plants subjected to wilting cycles during the preflowering and flowering stages, and a possible explanation for this lateral growth is discussed. However the pod swelling stage was more sensitive to water stress than other stages.

Changes in inhibitor levels alone, did not appear to be related directly to stomatal responses or any sensitivity of particular growth stages. Some possible reasons for these observations are presented on the basis of evidence available in the literature.

CHAPTER I

I REVIEW OF THE LITERATURE

A INTRODUCTION

Water stress in plants is probably one of the most common factors limiting maximum potential yields, and during the daytime, plants are always under some stress, no matter how well watered they are (Freeman et al, 1970).

Much of the early work, as the review by Salter and Goode (1967) illustrates, was investigating the effects of water stress on parameters of plant growth. From this earlier experimentation it became clear that some stages in the development cycle of plants were more sensitive to moisture stress than others. This phenomenon was shown to be widespread in both annual and perennial crops, generally the most sensitive stages being during flowering and the period of rapid development of reproductive organs. In the field situation, where plants are in a competitive situation, this sensitivity becomes even more important as Salter and Williams (1967) have noted.

Reviewing the effects of water stress on annual and herbaceous plants, Gates (1968) points out that the stress duration need only be for a short period, if the intensity is high enough, to have permanent effects in the plant, although apparently recovered.

In recent years, research has concentrated on the reasons for plant responses to water stress and now it is well known that one of the first responses is stomatal closure (Meidner and Mansfield, 1968), which can also cause a reduction in photosynthetic rates by reducing CO_2 uptake. Processes such as cell division and cell elongation are affected by relatively small changes in plant water status, the latter process being affected to a greater extent (Hsiao, 1973).

Perhaps the most exciting discovery has been the finding of a rapid increase in inhibitor β levels in leaves subjected to wilting stress (Wright, 1969) and the subsequent identification of abscisic acid (ABA) as

a major component of the increased inhibitor level (Wright and Hiron, 1969). This discovery has led to a whole new field of endeavour. Research has shown that ABA is not the only plant hormone to change in response to water stress, cytokinin levels decreasing markedly as Itai and Vaadia (1965) have demonstrated. Both ABA and cytokinins were shown to be involved in many plant processes which change in response to water stress e.g. stomatal opening (Horton, 1971; Cooper et al, 1972) and the water balance of tissues (Glinka and Reinhold, 1971). The involvement of these two growth regulators in particular, with the water relations of plants, has led to much speculation on their possible roles. As yet these roles have not been fully elucidated.

In the following sections, various aspects of plant response and some of the underlying factors, will be examined in relation to their possible involvement in the reaction of plants to water stress.

B. THE EFFECTS OF WATER STRESS ON VARIOUS PARAMETERS OF PLANT GROWTH AND YIELD

(1) ROOT GROWTH

Roots are generally the last to suffer the effects of water stress as they are nearest the major source of water for the plant i.e. the soil (Zahner, 1968). However if the soil becomes dry enough root growth can be markedly affected as Engin and Sprent (1973) demonstrated with clover plants. In the competitive crop situation depletion of soil moisture can be particularly important, as the reservoir on which the plant draws is limited (Salter and Goode, 1967).

Using osmotic solutions to impose the effect of a water deficit, Gonzalez-Bernaldez et al (1968) noticed a marked reduction in root growth, particularly at higher stress levels (-12 bars).

The effects on the root system are obviously dependent on the moisture supply to the root and the transpiration demand on the plant, both of which determine the degree of water deficit occurring in the plant.

(2) EXTENSION GROWTH, APICAL DOMINANCE AND TILLERING

Salter and Goode (1967) suggest that during the period of rapid vegetative growth, minimum loss of turgor is necessary to enable maximum growth rates, and Zahner (1968), in a review, concludes that water stress reduces stem fresh and dry weight increases, as well as elongation.

Working with tomato plants Gates (1955a, 1956) found water stress caused a redistribution of dry matter between leaves and stems, and on recovery a reverse pattern was noticed. In his review (Gates 1968) he comments that actively developing tissues were markedly retarded by a period of water stress but, if the duration of stress was relatively short, recovery resulted in a return to normal growth rates. From his work he concluded that the responses observed during the onset of water stress resembled a change to the senescent phase, whilst recovery suggested a return to a more juvenile state.

Looking at a particular example of the effects of water stress on stem growth, Maurer et al (1968) discovered that peas were tallest and had the highest haulm fresh and dry weights under optimum watering conditions. Internodes developing under moisture stress conditions were shorter. The effects on internode elongation were directly related to the degree of water stress acting on the plant, as internodes developing on plants relieved from stress elongated normally.

Regarding effects on the apical meristem, Morton and Watson (1948), interpreted their results as suggesting that the apex in sugar beet was less sensitive to moisture stress than other plant parts. However Gates (1968), from his work on Lupinus albus, considers the opposite is true, as primordial initiation was markedly retarded by relatively low plant water deficits. On recovery from stress rapid resumption of primordial initiation was observed. Associated with the above observation, McIntyre (1971, 1973) with peas and beans, noted that water stress enhanced apical dominance over basal nodes; leaf water deficits

had to be below a certain critical level before basal buds were able to shoot. Hussey (1973) suggests that tension on the apex may regulate primordial development.

These observations are best summed up by Gates (1968) who suggests that an important characteristic of juvenile tissues, even though highly sensitive to internal water stress, is that of being able to resume active development on relief from water stress. The function of the apical meristem is suspended, but not impaired, by sub-lethal water deficits.

(3) LEAF DEVELOPMENT

The leaf area of a plant is important with respect to final yield, due to its major role in photo-assimilation.

Morton and Watson (1948) observed marked reductions in the leaf areas of sugar beet plants subjected to moisture stress, part of the reduction being due to accelerated senescence of older leaves. The dry matter redistribution from leaves to stems noted by Gates (1968) supports these observations.

As the above workers point out however, leaf area on plants suffering water stress, can also be affected by decreased leaf expansion and decreased leaf initiation. Salter and Goode (1967) consider these reductions to be important as they reduce potential photosynthetic capacity and thus plant development and yield.

The sensitivity of leaf enlargement to moisture stresshas been demonstrated by Boyer (1973). Soybean leaf enlargement dropped sharply with relatively small decrease in leaf water potential. At a leaf water potential of -12 bars, leaf enlargement was essentially zero.

(4) FLOWERING AND FRUIT DEVELOPMENT

Because most crops are grown for their reproductive parts, the effects of water stress on flowering, fruit set and fruit development, are of major importance.

In a review article, Kaufman (1972) concluded that water stress had a variable effect on flower initiation.

Regarding flowering itself, plants have been found to be particularly sensitive to moisture stress at this stage (Salter and Goode, 1967) and Kaufman suggests that often water stress decreases fruit yield by enhancing flower and early fruit abscission. This suggestion is supported by Blackwall (1969) who observed increases in the number of flowers set on runner beans (Phaseolus multiflorus) under conditions of good moisture availability. This effect was reflected in the final yield of marketable pods.

Another point from the review by Salter and Goode (1967) is that the stage of rapid fruit enlargement is often sensitive to water stress.

One of the better examples illustrating the effects of water stress on final yield is the pea. Bartz (1960) and Salter (1962) noticed that low soil moisture in the post-flowering period caused a marked reduction in yield. In the period preceding flowering, low soil moisture levels had an effect on haulm growth but little effect on final yield. Looking at this further Salter (1963) noticed that the greatest final yield was obtained with low moisture stress conditions at both the early flowering period and the pod swelling stage. The increase in yield was due to both increased pod set and final pea weight. Maurer et al (1968) obtained similar results.

(5) DISCUSSION

The preceding brief resume illustrates that water stress can have marked effects on the rate of development and final status of various plant organs. Many of the above responses are only symptoms of the plant reaction to water stress and , in the following sections, some of the possible causes will be discussed.

C. THE EFFECTS OF WATER STRESS ON PHYSIOLOGICAL AND BIOCHEMICAL PROCESSES IN PLANTS

(1) CELLULAR DEVELOPMENT

Increases in the size of many plant organs depends on both cell division and enlargement and in relation to these processes, the laying down of new cell wall materials. Cleland (1967) points out that if cell turgor potential drops below a critical level, no elongation will occur. As the turgor pressure increases in excess of the critical potential, cell extension increases accordingly. Thus cell elongation depends to a large extent on high cell turgor.

Regarding cell elongation, several reports (Acevedo et al, 1971; Boyer, 1970) suggest that in intact leaves, a drop in leaf water potential to values less than -2 bars causes a reduction in elongation. Boyer found, with soybeans that leaf growth was negligible with a leaf water potential of -12 bars. An almost immediate resumption of elongation in midly stressed maize leaves was noted by Acevedo et al when plants were rewatered. Both Gardner and Neiman (1964) and Gonzalez-Bernaldez et al (1968), using osmotic solutions to induce stress in plant tissues, noted cell enlargement was markedly affected, even by a change to only -2 bars. Similar results were reported by Kirkham et al (1972).

The effects of water stress on cell division however, are not so clear. Hsiao (1973) comments that it has often been said cell division is less sensitive to water stress than cell elongation and the results of Gonzalez-Bernaldez et al (1968) support this. However the data of Gardner and Neiman (1964) Terry et al (1971), and Kirkham et al (1972) suggest that cell division, as measured by increases in DNA levels, is markedly affected by relatively small changes in water potential of only 1 to 2 bars. Further decreases in water potential, however, appeared to give little further effect.

Hsiao (1973) proposes that the effects on cell division may be indirect and that cells must reach an adequate size before a mitotic division takes place. The findings of Doley and Leyton (1968) support this idea. Cambial cells of Fraxinus excelsior had to be in the order of 6μ in diameter for a cell division to take place.

Cell wall metabolism is also apparently suppressed by water stress, but Hsiao (1973) suggests this change is also likely the indirect result of reduced growth than a direct effect of water stress. Support for this idea is obtained from the results of Cleland (1967) which show in Avena coleoptiles, while a marked reduction of glucose incorporation into cell walls occurs with a change in water potential of -3 to -4 bars, this same decrease causes an almost complete cessation of coleoptile growth.

Overall it appears cell elongation is the most sensitive of the three processes to water stress. The other two are affected, but the effects may be indirect, i.e. due to reduced cell elongation.

(2) STOMATA

As Weatherley (1970) stresses, stomata play a major role in the control of water loss from plants. Their effectiveness is due to their positioning in the gas phase of the soil-plant-atmosphere system, the point at which water potential differences are greatest.

Stomatal opening is closely dependent upon the hydrostatic pressure within the leaf epidermis and, as a water deficit develops beyond a critical level, partial or complete stomatal closure ensues (Meidner and Mansfield, 1968; Glinka, 1971). Meidner and Mansfield note that stomatal closure, when the water deficit is greater than the critical level, is hydroactive. The term hydroactive has to be clarified however. Stalfelt (1955) defined passive and active, in relation to stomatal movements, in the following way. Passive movement was totally dependent upon forces external to the guard cells, whereas active

movement involved the guard cells themselves. He illustrates the passive effects in terms of turgidity of the epidermis. In a fully turgid leaf there is a suboptimal water deficit where guard cells encounter a resistance, due to the epidermal cells, which prevents them from opening to their fullest extent. If epidermal cell turgor is altered rapidly then an initial passive opening movement occurs. Opening then reaches a maximum when there is an optimal water deficit in the epidermis i.e. the point where opening in the light meets little or no resistance from the epidermis. Then there is a supra-optimal water deficit which leads to partial or complete stomatal closure. This supra-optimal deficit induces hydro-active closure because the guard cells play an active part, due to loss of turgor, in producing the effect. Once this process is initiated, the stomata become less sensitive to light, opening to a lesser extent in a given light intensity. The fact that this closure process has been termed hydro-active does not necessarily mean that the movement is metabolically dependent, only that the guard cells are involved in the movement. Meidner and Mansfield (1968) suggest that closure may not indeed be metabolically dependent.

Meidner and Mansfield also noted that closure occurred during the early stages of a water shortage, often long before visible wilting occurs. Once visible signs of stress are seen, stomata are generally completely closed and remain so as long as the water shortage persists.

Looking at several examples it can be seen that stomatal closure can be relatively sudden. Turner (1973) observed this effect in tobacco with a leaf water potential of -12 bars, maize leaves at -16 bars and sorghum at -20 bars. Hsiao (1973) comments that once the threshold leaf water status for stomatal closure is reached, a sharp increase in leaf resistance occurs with a relatively small drop in water potential. He also observes that such a large increase in leaf resistance could be taken as almost complete stomatal closure but, in

a number of cases, further increases in water deficits resulted in increasing resistance with no signs of levelling off.

Not only does water stress affect stomatal closure it also delays full opening on rewatering. In relation to this Kramer (1950) observed that transpiration rates of several plant species took a number of days to recover to normal levels once water stress was relieved. Meidner and Mansfield (1968), discussing these after effects, noted that they were a relatively common occurrence and, in some species, several days passed after rewatering before normal apertures were attained. Fischer et al (1970) observed a delay of up to five days with respect to the stomata of Nicotiana tabacum, depending on the duration and intensity of the stress.

Maize stomata show a particularly marked reaction to water stress and may not ever recover full opening in leaves subjected to water stress (Glover, 1959). Meidner and Mansfield suggested that the delay in reopening was important because it guards against rapid consumption of the new supply of water to the plant.

Overall, stomata respond rapidly to water stress by hydroactive closure once a critical water potential is reached. This critical level depends on both the species and the growing conditions according to Hsiao (1973). During the recovery phase stomatal opening appears to be delayed, the intensity and duration of the effect depending upon both the intensity and duration of the stress and the species.

(3) PHOTOSYNTHESIS AND TRANSLOCATION

Water stress can affect photosynthesis in two major ways;

(a) indirectly by reducing carbon dioxide (CO_2) intake and

(b) directly by effects on the photosynthetic apparatus itself.

Reports in the literature indicate indicate that stomatal closure causes a major reduction in the rate of photosynthesis in plants suffering water stress. This would be expected as stomatal

resistance is only a minor component of the resistance to CO_2 uptake under normal conditions, but under conditions of water stress they become a major resistance. The model proposed by Charles-Edwards (1971) (Figure I) illustrates simply, the contributing factors relating to the diffusion of CO_2 into, and water vapour out of, the plant. Thus there are a greater number of resistances to CO_2 diffusion into the plant compared with water vapour diffusion out. Any reduction in stomatal aperture is likely to have a greater effect on the transpiration rate than CO_2 diffusion because of the different relative importance of stomatal resistance in the total resistance paths for the two different processes. This effect is illustrated by the photosynthesis: transpiration ratio (P/T). As stomatal aperture decreases due to increasing water stress, so the ratio increases showing that transpiration is affected to a greater degree by a given decrease in stomatal aperture, than photosynthetic rates. The steepness of the increase in the P/T ratio would depend on the relative proportion stomatal resistance is of the total resistance in the CO_2 diffusion pathway. Obviously in a situation where there is a high mesophyll resistance, as Kriedeman (1971) has described for orange leaves, the increase in the P/T ratio would be rapid as stress increased, whereas in a species where mesophyll resistance is low e.g. corn, the increase in this ratio would be less rapid. The latter example is a C_4 plant which is more efficient in its photosynthetic utilization of CO_2 (Hatch and Slack, 1970) and thus has a lower mesophyll resistance, stomatal closure being more important in the total resistance pathway.

However, because CO_2 uptake is also mainly via the stomata, oscillations in aperture due to change leaf water status, will also affect the uptake of CO_2. Thus the photosynthetic rates will also be affected. Several workers have observed oscillations in photosynthetic rates with oscillations in stomatal aperture (e.g. Kriedeman, 1971).

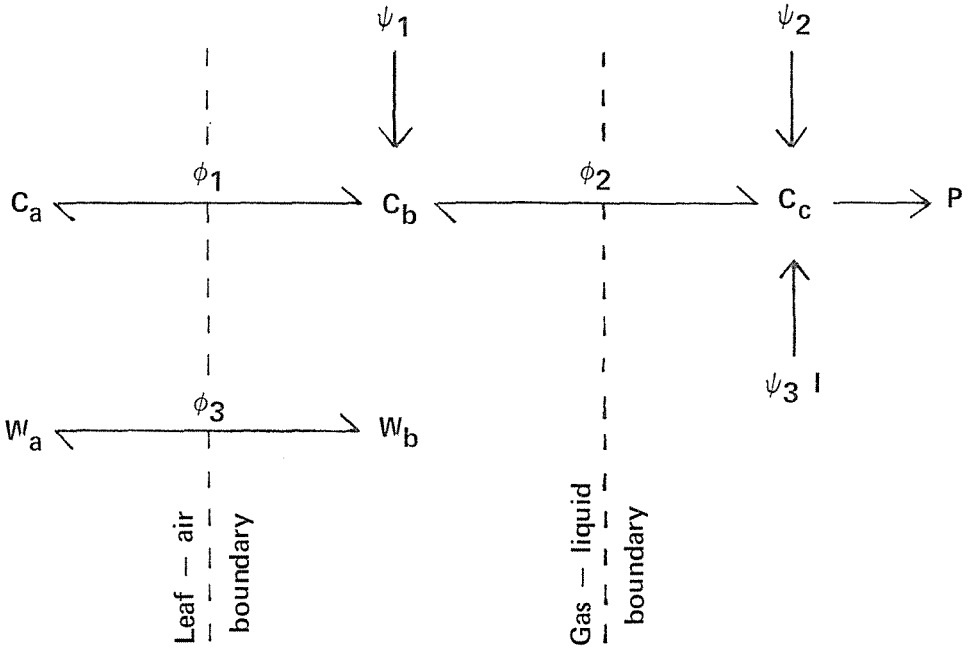


FIGURE 1 Scheme proposed by Charles — Edwards (1971) illustrating some of the major factors contributing to the water and CO_2 diffusion pathways between the air and the leaf. Key. C_a, C_b, C_c, ambient, intercellular, and intracellular CO_2 concentrations respectively. W_a and W_b ambient and intercellular water vapour concentrations respectively. ϕ_1, ϕ_2, ϕ_3, diffusive functions for CO_2 and water vapour. P, gross photosynthetic rate. ψ_1 and ψ_2 rates of basal respiration. ψ_3, light activated respiration. I, incident light flux density.

Downey (1971) has shown a close relation between leaf relative water content and photosynthesis in maize leaves, the rates being virtually zero with a drop of 30% in leaf water content.

Brix (1962) working with tomato plants has shown that their photosynthetic rate fell rapidly after leaf water potential had dropped to about -7 bars; by -9 bars the rate had dropped by 50% and at -14 bars was essentially zero. These results correlate well with the observations of Duniway (1971) showing a threshold value for stomatal closure in tomato leaves of about -7 to -9 bars.

Jones and Slatyer (1972) noted that another indirect effect of water stress on CO_2 diffusion was that of reducing intracellular transport by effects on cytoplasmic hydration.

An important effect of water stress is that of affecting photosynthetic capacity. Leaf area, as has already been mentioned (p5), is markedly affected by water stress and Morton and Watson (1948) point out that plant growth depends upon an effective leaf area. Crop growth rate (C.G.R.) is dependent upon two major parameters (Sestak et al, 1971), leaf area index (L) and net assimilation rate (E).

$$C.G.R. = L \times E$$

Obviously the net assimilation rate is going to be reduced in plants suffering water stress due to stomatal closure as described above, and possibly by other effects (see below). However leaf area can be markedly affected also, thus causing long term effects. Gates (1968), discussing his earlier work on the effects of wilting stresses on tomato plants, comments that leaves rapidly expanding at the time of stress imposition, were permanently retarded on recovery. This may be important in terms of potential photosynthetic production as particular leaves tend to contribute to different phases of growth e.g. in rice different leaves contribute to younger leaf development, tiller growth and grain filling, (Ishezuka 1971). Thus any effect

on a particular leaf could affect later plant development phases.

It is also well known that water stress reduces the rate of phloem transport (Crafts, 1968), whether the amount/unit time is also reduced is unclear. Evidence suggests that phloem sap concentration is increased to compensate for decreased translocation rates. (Hall and Milburn, 1973). If this is the case reduced rates of assimilate transport should have little effect on photosynthesis i.e. transport depends on photosynthesis rather than the reverse (Wardlaw, 1967). However Hartt (1967) concluded that in sugar cane, moisture stress had a primary effect on transport and transport was not directly dependent upon photosynthetic rates. In the short term, the evidence suggests it is unlikely that an accumulation of photosynthate in the leaves is causing a reduction in photosynthetic rates, in most species (Neales and Incoll, 1968).

There is evidence of non-stomatal inhibition of photosynthesis occurring in plants e.g. Boyer (1971). Hsiao (1973) points out that the effect depends on the species and generally most of the depression in photosynthetic rates in plants suffering moisture stress is due to stomatal closure. Graziani and Livne (1974) found that dessication of epidermis free tobacco leaf tissue (resulting in a 50% reduction in water content) and then recovery, resulted in only a small difference between recovered photosynthetic rates and control tissues. The results suggested that the drastic water deficit in the tissues impaired its capacity to synthesise light dependent intermediaries of photosynthesis, but on rehydration this capacity increased to 80% of normal. Leaves with the epidermis still intact, had zero photosynthetic rates at much smaller water deficits than levels required to reduce photosynthesis in leaves with the epidermis detached.

The literature still suggests, however, that there are some direct effects of water stress on photosynthesis and Todd (1972) and Hsiao (1973) point out that experiments have shown effects of water stress

on such processes as Hill reaction activity; however generally this occurs only at severe stress levels.

Some effect on the enzymes associated with photosynthesis has also been reported (Huffaker et al, 1970). Phosphoenol pyruvic carboxylase (PEP carboxylase) activity was markedly reduced in barley leaves subjected to a short drying cycle, but the major CO_2 fixing enzyme, ribulose 1.5 diphosphate carboxylase (RUDP carboxylase), showed little change in activity. A change in phosphoribulokinase activity was also noticed but was of little significance. The overall conclusion was that these changes would have little effect on potential photosynthetic rates. It was also noticed by these workers, that chlorophyll content of the leaves decreased during the drying cycle. However Hsiao (1973) proposes that generally, there is little effect of moisture stress on leaf chlorophyll content.

The greatest effect of water stress on photosynthesis, during a short term wilting cycle, would appear to be via stomatal closure. There may be effects on the photosynthetic apparatus itself but in the short term the effects of these changes appear to be of minor importance.

(4) PROTEIN SYNTHESIS, ENZYME ACTIVITY AND METABOLITE SYNTHESES

Obviously the synthesis of proteins and continuing enzyme activity are important for continued plant growth and development. The reviews of Crafts (1968), Naylor (1972), Todd (1972), and Hsiao (1973), all conclude that protein synthesis is markedly affected by water stress.

However the effects on enzyme activity vary. The findings of Huffaker et al (1970) have already been discussed. Todd (1972), in a comprehensive review, notes that several enzymes associated with catabolic processes increase in activity in plants i.e. ribonuclease and protease. Summarizing the data available Todd concluded water stress affected enzyme activity in the following ways:

(a) Severe water deficit generally cause an overall decrease in enzyme levels.

(b) Levels of those enzymes involving hydrolysis and degradation usually remain the same or increase slightly until severe dessication occurs.

(c) Levels of some enzymes associated with syntheses increase, others decrease, as a result of water stress.

(d) In some cases a change in isozymic form occurs.

Perhaps the most dramatic example of the effect of water stress on a particular enzyme is shown by changes in nitrate reductase activity. Matthas and Pauli (1965) observed this enzyme decreased in activity early in the drying cycle when little change in leaf relative turgidity occurred. At the same time nitrate accumulated in the plants. Similar results were found by Huffaker et al (1970), Shaked et al (1972), and Plaut (1973). Hsiao (1973) suggests that because this enzyme and phenylalanine ammonium lyase both have short half lives and respond rapidly to water stress, suppressed protein synthesis could account for their decreased activity. Furthermore, this rapid decrease in activity may have a role in a biochemical adaption for survival of plants during short periods of water stress.

Nitrate reduction is not the only aspect of nitrogen metabolism affected by water stress. Gates (1968) discovered protein synthesis decreased with increasing water stress in Lupinus albus. Rapid recovery was noticed on rewatering, suggesting little impairment to the protein synthetic machinery, rather a suspension of activity. Further commenting on this, Naylor (1972) concludes that under mild to moderate stresses, little proteolysis occurs. Many enzymes are turning over rapidly and so continued synthesis is required to enable continued activity.

Naylor (1972) also reports that water stress tends to initiate a rise in soluble nitrogen levels. The evidence suggests that the increase in free amino acids in plants under water stress is due to lack of incorporation into protein. Of the amino acids accumulating, however, proline levels increased to a much greater extent due to de novo synthesis, (Stewart, 1973). The increase in proline levels may be involved in the protection of plants suffering moisture stress (Hsiao 1973).

While looking at possible changes in the metabolism of plants suffering water stress, Stewart (1971) found wilting caused an accumulation of sucrose in excised bean leaves in the dark. This accumulation was accompanied by accelerated starch loss. Some protection of chloroplasts can occur by increased sucrose concentrations due to the binding ability of sucrose for water (Todd, 1972; Santarius, 1973).

As a final comment, the postulation by Todd (1972) that at mild to moderate stress levels, enzymes may not be denatured as such, but their activity may be lost due to decreased availability of substrates or alterations in the organelles in which the enzymes are located e.g. membrane systems such as mitochondria (Miller et al 1971), should be noted. Thus extractable activity as reported by various workers, may be higher than enzyme activity in vivo.

In conclusion, changes in enzyme activity, protein, nitrogen and carbohydrate metabolism would be expected to have profound effects on plant growth during water stress. On recovery, because of suspension rather than impairment of these processes under mild to moderate stresses, growth could be expected to rapidly return to normal.

(5) NUCLEIC ACIDS

Nucleic acids are of major importance in cellular development and any effects of water stress on these compounds would be expected to elicit marked changes in the growth response of plants. However as

Hsiao (1973) comments, the reported effects are open to criticism due to the methods of metermination employed.

Gates and Bonner (1959) reported DNA levels in moisture stressed tomato leaves remained constant over time whereas control levels increased. They suggested this was due to the cessation of chromosomal multiplication. Increases in RNA content were also suppressed, radioisotope experiments suggesting a greater rate of destruction occurred in moisture stressed plants. Data reported by Shah and Loomis (1965) shows actual decreases in RNA and DNA levels in stressed plants.

Shah and Loomis also discovered changes in base composition of RNA in plants subjected to water stress. Uridylic bases increased whilst guanylic basis decreased. They suggested the observed changes in plant growth may be related to changes in RNA and protein metabolism. In fact Crafts (1968) points out the changes in the base ratios were the same as those associated with ageing.

Different RNA forms are affected differently, soluble RNA increasing in response to moisture stress, whilst that in ribosomal and other cell fragments decreased. RNA levels were affected before wilting was observed, as were protein levels (Crafts 1968).

Todd (1972) examined the effects of water stress on nucleic acids further and found that reports suggested the protein synthetic machinery itself was affected by water stress. Drought caused the disappearance of polysomes (4 to 10 ribosomes) and the appearance of free ribosomes and dimers. At the same time relative protein content decreased. Ribosomes isolated from water deficient leaves were observed to have a reduced capacity for amino acid incorporation suggesting their functioning had somehow been imparied.

The results of Shah and Loomis (1965) and the finding of a decrease in arginine containing proteins (nucleoproteins and ribosomal proteins

being rich in arginine) as mentioned by Naylor (1972) suggests there is some decrease in nucleic acid levels. Todd (1972) has noted under mild stress conditions, the effects may be readily reversible as it was noticed that declining RNA levels were accompanied by an accummulation of the intermediaries of nucleic acid metabolism. Thus, under mild to moderate conditions of water stress of short durations little major effect on nucleic acid structure is likely to occur, perhaps more a suspension of synthesis as for proteins.

(6) TRANSPORT

Regarding nutrient transport, the review of the literature by Viets (1972) conveys the impression there is a general decrease in nutrient uptake by plants subjected to water stress. However Hsiao (1973) points out these differences may be due to reduced availability under drier soil conditions or reduced demand because of the slowing of growth within the plant. He suggests uptake itself may be affected but this could be due to a lack of substrates required for uptake.

Looking at the transport of photosynthates and developing a little further on that mentioned previously, it is difficult to determine whether the total amount of assimilates transported in the phloem is reduced directly by water stress, or indirectly by effects on the source of supply and/or the sinks at which they are utilised. Wardlaw (1969) suggests the phloem transport pathway appears able to function efficiently even under stress. Crafts (1968) supported this by pointing out that the phloem is also responsible for providing water to such tissues as meristems, even when a high stress is apparent in the adjacent apoplastic pathway. It would appear, from the literature available, that any effects on the amount of phloem transport in plants suffering water stress, are indirect i.e. due to decreased loading and unloading of assimilates.

Water stress can also influence the pattern of water movement throughout the plant (Kozlowski, 1972) and it has been noticed that older leaves tend to wilt first and their relative water contents decrease first during a stress cycle (Crafts, 1968). It appears some relation between leaf age and possibly solute concentration exists, with younger leaves having a greater capacity to retain their moisture supply than older leaves (Turner, 1974).

It is important to note that there are two paths of water movement within the plant. Crowdy and Tanton (1970) in a unique experiment, demonstrated that most water movement was through a low resistance intercellular pathway, with much less flow through a high resistance intercellular pathway. More recent work by Boyer (1973) supports this concept. He postulates that, under conditions of low evaporative demand, much of the water flow to the point of exit from leaves is through the high resistance pathway. When evaporative demand increases, the proportion moving through the low resistance pathway increases rapidly. These reports tie in with the theory proposed by Weatherley (1970) which suggests that, rather than the bulk of the transpiration flow being determined by cellular water potentials authors such as Knight (1965) have described, the bulk of the water flow in transpiring plants occurs through the low resistance intercellular pathway. His theory suggests, in fact, that the water potential gradients observed in plant tissues are the result of changes in water potential in the low resistence pathway due to transpirational losses, and not the cause. As the transpiration demand on the plant increases and root uptake becomes limiting, water is drawn off from the surrounding tissues into the rapid flow pathway thus causing decreased potentials in these tissues. Obviously greater losses will occur from those tissues with a higher water potential e.g. older leaves because they have a lower solute concentration as Turner (1974) has reported.

(7) DISCUSSION

These preceding sections illustrate the fact that water stress can affect many fundamental processes such as photosynthesis, protein metabolism and nucleic acid levels and types. These effects may explain some of the obvious symptoms in the plant response, such as yield reductions.

Of particular interest recently, has been the finding that plant growth regulators are also affected by water stress and therefore may be playing a regulatory role in some of these processes.

D. WATER STRESS AND PLANT GROWTH REGULATORS

(1) THE GROWTH REGULATORS INVOLVED AND CHANGES IN THEIR ACTIVITY IN RESPONSE TO PLANT WATER STRESS.

(a) INHIBITORS

(i) Introduction

Since the development of techniques for the chromatography of plant extracts, and thus the discovery of inhibitor 'β' (Bennett-Clark and Kefford, 1953), there has been a large amount of research into this inhibitory complex. Much of the earlier work was related to domancy in woody plants and it was in 1965 that Cornforth et al found the properties of dormin, an inhibitory material isolated from the inhibitor 'β' complex, to be identical with that of abscisin II, an abscission accelerant isolated from young cotton bolls (Okhuma et al, 1963).

The presence of abscisin II was demonstrated in many different species and in many cases, changes in abscisin II levels followed those in inhibitor 'β' (Milborrow, 1967). Much of Milborrow's work suggested that the bulk of inhibitor 'β' was in fact abscisin II. Because of confusion over nomenclature it was decided to rename abscisin II as abscisic acid (ABA) (Addicott et al, 1968).

Wright (1969) then discovered that wilting excised wheat leaves caused a marked increase in their inhibitor β content. Further

examination by Wright and Hiron (1969) determined that the principal inhibitor in this zone, which was changing in response to the imposition of wilting, was ABA. Their results showed a 40 x increase in inhibitor levels by the time the leaves had lost 9% of their fresh weight.

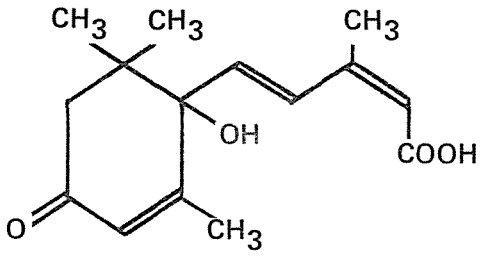
Marked increases in leaf ABA levels were observed when intact plants were subjected to moisture stress (Zeevart, 1971; Most, 1971). Mizrahi et al (1971) also observed that under conditions of salinity stress and low humidity ABA levels in tobacco increased markedly.

Recently it was reported by Wright and Hiron (1972) that inhibitor levels appeared to take some time to decline after rewatering in the species they examined and it has now been determined that the plant water potential has to drop below a certain critical level before any detectable increase in ABA levels occurs (Beardsell and Cohen, 1973, Zabadal, 1974).

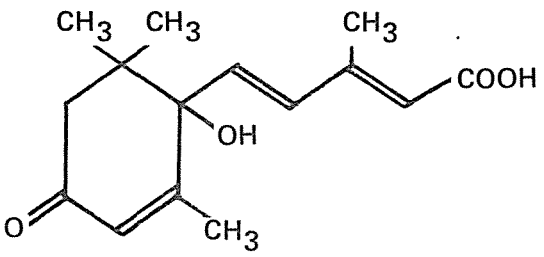
(ii) Chemistry of Abscisic Acid and Related Inhibitors

Some discussion on the chemistry of ABA and related inhibitors is necessary to enable definition of the requirements for activity within the plant. The chemistry of ABA is complicated by the fact it has several types of isomers.

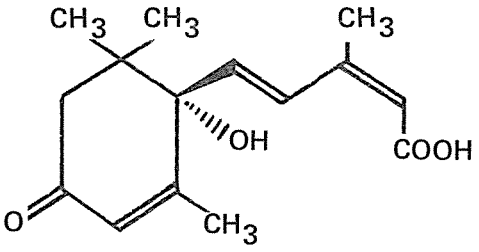
The first synthesis of abscisic acid, at the time named abscisin II, was described by Cornforth et al (1965). The formula assigned to ABA was shown in figures 2a and 2b. In a further paper Cornforth et al (1967) demonstrated that there can be two enantiomers around the centre of asymmetry. Spectropolarimetric determinations showed that the (+) enantiomer was the natural form. In fact the original (+) ABA form illustrated by Cornforth et al (1966) and confirmed (Cornforth et al 1968) was the opposite (-) ABA enantiomer. It had been determined incorrectly from rules proposed by Mills (1952). Ryback (1972) proposed the corrected formula as shown in figure 2c.



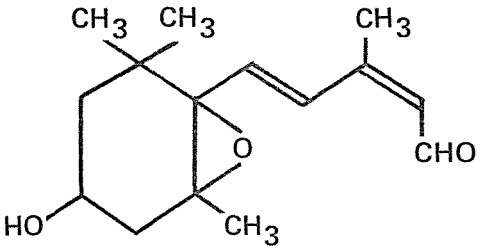
(a) Cis – trans abscisic acid



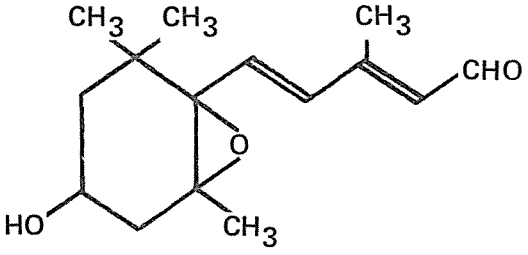
(b) Trans – trans abscisic acid



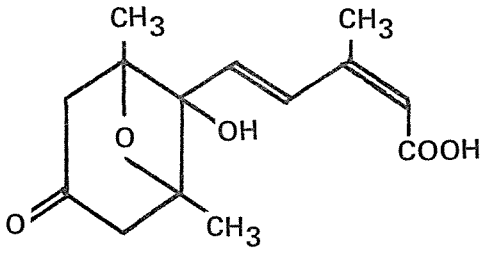
(c) (+) cis – trans abscisic acid



(d) Cis – trans xanthoxin



(e) Trans – trans xanthoxin



(f) Phaseic acid

FIGURE 2 The chemical structure of abscisic acid and related inhibitors

Because of the strong effect of (+) ABA in a spectropolarimeter (Cornforth et al, 1966), quantitative analysis could be made on fairly pure samples.

Addicott et al (1968) point out there are also stereoisomers i.e. R and S forms. The S form is the natural form and corresponds with diagram 2c.

Regarding the activity of the various isomers in plant systems, Addicott and Lyon (1969) and Dorffling (1972) note that the trans -trans form has much less effect in plant assays than the cis-trans form. Milborrow (1970) points out that there is little trans-trans ABA in most plant species and often identification of it in plant extracts is due to breakdown of the cis-trans form in the light.

With respect to the S and R forms (S (+) ABA being the natural form), Sondheimer et al (1971) found there is little difference between them in their effects on most assay systems. However there was a suggestion that the R form may be less active, but they concluded a change from cis-trans to trans-trans ABA was more deactivating than a change from S to R form. A change from S to the R isomer appeared to have a greater effect on metabolic rather than hormonal activity of ABA.

(iii) Biosynthesis and Metabolism of Abscisic Acid

Milborrow (1970, 1972) discussed two theoretical pathways proposed for ABA biosynthesis. The pathways were

(a) direct synthesis and

(b) a system involving carotenoid degradation.

Experiments with wilting plants and labelled mevalonic acid suggested that virtually all the increase in ABA levels was due to biosynthesis rather than release from bound forms. No evidence to support the theory that intermediaries in ABA biosynthesis are derived from carotenoids, was obtained. Milborrow also postulated there may be some feed-back mechanism operating by which ABA, or a close precursor, may inhibit ABA

biosynthesis once it reaches high levels.

Evidence supporting the carotenoid theory was proposed by Taylor and Burden (1970) who discovered xanthoxin (figures 2d and 2e), a photo-oxidation product of a common xanthophyll, violaxanthin, had as much activity in the wheat coleoptile bioassay as ABA. In a later paper, as Dorffling (1972) notes, these workers were able to chemically convert xanthoxin to ABA. Wain (1972) comments that there may be a relation between xanthoxin and ABA production and more recently (Wain, 1973) he infers xanthoxin in the plant can be slowly metabolised to ABA.

However in a recent paper, Milborrow (1974) provides more evidence to support the idea that ABA is newly synthesised by showing that chloroplast preparations from avocado fruit were able to convert mevalonate to ABA. He also notes that ABA may be formed outside the chloroplast as well.

In relation to the metabolism of ABA, Milborrow (1970, 1972) found that trans-trans ABA was converted to abscisyl-B-D-glucopyranoside at a ten times greater rate than (+) cis-trans ABA. The latter form was converted slowly to this glucoside and at the same time produced two metabolites designated 'A' and 'C'. It was also noticed that the unnatural enantiomer (-) ABA was also converted to the glucoside but not metabolite 'C'. It was not determined whether the glucose ester could be neutilized. In the later paper Milborrow determined that metabolite 'C' is converted to phaseic acid (figure 2f) and it is of interest to note that more recently Walton et al (1973) have identified an ABA metabolite as 4 dihydrophaseic acid.

The above proposed metabolites are pertinent to water stress responses when related to several reports in the literature. Zeevart (1971) observed an increase in the glucoside in wilting spinach leaves and Most (1971) found an increase in the phaseic acid level in moisture stressed sugar can leaves. Recently it was reported that phaseic acid itself may affect stomatal opening (Kriedeman and Loveys, 1973) and it has been noted to play a similar role to ABA in the abscission of cotton (Davis et al, 1972).

Bearing in mind the results of Milborrow (1970, 1972), it would be expected that levels of both the glucoside and phaseic acid would rise as ABA levels increase to high levels in response to water stress.

(iv) Distribution of Abscisic Acid

Milborrow (1967) determined the presence of ABA in twigs of several species, broad bean shoots, leaves of various plant species, buds, potato tubers, and yellow lupin pods. Milborrow (1974) also mentions that avocado fruits are a rich source of ABA. That ABA is found in leaves is beyond doubt e.g. Wright and Hiron (1969), Zeevart (1971) and Most (1971), and much of the evidence suggests this is a major site of ABA synthesis e.g. Milborrow (1970), Milborrow and Noddle (1970).

An inhibitor has been found in corn and pea roots but no identification was made (Shaw and Wilkins, 1973). Melhuish (1973) suggests ABA may be produced in plant roots and transported upwards in the xylem. Davison and Young (1973) have also detected ABA in the xylem sap of various plant species.

Regarding ABA transport, Ingersoll and Smith (1971) found it moved in a similar pattern to auxin in excised cotton tissue. However no polarity was observed and the capacity for transport appeared greater for ABA than indoleacetic acid (IAA). In a recent report Hocking et al (1972) demonstrated basipetal movement of ABA towards bean plant root systems. In relation to transport it is of interest to note that Most (1971) detected ABA in the immature meristem of sugar cane. However there is no data relating to the transport and distribution of ABA in water stressed plants.

(v) Discussion

ABA in particular increases rapidly in plants subjected to water stress with many postulated effects. However it is important to note that ABA and related inhibitors are only part of the growth regulating

complex in plants and when discussing plant responses to water stress, the manner in which other hormones are affected should be known.

(b) CYTOKININS

It was first noticed by Itai and Vaadia (1965) that water stress decreased cytokinin levels in the exudate of decapitated plants. Further examination demonstrated a decrease in protein synthesis and cytokinin levels in salinized plants, kinetin treatment being able to partially reverse the depressive effects of the stress on protein synthesis (Itai et al 1968). On recovery from stress cytokinin levels in the exudate of decapitated plants actually increased to greater levels than controls for a short period. Examining changes in the cytokinin like activity in excised leaves themselves, Itai and Vaadia (1971) discovered rapid decreases in activity on wilting. However recovery of activity was obtained by a relatively short period of exposure of leaves to a humid atmosphere. This suggested a chemical transformation of the cytokinin molecule.

More recently Back et al (1972) suggested ABA limits the rate of conversion of kinetin to adenine. The implication of this was that cytokinins had to be metabolized in the plant to enable regulatory action. The depression in the rate of cytokinin turnover in the presence of ABA was suggested to be an explanation for some of the depressive effects of water stress on various metabolic processes within the plant.

(c) AUXINS

Darbyshire (1971a) found water stress increased IAA oxidase activity in etiolated pea seedlings and suggested that this increase may result in decreased auxin levels (Darbyshire, 1971b). But no determination of auxin levels was made. Work by Phillips (1964) on flooded sunflower plants suggested there was a slight increase in auxin levels as the duration of stress increased. Reid and Railton (1973) also noticed

a marked increase in IAA responses in flooded tomatoes but applications of benzyladenine (BA) reversed the effect suggesting there was an imbalance of hormones rather than an increase in IAA levels. Jenkins (1973) could find no consistent evidence of changes in auxin levels in water stressed plants.

As yet no clear indication of how auxin levels respond has emerged. However as Tal and Imber (1971) contend, it would be to the advantage of the plant if auxin levels remained relatively high as they appear to play a protective role during water stress.

(d) GIBBERELLINS

Reid et al (1969) and Reid and Crozier (1971) reported flooding of tomato plants reduced gibberellin levels. More recent papers by Reid and Railton (1973, 1974) suggest decreased gibberellin levels in this situation may be related to decreased cytokinin levels, i.e. as the cytokinin levels decreased so did the endogenous gibberellin levels. If this relationship exists, it would be postulated that gibberellin levels in drought stressed plants may change in a similar manner. However as yet there is no evidence to support this contention.

(e) ETHYLENE

Recent reports have presented evidence indicating water stress induces a marked increase in ethylene levels in cotton leaf petioles (Jordan et al 1972; McMichael et al, 1972). These increases enhanced leaf abscission, depending on their physiological age. Kawase (1972) observed increases in the endogenous ethylene levels of various plant parts subjected to flooding and more recently El-Beltagy and Hall (1974) noted marked increases in endogenous ethylene levels of Vicia faba tissue subjected to drought or flooding stresses.

(f) DISCUSSION

Water stress markedly affects plant hormone levels, in particular ABA and cytokinins. Ethylene levels also appear to be affected. Evidence

relating to changes in auxin and gibberellin levels is scant. The changes in the balance of these growth regulators may be of great importance in relation to the changes in the growth pattern of plants subjected to drought stress.

The following discussions centre upon the association of growth regulators with some of the major processes which are affected in stressed plants.

(2) THE ASSOCIATION OF PLANT GROWTH REGULATORS WITH THE MAJOR PLANT PROCESSES AFFECTED BY WATER STRESS

(a) STOMATA

During the past few years it has been discovered that stomatal functioning can be influenced by various plant hormones.

Some of the first work with ABA determined that, at physiological concentrations, this inhibitor promoted stomatal closure in excised leaves (Mittelheuser and Van Steveninck, 1969; Jones and Mansfield 1970) and prevented stomatal opening in epidermal strips of Vicia faba (Horton 1971). The effect was shown to direct on the guard cells as opening could not be induced by CO_2 free air (Jones and Mansfield 1970; Horton 1971).

Tal et al (1970) and Tal and Imber (1970, 1971, 1972) demonstrated that the wilty nature of the flacca mutant of tomatoes was due to an inability to close their stomata, even when the guard cells were plasmolysed. On analysis of the hormone levels in the mutant and normal plants, ABA levels were found to be much lower in the former, and spraying the mutants with ABA at physiological concentrations enabled closure. These observations suggested a major role for ABA in stomatal closure.

Looking at the effect of ABA on stomatal closure in more detail, Kriedeman et al (1972) determined ABA concentrations need only rise two to three times greater than the normal endogenous levels to induce closure.

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During recovery from wilting stress it has been observed there is a delay in the recovery of stomata and Allaway and Mansfield (1970) found the delay in Rumex sanguineus was consistent with either the accumulation of an inhibitor preventing opening or a lack of substrate promoting opening. Mizrahi et al (1970) suggested this inhibitor may be ABA due to the high level of accumulation occurring in water stressed plants. This idea is further supported by evidence from Cummins et al (1971) and Kriedeman et al (1972) showing that stomatal closure can be reversed by removal of the ABA source, thus suggesting continued closure depended on continued presence of ABA. It was demonstrated by Wright and Hiron (1972) that in whole plants ABA levels did not decrease to control levels until several days after rewatering suggesting its persistence could explain the delay in the recovery of stomata. More recently Beardsell and Cohen (1973) have suggested that, at least in maize, the relationship between ABA levels and closing and opening is not as clear cut as was first imagined.

Possible mechanisms which ABA might affect, in relation to the induction of stomatal closure, were examined by Mansfield and Jones (1971). Experimental results indicated ABA limited potassium uptake by guard cells and acted preferentially on these cells compared to other leaf cells. In addition an increase in guard cell chloroplast starch content was apparent. Both these changes are associated with stomatal closure. Kriedeman et al (1972) also favour a direct effect of ABA on guard cell processes rather than a permeability effect on guard cell turgor, because as Fischer et al (1970) discovered, the water status of the plant returned virtually to normal in a much shorter time interval than that required for full recovery of stomata. Also Cummins (1973) comments that if a

differential turgor effect due to changed permeability was occurring, it would be difficult to explain the reopening of stomata on isolated epidermal strips when the ABA solution was removed, because many of the epidermal cells would be ruptured. However, as Hsiao (1973) points out, the exact relationship between ABA levels and stomatal closure in the intact plant has yet to be fully resolved.

Regarding a possible association of cytokinins with stomatal opening, Livne and Vaadia (1965) discovered kinetin stimulated the transpiration rate of excised barley leaves. However Luke and Freeman (1968) noted this effect was not universal and tended to be associated with monocotyledonous species only. Meidner (1967) determined that the effect was at least partly due to stimulated photosynthetic utilization of CO_2 and thus increased stomatal opening. However, by adjusting the intercellular CO_2 levels, he also observed an opening effect due to kinetin in excess of that explained by reduced internal CO_2 levels. More recently Cooper et al (1972) noted a direct kinetin effect even in the presence of ABA. Previously it was accepted that ABA exerted a non-competitive effect on stomatal apertures (Horton, 1971). Pallas and Box (1970) provided a possible explanation for this direct effect when they observed that kinetin reduced the turgor potential of the surrounding epidermal and mesophyll cells thus enabling the guard cells to exert a greater influence in determining the aperture. These authors suggested that kinetin lowered the concentration of pooled osmotically active compounds in excised leaves, tending to decrease the osmotic potential.

The direct effect of cytokinins appears to be confined to particular plant species, in the main monocots. In other plant species the effect of ABA on stomata is not overcome by cytokinins, and co-application with ABA results in complete dominance of ABA in any effects on stomata.

With respect to the other plant hormones, the role of auxins has

not yet been clarified. Mansfield (1967) found that synthetic auxins reduced photosynthetic activity thus indirectly causing stomatal closure. However Tanas et al (1972) noted IAA stimulated photosynthetic activity in isolated chloroplasts.

Gibberellic acid was observed to stimulate the transpiration of excised leaves (Livne and Vaadia 1965). But the effect appears to be indirect as Horton (1971) has demonstrated that exposure of epidermal strips to a solution containing both ABA and gibberellic acid results in an ABA effect only. Also Treharne and Stoddart (1968) have shown that the effect of gibberellin on photosynthesis in clover is related to increased CO_2 assimilation.

No effects of ethylene on stomatal functioning were observed by Pallaghy and Rashke (1972) or Dozier and Barden (1971), the latter workers finding no effect on apple stomata at concentrations up to 40000ppm.

(b) WATER AND IONIC BALANCES

Apart from affecting plant water status by effects on stomata, growth regulators have also been shown to affect directly, the water balance of plant tissues.

In their work with the flacca mutant of tomatoes, Tal and Imber (1971) observed that kinetin reduced the rate of sap exudation from decapitated plants whilst ABA and to a lesser extend IAA, enhanced exudation. The results suggested these hormones were affecting root resistances directly. Tal and Imber (1971) hypothesised, because cytokinins decrease stomatal resistance and increase root resistance to water flow, a general reduction in turgor results, and recent papers by Glinka and Reinhold (1971) and Collins and Kerrigan (1974) have also described this effect.

Looking at the ABA effect in a little more detail Glinka and Reinhold (1971) observed that the increase in permeability of tissues to water was not accompanied by changes in plant ionic levels, suggesting a

selective effect. This effect was suggested to be a metabolically dependant action of ABA (Glinka and Reinhold 1972) and was shown to be true for intact roots (Glinka 1973). Conflicting with these reports are the results of Cram and Pitman (1972) showing no increase in root permeability to water flow when treated with ABA.

However, overall it appears that cytokinins tend to decrease plant turgor whereas ABA tends to increase plant turgor (Mizrahi and Richmond, 1972). Tal and Imber (1971) suggest that oscillations in plant water status, even in the short term, may be regulated to some extent by growth regulator levels.

Ionic balances can also be affected by growth regulators. It has already been noted that Mansfield and Jones (1971) found ABA restricted potassium uptake into guard cells. Van Steveninck (1972) also observed ABA treatment of beetreet tissues altered the selectivity of these tissues to ion uptake. Sodium ions were taken up in preference to potassium ions, the reverse of the normal situation. Overall uptake of sodium, potassium and chloride ions by these tissues, was stimulated. However Cram and Pitman (1972) discovered that ABA did not inhibit uptake into the roots of intact plants but limited long distance transport of both potassium and chloride ions. This suggested ABA inhibited secretion of these ions into the xylem. In response to water stress in the aerial parts of the plant, it was suggested ABA is translocated to the roots where it exerts some control over the rate of secretion of ions into the xylem. The authors postulate this may be a protective mechanism preventing build up of salts in the leaves and other upper plant tissues, due to decreased demand. At the same time this restriction in ion uptake allows some recovery of turgor by preventing any increase in osmotic potentials.

It appears ABA is effecting more than just a general reduction in ion uptake. Some selectivity is involved and more evidence to support this

is provided by Reed and Bonner (1974) who found ABA decreased potassium uptake and, to a lesser extent, chloride uptake.

(c) PHOTOSYNTHESIS AND TRANSPORT

Hormonal changes can affect photosynthesis both directly and indirectly. Indirect effects on photosynthesis basically relate to changes in stomatal aperture and this has already been examined in a previous section.

Direct effects can be due to association with the development of the photosynthetic apparatus and continued operation thereafter. Gates (1968) has observed that on recovery from water stress, tissues growing rapidly at the time of stress imposition may be permanently retarded. Reduced turgor potential obviously has some role as do many of the previously mentioned processes. However young actively growing leaves have associated high hormonal activity which is related to the development of cells and cell organelles. Feierabend (1970) discovered that auxins and cytokinins had opposing effects on the formation of enzymes associated with the photosynthetic apparatus in rye seedlings, cytokinins being promotory as also reported by Fletcher and McCullagh (1971, 1972). Auxins, on the other hand were inhibitory. Steer (1973) postulates that auxin may inhibit RuDP carboxylase activity in expanding leaves of Capsicum frutescens whereas cytokinins may act in a promotory role. Wellburn et al (1973) discovered gibberellin treatment enhanced RuDP carboxyglase activity in greening leaves whereas ABA had a marked depressive effect.

The evidence suggests that the development of the photosynthetic apparatus is promoted by both cytokinins and gibberellins, whereas ABA and auxins tend to inhibit chloroplast development.

In mature leaves Sweet and Wareing (1966) hinted that auxins may act directly to stimulate the photosynthetic apparatus and experiments by Tanas et al (1972) provide evidence to support this idea.

With respect to cytokinins, Romanko et al (1969) observed that

functioning of chloroplasts was dependent upon the presence of cytokinins, and Wareing et al (1968) suggested that RuDP carboxylase activity in leaves was in fact limited due to competition between leaves for these growth regulators. Although Treharne et al (1970) could not prove that cytokinins enhanced synthesis of RuDP carboxylase, is was suggested they were responsible for maintaining the activity of this enzyme (and increasing it when applied exogenously). However Richmond et al (1971) suggested that the cytokinin effect on chloroplasts may in fact be related to effects on the hydration and permeability of chloroplasts and their membranes, and not directly upon its machinery for protein synthesis.

Gibberellic acid was also seen to stimulate RuDP carboxyglase activity in Trifolium pratense L (Treharne and Stoddart, 1968). But again no proof was obtained to suggest that gibberellins enhanced synthesis of this enzyme (Treharne et al, 1970). Both Itai and Vaadia (1971) with cytokinins, and Whyte and Luckwill (1966) with gibberellins, have noted that these hormones delay senescence in leaves and, in fact stimulate amino acid incorporation into protein. So increased sythesis of RuDP case may be occurring, but not in the system used by Treharne et al (1970).

Regarding transport, it is generally accepted that the distribution of assimilates depends on sink demand and this in turn depends on hormonal balances e.g. Booth (1959), Lovell and Booth (1967), Booth and Lovell (1972), Buttrose and Mullins (1968), Hansen (1971), and Habeshaw (1973). It seems reasonable to expect that changes in the hormonal balance reported for plants suffering water stress, play some role in the observed responses e.g. retarded growth, slowing down of primordial initiation, and flower and fruit abscission. There could also possibly be a direct effect of hormones on the transport system (Patrick and Woolley, 1973, Patrick and Wareing, 1973).

(d) CELL GROWTH AND EXTENSION

Cleland (1967) determined that cell extension was dependant upon turgor pressure greater than a critical level to produce the actual extension, and auxin to induce cell wall loosening for the turgor pressure effect to take place. Cell extension could not take place, even in the presence of auxin induced cell wall loosening, if the turgor potential was below the critical level. This was also observed by Doley and Leyton (1968). These latter workers however also noted the stimulation of cell division in the presence of gibberellin was much less affected by changes in water potential.

Darbyshire (1971b) postulated that increases in IAA oxidase activity in water stressed plants caused reduced auxin activity thus reducing the potential for cell elongation. But Hsiao (1973) comments that IAA oxidase activity appears less sensitive than cell elongation. Also no one as yet has demonstrated any decrease in auxin levels in water stressed plants.

Regarding the effects of ABA, Hsiao (1973) and Dorffling (1972) note that ABA retards internode elongation. Isaia (1971) discovered that ABA strongly inhibited leaf growth, the presence of gibberellin apparently enhancing this effect. It was determined by Pilet (1972) that the inhibitory action of ABA on cell elongation appeared to be due to an antagonistic effect on auxin.

Looking in more detail at the depressive effect of ABA on elongation, Phillipson et al (1973) noticed that ABA inhibited Avena coleoptile growth after a short time lag. Removal of ABA did not result in a full recovery of IAA induced extension rates. ABA did not inhibit IAA uptake into coleoptile segments and so it appeared it was acting essentially as an antagonist from within.

In a more applied situation, Jenkins (1973) observed that small diameter increases in pinetrees in drought years could be related to an

ABA effect on cell enlargement. ABA levels increased in trees under moisture stress whereas auxin levels did not change consistently. The increase in ABA thus resulted in a decrease in cell number and size, whilst auxin levels appeared to remain fairly high because of the increases in tangential wall thickness which were observed.

The effect of ABA on cell elongation is rapid as shown by the results of Newton (1974). He found 2 ppm of ABA had an effect on the root growth of Lemna minor within an hour.

It has already been mentioned that ABA is non-competitively antagonistic to IAA and Reed and Bonner (1974) suggest that reduced cell elongation in the presence of this inhibitor may be due to effects on potassium ion balancing of a possible proton pump involved in cell elongation.

Ethylene also depresses cell division and elongation as Burg et al (1972) point out. In many situations it is antagonistic to auxin effects and so it too may play a role in water stress responses.

(e) ENZYMIC PROCESSES AND NUCLEIC ACID STRUCTURES AND FUNCTIONS

The effects of ABA on DNA and RNA, as reported, have to be interpreted with care (Addicott and Lyon, 1969; Dorffling, 1972). Often there is a marked lag between ABA treatment and observed changes in nucleic acids suggesting that the effects may be indirect.

Regarding anzyme activity Addicott (1970) notes that ABA can induce increases in many degradative enzymes e.g. ribonuclease and cellulase, particularly in relation to abscission. However the role of these enzymes in growth inhibition is not clear because as Bex (1972a) notes, increases in ribonuclease activity did not occur until 8 hours after ABA treatment. Both Bex (1972b) and Dorffling (1972) comment that growth inhibition effects are almost immediate.

It has also been noted ABA inhibits the development of RuDP carboxylase activity in greening leaves (Wellburn et al, 1973).

After a lag period both RNA and DNA appear to be markedly affected by the presence of ABA. Van Overbeek et al (1968) found that ABA treatment produced changes in the RNA and DNA fractions in the opposite manner to the cytokinin, benzyladenine. They postulated that the ABA effect was due to an allosteric enzyme inhibition, DNA polymerase being suggested as the enzyme involved with DNA. Saunders and Poulson (1968) postulated an allosteric effect also, only in their case they suggested invertase. Addicott (1970) concluded that the effect of ABA on RNA is mainly by inhibition or modification of the synthesis of one or more major fractions of RNA, and Pilet (1972) using several tissue systems, noted strong inhibition in RNA increases when ABA was present. Ribonuclease activity increased markedly. However Bex (1972a) noted that ABA inhibited total RNA synthesis after 30 hours. The effects of ABA were different for different RNA fractions, ribosomal RNA being inhibited to a greater extent, but as Bex (1972b) comments, growth is affected much more rapidly than ribonuclease, RNA polymerase, and RNA levels, suggesting that ABA may have more than one mode of action.

In relation to DNA, Mondal and Biswass (1972) discovered ABA at high concentrations, can induce cross-linking of DNA strands thus preventing RNA synthesis by RNA polymerase systems. It was also suggested cell division may be affected because of prevention of strand replication. But their comment that there may be another mechanism involving specific protein acceptors which would give a more precise control in vivo should be noted because, as Van Overbeek et al (1968) point out, materials which act directly on DNA strands are generally regarded as cell poisons.

At this stage no real clarification of the effects of ABA on nucleic acids is available. However the literature suggests that there are

depressive effects on the nucleic acids when the inhibitor is present. But, as Addicott and Lyon (1969) point out, it is difficult to determine whether the effects are direct or indirect. The data from Bex (1972a) suggests that with such a long lag period, the effect must be indirect.

It has already been reported that cytokinins maintain protein levels in leaves e.g. Itai et al (1968).

The literature reviewed suggests that ABA generally inhibits metabolic activity and depresses protein synthesis whereas cytokinins and possibly gibberellins (Whyte and Luckwill,1966) are responsible for maintaining leaf protein levels. Depression of protein synthesis in the leaves of plants subjected to water stress is accompanied by much increased levels of ABA and depressed cytokinin activity in particular.

(f) INTERACTIONS BETWEEN GROWTH REGULATORS

As Van Overbeek et al (1968) state, it is difficult to explain plant responses to a situation in terms of one hormone alone. Changes in relative importance are of greater significance.

Summarizing the literature, Addicott (1970) concludes that ABA appears to be a non-competitive antagonist of auxins and gibberellins. Cytokinin action however, was suggested to be competitively inhibited at lower ABA concentrations, but at higher concentrations non-competitive inhibition occurred.

In relation to growth regulator effects on stomata, Horton (1971) observed ABA was acting non-competitively, when inducing closure of stomata, in the presence of either Kinetin or gibberellic acid. However Cooper et al (1972), working with a species which shows a direct effect of cytokinins on stomatal aperture, found a suggestion of some competitive effect at higher ABA and Kinetin levels. Itai and Vaadia (1971) and Back et al (1972) suggested that ABA was having a direct effect on the kinetin molecule in leaves indicating that a direct

antagonistic effect may be occurring.

Barnes and Light (1969) report ABA was a gibberellin inhibitor in lima beans.

As Addicott (1970) points out, much of the evidence suggests ABA is strongly inhibitory but biochemically non-competitive with the growth promoting hormones in most situations.

E THE EFFECTS OF WATER STRESS ON THE WHOLE PLANT

Water stress effects on the various plant processes previously described result in the expression of typical symptoms by the plant (Treshow, 1970). In a short stress cycle, the effects on the general appearance of the plant are less profound than for a continuous or severe stress, because the plant tends to regain much of its lost growth during the recovery phase (Gates 1968). Gates explains these changes in terms of the plant being thrown into the senescent phase during the development of moisture stress, and a more juvenile phase on recovery.

Regarding the onset of stress, the changes in ABA and cytokin levels in particular, fit with the above idea, as do changes in protein metabolism (Itai et al, 1968), enzyme activity (Todd 1972), the redistribution of dry matter and nutrients (Gates 1955a, 1955b), and the acceleration of leave senescence (Morton and Watson 1948). The increase in ethylene levels associated with leaf senescence observed by Jordan et al (1972) and McMicheal et al (1972) also support this idea.

However the alterations in the plant reflect more than just a change to a senescent phase. These changes also play a role in enabling the plant to survive a period of adverse conditions. Stomatal closure, changes in ion uptake (Cram and Pitman 1972), reduced photosynthesis (Downey 1971), decreased protein synthesis and enzyme activity, in particular nitrate reductase (Todd, 1972; Naylor 1972), all point toward a shutting down of processes associated with growth. In

addition changes in the hormonal balance in plants suffering stress favour the maintenance of as higher turgor as possible under the conditions prevailing (Tal and Imber 1971).

On recovery from short term stress, growth rates are often greater than control rates (see Gates, 1968). Much of the depression in various growth processes, occurring during the induction of stress, is compensated for by this rapid growth on recovery. However rapidly expanding tissues such as young developing leaves and internodes may never fully recover if the stress is severe enough. Partial explanation of the greater growth rate on recovery may be associated with the observed increase in cytokinins to levels greater than controls, in plants relieved from stress. Rapid resumption of protein synthesis (Gates 1968) and utilization of free nitrogenous compounds (Marton and Watson 1948) have also been observed suggesting, as Gates (1968) proposes, that many processes are suspended, at least during mild stresses, and are capable of rapid recovery. However not all processes recover rapidly, stomatal opening being one of the most notable. Meidner and Mansfield (1968) have suggested that this slow recovery may be related to a protective role, i.e. preventing against the loss of newly gained turgor. Wright and Hiron (1972) postulate that ABA may possibly be associated with this delay in stomatal opening and have provided evidence suggesting that ABA may be involved in the adaptation of plants to stress situations (Hiron and Wright 1973).

Looking at another aspect of the whole plant response, Salter and Goode (1967) have reported that many crop species are particularly sensitive to stress at certain developmental stages, in particular, during flowering and the period of rapid fruit swelling. Examining the ontogeny of peas Salter and Drew (1965) observed there was a change in the pattern of root development associated with the physiological state of the plant, root growth at the flowering and pod swelling stages being

virtually non-existent. Prior to flowering root growth was rapid and during the flat pod stage some resurgence in root growth was noticed. This reduction in root growth observed by Salter and Drew appears to be related to the development of reproductive organs. At this stage a strong competitive demand is likely between the sinks created by the reproductive organs and the roots for available assimilates. It has been found by Jeffcoat and Harris (1972) that removal of the apical flower bud on carnation shoots causes a marked increase in the proportion of assimilates moving downward to the roots from a particular source leaf. Ryle and Powell (1972) also noted that a change to the reproductive phase in Lolium temulentum resulted in an increased proportion of assimilates produced in all leaves, being translocated to the apical meristem.

Having discussed the literature relating to water stress and the various processes which may be affected, a basis for experimental work can now be presented.

CHAPTER II

II. RATIONALE FOR EXPERIMENTAL WORK

Major increases in inhibitor β and ABA levels in the leaves of water stressed plants are now well established. It has also been demonstrated in various experiments that ABA can induce stomatal closure (Jones and Mansfield, 1970), or prevent opening of stomata which are already closed (Horton, 1971). An examination of the literature also shows that the ABA levels do not return to control levels immediately upon rewatering, and that there may be some relationship of this to the delay in stomatal opening been after the relief of water stress in plants (Wright and Hiron, 1972).

From the review by Salter and Goode (1967) it has also been deduced that plants are generally more sensitive to water stress at certain stages. The plant responds to water stress in many ways and these responses are likely to influence plant growth and final yield.

With these points in mind, it was decided to see if any effects on pea yield, due to subjecting pea plants to wiltingstress cycles at different stages of development, in particular the reported stress sensitive stages of flowering and pod swelling, were related to changes in the endogenous inhibitor levels. In conjunction with this, it was proposed to examine the changes in stomatal aperture as affected by stress cycles at these different stages, and see what relationship existed with changes in inhibitor levels and stage of growth of the pea plants.

An experiment was designed to compare the levels to which inhibitor β rose in pea plants at the various sensitive and insensitive stages of growth. Inhibitor levels during recovery would also be examined to determine how long it took to decrease, and whether there were any differences between stages. At the same time, measurement of stomatal aperture would be undertaken to determine the changes occurring during the onset of, and recovery from, water stress.

A valid criticism of much of the previous work which Hsiao (1973) makes, is that little indication of the water status of plant tissues during the estinations of inhibitor levels was given. Therefore attention was given to measurement of plant water status during the stress and recovery phases of the cycles.

To check on the reported sensitivity of peas to moisture stress at different physiological stages, several series of plants stressed at these various stages were planned to provide data on various parameters of growth and final yield. Thus the results of the other determinations could be related to some observable responses of the plant.

Much of the work on the relationship between water stress and inhibitors has involved the direct analysis of presumed ABA levels by gas chromatography. It was felt that it was important to study the relationship between stress and the total inhibitor β complex as well as that portion actively attributable to ABA. Therefore inhibitory activity was to be assessed by two different bioassays as well as by using gas chromatography.

CHAPTER III

III MATERIALS AND METHODS

A INTRODUCTION

Peas are a major process crop in New Zealand and show marked responses to water stress at the sensitive flowering and pod swelling stages, in relation to final yield (Salter and Goode, 1967). The main processing variety 'Victory Freezer), a semi-determinant variety, was thus chosen for experimental purposes. The experimental work was divided into two stages; firstly several preliminary experiments were run for observation and initial proceedural work, and finally, an experiment under controlled environmental conditions was carried out to determine the effects of water stress cycles at the preflowering, flowering, flat pod, and pod swelling stages, on various plant processes.

B EXPERIMENTAL DESIGN

1 PRELIMINARY EXPERIMENTS

These were carried out in glasshouses with heating programmed to come on when the temperature dropped below 10°C and ventilation at 20°C. Two preliminary experiments were carried out, the first during the late winter to spring period and the second during summer.

A relatively rapid drying soil substitute medium was required to facilitate the imposition of short stress cycles, and so a North Carolina State University (N.C.S.U.) mix consisting of 70% 3mm graded metal, 15% vermiculite, and 15% peat, was chosen. Two litre black polythene bags were used as containers.

Graded pea seed was surface sterilized with a 5% sodium hypochlorite solution for 15 minutes and then thoroughly rinsed in running tap water for several hours (Morris and Kadir, 1972). The seeds were then germinated in trays of vermiculite in a darkened room at 25°C. On the third day after being placed in the trays the germinated seed were

selected for evenness of radicle length and planted two per container. After several weeks they were finally thinned down to one plant per pot.

Water and nutrient requirements were supplied by using a modified NCSU phytotron nutrient mix (see Appendix I) equivalent to approximately quarter strength Hoagland's solution. This was applied as required.

As the plants grew taller it was found necessary to stake them individually.

The first experiment was used mainly to determine the effectiveness of the medium and nutrient feed, as well as for observational work. Determinations of the time of flower initiation, the various stages of development, and time required to induce wilting were carried out. Some samples were taken for initial trials with the bioassay systems. Plants from the second experiment were used for further bioassay characterisation, testing of materials for stomatal impressions and trials with methods of plant water status measurement.

2 MAIN EXPERIMENT

Two Climate Rooms, kindly provided by the Plant Physiology Division of the New Zealand Department of Scientific and Industrial Research, enabled standardized environmental conditions to be used and maintained.

The room conditions were as follows:

Light intensity	160 watts m^{-2}	
Light duration	12 hours	
Air temperature	17.5°C day 12.5°C night	(Dolan, 1972)
Relative humidty	60% day 80% night	
Air flow	0.3 - 0.5 m sec^{-1}	
CO_2 level	ambient concentration	

The same medium used in the preliminary experiments was chosen and contained in 6" pots. Water and nutrients were supplied using the same

feed as in the previous experiments. Initially two 120 ml feeds were provided each day, but as the plants became larger the frequency was increased. Feeds were applied automatically, one always being applied at 1 p.m. prior to sampling

The seed was germinated as previously described, and planted at the rate of four per pot, after selecting for radicle length. After several weeks they were thinned to two per pot for the preflowering sampling series, and one per pot for later stages.

A total of 396 6" pots could be accommodated on trolleys in the two rooms and allocations for the various determinations were made in the following way.

At each of the four stages (i.e. pre-flowering, flowering, flat pod, and pod swell) a group of plants was subjected to a wilting cycle. Stomatal impressions, water status determinations, and bioassay samples were taken at five different intervals during this cycle, i.e. initially (control), a mid-stress stage (determined approximately from preliminary experiments), maximum stress (when the plants were obviously wilting), one day after rewatering, and four days after rewatering. At this last stage a further group of unstressed plants was analysed as well.

Regarding sampling, this was always begun at 1.30 p.m. (equivalent to midday in the climate room) and completed within an hour. The first mature leaf was selected for bioassay material, and the leaf immediately below for water status determinations. Stomatal impressions were also taken from the first mature leaf. For bioassaying two separate samples of leaf tissue were taken at each sampling time for different assay systems. In addition, at the maximum stress stage in each cycle, a sample of the remaining leaf tissue was taken for gas-liquid chromatography work. During the preflowering stage samples of the apical tissues (apex plus unexpanded leaves) were also taken for bioassaying during the stress

cycle.

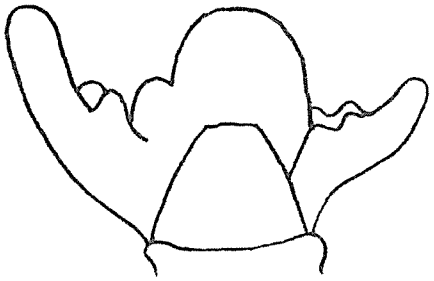
To provide enough material for these determinations, 24 plants were required at each sampling time during the pre-flowering stress cycle, and 12 plants for sampling times at the later developmental stages. 108 plants remained and of these, six series of ten plants were set aside for a final yield analysis. One series was left unstressed, a further four stressed at either the preflowering, flowering, flat pod, or pod swell stage, and the final series was subjected to stress at both the flowering and pod swelling stages. The balance of the plants were set aside to provide reserve material.

To induce water stress in the plants the water supply was simply disconnected. For recovery the plants were thoroughly watered immediately after the maximum stress sampling time in each cycle, and the automatic nutrient feed system reconnected.

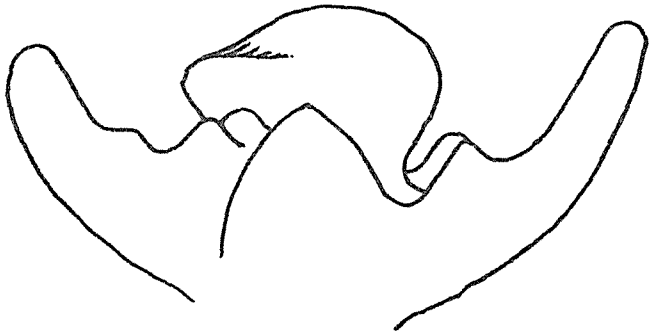
C OBSERVATIONS ON THE STATUS OF THE APICAL MERISTEM

Being a semi-determinant variety, once initiation of flowering has occurred, all nodes formed after this stage would produce flower buds until growth finally ceases (Haupt, 1969). Some indication of when the meristem changed from the vegetative to the floral state was required to determine when to commence the first wilting cycle. In the first preliminary experiment two plants were selected every two to three days from a period starting 19 days after the seed was sown. The apical meristem was examined under a disecting microscope. The observed changes are shown in figures 3a to 3d. The first signs of an apparent change to the reproductive phase occurred after about 25 days from seed sowing. At this stage the pea plant had initiated 15 leaves of which 6 to 7 had expanded. On this basis it was decided to pick a similar stage of development for the preflowering sampling series in the main experiment.

FIGURE 3 Observations on the status of the apical meristem of *Pisum sativum L.*



(a) 19 days after sowing



(b) 25 days after sowing

(c) 34 days after sowing

(d) 49 days after sowing

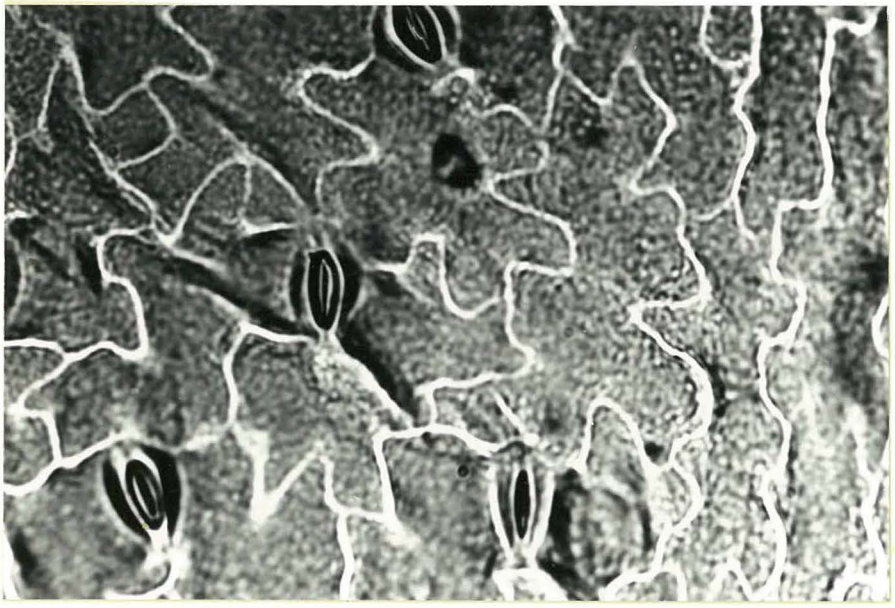
D STOMATAL IMPRESSIONS

It was originally planned to use silicone rubber as described by Sampson (1961), for making stomatal impressions, but at the time it was difficult to obtain. Several recent papers (Waisel et al, 1969; Williams, 1973) reported effective stomatal impressions could be obtained using a plastic cement, the latter author commenting that is appeared nondestructive. On this evidence, an attempt to find a similar material, which was readily available and gave good impressions, was made.

A number of materials were first tested on leaves of Commelina communis. Two brands of aerosol spray-on-plastics were immediately eliminated because of their high solvent content, damage to the leaves being instantaneous. Cellulose-acetate gave variable results with definition being poor at times, but it was apparently non-toxic. Polyvinyl acetate was non-toxic and gave good clear impressions but the drying time was too slow, being in the order of 30 minutes or more. Selley's 'Plastic Glue' and 'Instant Grip' both gave good results with a short drying time of about 3 to 10 minutes, the latter being the slower drying material. Both were non-toxic.

Using those materials which had proven non-toxic in the above tests it was found that Selley's 'Plastic Glue' gave very good epidermal impressions of pea leaves. It appeared non-toxic and repeated applications to the same area of the leaf appeared to have no deleterious effects (see Table I). The plants were well watered and placed in the sun during the day, stomatal imprints being taken several times each day over two days.

The natural aperture, as measured in epidermal strips from leaves themselves under similar conditions as above, was $4.5 \pm 0.2\mu$. This in combination with the above data, suggested that the plastic glue was having little effect on stomatal functioning, even when applied a number of times to the same area on the leaf. In addition, little stretching of the impression appeared to occur when it was removed, so long as care was taken.



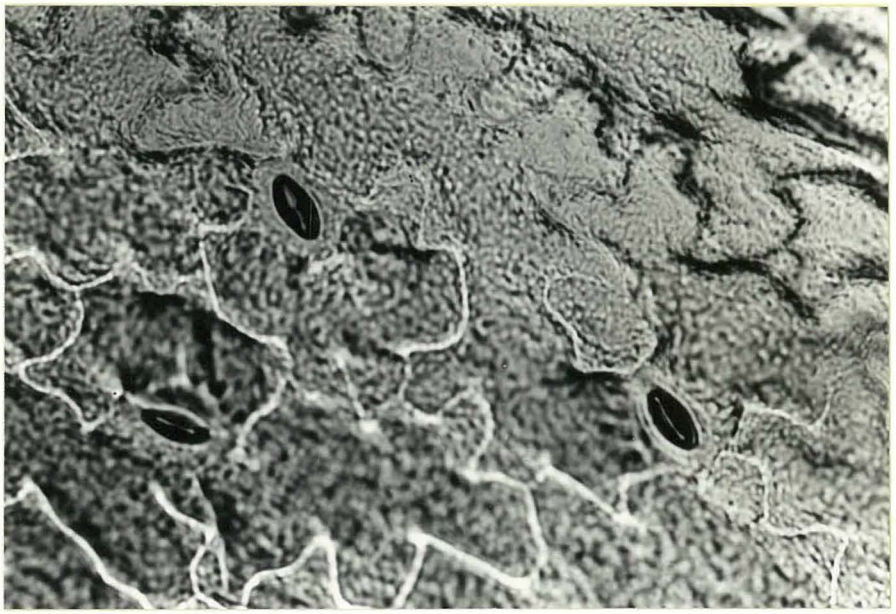


Figure 4. Stomatal impressions of the abaxial epidermis of Pisum sativum made with plastic glue. Upper photograph shows open stomata, the lower closed.

If a thin film of glue was applied it dried in 3 to 5 minutes and could be removed by gently lifting with a razor blade. The impression could then be placed on a glass microscope slide and examined at leisure. All aperture measurements were taken under a light microscope at 1000 x magnification. Photographs of these plastic inpressions can be seen in figures 4a and b.

These above findings suggested this material could be used quite safely for obtaining an estimate of stomatal aperture.

E MEASUREMENTS OF PLANT WATER STATUS

1 LEAF WATER DEFICIT

To gain some indication of the degree of water stress the plants were suffering, and a basis for correction of bioassay results, some measurement of leaf water content was required. Originally it was thought that the relative turgidity technique as described by Weatherley (1950), could be used. However on studying the comments of Barrs and Weatherley (1962), Slatyer and Barrs (1965) and Sponer (1972), it was decided that, with soft tissue such as pea leaves, the injection errors were likely to be high. Therefore the method devised by Stocker (detailed by Kramer and Brix, 1965; Slatyer, 1967), for determining leaf water deficit, was chosen.

The water deficit was calculated using the following formula.

$$W.S.D. = \frac{Ws - Wf}{Ws - Wd} \times 100$$

W.S.D. is the water saturation deficit, Ws is the saturated weight of the leaf, Wf is the original fresh weight of the leaf, and Wd the leaf oven dry weight. This technique can also be used successfully for leaf parts only e.g. Kassam (1972) with Vicia faba leaflets.

The first problem was to determine the time required for full saturation of the leaf tissue. Harvey (1970) suggested it took one hour for pea leaves. He used whole pea leaves, lightly crushing the

TABLE I. The effect of repeated applications of Selley's 'Plastic Glue' on the stomatal aperture in the same leaf on two different plants.

Number of appl's of plastic glue		Stomatal apertures in the same area of application (μ)	Stomatal apertures in the same leaflet but in a new area (μ)
Day I			
1	Leaf 1	4.0 (±0.2)*	-
	Leaf 2	4.7 (±0.3)	-
2	Leaf 1	4.3 (±0.2)	4.2 (±0.2)
	Leaf 2	3.8 (±0.2)	4.1 (±0.3)
3	Leaf 1	3.8 (±0.3)	3.5 (±0.2)
	Leaf 2	4.2 (±0.3)	4.5 (±0.2)
Day II			
4	Leaf 1	4.0 (±0.4)	4.2 (±0.3)
	Leaf 2	3.7 (±0.2)	3.9 (±0.3)
5	Leaf 1	3.9 (±0.2)	3.7 (±0.2)
	Leaf 2	3.7 (±0.2)	3.8 (±0.1)

*Figure presented is the mean of 10 apertures
Standard errors are shown in brackets.

petiole before placing it in water in a sealed container. In initial testing similar methods were used. However it was often observed that larger leaves, or slightly immature leaves, produced no distinct change in the uptake curve over time, rather a gradual change. Catsky (1965) commented that immature tissues take up water for a much longer period without giving a distinct two phase curve. It was also thought, because some of the larger leaves carried up to six leaflets, this may partly explain why poor curves for water uptake over time were obtained. Therefore the procedure was modified.

Leaves were separated into the proximal two leaflets plus petiole and the distal leaflets plus petiole. Comparisons of the water uptake curves were made for the two different sections and little difference was observed (see Table II).

The procedure for the main experiment was designed as follows. The distal two leaflets plus associated petiole and tendrils were excised from the leaf chosen for water deficit determination. This tissue was immediately weighed (Wf) and placed, with the petiole immersed in about 1 cm. of water, in a glass jar which was tightly sealed. After five hours (the time course curve in figure 5 shows this is the time at which the leaf water deficit appears to be saturated) the leaf tissue was removed from the jar and thoroughly dried, the petiole being split longitudinally with a razor blade to enable the inside of the petiole to be dried also. It was then quickly reweighed (Ws) and placed in a hot air oven for 24 hours at 80°C and reweighed to give the oven dry weight (Wd).

One problem associated with the main experiment was that water deficit determinations would have to be carried out on the leaf immediately below the one actually taken for bioassay samples, because of the limitation on the number of plants which could be accomodated in the two Climate Rooms. However, although differences in the level of the water deficit

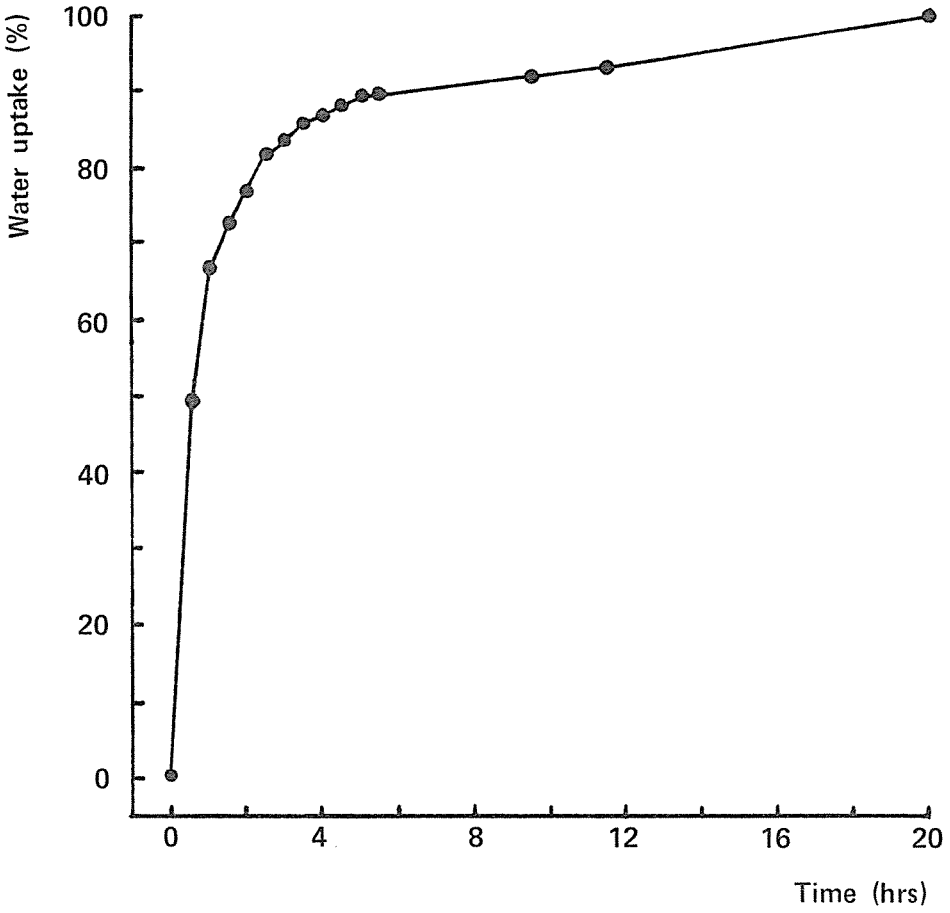


FIGURE 5 Stocker's determination. Time course for water uptake by mature pea leaves. Each point the mean of 4 determinations.

between the first mature leaf and the one below it may be of significance when the plant has only a few leaves (as at the preflowering stage) the data in Table III show that there is apparently little difference between these leaves at later stages of development.

Thus it was considered this technique could be usefully employed to give some indication of the level of water stress the plant was suffering.

2. WATER POTENTIAL

Scholander et al (1964, 1965) commented that if there is negative hydrostatic pressure in plants, and if a piece is excised, the sap would recede from the cut until it reached a cross wall in the vascular system. Therefore, they theorized, the hydrostatic pressure in the system could be determined by increasing the ambient pressure to a level which just allowed the vascular system to yield liquid. They achieved this by placing excised twigs in a pressure chamber with the cut end protruding through an aperture in the top. Gas pressure was increased inside the chamber until the first signs of exudation occurred, indicating the hydrostatic pressure within the plant tissues. This early work was confined to the use of twigs from trees as was that of Waring and Clearly (1967). However, more recently reports have demonstrated that the pressure bomb can be used on softer leafy tissues e.g. Boyer and Ghorashy (1971) with soybeans, and Kassam (1972) with Vicia faba.

Kassam notes that water potential depends on a number of different parameters including solute concentrations, matric potentials, and tissue characteristics, so its relation to such measurements as relative water content is likely to vary with different plant tissues. It was decided to include measurements of water potential using the pressure bomb, as an additional indicator of plant water status.

Initially, for the preflowering stage, readings were taken using a cap on the bomb with a small circular hole (3mm) through which the petiole

TABLE II The use of different pea leaf sections to determine leaf water deficit by Stocker's method

Water deficit in whole leaves	Water deficit in proximal sections	Water deficit in distal sections
12.5% (± 0.6)*	12.5% (± 0.7)	12.2% (± 0.9)

*Each value the mean of the value for the first mature leaf from four different plants. Standard errors in brackets.

TABLE III The effect of water stress on the development of water deficits in the first mature pea leaf and the leaf immediately below it, as determined using Stocker's method.

Days without water supply	Water deficit in first mature leaf	Water deficit in leaf immediately below
0	10.5% (± 0.6)*	10.1% (± 0.9)
2	23.2% (± 2.2)	23.1% (± 2.2)
4	31.0% (± 3.0)	33.1% (± 3.1)

*Each value the mean of five leaves from five different plants. Standard errors in brackets.

of an excised leaf was inserted. A steel insert was positioned in the hollow petiole to prevent it collasping. However as the plants grew larger the petiole diameter became too large for this cap and so the petiole was slit with a razor blade and a section of petiole plus a basal leaflet was inserted through a cap with a slit (as used for determinations of water potential of maize and wheat leaves). The results from leaf sections appeared comparable with those of whole leaves.

F EXTRACTION AND PURIFICATION PROCEDURE FOR AUXINS AND INHIBITORS

10 gram samples of the first mature leaf (plus adjacent leaves if necessary), or the apex plus unexpanded leaves in the case of apical samples, were macerated in a blender with 80% methanol for 5 minutes at 0°C. The resulting suspension was transferred to a 500 ml conical flask and together with several washings, used a total of 100 ml of 80% methanol to give a 10:1 $^{V}/_{W}$ ratio of extracting solution to plant material. This was placed under refrigeration at 1°C for 18 hours and the flask was agitated several times during this initial extracting process. At the end of this period the suspension was filtered through a Buchner funnel and the residue resuspended twice in 50 ml of 80% methanol for four hour periods, giving a final total volume of extractant of 200 ml.

To purify the extract it was first reduced to the water phase on a rotary evaporator using a 500 ml spherical flask. This process was carried out under vacuum with dry ice traps to speed the process, and recover the methanol. The flask was rotating in a water bath at 25°C and shielded from direct light. The water phase remaining in the rotary evaporating flask (about 25 to 30 ml) was then transferred to a 250 ml centrifuge bottle, several aliquots of distilled water being used to thoroughly rinse the flask, giving a final volume of 50 ml. This was left overnight under refrigeration at about 1°C and then centrifuged at 1000 r.p.m. for 30 minutes at 0°C. The supernatant was

carefully poured off into a beaker and the pH adjusted to 2.5 ml with 50% HCl. It was then placed in a 250ml separating funnel and shaken vigorously for 5 minutes with three separate equal volumes of diethyl ether. The bulked ether fractions were shaken for 5 minutes with 15 ml of distilled water (at pH 2.5) to remove any remaining cytokinins (Park, pers. comm.) The aqueous phase from this backwash was combined with that from the initial partitions and dried down under vacuum at 35°C. this was stored for possible future use.

The ether fraction was then extracted twice with 50 ml aliquots of 5% sodium bicarbonate (pH 8.5) using a 500 ml separating funnel, the ether phase retained being the neutral and basic ether fraction.

It should be noted that with the partitions up to this stage, it was often difficult to separate the two phases. A number of different methods were used to try and speed up the separation but, in the end, centrifugation seemed the only answer. Where this difficulty arose the particular partition was transferred to centrifuge bottles and centrifuged for 5 minutes at 10,000 r.p.m. This overcame the problem to a large extent.

The bulked aqueous phase (100 ml) remaining after the bicarbonate/ether partitions, was acidified to pH 2.5 using 50% HC_1 and extracted three times with equal volumes of diethyl ether. These ether extracts were combined and retained as the acidic ether fraction.

The above procedure is essentially that described by Yadava and Dayton (1972). A summary of the extraction procedure is shown in figure 6.

The two ether extracts were dried over anhydrous sodium sulphate for several hours, then filtered and taken to dryness on a rotary evaporator. The residue was dissolved in several mls. of methanol and transferred to a vial for a final drying down under vacuum. Once dry

FIGURE 6 Summary of extraction procedure for auxins and inhibitors

the vials were capped and stored in a deep freeze until required.

For bioassay purposes further purification was obtained by use of paper chromatography. The residues of the neutral or acidic ether fraction were taken up in 0.5 ml of 50% acetone/50% methanol. A further 0.25 ml was used to rinse the vial and this was also taken up in the same pipette. This was then applied to give a 15 cm streak across a sheet of prewashed Whatman No 1 chromatography paper. (Pre washing was carried out using the same solvent as that used during running). Marker spots of ABA and IAA were applied as required. The paper was developed in a descending manner using 10:1:1 v/v isopropanol, 880 ammonia, and water (Wright and Hiron, 1969). This process was carried out with the tank in the dark, and the solvent allowed to run about 30 cm. At this stage the chromatograms were carefully removed and the solvent front marked immediately. After drying in the air for about one hour they were dried under vacuum (in the dark overnight). The papers were cut into strips, the number depending on the procedure for which the samples were to be used.

G BIOASSAY PROCEDURES

1 TRITICUM COLEOPTILE BIOASSAY

The procedure followed was essentially that described by Nitsch and Nitsch (1956).

Seed of the wheat variety 'Aotea' was soaked in tap water for two hours and then placed on moist filter paper in plastic trays. Glass covers were placed over the trays and the wheat germinated in the dark at 25°C. On the third day after planting the coleoptiles were 2 to 3 cm. long and ready for use. 10 mm sections 3 mm behind the tip, were cut from the coleoptiles, with the device shown in figure 7, under a green safe light. The coleoptiles were placed in distilled water until used several hours later.

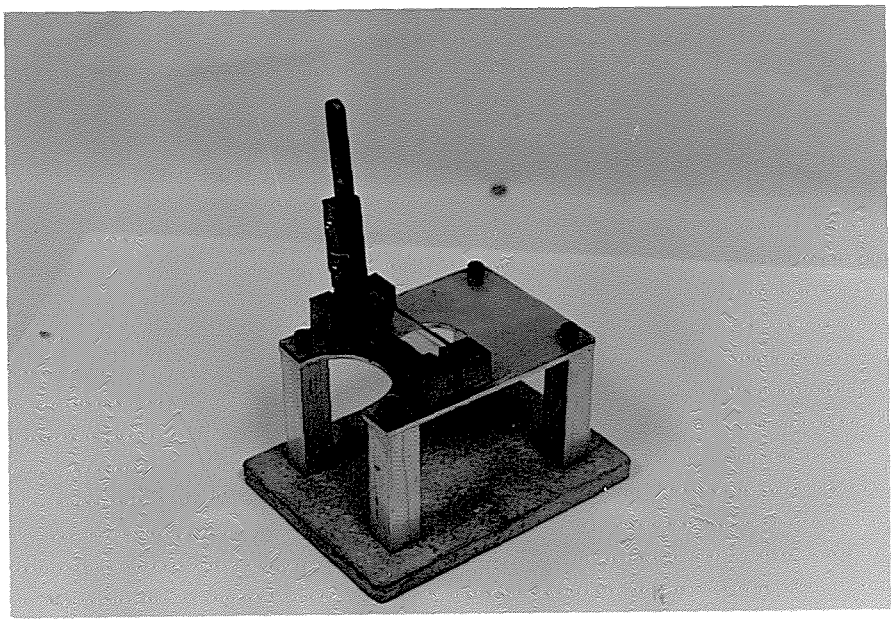


Figure 7. Apparatus for coleoptile cutting.

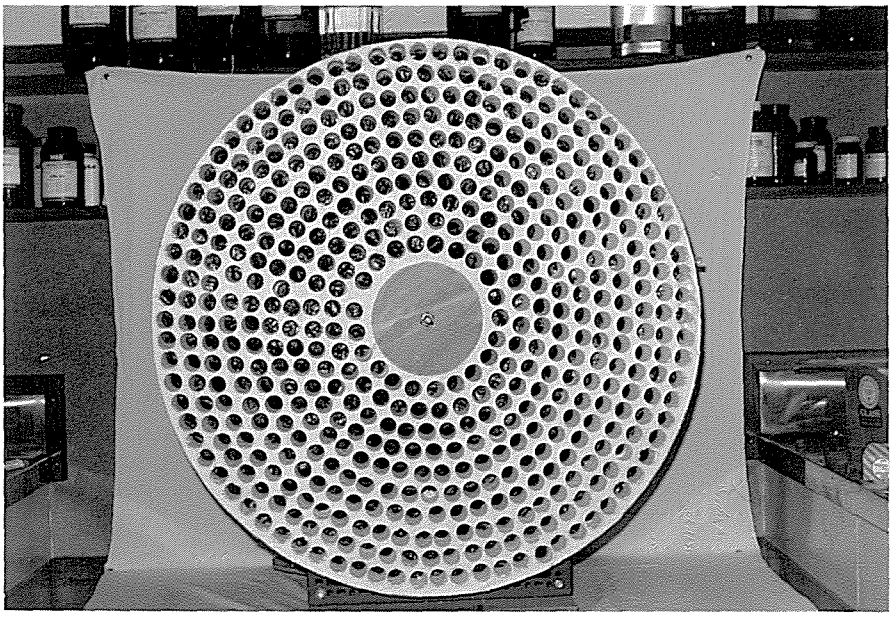


Figure 8. Rotating wheel for coleoptile bioassays

The chromatograms were cut into 20 strips plus a control strip (from behind the base line), in the case of acidic ether chromatograms, or 10 strips plus a control strip for basic ether chromatograms. Each strip was lightly rolled and placed inside a 40 x 25 mm glass vial so the paper was in contact with the wall. 2 ml of phosphate-citrate buffer (see Appendix I) were added to each vial. Twelve coleoptiles were introduced carefully into each vial under the green safelight and each vial closed with a plastic cap with a needle hole in the centre. The vials were then placed in a turntable (figure 8), which rotated at approximately 1 r.p.m., for 20 hours. The vials were then removed and 1 ml of 10% methanol added to each to kill the coleoptiles. The coleoptiles were then measured on a photographic enlarger at 3 x magnification.

As well as the above, a standard series of ABA was included with each bioassay run. The ABA was made up in the concentration range of 20 µg/ml. down to 0.002 µg/mlin a log dilution series. 1 mL of standard plus 1 ml. of double strength buffer were added to each vial giving a final concentration range of 10 µg/ml. to .001 µg/ml. Two replicates of each concentration were always used and a strip of washed chromatograph paper was also included during sample bioassay runs to allow for any effects of the paper on the bioassay. A typical standard curve is shown in figure 9.

2. COMMELINA COMMUNIS BIOASSAY

Tucker and Mansfield (1971) reported a sensitive bioassay for ABA using epidermal strips of Commelina communis, and it was attempted to run this bioassay along similar lines.

Commelina communis seed was germinated at 25°C and then planted into pots in a glasshouse with a temperature range of 15°C minimum to about 20°C maximum. For bioassay purposes, the abaxial epidermis was removed from the youngest mature leaf (Willmer and Mansfield, 1969) from plants

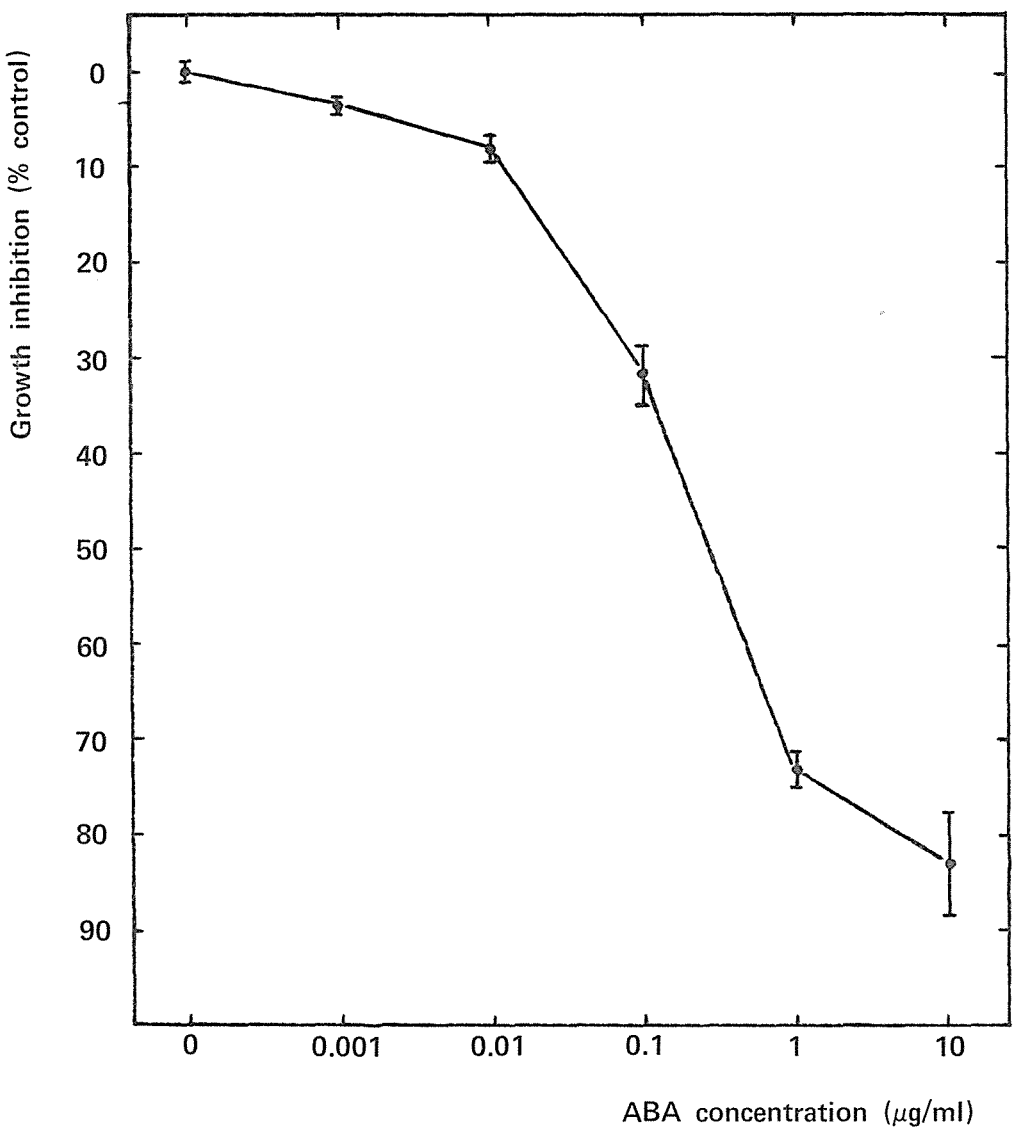


FIGURE 9 Standard curve for wheat coleoptile response to abscisic acid. Standard errors shown.

which had been kept in the dark before use. This would ensure that the stomata were closed.

For standard determinations a log dilution series of ABA was made as described i.e. 20 µg/ml. to 0.002 µg/ml. 2 ml. of double strength phosphate buffer (see Appendix I) at pH 6.9 were placed into a 25 ml. rotary evaporating flask containing 2 ml. of standard, giving a final total volume of 4 ml. Four sections of epidermis about 2 mm. square were floated on the solution in each flask and the flask connected to the apparatus as shown in figure 10. The flasks were immersed in a glass water bath at 25°C and CO_2 free air bubbled through the solution within the flask. CO_2 had been removed by passing the air through a concentrated sodium hydroxide solution and lighting was provided by two 40 watt warm white fluorescent tubes situated beneath the water bath, and a 75 watt incandescent bulb above. The bioassay was run for three hours and then 15 to 20 stomatal apertures were measured on each of three strips from each flask, under a light microscope of 1000 x magnification. The standard curve is shown in figure 11.

Regarding plant extracts, the acidic fraction from the partitioning series was chromatographed as described previously but only run about 15 cm. Rf values 5 to 7 (determined by an ABA marker spot) were cut from the chromatogram and eluted with 3 ml. of methanol for one hour. This was poured off and another 3 ml. added and allowed to elute for a further 12 hours on the rotating wheel described previously. This was added to the initial 3 ml. as was a further 3 ml. used as a final rinse. The bulked eluate was dried down under vacuum. The residue was taken up in 2 ml. of .880 ammonia and reduced to about 1 ml. on the rotary evaporator under vacuum (to remove the ammonia). The amount remaining was taken up in a pipette and the difference between this and a total of 2 ml. was used to rinse the flask before transferring the lot to an incubating flask.

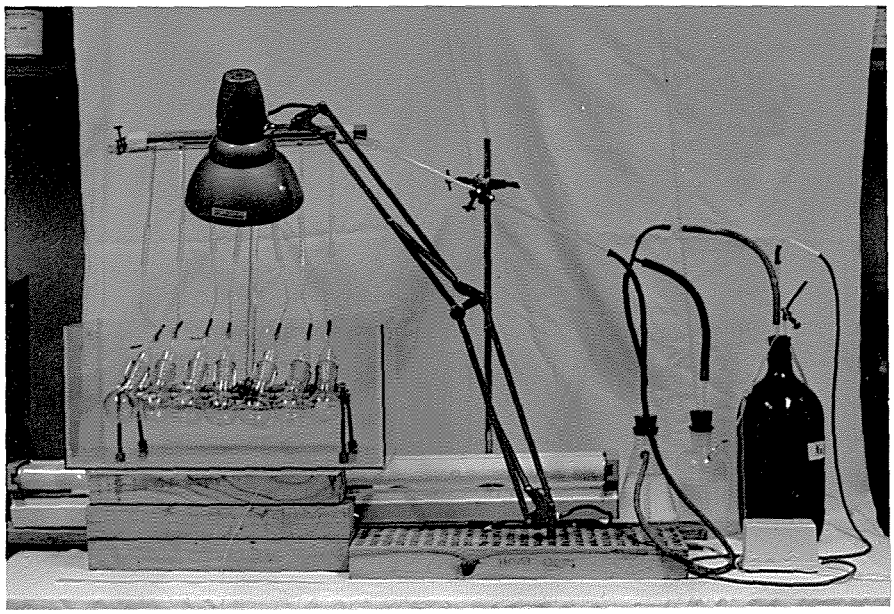


Figure 10. Apparatus used for Commelina communis epidermal strip bioassay. Shown from right to left are the air pump, CO_2 scrubber, pressure head regulator, washing bottle, and combined manifold and incubating flask holder immersed in a glass water bath. Above the bath is the incandescent light source and below, the fluorescent tubes.

2 ml. of double strength buffer were then added and procedure as described for the ABA standards was carried out.

Each run included a control, three different standard concentrations, and three samples.

3 AVENA COLEOPTILE BIOASSAY

At first, attempts were made to obtain a standard curve for responses of 'Aotea' wheat coleoptiles to log concentrations of IAA in the range of 10 µg/ml. to 0.001 µg/ml., using the method described by Nitsch and Nitsch (1956), but poor responses were obtained time after time, even with different sources of IAA. However, using the technique from a recent paper by Burström (1973) good responses were obtained. Initial experimentation suggested that the oat coleoptile was more sensitive than either the wheat coleoptile which Burström used, or the oat mesocotyl (Tustin, pers. comm.).

The procedure was carried out as follows:

Seeds of the oat cultivar 'Brighton' were soaked for 15 hours in 0.1% hydrogen peroxide. They were then washed and spread evenly on moist filter paper in a plastic tray. A glass cover was placed over the tray which was then placed under red light for 24 hours. The tray was then placed in the dark at 25°C for a further two days until the coleoptiles were 2 to 3 cm. long and ready for use. 5 mm. sections were cut 3 mm. behind the tip using the same apparatus as shown in figure 7. The coleoptile sections were placed in Burström's buffer (see Appendix I) for one hour before use. From the chromatograms run for the Commelina bioassay the Rf values 2 to 4 (as determined by IAA marker spots) were individually cut from the paper and placed into 40 x 17 mm. glass vials as described for the wheat coleoptile bioassay. A control strip was also taken in each case. 1 ml. of Burström's buffer was then added to each vial. Ten coleoptile sections were placed in each vial which was

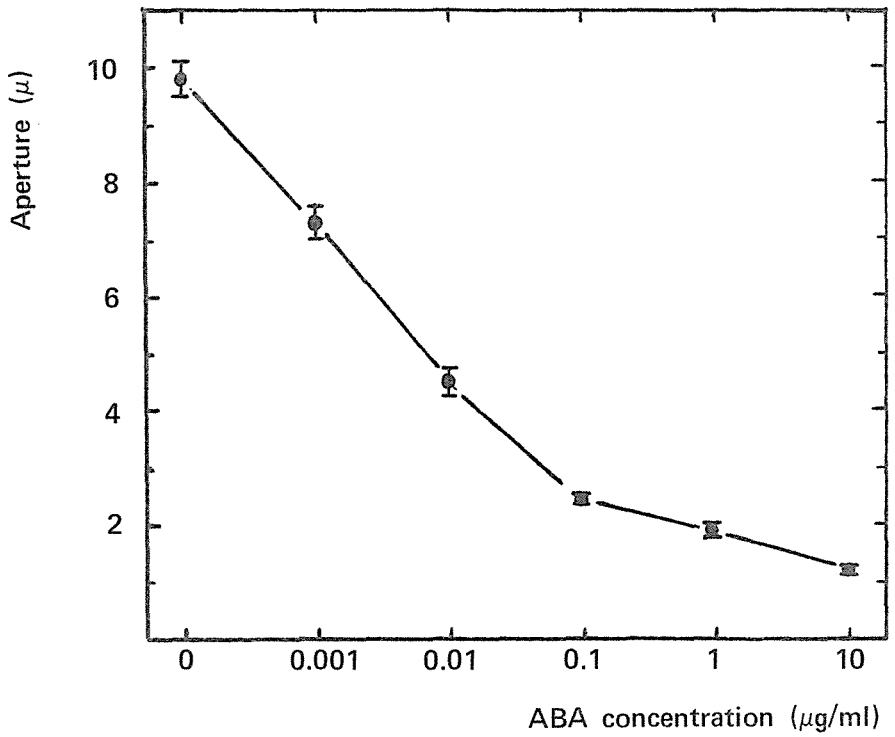


FIGURE 11 Standard curve illustrating the response of stomata, in epidermal strips of *Commelina communis,* to abscisic acid. Standard errors shown.

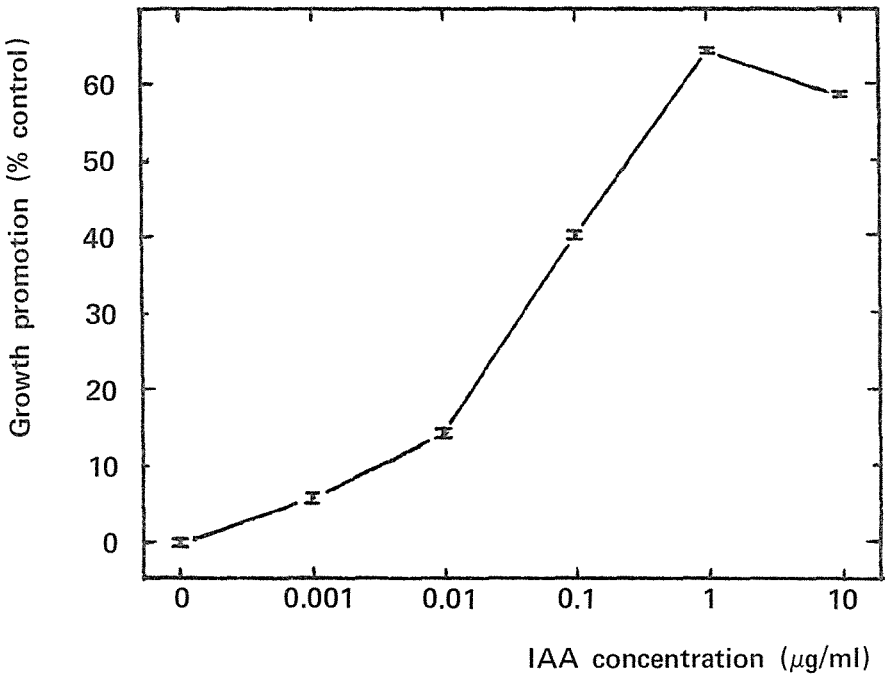


FIGURE 12 Standard curve for *Avena* coleoptile response to IAA. Standard errors shown.

then capped. The vials were loaded on to the turntable as shown in figure 8 and run at 1 r.p.m. for six hours. At the end of this time the vials were removed and 1 ml. of 10% methanol added to each to kill the coleoptiles. Measurements were carried out under the enlarger as described previously.

Standards were made in a similar manner as for ABA i.e. both buffer and IAA standards were made double strength and 0.5 ml. of each were added to vials to give a final concentration range of 10 µg/ml. down to 0.001 µg/ml. A strip of washed chromatogram paper was included in each vial to standardise the procedure. For each bioassay run two replicates of each standard concentration were included. A standard curve is shown in figure 12.

H. PROCEDURE FOR GAS-LIQUID CHROMATOGRAPHY (G.L.C.)

Simpson and Saunders (1972) examined the ABA levels in extracts from pea plants using G.L.C. For purification of their samples, they adopted a procedure devised by Lenton et al (1971). This same procedure was used for the purification of samples in these experiments.

The first step required the use of a column of Polyclar A.T., an insoluble form of the polymer polyvinylpyrrolidone (PVP). The PVP was prepared in the manner described by Glenn et al (1972). The dry powder, from which the larger particles had been graded out, was suspended in five times its own volume of distilled water and left to settle for 15 minutes, the supernatant being decanted at this stage. This was repeated six times resulting in an even particle size of about 60 to 120 mesh.

To pack the column the PVP was suspended in an equal volume of distilled water and poured into a glass column of an internal diameter of 1.5 cm. and with a sintered glass disc in the base. The excess water was run off the column and the suspension added again several times until the final length of the packed column was 10 cm. A pad of glass

wool was placed on top of the column which was then washed thoroughly with 0.1M phosphate buffer (see Appendix I) at pH 8. The flow rate of a packed column was in excess of 100 ml. an hour.

Characterisation of the column was done in the following manner. Approximately 1 mg. of ABA was dissolved in 2 ml. of .880 ammonia and the ammonia taken off, under vacuum, on a rotary evaporator. The aqueous residue was introduced on to the top of the column using a Pasteur pipette, and the buffer flow commenced. The flow of buffer into the column was automatically regulated by the flow out of the basal end. 5 ml. fractions were collected, some idea of where the ABA would be located being obtained from the data of Glenn et al (1972).

The fractions collected were then analysed on a Unicam S.P. 800 Spectrophotometer to determine where the ABA was located. On fractions from two different columns, the first detection of ABA was in fraction 5, the bulk was in 6 and 7, and lesser amounts were detected in 8 and 9. There was no detection in fraction 10.

These columns, once characterised, were used for purifying the acidic ether extracts obtained from partitioning. The residue was dissolved and introduced to the column as described for ABA above. Once the column was running, fractions 5 to 9 were collected and bulked. This was partitioned three times with equal volumes of diethyl ether. The combined ether partitions were then taken down almost to dryness on a rotary evaporator. The remainder was transferred (together with several washings with ether) to a small pointed vial and dried under vacuum. The residue was taken up in 250 µl of 50% acetone/50% methanol and spotted on to a thin layer plate. (This plate was coated with Silica gel GF 254 and had earlier been pre-run in 98:2 v/v ethanol and acetic acid. It was then heat activated for 30 minutes at 100°C). A marker spot of ABA was included and the loaded platedeveloped in a 50:5:2 v/v mixture of

benzene, ethyl acetate and acetic acid. To achieve adequate separation from the base line it was necessary to run the plate three times in the same solvent system. The zone corresponding with the ABA marker spot was scraped off the plate after the final run, before the solvent had evaporated completely from the plates. Simpson and Saunders (1972) reported that this produced a better recovery of ABA.

The scrapings were placed in a centrifuge tube and eluted with 2 ml. of methanol for two hours. After centrifuging the eluate was removed (using a pipette) and transferred to a small pointed vial. The silica was then resuspended in a further 2 ml. of methanol and again centrifuged, the supernatant being transferred to the same vial as above. The eluate was then dried down under vacuum.

Methylation was achieved by taking up the purified extract (or ABA for standard purposes) in 100 µl. of methanol and adding 1 ml. of diazomethane. These solvents were then evaporated off under a jet of nitrogen. The residues were taken up again in a small quantity of methanol in preparation for injection.

The G.L.C. work was carried out in a Pye Series 104 Gas Liquid Chromatograph. The column used contained 3% OV-I (stationary phase) coated Gas Chron Q, 80-100 mesh (support phase). (Park, pers. comm.). The following operating procedure was used.

The column was first heated to 160°C and the sample injected. After 4 minutes the temperature was increased to 200°C for $1\frac{1}{2}$ minutes and thereafter was set to automatically increase by 3°C per minute. The carrier gas flow (nitrogen) was 50 ml./minute. The machine was callibrated using ABA standards. The attenuation used was 20×10^2 or 10×10^2 depending on the quantity injected.

For plant extracts a known quantity was injected into the column. To determine whether a particular peak corresponding with the ABA

standard peak, was in the same position, coinjection with a known quantity of ABA was carried out. No attempt was made to accurately quantify the amount of ABA present in the plant extract.

I FINAL YIELD ANALYSIS

As a check on the response of peas to water stress at different physiological states and, also as a basis for the discussion of the results obtained by the preceding analyses, it was necessary to carry out a final yield analysis. The plants were analysed, two weeks after the final stress cycle, when virtually all the pods were maturing. The following procedures were carried out for each plant in each series.

Leaf area was measured using a Hayashi Denko WAM-5 Leaf Area Meter. The number of tillers, plant height, and fresh and dry weights of leaves, pods, peas, and stems were also determined. The numbers of peas and pods were also counted. Other records made included the number of leaves which were alive or in an advanced stage of senescence, the division being arbitrary.

These measurements were to give some indication of the effects of water stress on the yield of the plant and the yield of various plant parts, and also some indication of the effect of water stress on the physiology of the plant.

CHAPTER IV

IV RESULTS

A INTRODUCTION

The main experiment took a total of twelve weeks from seed sowing to the time of final yield analysis. The preflowering stress cycle was commenced 27 days after seed sowing, the plants, at this stage, having 7 expanded leaves. An examination of the apex showed that flower initiation had already occurred and the meristem was in a similar state to that shown in figure 3c., however there was not enough material for the various analyses prior to this point. As well the data of Salter and Drew (1965) showed that, with their variety at least, root growth was still relatively rapid at this point. It took 3 days after the water supply was disconnected before the first signs of wilting were apparent. Another 3 days elapsed before marked wilting was observed, and so the total length of this cycle, including the recovery phase, was 10 days.

The second cycle commenced 51 days after sowing when the plants had 15 expanded leaves and, at this stage, the first flowers were opening on the lowest flowering node. It took only 24 hours after the water supply was disconnected for the first signs of wilting to become apparent, and then a further 24 hours for marked wilting to occur. Thus the total length of this cycle, including the recovery period, was only 6 days. Figure 13 shows plants at this stage, in both the turgid and wilted states.

The third cycle commenced 64 days after sowing when the plants had 18 expanded leaves and the largest pods were about 6 cm. long. Because of the rapid onset of wilting in the second cycle, it was decided to delay disconnection of the water supply until 12 hours after the first sample was taken. After 12 hours without water the plants were already showing the first signs of water stress and, in a further 24 hours they were wilting markedly. This cycle, including the recovery phase, was actually only $5\frac{1}{2}$ days, although the time from the first to last sampling was 6 days.

Figure 13. Wilted and unwilted plants from the flowering wilting stress cycle.

The fourth cycle was initiated 72 days after sowing. At this stage the plants had 20 expanded leaves, apical growth had essentially ceased, and the lower pods were swelling noticeably. It was decided to use the same timing as for the third cycle, with respect to the induction of wilting. However, 12 hours after the water supply was disconnected, no sign of water stress was obvious. After a further 24 hours only mild wilting occurred, and so the plants were left without water for another 24 hours when wilting, similar to that in the earlier stages, was finally achieved. This cycle took $6\frac{1}{2}$ days (7 days from the first sampling to the last). The reason for the longer time to obtain a similar degree of visible wilting at this stage of development may have been related to the cessation of rapid leaf development, as mentioned above.

The results of the various analyses carried out during these cycles are not presented. In the diagram, S represents the start of the stress phase period, and R the beginning of the recovery phase. The additional point plotted at the 4 days after rewatering period on the graphs in figures 14 to 18 represents the control value determined at this same sampling time. This point is plotted only where possible, i.e. when markedly different from the value for recovered plants.

B STOMATAL APERTURE

In all cases 20 stomata on each of three separate impressions from different leaves, were measured.

Changes in stomatal aperture follow a similar pattern in each of the four wilting stress cycles. At the time of the maximum wilting, stomatal apertures were markedly reduced as shown in figures 14 to 17, and there was also a delay in reopening upon rewatering. However by four days after rewatering, apertures appeared to be normal.

An exception to these general trends was observed for apertures during the flat pod stress cycle when stomatal closure occurred more

rapidly, with the aperture being noticeably reduced by the time of second sampling. In the other cycles only a small decrease in the aperture had occurred by this stage. Also the stomata did not appear to recover fully by four days after rewatering.

C WATER STATUS DETERMINATIONS

1. LEAF WATER SATURATION DEFICIT

Again similar trends were seen for each of the four cycles in figures 14 to 17. The deficit at the maximum stress stage of the flowering cycle appeared greater than for the others, although the error was larger also. The water deficit returned to normal, without exception, by 24 hours after rewatering.

A point to note is that the measured water deficit taken during the initial sampling stage in both the flat pod and pod swelling cycles appears to be slightly more than expected. It may be that the leaves were not fully mature and the observed deficit was exaggerated as Catsky (1965) explains.

The important fact emerging from these results is the rapid recovery from leaf water deficits upon rewatering.

2 PRESSURE BOMB

Figures 14 to 17 show that water potential changes in a similar manner for the different cycles. Several interesting points are apparent. The most negative potential reached (in the flat pod stress cycle) was only -14.7 bars. It would have been expected to drop further than this. However, as Jordan and Ritchie (1971) point out, smaller decreases in water potential are often observed under growth room conditions compared to the field situation. Perhaps another point of interest is the more rapid decrease in water potential and more negative final value obtained during the flat pod wilting cycle. The onset of stress, as measured by this means (and stomatal aperture measurements), appears more rapid than

for any other stage.

Decreases in stomatal aperture also appear to have followed a similar pattern to decrease in water potential. However, on recovery water potential was back to control levels by one day after rewatering whereas stomatal apertures were still markedly different.

D BIOASSAY DETERMINATIONS

1 WHEAT COLEOPTILE

The results of coleoptile response to chromatogram sections have been plotted as histograms (Appendix II, figures 21 to 25). Confidence limits at P = 0.01 have been calculated according to the procedure in Appendix III.

Regarding the acidic ether fractions, marked inhibitory activity was observed in the Rf 5 to 7 range, the inhibitor zone described by Bennet-Clark and Kefford (1952). ABA marker spots also covered a similar range of values.

Values of inhibitor concentration, expressed as equivalents of ABA, were calculated from the significant peaks using data from the appropriate standard curve, and corrected using the data in figure 32 (Appendix II) to give values on a gram dry weight basis. Plots of these values are shown for the four different wilting cycles in figures 14 to 17. It is interesting to observe that there is no obvious increase in inhibitor B levels until the maximum stress sample in all except the flat pod series. In this particular series levels had increased markedly by the second sampling stage, only 12 hours after watering was stopped.

Another interesting result is the marked decrease in the maximum value attained as the flats became physiologically older. In fact the maximum value is approximately halved with each increase in physiological age. Because the strong coleoptile inhibitions obtained in the bioassay may have fallen outside the ABA standard series range, 1/10th of the

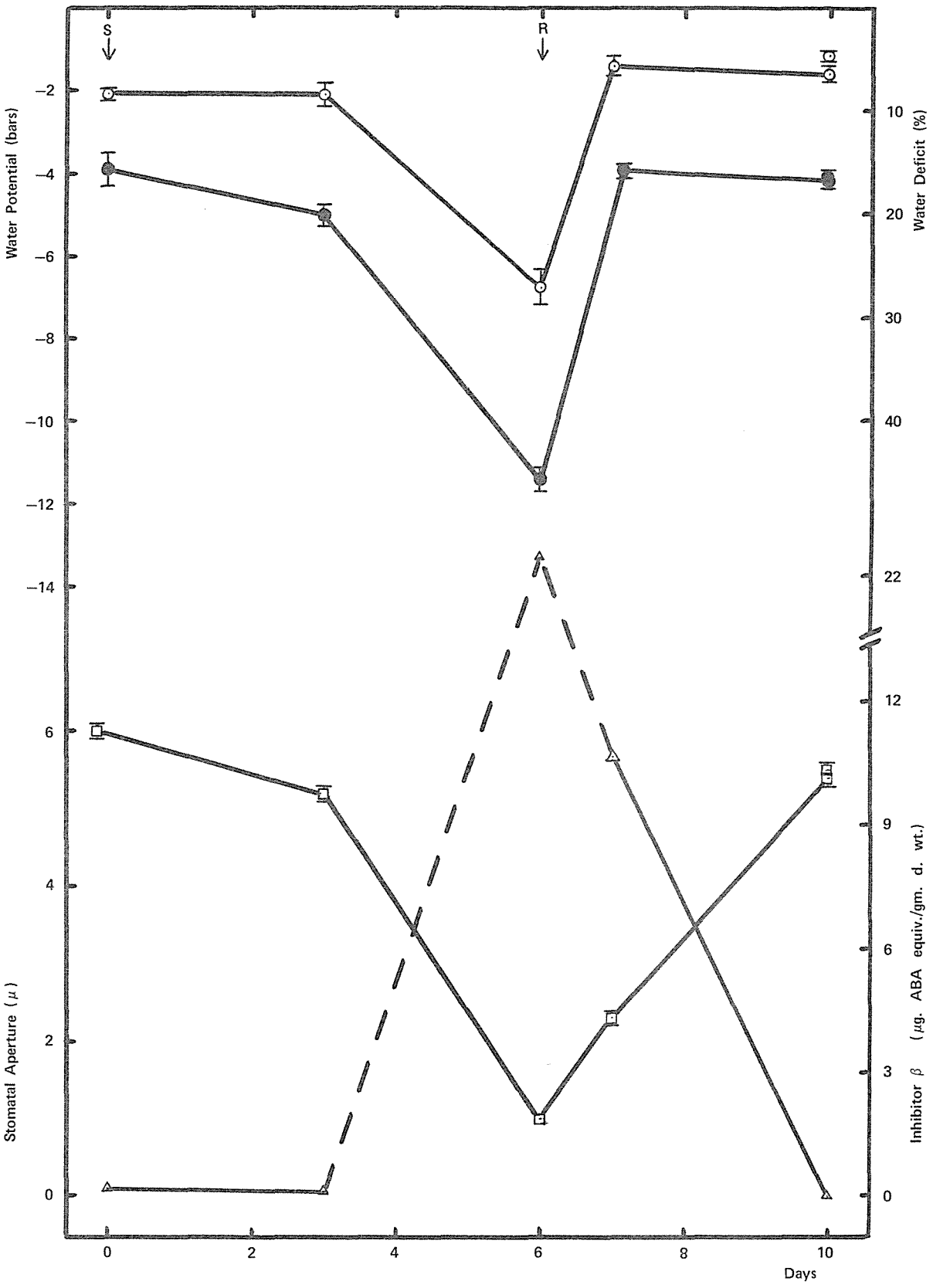


FIGURE 14 Trends in water potential, water deficit, stomatal aperture, and inhibitor β levels in the first mature leaf of pea plants subjected to a wilting cycle during the pre-flowering stage. Key. ○——○ water deficit, ●——● water potential, □——□ stomatal aperture, △——△ inhibiter β. Standard errors shown where applicable.

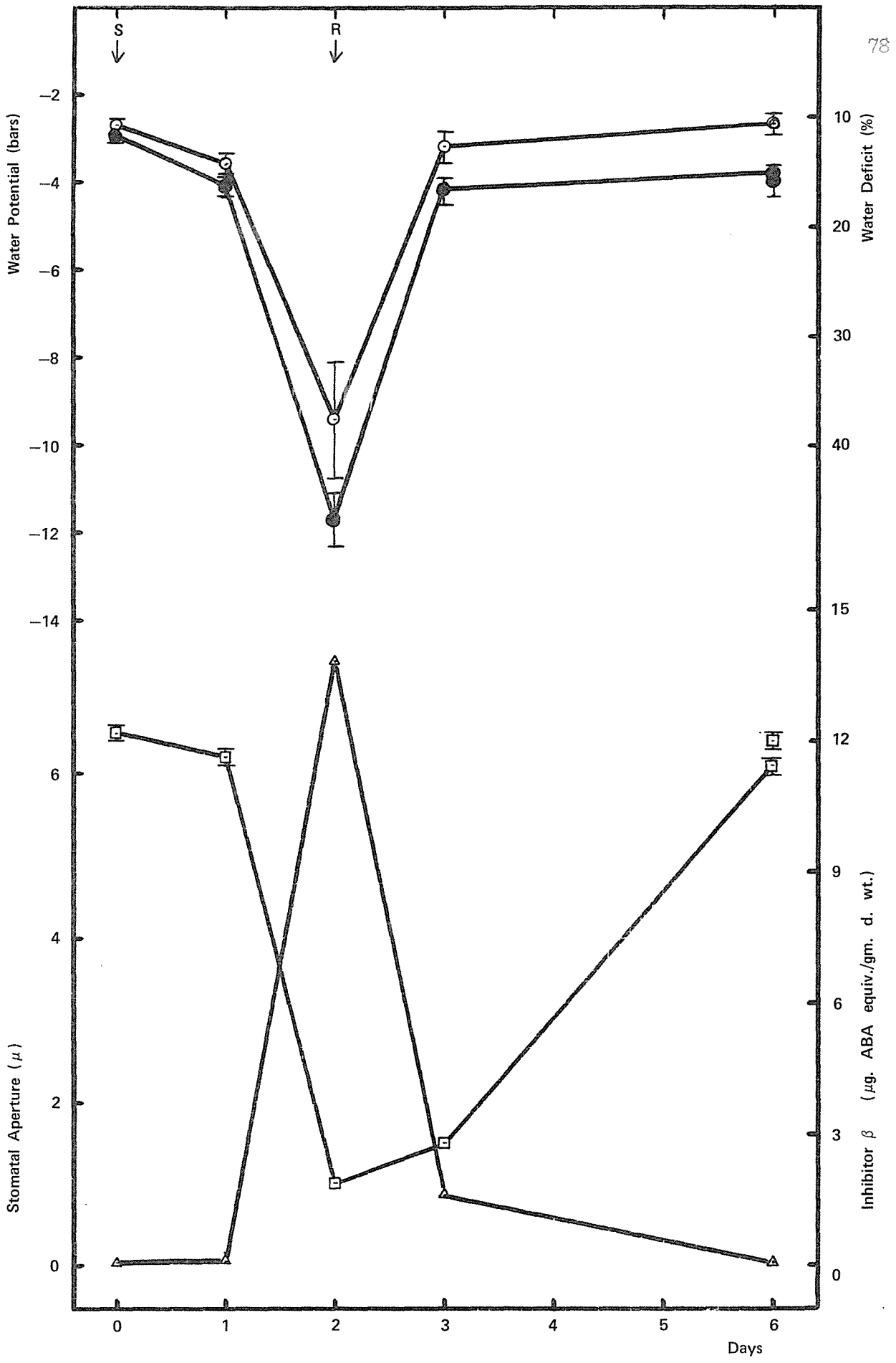


FIGURE 15 Trends in water potential, water deficit, stomatal aperture, and inhibitor β levels in the first mature leaf of pea plants subjected to a wilting cycle during the early flowering stage. Key as for Fig. 14. Standard errors shown where applicable.

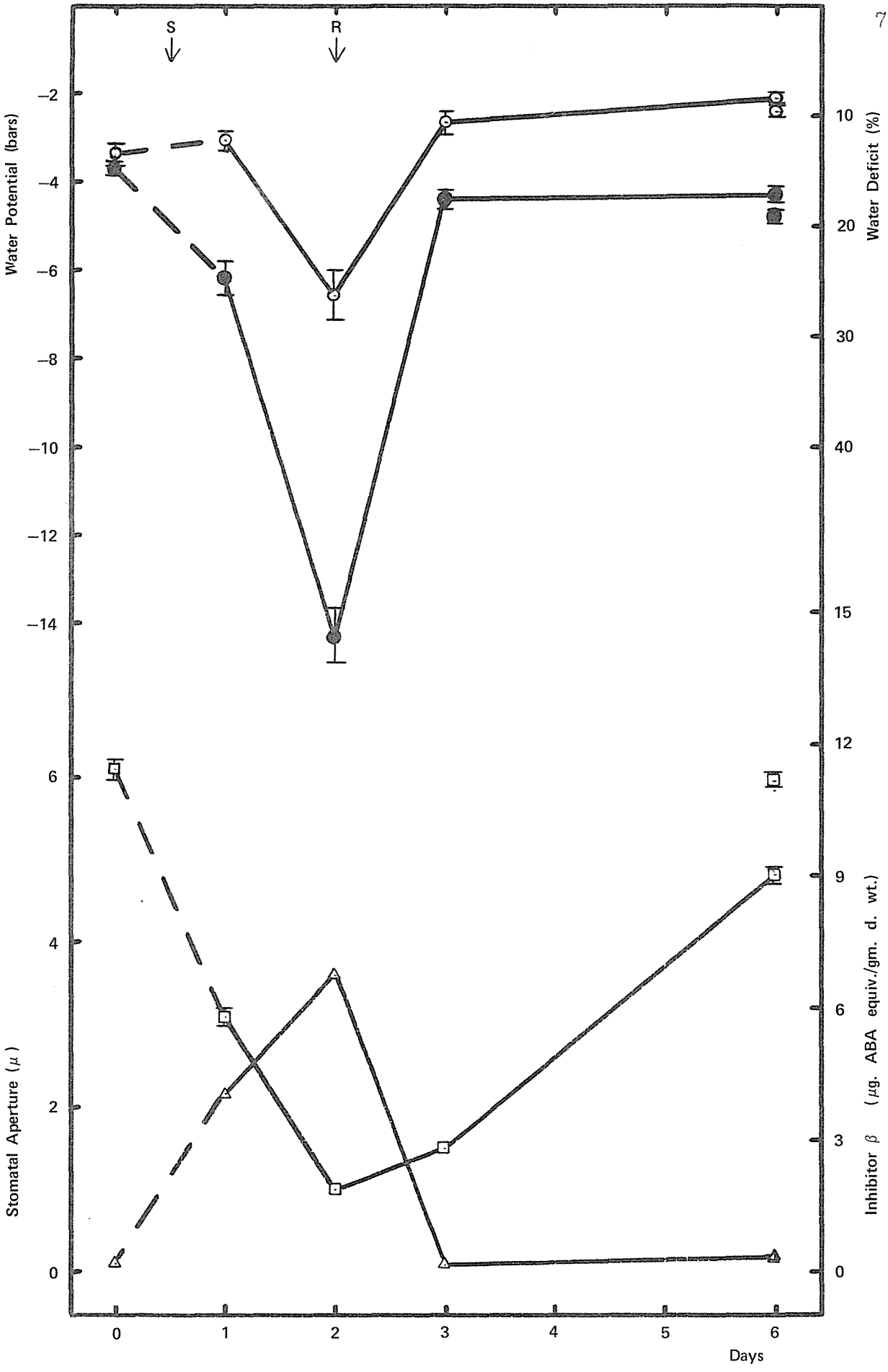


FIGURE 16 Trends in water potential, water deficit, stomatal aperture, and inhibitor β levels in the first mature leaf of pea plants subjected to a wilting cycle during the flat pod stage. Key as for Fig. 14. Standard errors shown where applicable.

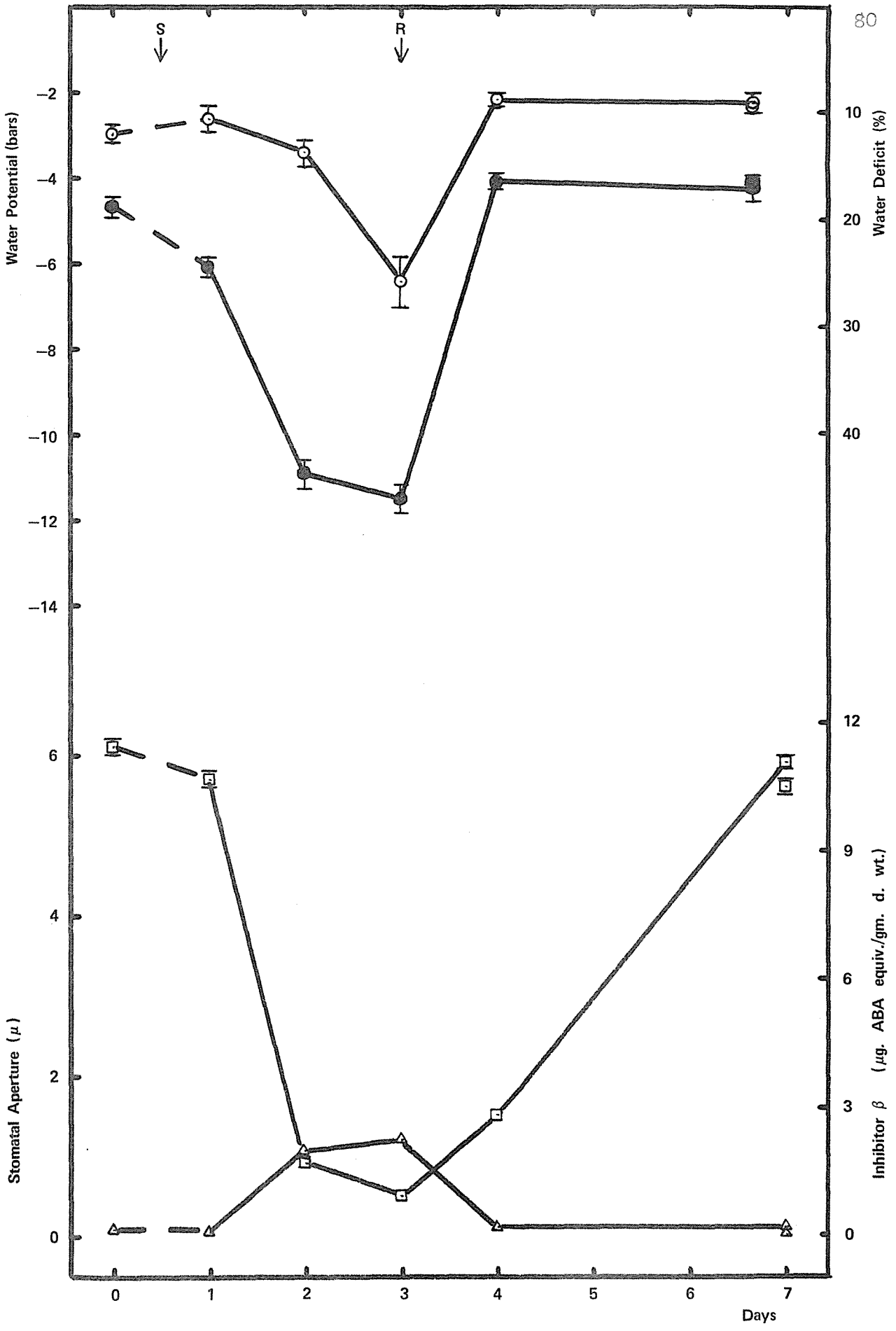


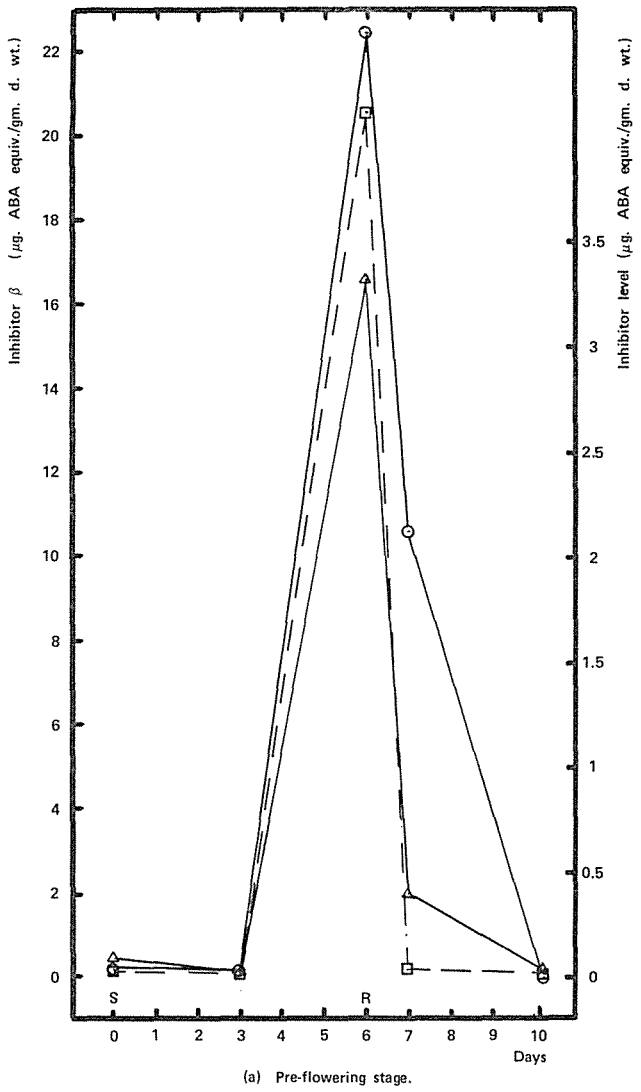
FIGURE 17 Trends in water potential, water deficit, stomatal aperture, and inhibitor β levels in the first mature leaf of pea plants subjected to a wilting cycle during the pod swelling stage. Key as for Fig. 14. Standard errors shown where applicable.

sample to be chromatographed was treated separately and the same trend of values emerged.

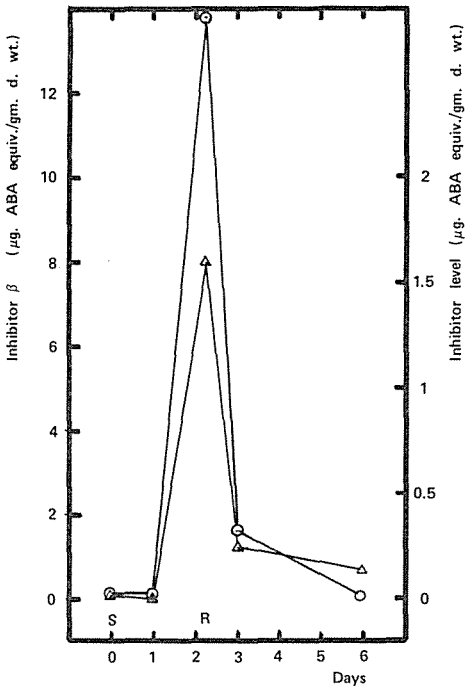
A final point of significance is the differences in inhibitor levels detected during the recovery phase. In the preflowering series the inhibitor B levels had decreased only by about 50% 24 hours after rewatering. However three days later it was essentially the same as control levels. A more rapid decrease was observed for the flowering stage with a decrease to about 15% of the maximum level being observed one day after recovery. Again by four days after recovery, the detectable level was essentially the same as the control. At the final two stages the detectable levels had dropped to similar levels as controls within 24 hours.

Looking at a different aspect of these results, figure 18a shows the inhibitor level of extracts of apical tissues taken during the flowering stage increased to a similar value at the maximum stress sampling time as for leaf tissues. However the value dropped back rapidly being virtually the same as control levels 24 hours after rewatering, whereas the inhibitor level in leaf tissues was still high at this point of time.

The responses of wheat coleoptiles to chromatogram sections of the basic ether extracts are shown in figures 26 to 29 in Appendix II. A strongly inhibitory material at Rf 8 to 10 was apparent. However trends in this inhibitory zone are hard to determine. During the preflowering and flowering water stress cycles, relatively low values of this inhibitor were present in the first four samples. However at the sampling time 4 days after recovery, in both cases, the samples from recovered plants and control plants show much higher levels of this inhibitor. For the flat pod stress cycle a reverse pattern seems to occur values starting off high during the first four sampling stages but dropping off at the last sampling stage for both recovered plants and controls. Values for the

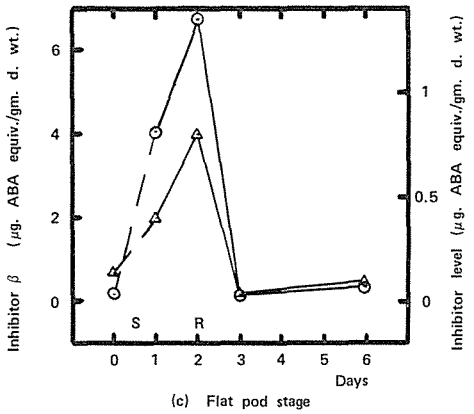


(a) Pre-flowering stage.

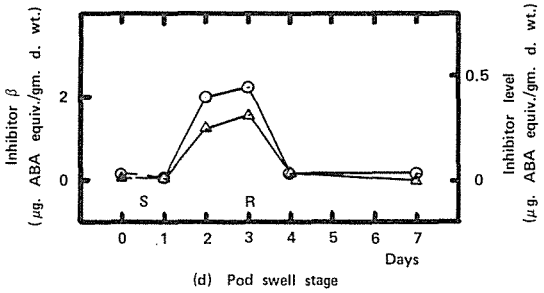


(b) Early flowering stage

FIGURE 18 A comparison of trends and values for inhibitor levels as determined by the wheat coleoptile and *Commelina communis* epidermal strip bioassays. Key: ○——○ inhibiter β levels in the first mature pea leaf as determined by the wheat coleoptile bioassay. □——□, inhibitor β levels in pea apical tissues as determined by the wheat coleoptile bioassay. △——△, inhibitor levels as determined by the epidermal strip bioassay.



(c) Flat pod stage



(d) Pod swell stage

pod swell cycle are somewhat more variable. The significance of the presence of this inhibitor cannot really be interpreted from the results. Dorffling (1972) suggests that the neutral inhibitor at this Rf in the solvent system used, could be xanthoxin.

2 COMMELINA COMMUNIS EPIDERMAL STRIPS

Comparisons of inhibitor levels determined using this bioassay and the wheat coleoptile bioassay are shown for the four stress cycles in figures 18a to d. The pattern of change in inhibitor values is similar to that for the wheat coleoptile bioassay except that the amount persisting 24 hours after rewatering, in the preflowering stress cycle, is a lesser proportion of the maximum value.

However, the magnitude of values is quite different, particularly at the points of maximum stress where they are about 1/7th of the values determined by the wheat coleoptile bioassay. Obviously the Commellina assay system was responding to only a portion of the inhibitor β complex. Tucker and Mansfield (1972) designed this bioassay for the assessment of materials which influenced directly, stomatal aperture. In particular the assay was demonstrated to be sensitive to a range of concentrations of ABA. In these experiments it appears quite conceivable that the response of the assay system to plant extracts, in particular, the eluates of Rf 5 to 7 (the inhibitor β zone), is due to ABA (and possibly phaseic acid, if present, as Kriedeman (1973) has mentioned this material can induce stomatal closure also). Evidence to support this contention comes from the report of Simpson and Saunders (1972) which shows that the ABA levels in young wilted pea plants were in the order of 3.5 μg/10gm. fresh weight of tissue. A similar value can be seen in figure 18a for the maximum stress sample during the preflowering stress cycle as determined by the Commelina bioassay. Also rough comparisons of the sample peak sizes and ABA standard peak sizes, during gas-liquid

chromatography, suggested the amount of ABA present in the original samples was much nearer the Commelina value than the wheat coleoptile value for inhibitor levels.

3 AVENA COLEOPTILE BIOASSAY

As with previous workers, e.g. Jenkins (1973), no consistent pattern of change in IAA values was observed during water stress cycles (figure 30). However the values for samples taken during the flat pod stress cycle are consistently higher than in any of the other three wilting cycles.

It is possible sample material may have required further purification to remove any materials which could be masking the IAA response.

Another point of interest is to note that coleoptile responses to IAA concentrations is much less in vials containing washed chromatograph paper compared to those without (compare the promotion responses in figure 30 with those in figure 12).

E G.L.C. DETERMINATIONS

Injection of samples eluted directly from the chromatogram run with 10:1:1 v/v isopropanol, ammonia, water, and methylated without further purification, showed several large contaminant peaks were present which partially masked the ABA peak (as determined by co-injection). Although further purification, as described earlier, markedly reduced the levels of these contaminants, there was still some distortion of the ABA peak (figure 19). However coinjection with authentic ABA does correspond. This procedure was repeated a number of times with similar results for samples taken at the maximum stress stage of all wilting cycles. However the magnitude of this peak for a known injected volume, decreased as samples were taken at an older physiological age. Although no accurate quantification was attempted, the size of the peaks present, compared to standard peaks, suggested the quantities present in the original sample (allowing for losses during purification) were similar in

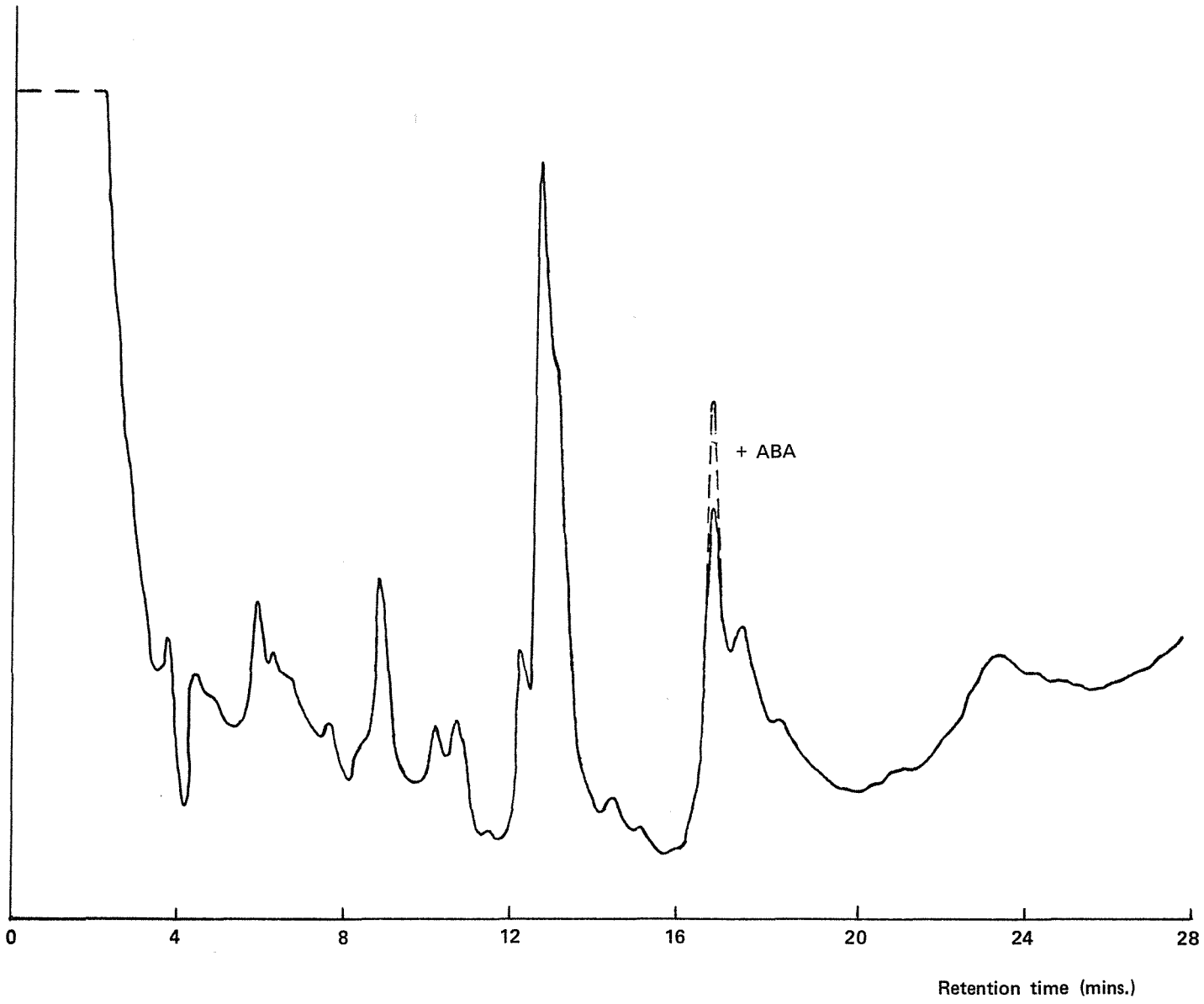


FIGURE 19 Determination of the prescence of abscisic acid in the purified acidic ether fraction, from extracts of pea tissue, using gas – liquid chromatography.

magnitude to the inhibitor levels obtained by the Commelina communis epidermal strip bioassay.

F FINAL YIELD DATA

Some of the more important results are shown in Table IV. Other data is shown in Table V (see Appendix II).

Perhaps of greatest importance is the effect of wilting stress cycles on the number of basal shoots per plant, because this effect has influenced many of the other results. The number of basal shoots per plant is significantly higher than the control for plants from all stress cycles except the pod swell cycle. The greatest effect appears to have occurred on plants stressed at the preflowering and flowering stages, this effect even showing in plants stressed at both the flowering and pod swelling stages.

Haulm dry weight is not markedly affected by water stress during the preflowering cycle but it does increase significantly for plants stressed during flowering, this being due to the marked tiller growth. However plants stressed at the flat pod, pod swell and both flowering and pod swell have significantly smaller haulm dry weights than control plants, the plants stressed during the pod swelling stage having the least values.

Regarding leaf area, the influence of tillering again becomes apparent in relation to the values for plants subjected to stress during the preflowering or flowering periods. Leaf area for plants stressed at the latter stage is, in fact, significantly higher than for control plants. However plants stressed at the later stages and both flowering and pod swell showed marked decreases in leaf area. Looking at the effects of water stress on leaves from a different angle, the percentage of leaves alive on each plant falls significantly as plants are subjected to wilting stresses at later physiological stages.

TABLE IV The effects of water stress, applied at different stages in the pea plant life cycle, on some parameters examined in the final yield analysis.

Series	Haulm dry wt. (gm)	Tiller no.	Leaf area (cm^2)	% leaves alive	Height (cm)	Pod no.	Pea no.	Pea yield	
								Fr.wt.(gm)	D.wt.(gm)
Control	29.53b*	0.4d	1475bc	55.5a	107a	15.0bc	68.0b	27.98bc	5.61bc
Stress preflowering	29.71b	1.1bc	1549ab	59.7a	1069	16.7ab	70.4ab	35.87ab	7.48ba
Stress preflowering	34.74a	1.5a	1820a	48.0b	102ab	19.6a	83.2a	41.24a	8.30a
Stress flat pod	23.19cd	0.9bc	1165cd	48.1b	87c	14.2bc	58.8bc	28.21bc	5.69bc
Stress pod swell	19.63d	0.3d	771e	38.4c	101ab	12.6c	50.7c	21.40c	4.0bc
Stress flowering and pod swell	26.11c	1.2ab	1067de	36.9c	94bc	16.7ab	61.1bc	32.246	6.88ab

* Figures followed by the same letter not significantly different at P = 0.05 (Duncan's Multiple Range Test). Each value the mean of 10 determinations.

Pod number is highest for plants which were subjected to a wilting stress at the flowering stage, again reflecting the influence of tillering. The least pod number was observed for plants stressed at the pod swelling stage. However this value was not significantly different from the control value. Mean pea number was also highest for plants stressed at the flowering stage whereas the lowest yield was obtained for plants stressed at the pod swelling stage. The number of peas per plant was also lower than controls for plants stressed at the flat pod and both the flowering and pod swelling stages, but not significantly so. The yield of peas as measured by fresh and dry weight per plant again is highest for plants stressed at the flowering stage. Lowest yields were obtained from plants stressed at the pod swelling stage but these were not significantly different to control plants.

These results demonstrate that water stress, at the different developmental stages, has differentially affected the physiology of the plant, which in turn has affected final growth and yield.

CHAPTER V

V DISCUSSION

Because of the different aspects investigated in these experiments the discussion of the results will be undertaken in sections, with the whole being critically examined as a final analysis.

A INHIBITOR LEVELS IN PEAS SUBJECTED TO WATER STRESS CYCLES

The data in figures 18a to d show that although similar patterns of change in inhibitor levels are obtained using the wheat coleoptile bioassay or Commelina epidermal strip bioassay, the actual values are markedly different. Maximum values obtained in the latter assay system was only about 1/7th of those attained using the former and, as already mentioned, the maximum values determined by the Commelina assay were similar to the ABA levels estimated approximately by G.L.C. As well, the maximum level of ABA reached during the preflowering stress cycle was similar to that detected by Simpson and Saunders (1972) for pea plants at an early stage of development. One can deduce from this that there must be a difference in the number of inhibitory materials affecting coleoptile growth compared to those affecting stomatal aperture in epidermal strips. The bulk of the inhibitor determined by the Commelina assay, is probably ABA, while the coleoptile bioassay is presumably detecting a number of other compounds which contribute to the so-called inhibitor β zone.

Milborrow (1967) identified ABA in a number of different plant parts and species, and the Rf of ABA marker spots corresponded well with that of inhibitor β, suggesting ABA was a major component of the inhibitor β complex. Wright and Hiron (1969) determined that the bulk of the inhibitor zone which changed in excised wilting wheat leaves, was in fact ABA. However their purification was somewhat more extensive than that carried out in these experiments for bioassay work, thus it is conceivable that in their case a number of materials had been removed from the inhibitor β zone.

It is of interest that the apparent change in these other materials in the inhibitor β zone follows a similar pattern to ABA (if it is accepted that the Commelina assay is responding mainly to ABA). That the inhibitor β zone can contain other materials is well proven, and Dorffling (1972) comments that in some lower plants this zone does not even contain ABA. Robinson et al (1963) mentioned that several phenolic materials had been isolated from the inhibitor β zone, including coumaric acid, salicylic acid, and possibly ferulic acid. But, on further purification by running in a different solvent system to the isopropanol:ammonia:water mixture used in these experiments, these phenolics disappeared from this zone. These phenolics could produce inhibitory effects in the wheat coleoptile bioassay. If phenolics do accumulate in water stressed plants (as they do under other stress situations and senescence; Uritani, 1971), this could explain the differences between the two bioassays and also the similar pattern of accumulation. Evidence to support the idea that these materials are different from ABA is shown in the inhibitor levels remaining one day after rewatering in the preflowering stress cycle. The decline, as detected by the wheat coleoptile assay, is much slower than that detected by the Commelina epidermal strip assay. This suggests that the rate of removal of the inhibitory materials detected by the former assay is slower than that for the latter.

Stomata are unlikely to respond to these phenolics, evidence to support this being provided by Ogunkanmi et al (1973). They show that the Commelina epidermal strip assay, will not respond to various phenolics, even as relatively high concentrations. Coumarin at 6.1×10^{-5}M or xanthinin at 3.75×10^{-4}M, in solution with ABA, caused no difference in the aperture in epidermal strips as compared to ABA alone. Similarly, these two materials had no effect on their own. Scopoletin at 10^{-3}M and 10^{-4}M had no effect and chlorogenic acid gave only a slight effect at 10^{-3}M.

Thus it seems likely that the difference between the two assays is due to the presence of phenolics in the inhibitor β zone using the purification procedure adopted for these experiments.

Unfortunately it was difficult to determine the actual number of times the inhibitor levels increased from the control to maximum stress stage because of the limitation of the bioassays in detecting low levels of these inhibitors accurately with the small samples used. However to give an indication of trends, the increase between the initial sample and the maximum stress sample was 120 x (wheat coleoptile) and 36 x (Commelina) for the preflowering cycle. For the last cycle the values were 29 x and 10 x respectively showing that some decline in the magnitude of increase, as well as the maximun value, appeared to have occurred with increasing age.

B EFFECTS OF WATER STRESS ON STOMATA

Looking at the response of stomata to wilting stress cycles, it is observed that they close fairly rapidly once water stress within the leaf passes a certain level. There was also some delay in reopening upon rewatering, even though the plant water status as measured by leaf water potential or leaf water saturation deficit, returned to fairly normal levels within 24 hours.

Dealing with the closure phase, it is well known that once a critical leaf water potential is reached stomatal closure occurs fairly swiftly. (Hsiao, 1973). Several authors (Stalfelt, 1961; Willis and Balasubramanian, 1968; Graziani and Livne, 1971) observed that this could happen rapidly with leaf water deficits of only 2 to 4%. The response probably depends on the rapidity of the imposition of water stress, these latter examples being related more to rapid changes in water content. Obviously the conditions of growth will also affect the degree of stress developing (Jordan and Ritchie, 1971). (see p 75).

More recently it has also been reported that leaf water potential has to drop to a particular level before any increases in ABA levels occur. Zabadal (1974), examining this relationship in several species of Ambrosia, determined a critical point of about -10 to -12 bars; for one species this represented a drop in water potential of about 2 bars while in the other about 6 to 8 bars, as compared to the water potential of control plants. Beardsell and Cohen (1973) observed a rapid increase in ABA production in water stressed maize leaves when the leaf water potential fell in the range of -6 to -10 bars, -6 bars being the approximate control leaf water potential. However the exact point was not determined.

Similarly the stressing of plants at different physiological stages by wilting, suggests that there is a critical drop in water potential required to initiate stomatal closure and large increases in inhibitor levels. From figures 14 to 17, it can be seen that water potential drops for the preflowering, flowering, and pod swell cycles were in the order of 1 bar between the initial and second samples. These differences were all significant as shown in Appendix III. At the same time significant decreases in stomatal apertures were observed. (Care has to be taken in interpretation since no control was taken to compare the value determined at the second sampling time. However in a constant environment and with constant sampling times it was considered that a control value at the start and the finish of the cycle would be sufficient. It should also be noted there is some decline in the control value between the initial and final samplings and that this is probably related to an ageing factor as described by Fischer et al, 1970). This suggested that perhaps the stomata were already responding to a change in the plant water status.

During the flat pod wilting cycle, leaf water potential dropped by 2.5 bars between the initial and second sampling times. At the same time stomatal aperture had decreased by nearly 50% and a rapid increase in

inhibitor levels (as measured with either assay system) had occurred suggesting the critical leaf water potentials, for both rapid stomatal closure and the induction of increases in inhibitor levels, had been passed.

The fact that stomata may have been closing in the other cycles, with smallish decreases in water potential and no rapid increases in inhibitor levels, supports the contention of Hsiao (1973) that possibly stomata are closing before any increase in ABA has occurred. Zabadal (1974) also comments that closure of stomata, such as Graziani and Livne (1971) have observed, can occur within ten minutes in an excised leaf. However ABA, although increasing significantly within 15 minutes of the onset of water stress (Loveys and Kriedeman, 1973), may not be increasing rapidly enough to explain the rapid decreases seen in stomatal aperture and he suggests that at least under conditions of rapid desiccation, loss of turgor alone may be the predominating factor. However he also suggests that under slower drying conditions, the increase in ABA levels may be of more significance with respect to stomatal closure.

Regarding the rapidity of ABA increases, Hiron and Wright (1973) observed increases in ABA levels within 10 minutes of supplying warm air to bean leaves. In fact, the curve of increase in ABA levels follows a similar pattern to the curve of increase in stomatal resistance. However this does not necessarily establish a cause and effect relationship, simply that both are changing in a similar pattern. Also stress induction was rapid and so it would be difficult to separate the two processes over such short time intervals.

There has been a large amount of work carried out in recent years relating to the effects of ABA on stomata. However one has to examine this fairly critically as there are several important points which appear to have been overlooked.

With regard to entire leaves, Little and Eidt (1968) were among the

first to report that the transpiration of tissues standing in ABA solutions, was markedly reduced. Mittelhouser and van Steveniek (1969) observed that, within six hours of standing excised wheat leaves in a 3.8×10^{-6}M ABA solution, stomatal apertures were near zero and in a later paper they determined an increase in stomatal resistance within 10 minutes of supplying ABA to detached leaves. However the effect was less rapid when supplied to the roots of intact plants because of the greater distance ABA has to be transported to its site of action. Jones and Mansfield (1970) obtained stomatal closure in leaves of Xanthium pennsylvanicum by painting leaf surfaces with ABA at the rate of 0.02 $\mu g/cm^2$. In addition, supplying 10^{-4}M ABA via the petioles of tobacco leaves also caused abrupt stomatal closure. In a later paper (Jones and Mansfield, 1972) they determined that painting leaves with concentrations of less than 10 μM of ABA gave little effect on stomata and that any effects that were observed occurred only in treated areas. Cummins et al (1971) also supplied 10^{-7}M ABA to excised barley leaves by standing them in this solution and detected a response (as measured by diffusive resistances) within 10 minutes of application. Kriedeman et al (1972), supplying ABA by standing the petioles of excised leaves in 50 μM solutions, noted that stomatal responses occurred within 5 to 20 minutes, depending on the species. They suggested that only a doubling of the endogenous concentration of ABA was required to initiate stomatal closure.

A major weakness in all these experiments is that the ABA supply is exogenous. Supplying ABA via petioles or root systems means that the bulk of its transport will be in the apoplastic system thus leading to accumulation in the region of the stomata so that the concentration of ABA in the vicinity of the stomata could be relatively high compared to the rest of the leaf tissue. This is a major criticism of the conclusion of Kriedeman et al (1972) that endogenous ABA levels need only double to

initiate stomatal closure, because the actual level near the stomata due to their method of applying ABA, could be higher than for the entire leaf tissue. Also applying ABA to leaf surfaces as Jones and Mansfield (1972) have done, again places high concentrations of ABA in close proximity to the stomata.

Further to the above, there is the finding that ABA will not close stomata which are already open on epidermal strips, but will only prevent closed stomata reopening under conditions of light and CO_2-free air (Horton, 1971). Similarly, a basic requirement for the Commelina communis bioassay is that stomata in the epidermis must be closed prior to incubation with ABA (Tucker and Mansfield, 1971), suggesting that ABA will only inhibit reopening.

We could deduce from this that either the presence of CO_2 and/or mesophyll tissue is necessary for the induction of stomatal closure in the presence of ABA.

Milborrow and Noddle (1970) have reported that ABA is a leaf produced hormone and more recently it has been reported to be produced by avocado chloroplasts (Milborrow, 1974) and associated with pea leaf chloroplasts (Railton et al, 1974). If this is the case, is ABA produced within the mesophyll and transported to the guard cells where it affects aperture, or is it produced in the guard cells themselves, or is it actually causing an indirect effect? A problem with the assumption that ABA is causing stomatal closure directly is the apparent lack of effect on open stomata in epidermal strips. Because the differences between the epidermal tissue system and the whole leaf system are basically the presence of mesophyll tissue and CO_2, one could hypothesize that ABA induced closure of stomata in entire leaves is due to changes in internal CO_2 levels and/or sensitivity to CO_2.

Looking at the possibility of an indirect effect of ABA on stomata,

Jones and Mansfield (1970) reported that the CO_2 compensation point increased by 15% in ABA treated leaves but concluded that this could not account for the closure induced by ABA. Their argument that flushing leaves with CO_2 free air was not reversing the closure effect does not necessarily mean ABA is causing closure directly, simply that re-opening is inhibited. Meidner and Mansfield (1968) have stated that the opening and closing movements of stomata are quite different. Heath and Meidner (1961) have observed that the compensation point increases in water stressed leaves. This increase could be due either to effects on the photosynthetic enzyme system itself (Wellburn et al, 1973, have reported ABA inhibits RuDP carboxylase activity) or on intracellular CO_2 transport (Jones and Slatyer, 1972). The finding that ABA is associated with leaf chloroplasts by Railton et al (1974) could also be of importance in relation to these observations. However, the effects of short term water stress on the photosynthetic apparatus itself appear to be slight (Graziani and Livne, 1974) and so if ABA is causing stomatal closure it seems unlikely that the effect is due to increased internal CO_2 levels alone.

In relation to this we have the observation of Heath and Mansfield (1962) that the sensitivity of stomata to CO_2 increases markedly in water stressed leaves. The results of Jones and Mansfield (1970) infer that ABA can induce this sensitivity effect and more recently, Raschke (1973) has presented evidence to show that ABA does increase the sensitivity of stomata to CO_2. Another interesting observation is that the time course for stomatal closure due to increased CO_2 levels in the air (Loveys et al, 1973) is similar to that for ABA induced stomatal closure (Kriedeman, et al, 1972). Thus it could be possible to explain the differences between the response of stomata on isolated epidermal strips and intact leaves in terms of the requirement for the presence of CO_2, upon ABA treatment, to enable closure to occur.

With this evidence in mind it could be postulated that any effect of increased endogenous ABA levels on stomata could be due to a minor extent on increased internal CO_2 levels in leaves, but predominantly due to increased sensitivity of stomata to CO_2. How this increased sensitivity comes about has not yet been fully elucidated but it may be related to the finding of Willmer and Dittmer (1974) that CO_2 fixation in stomata appears to be quite different to that in the mesophyll tissues and can continue in the light and the dark. This observation will be developed a little further later in the discussion.

Regarding the reopening phase (which is a different process to closing according to Meidner and Mansfield, 1968) the data in the experiments show a consistent delay in the reopening of pea stomata upon rewatering (figures 14 to 17). For all wilting stress cycles, the water status of the plants has returned to normal within 24 hours of rewatering. However stomatal opening has increased only a little. These results compare favourably with the observations of Fischer et al (1970) and Allaway and Mansfield (1970). The fact that stomatal apertures are still significantly smaller at the final sampling time in the flat pod stress cycle is probably related to the fact that the intensity of the stress, as measured by leaf water potential, was greater than for any other cycle and as Fischer et al (1970) reported, the delay in recovery was dependent upon the duration and intensity of the moisture stress which the plant suffered.

It has been suggested that the build up of ABA during a period of water stress could be responsible for the delay in stomatal opening (Horton, 1971). Allaway and Mansfield (1970) reported that the delay in reopening was consistent with either the absence of a substance promoting opening, or an inhibitor preventing reopening.

Wright and Hiron (1972) and Hiron and Wright (1973) reported that ABA levels do not decline to normal upon rewatering, and Horton (1971), and

and Tucker and Mansfield (1971) demonstrated that ABA certainly inhibits opening in epidermal strips. It has been reported that the continued presence of ABA is necessary to prevent stomatal opening (Tal and Imber, 1971; Cummins et al, 1971; Kriedeman et al, 1972). However recent work questions the possibility of the continued presence of ABA preventing stomatal opening in intact leaves. Firstly Cummins (1973) reported that the metabolism of ABA was not rapid enough to explain the recovery of stomata observed in his experiments and some localization must have been occurring. Secondly, Beardsell and Cohen (1973) observed that ABA levels in maize leaves decreased more rapidly than stomatal resistance and that stomatal resistance was still higher than control values 4 days after rewatering, even though ABA levels appeared normal.

The results obtained from stressing peas at different stages during their growth cycle show that inhibitor levels, as detected by either bioassay system, were still relatively high 24 hours after rewatering during the preflowering cycle. However during the flowering cycle the proportion persisting was much less, and in later cycles no persistence was observed 24 hours after rewatering. Also, the maximum levels of inhibitor attained, on subjecting plants to moisture stress, decreased with increasing physiological age. However, the recovery of stomatal apertures followed a similar pattern in all cases suggesting that a direct reliance on the persistence of ABA was not in fact occurring. Also the degrees of failure to recover did not appear to be related to the maximum inhibitor level which was attained because stomata in the flat pod cycle showed the greatest delay in reopening yet the inhibitor levels during this cycle were markedly less than previous cycles. The delay in reopening appeared more related to the decrease in water potential in this case, than ABA levels.

Cummins (1973) suggested that because metabolism of ABA could not explain the recovery of stomata on removal of the ABA source, ABA must be removed to

sites where it cannot act on stomata. One must ask how delays in reopening are occurring if the continued presence of ABA is required to maintain closure, and if it is not persisting as both Cummins and the present work suggests one could postulate that ABA could be localized in guard cells, especially when higher endogenous levels are produced, and perhaps it could be exerting an effect here for a longer period although levels in the whole leaf have fallen. The major problem at the moment is to determine whether ABA is produced within guard cells as well as in mesophyll tissue or whether it is transported from the mesophyll to guard cells, and if so, what happens to it with respect to persistence within these guard cells. There seems no doubt that guard cells are more sensitive to ABA than other leaf cells, at least with respect to ion uptake, because Mansfield and Jones (1971) have demonstrated a reduction in potassium ion uptake into guard cells of a greater magnitude than the ion uptake of whole leaf sections.

This leads to the possible mechanisms by which ABA could prevent the reopening of stomata. It is first necessary to briefly discuss the more important processes associated with the opening of stomata.

Recently, it has been observed that potassium ion uptake by guard cells, is necessary for opening. Conversely, closure was associated with a reverse flow of K^+ ions (Humble and Hsiao 1970; Raschke and Fellows, 1971; Pallaghy, 1971; Humble and Raschke, 1971; Hsaio et al, 1973; Willmer and Pallas, 1973). This movement occurs generally between the guard cells and subsidiary cells. Associated with this may be changes in organic acid levels. Pallas and Wright (1973) suggested organic acids may be produced in the leaf epidermis as possible counter ions to K^+ when the stomata closed and as stomata opened K^+ uptake into guard cells would decrease the pH of the epidermis. This could explain the changes in guard cell pH which have been observed with changes in aperture, being

around 7 when open and about 5 when closed (Meidner and Mansfield, 1968). Pallas and Wright found that, as stomatal resistance decreased, total titratable acidity in the epidermis increased as did the levels of the organic acids, glyceric, malic and citric acid. As the stomatal resistance increased, organic acid concentrations decreased in the epidermis suggesting K^+ was neutralizing the acid radicals. The changes in organic acid concentrations were ample to offset K^+ uptake into guard cells, the increased K^+ concentration in the guard calls as opening occurs resulting in decreased acidity in the guard cells and increased acidity in epidermal cells.

Further to this Allaway (1973) noted that malate levels also increased within the guard cells. The amount of increase in light induced opening was enough to balance half the observed K^+ uptake. Where this was derived was not determined, but it was postulated that it may be formed from either the conversion of starch to sugar (a process associated with opening in many situations as Meidner and Mansfield, 1968, have reported) and further metabolism, or perhaps directly by guard cell chloroplasts themselves in the light. However opening in CO_2 free air would be hard to explain in these terms.

In relation to these findings, Mansfield and Jones (1971) reported that 100 µM ABA restricted stomatal opening on epidermal strips when they were immersed in solution. This was due to a fall in guard cell osmotic pressure. ABA prevented K^+ uptake to a great extent. Also the starch content of ABA treated guard cells was higher than untreated open stomata. These authors note that starch disappearance seems to occur simultaneously with K^+ ion accumulation in guard cells. Henberg (1967) reported that ABA inhibited α amylase activity and Leshem (1971) concluded that this effect may have been due to the induction of ribonuclease activity. Thus ABA, by its effect on α amylase, could inhibit starch hydrolysis

necessary for stomatal opening. The breakdown of starch could be associated with the ion flux by providing an energy source for an ion pump or by producing organic acids for balancing the K^+ influx. They also suggested that organic acid production is unlikely to depend on CO_2 because opening is inversely related to CO_2 levels in the light and dark although more recent work by Willmer and Dittmer (1973) disagrees with this contention. Another point these authors made was that a difference in sensitivity to ABA between guard cells and the mesophyll is obviously important for effective hormonal control of transpiration because it is desirable the mesophyll should continue to function efficiently under the protection offered by closed stomata, (although it has not been proven conclusively that ABA does not affect the functioning of mesophyll tissue).

It may be possible that ABA influences organic ion production associated with stomatal opening. Huber and Sankla (1974) have reported that, in vitro, ABA actually stimulates malate dehydrogerase activity and this may result in an alteration of malate levels in the guard cells which might affect stomatal opening, but as Willmer and Dittmer (1974) have reported, the role of malate within guard cells has not yet been fully elucidated.

Several groups of workers have also reported that ABA increases the permeability of plant tissues to water (Glinka and Reinhold, 1971, 1972; Glinka, 1973; Collins and Kerrigan, 1974), although this conclusion is not universal (Cram and Pitman, 1972). As well, alterations in ion uptake patterns by tissues occurred in ABA treated tissues. Van Steveninck (1972) observed a marked change in the preference between K^+ and Na^+ in beet root tissues, and Collins and Kerrigan (1974) observed a decrease in K^+ ion levels in the root exudate of ABA treated root systems. These results provided further evidence to support the observation of Mansfield and Jones (1971) that ABA affected K^+ uptake into guard cells. It also

suggests that ABA could affect the permeability of guard cells. However Cummins (1973) suggests it would be difficult to explain the reopening of stomata on epidermal strips when the ABA source is removed as observed by Horton (1971), because most cells in the epidermis would be ruptured, except the guard cells (and presumably the subsidiary cells) thus making it difficult to be due to a differential turgor effect.

Thus at this point in time, the role of endogenous ABA in stomatal functioning is not really clear. Further discussion on this will be made in a later section.

C EFFECTS OF WATER STRESS ON THE PHYSIOLOGY AND YIELD OF PEA PLANTS

1 CHANGES IN APICAL DOMINANCE

Contrary to expectations, the pattern of plant responses to wilting stress cycles at different stages of growth were somewhat different to those observed by Salter (1963) because lateral growth initiated during the earlier wilting cycles grew rapidly and affected a number of the parameters of the final yield analysis. As Table IV illustrates, basal shooting has been markedly stimulated by stress cycles imposed during the preflowering and flowering stages, and to a lesser extent at the flat pod stage. It is well known that such environmental factors as nutrition (Gregory and Veale, 1957) and water relations (Remy, 1968; McIntyre, 1971, 1973) can affect the apical dominance of plants. The relatively high nitrogen nutrient feed, and low stress conditions (as compared with the data of McIntyre, 1971) would have been expected to have been conducive to lateral growth. However, control plants and plants stressed at the pod swell stage had markedly less lateral growth compared to other stages. This suggested that, at some stage during the stress cycle, a trigger action releasing these basal buds occurred, particularly for the first two water stress cycles.

Gregory and Veale (1957) reported a growth potential gradient existed in the stems of flax plants, the lower sections having a greater potential

for growth. This suggested some hormonal gradient may be involved and the work of Thomas (1972) supports the idea of gradients of growth regulators in stems of clover. Much of the earlier work on apical dominance was reviewed by Phillips (1969) and the hormonal role was based on the assumption that IAA was the predominating factor in determining apical dominance. However, more recently Shein and Jackson (1971) disputed this and considered the phenomenon of apical dominance depended upon a delicate balance of various hormones within the plant, in their case IAA, gibberellins and cytokinins in particular. ABA was also suggested to have a possible role on the basis of the report by Arney and Mitchell (1969). Tucker and Mansfield (1972) also demonstrated a possible involvement of ABA. An even more interesting report is the discovery of Firn et al (1972) of high levels of a potent neutral inhibitor, xanthoxin, in young tissues of plants. This level appeared to decline as the tissues become physiologically older, in particular in leaves and stems. Pratt and Goeschl (1969) mention ethylene has also been reported to affect apical dominance.

This concept of hormonal balance is important in relation to water stress effects on apical dominance, because there are major changes in the levels of several hormones in plants subjected to water stress, in particular ABA, cytokinins, and possibly also ethylene.

A possible explanation for the basal shooting observed may be related to the increase in endogenous cytokinin levels to levels greater than controls, observed in the exudate of tobacco root systems (Itai and Vaadia, 1965). If this boost also occurred in peas, it may have been enough to release these basal buds from apical dominance. Differences between the effects of water stress at different growth stages may have been attributable to either the magnitude of this boost or the changing relationship of this increase in cytokinin levels, upon rewatering, with respect to the other hormones present.

Evidence to support the possible role of a change in hormonal balance to favour cytokinins such that it stimulates basal shooting comes from several sources. Firstly, the data of Itai and Vaadia (1965) and the review by Hall (1973) suggest that a major source of cytokinins is plant roots. Also Thomas (1972) contends that the basal factor, playing an important role in stem development in the apical region, includes cytokinins and gibberellins. It has also been observed that cytokinins will release lateral buds from apical dominance (Kulasegaram, 1969a, 1969b; Shein and Jackson, 1971; Usciati et al, 1972), and also play a role in their determination (Woolley and Wareing, 1972a, 1972b). Gibberellins have also been observed to promote lateral outgrowth (Katsumi and Ikeo, 1970; Kulasegaram, 1969a; Shein and Jackson, 1971); however, as Woolley and Wareing (1972c) suggest, a delicate balance between auxins, cytokinins, and gibberellins probably exists in determining lateral outgrowth. Thus it may be a balance of both cytokinins and gibberellins which is required, possibly with cytokinins as the predominating factor for the initiation of growth and gibberellins more important in continued growth. Reid and Railton (1974) have reported that there appears to be some mutual relationship between cytokinins and gibberellins, at least in plants subjected to flooding stresses where endogenous levels of cytokinins and gibberellins are low, as applications of synthetic cytokinins stimulated gibberellin levels.

Since it also appears that cytokinin levels may depend on root activity (see Hall, 1973) the size of the cytokinin boost upon rewatering, if it occurs in peas, might be determined by root activity and this, according to Salter and Drew (1965), drops off rapidly after the flowering period in pea plants.

It seems quite reasonable to expect that some modification of the endogenous hormonal balance, possibly to favour cytokinin levels and

thus availability to the lower buds, could explain this enhancement of basal shooting on pea plants, particularly during the earlier stages of the growth cycle.

2. SENESCENCE

The data showing the proportion of the leaf area which has senesced (Table IV) illustrate the effect of wilting cycles at different growth stages. Little effect resulted from stressing at the preflowering stage but plants stressed at the flowering stage show a significant increase in the proportion which have senesced. Plants stressed at the flat pod stage show a similar level of senescence to those stressed at the flowering stage. However water stress at the pod swelling stage caused a further marked increase in the proportion of leaves which have senesced. This progression agrees well with the conclusion of Salter and Goode (1967) that the flowering and pod swelling stages of the pea life cycle are the most sensitive to moisture stress. These leaves senesced rather than abscised. In fact few leaves actually separated from the plant, hence the ability to determine the percentage of senesced leaves.

Obviously, as senescence is hastened by ABA (Addicott and Lyon, 1969), and ethylene (Pratt and Goeschl, 1969), and several groups of workers (Jordan et al, 1972; McMichael et al, 1972) have shown increases in the endogenous ethylene levels in cotton leaf petioles on plants subjected to water stress, alterations in the levels of these two hormones relative to the overall hormonal balance, are probably of major importance in the hastening of leaf senescence. ABA levels in these experiments have increased markedly in leaves in response to stress.

The senescence effect, as mentioned, probably depends on a hormonal balance as ABA levels have risen to high levels in leaves which did not senesce. Also Jordan et al (1972) determined that ethylene levels in the petioles of upper leaves, as well as lower leaves also increased

markedly.without senescence or abscission occurring. This suggests that auxin levels, which are higher in these upper leaves, are high enough to prevent triggering of senescence. Decreases in cytokinin levels probably also contribute to the hastening of senescence as Itai and Vaadia (1971) have pointed out. Similarly a decrease in gibberellin levels (if it occurs in water stressed plants) would also be expected to hasten this process.

3 YIELD OF HAULM AND PEAS

The total haulm dry weight, as affected by water stress at different developmental stages, does show some trend even though affected by basal shooting. Little change occurred due to the preflowering wilting cycle but a significant increase compared to control values, was observed for plants stressed at the flowering stage. Stress at the flat pod and pod swelling stages resulted in significant decreases. Stressing at both the flowering and pod swelling stages resulted in some effect of the latter cycle being imposed on the promotory effect of the former, however the overall value is not significantly different to the control value. It appears that at the later stages, in particular pod swelling, marked reductions in haulm weight due to water stress have occurred.

The number of peas per plant follows a similar pattern being least for plants stressed at the pod swelling stage, and most for plants stressed at the flowering stage. Trends in pea yield expressed as fresh or dry weight also show this pattern because pods developing on the laterals were also maturing at the time of final harvest.

Overall it appears under the controlled environmental conditions used for this experiment and with the variety 'Victory Freezer' there would be some advantage in subjecting these plants to a wilting stress cycle at the preflowering or flowering period, thus resulting in increased pea yields. However one must be wary in comparing this with the field situation

because environmental conditions in the field are likely to favour apical dominance to a greater degree (due to greater stresses on the plant) and so the effects of wilting stresses at these different developmental stages would likely be different, perhaps more in line with the findings of Salter (1963).

D WHAT ROLE DO ENDOGENOUS PLANT HORMONES PLAY IN RESPONSES TO WILTING STRESS CYCLES

There certainly are marked changes in hormone levels of plants subjected to water stress and a number of workers consider these changes play an important part in the protection of the plant during periods of water stress (Tal and Imber, 1971; Livne and Vaadia, 1972; Mizrahi and Richmond, 1972b; Hiron and Wright, 1973). By what means they provide protection is not entirely clear as yet but some interesting possibilities could be proposed.

One possibility is that plant hormones may be playing a role in stomatal functioning (Imber and Tal, 1970). However, the contention of Tal and Imber that auxins may be involved was based on little evidence. The data of Ferri and Lex (1949), Bradbury and Ennis (1952) and Mansfield (1967), all relate to the use of synthetic auxins at relatively high concentrations. Zelitch (1961) observed that IAA, even at concentrations as high as 10^{-2}M, had little effect on stomatal aperture in tobacco leaves. Some evidence of indirect effects was provided by French and Beevers (1953) who have shown that 10^{-5}M. IAA stimulated respiration in leaves thus increasing internal CO_2 levels which might have some part in inducing stomatal closure. However, Tamas et al (1972) observed that IAA stimulated photosynthesis of isolated chloroplasts thereby reducing internal CO_2 levels, and so even indirect effects seem uncertain.

Cytokinins were shown to cause increases in stomatal opening, but as discussed in the preceding literature review, in most cases the effects

were indirect due to stimulation of photosynthesis and thus reduced internal CO_2 levels. Some workers observed direct effects in excess of the above indirect effect but they were confined to monocotyledonous species (Luke and Freeman, 1968). The explanation of Pallas and Box (1971) that kinetin reduced the free pool of osmotically active materials in leaf epidermal cells suggested two things. Firstly, the supposed direct effect could have been hydropassive due to the reduced turgor of epidermal cells, and secondly, because only some species responded, there may have been some relationship with endogenous cytokinin levels.

Gibberellins were also reported to have stimulated transpiration, probably due to increased stomatal aperture (Livne and Vaadia, 1965). However, the effect was likely to have been indirect as Treharne and Stoddart (1968) demonstrated that gibberellins can stimulate photosynthesis, thus reducing internal CO_2 levels and increasing stomatal aperture. Also, Horton (1971) determined neither gibberellins nor cytokinins stimulated stomatal opening in epidermal strips thus eliminating any possibility of direct effects of these hormones on stomata in his experiments.

Even the role of endogenous ABA in the stomatal functioning of plants subjected to water stress seems unclear. Many experiments have dealt with exogenous applications of ABA and, where endogenous levels have been examined, there appear to be some areas of uncertainty. In the experiments with peas, there was a suggestion that stomata were closing even before ABA levels increased, an occurrence which Hsiao (1973) also suspects. Also, these same experiments have shown that during the recovery phase, ABA levels appear to return to normal long before stomatal apertures do. As well, a similar pattern of reopening appears to occur with each stress cycle whereas the maximum level of inhibitor decreases with each later developmental stage subjected to a wilting cycle.

One could postulate that because leaf water potential would decrease

more rapidly around the points of exit of the transpiration stream (i.e. stomata), localized increases in ABA could occur. Stomatal cells are such a small proportion of the total cell number in leaves so that some effect on their ABA levels may be occurring with little change in the overall endogenous levels measured in the whole leaf tissue. During the recovery phase, it could be postulated that some localized persistence could occur in the vicinity of the stomata and that it may also be difficult to detect when using whole leaf sections. However, evidence to support these ideas has yet to be forthcoming. Cummins et al (1971) considered for continued stomatal closure the continued presence of ABA was necessary. One wonders if ABA is persisting in sufficient quantities to explain the magnitude of the delays in reopening observed in these experiments, particularly for the later stress cycles when the maximum levels of ABA attained were so much lower. Another consideration, however, is that higher levels of ABA could possibly cause some persistent effect on reopening by affecting some process which takes some time to recover in the absence of ABA because Willmer and Dittmer (1974) note that CO_2 fixation in stomata appears to be quite different to that of mesophyll tissue because of the possible involvement of Crassulacean acid metabolism. Thus it could be possible that ABA is affecting some point in this metabolic system.

Another possible role of these changes in endogenous hormone levels in plants subjected to water stress may be related to regulation of internal processes. It has been reported that ABA will inhibit cell enlargement (Arney and Mitchell, 1969; Isaia, 1971; Dorffling, 1973; Newton, 1974), and possibly also cell division (Nagl,1972). Also, subjecting pea plants to wilting stress cycles resulted in a marked enhancement of senescence of lower leaves. This is also probably related to changes in hormone levels, in particular ABA. However, effects on cell expansion are also

noted with small decreases in water potential suggesting that effects may be occurring before any increases in ABA occur. Any permanent effects such as leaf senescence, probably do relate to alterations in hormonal balance, the effect depending on the organ and the physiological age.

In short term stresses however, where much of the growth suppression is recovered upon rewatering (Gates, 1968; Acevedo et al, 1971), these hormonal changes probably play a more dominant role in protection, particularly in the younger tissues which are responsible for maintaining organization for survival (Gates, 1968). To support the concept that these changes are related to a modulating role, it was observed that during the experiments with peas, the maximum ABA levels were relatively low, even at the stage of greatest increase being only about 0.3 ppm (on a fresh weight basis). At these low levels, to maintain an effect e.g. on stomata (Cummins et al, 1971), or cell vacuolation (Arney and Mitchell, 1969), the continued presence of ABA is required. Thus a suspension of synthetic processes, but not impairment (as Gates, 1968, suggests), could be explained in terms of a modulating role.

Because of the finding of ABA associated with chloroplasts (Railton et al, 1974) and possible localisation there, plus the observation of Jones and Mansfield (1972) that ABA painted on to leaves had only a localized effect, it could be postulated that ABA is having a localized action on some of the processes associated with leaf chloroplasts, and what is more, in a reversible manner. The conclusion that ABA generally depresses enzyme activity (Addicott and Lyon, 1969) and, in particular, the suggestion that it can depress RuDP carboxylase activity (Wellburn et al, 1973) suggests it may be involved in a temporary shutting down of such anabolic processes as photosynthesis and protein synthesis. Decreases in cytokinin and gibberellin levels would be expected to hasten this suspension because of their role in the maintainance of protein synthesis (Romanko et al, 1969;

Whyte and Luckwill, 1966), and possibly also photosynthesis (Wareing et al, 1968; Treharne and Stoddart, 1968). ABA could shut down these processes by effects on permeability and/or ionic balances (Reid and Bonner, 1974), via the induction of ribonuclease activity (van Overbeek et al, 1969), or due to effects on the activity of other hormones e.g. cytokinins (Back et al, 1972) and gibberellins (Barnes and Light, 1969). The fact that Graziani and Livne (1974) observed little effect of desiccation on the rate of photosynthesis of epidermis-free leaves was probably related to the rapidity of desiccation, allowing little time for alterations in hormonal balance to occur and exert any effect. However, if stress induction does cause a marked change in endogenous hormone levels, one could imagine a slowing down of processes which required continued protein synthesis for maintainance of action, such as Romanko et al (1964) have described for chloroplast action.

As well, photosynthetic rates could be affected by decreased intracellular CO_2 transport, and Jones and Slatyer (1972) have demonstrated that this can occur in plants subjected to water stress. Unfortunately, no measurement of photosynthetic rates or associated diffusive resistances was made during these experiments on peas.

Not all ABA effects may be localized as ABA has been found in meristems (Most, 1971), and also in roots (Tietz, 1971; Kundu and Audus, 1974). Thus the possibility of transport of ABA to other plant parts during conditions of water stress cannot be discounted. Ingersoll and Smith (1971) have demonstrated ABA transport is possible in excised plant segments and more recently, Hocking et al (1972) observed marked basipetal transport in bean plants. Davison and Young (1973), 1974) have also detected the presence of ABA in xylem tissues.

The movement of ABA to roots may have some significance in relation to the reported effects of changes in hormone levels on plant water status. It has already been mentioned that ABA enhances tissue permeability to

water. Glinka and Reinhold (1971) also observed that kinetin decreased tissue permeability to water. Tal and Imber (1971) observed similar effects of ABA and kinetin on the sap flow of decapitated plants as those reported above. Hasmann and Onder (1972) however, reported a slight increase in tissue permeability in the presence of kinetin contrary to the above. Tal and Imber (1971), and Hasmann and Onder (1972) reported that auxin also increased tissue permeability to water (although recently Dowler et al, 1974, suggested auxin had no effect on the permeability of pea segments to tritiated water). Another important observation associated with these hormonal effects was that the effects on tissue permeability to water occurred independently of any effect on osmotic potentials.

As well as permeability effects, alterations in ionic balances have been observed, particularly with respect to potassium (van Steveninck, 1972; Reid and Bonner, 1974), in the presence of ABA. Cram and Pitman (1972) suggested a more general decrease in ion uptake occurred thus preventing the build up of ions in water stressed plants and another important observation of theirs was that the ABA effect appeared to be related to ion secretion into the xylem, rather than uptake itself, thus ABA transport to the roots of plants subjected to water stress, if it occurs in water stressed plants, may be playing a role here.

What becomes important from this discussion is the fact that ABA, which increases tissue permeability to water, increases in quantity in stressed plants, whilst cytokinins which cause the reverse, decrease. Thus the rapid recovery of leaf water status observed in pea plants could possibly be related to these alterations in hormonal balance. Hiron and Wright (1973) observed that bean leaves subjected to a mild stress at first wilted, but as endogenous ABA levels increased they recovered even though the conditions persisted. This suggested ABA was playing a major role in turgor recovery. Similar views were expressed by Mizrahi and Richmond

(1972b). In fact the work of Mizrahi et al (1970, 1971, 1972) and Mizrahi and Richmond (1972a) suggested ABA may be playing a protective role in the adaptive response of plants to any factor affecting plant water status.

Thus in short term water stress cycles, as in these experiments with peas, where marked recovery of plants occurs upon rewatering the hormonal balance may be altered to produce two major effects. The first could be a suspension of synthetic processes as growth slows down due to increasing water stress, and the second could be the provision of conditions favourable for maximum turgor under the conditions prevailing, and also for rapid turgor recovery upon rewatering. The fact that some lower leaves senesced on pea plants during these cycles was probably related to changes in their internal hormonal balance which set senescent processes in motion, whilst in upper leaves similar changes are related to a protective role during the stress period allowing plant survival and rapid recovery when favourable conditions are again prevailing. Differential sensitivity of different stages of plant growth could be at least partially related to changes in endogenous hormone levels, particularly during the periods of flowering and rapid pod development. In addition photosynthesis can be affected differentially at different stages of growth (Ghorashy et al, 1971). Thus effects on this process may also partially explain the different responses which have been observed. At the pod swelling stage of soybeans, photosynthetic rates were particularly sensitive to moisture stress and this effect has also been observed for peas, only in the latter case in relation to root growth (Salter and Drew, 1965). A lack of photosynthates at a time of high sensitivity to water stress could result in yield losses due to flower abscission, embryo abortion, or reduced rate of growth of reproductive parts (Kaufman, 1972). Because of alterations in photosynthate distribution, e.g. at the time of lower initiation (Jeffcoat and Harris, 1972), it appears that a lesser proportion of photo-assimilates is available

for the root system. Hence at times of high demand by upper plant parts root growth suffers as observed by Salter and Drew (1965). Thus differential sensitivity of different stages of plant development is probably a reflection of relative sink demand which in turn depends on hormonal balances.

A hypothetical scheme associating changes in growth regulator activity in plants suffering short term moisture stress with various plant processes is shown in figure 20.

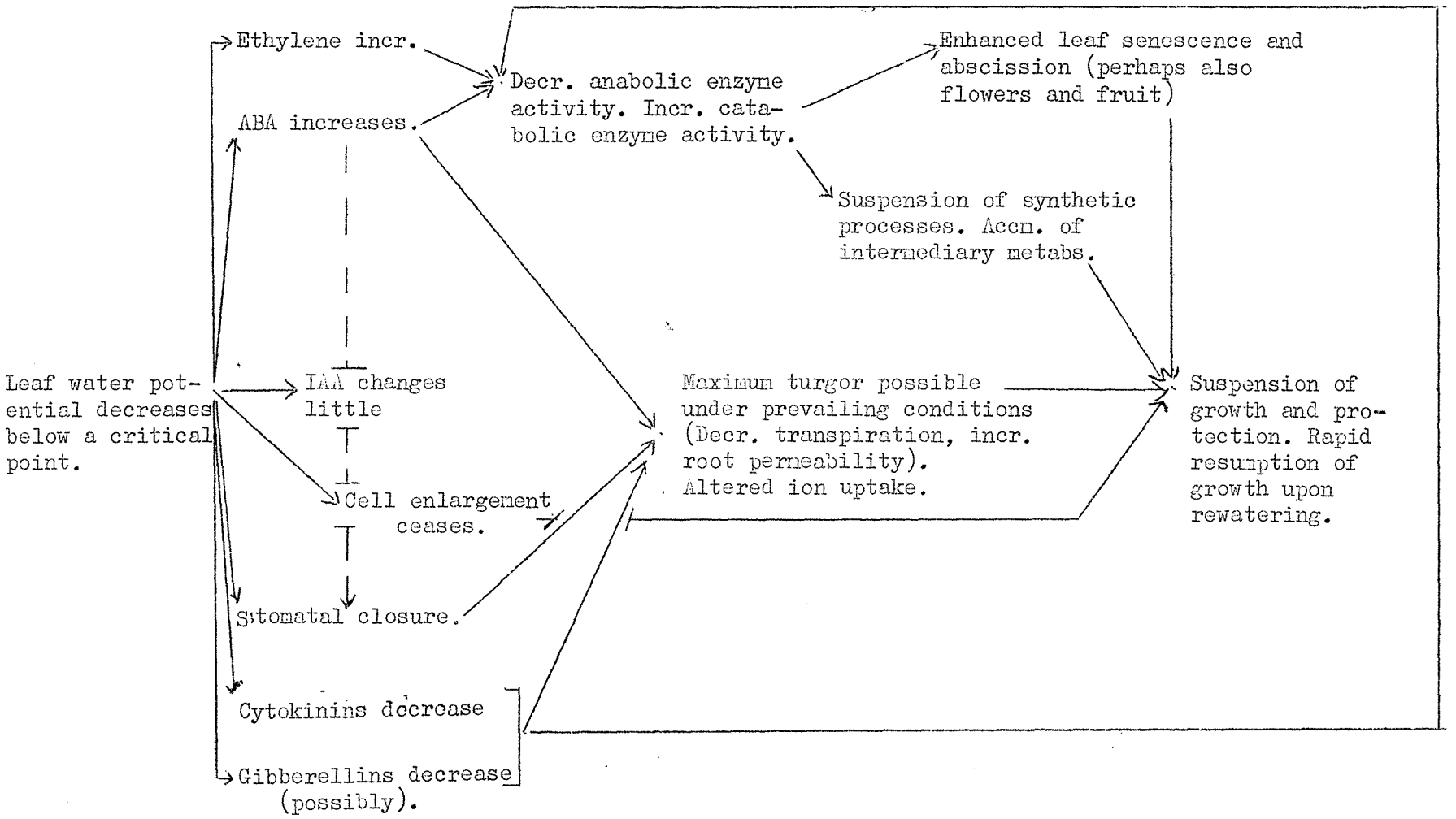


FIGURE 20. Hypothetical scheme for the association of growth regulators with growth processes affected by water stress during short term wilting stresses from which plants can recover rapidly

CHAPTER VI

VI FINAL COMMENT

These experiments, using pea plants, have shown that marked hormonal changes can occur within water stressed plants, in this case inhibitors. Different developmental stages have also shown different responses with respect to inhibitor changes and yield. Changes in the physiology of the plant, as reflected by apical dominance and leaf senescence, also occurred due to the imposition of wilting cycles. However no evidence for explaining the differing sensitivities of different growth stages has been obtained in these experiments and further research into this aspect of plant response to water stress is necessary.

One question which these experiments has raised is what exactly is the role of endogenous ABA in the plant response to water stress, in relation to changes in the activity of other hormones? Future research will have to examine more closely the relationship of ABA levels to stomatal function and determine whether there is any localized effect in or near the guard cells to explain the closing response and delay in re-opening observed during wilting cycles. Are the effects of endogenous ABA entirely direct, and if so, is ABA produced within the guard cells themselves. As yet this has not been fully elucidated either.

Apart from stomatal effects more investigative work is required on the basic modes of action of plant hormones such as ABA and cytokinins. Only then will the real roles of these hormones in plant responses to water stress be known. In addition, it has yet to be shown that these changes are affecting anabolic processes such as protein synthesis in water stressed plants and it would be interesting to determine whether these hormonal changes are associated.

A final point requiring more research is whether ABA is produced in plant parts other than leaves and whether it is transported to other areas in plants subjected to water stress.

At this stage there are still many aspects of the plant response to water stress which require much investigation.

APPENDIX I

APPENDIX I

A Buffer Solutions

1. Phosphate-citrate Buffer.

The following buffer was used for the wheat coleoptile bioassay (Nitsch and Nitsch, 1956)

Material	Quantity
K_2HPO_4	4.485 gm
Citric acid monohydrate	2.547 gm

Dissolve the above in 250 ml of distilled water. For use, dilute 1 in 10 and add 2 gm of sucrose per 100 ml. pH should be 5.3. (Check with pH meter and adjust if necessary).

2. Phosphate Buffer for Commelina communis Bioassay

The composition of this buffer was determined from Willmer and Mansfield (1969).

Two solutions were made

(a) Basic phosphate solution; 2×10^{-2}M

K_2HPO_4	871 mgm/250 ml

(b) Acidic phosphate solution; 2×10^{-2}M

KH_2PO_4	681 mgm/250 ml

Solution (b) was added to (a), using a pH meter, until the final pH was 6.9. This was the double strength buffer used in the bioassay system.

3. Burström's Buffer

To obtain good promotion responses in the Avena coleoptile bioassay it was found necessary to use this particular buffer solution as described by Burström (1973).

Material	Concentration
K_2HPO_4	10^{-3} mol/litre
$Ca(NO_3)_2$	10^{-4} mol/litre
$MgSO_4$	10^{-5} mol/litre
Glucose	16 gm/litre

To this solution 0.1M citric acid was added to give a final pH of 5.6

4. Phosphate Buffer for Polyclar Columns

This buffer is essentially the same as that described in (2) above, except that both solutions (a) and (b) were made up as 0.1M solutions and the acidic solution was added to the basic solution to give a final pH of 8.0.

B. Nutrient Feed

The following is the modified N.C.S.U. phytotron nutrient feed as used in the Climate Laboratory of the Plant Physiology Division of the New Zealand Department of Scientific and Industrial Research, Palmerston North.

Solution A.

Material	Gms/litre stock solution
$NH_4 NO_3$	80.05
$CaNO_3 4H_2O$	159.25
Iron chelate (7% Fe)	29.80

Solution B

Material	Gms/litre stock solution
KNO_3	63.90
$Mg SO_4.7H_2O$	30.81
$Na_2 SO_4.10H_2O$	80.50
$ZnSO_4.7H_2O$	0.025
$MnCl_2.4H_2O$	0.26
$CuSO_4.5H_2O$	0.01
H_3BO_3	0.35
Na_2MoO_4	0.002

Stock solutions are used at a dilution rate of 1 in 500 i.e. 100 ml of each of solutions A and B per 50 litres of water.

APPENDIX II

FIGURE 21 Growth response of wheat coleoptiles to chromatograph sections of the acidic ether fractions from extract of the first mature leaf of pea plants taken during the pre-flowering stress cycle. Shaded areas significant at P = 0.01.

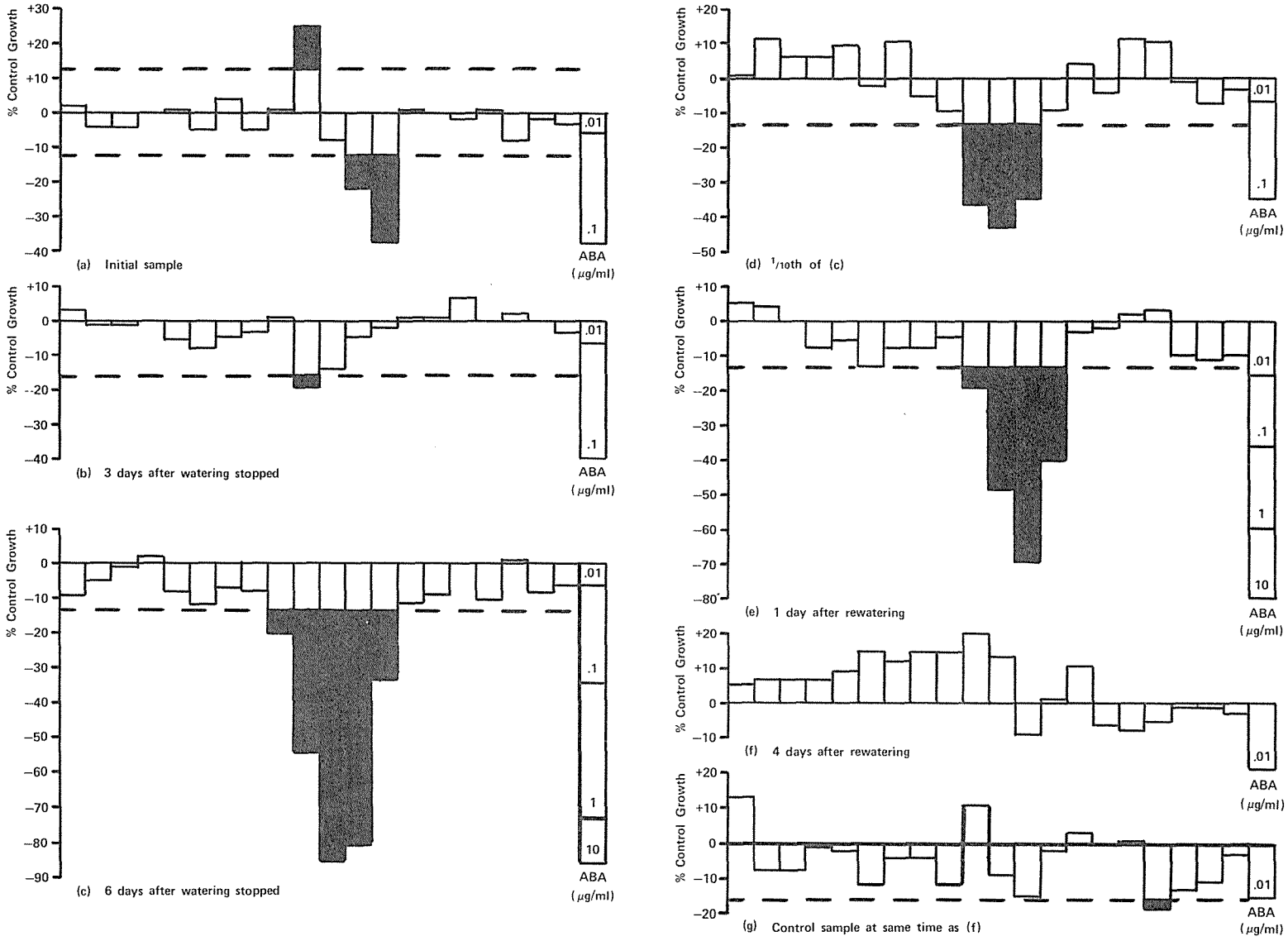


FIGURE 22 Growth response of wheat coleoptiles to chromatograph sections of the acidic ether fractions from extracts of the apical tissues of pea plants taken during the pre-flowering stress cycle. Shaded areas significant at P = 0.01.

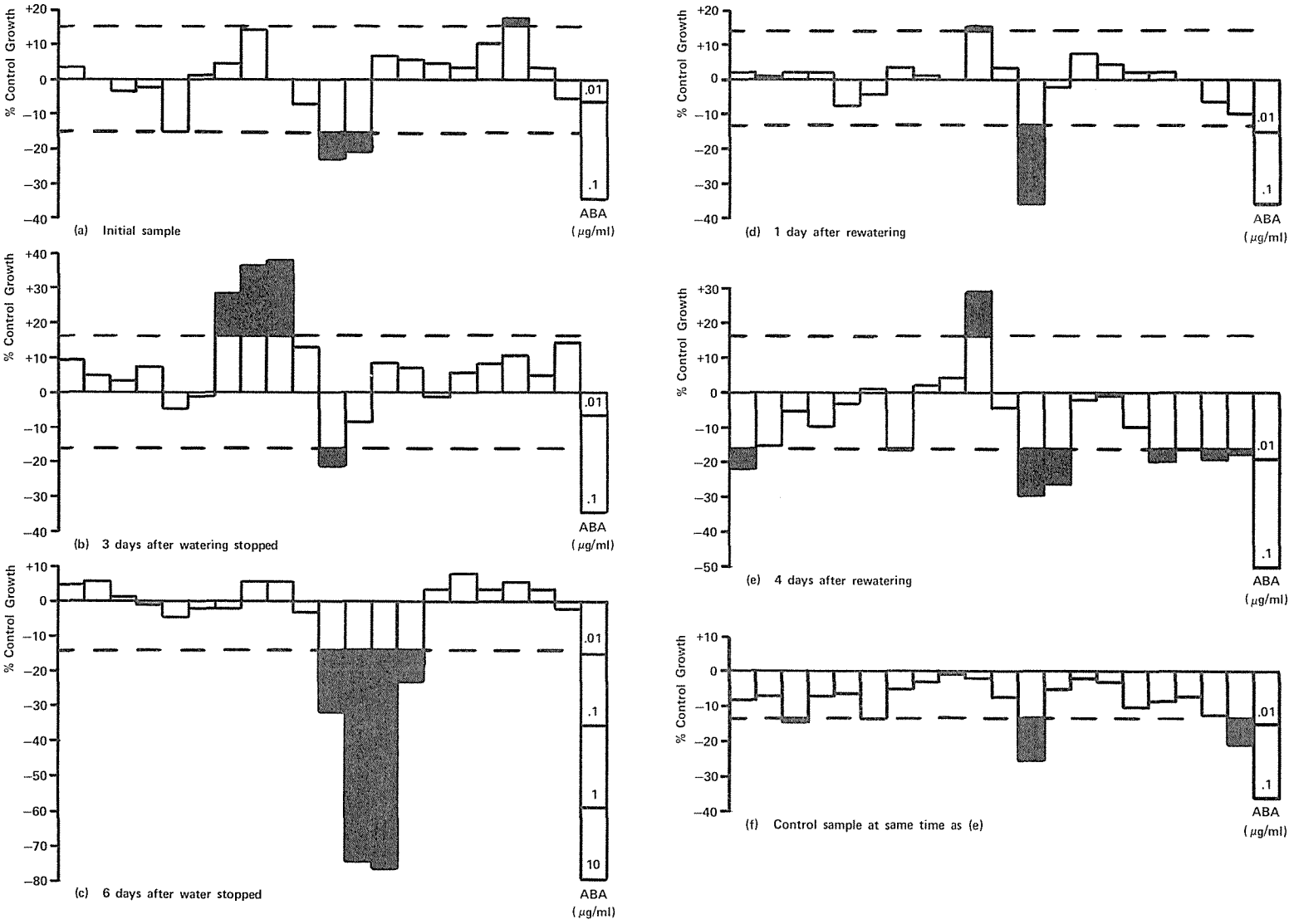


FIGURE 23 Growth response of wheat coleoptiles to chromatograph sections of the acidic ether fractions from extracts of the first mature leaf of pea plants taken during the early flowering stress cycle. Shaded areas significant at P = 0.01.

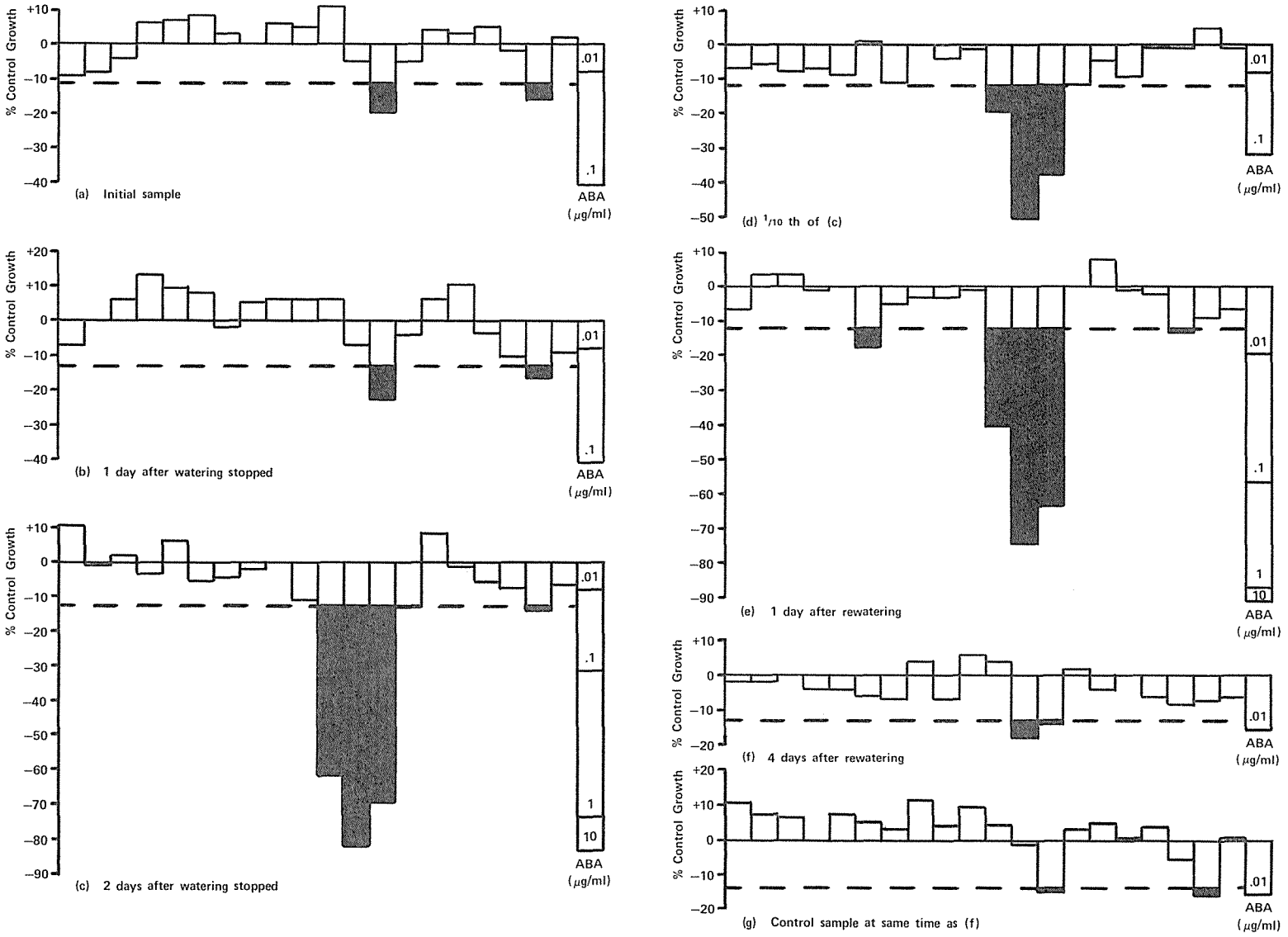


FIGURE 24 Growth response of wheat coleoptiles to chromatograph sections of the acidic ether fractions from extracts of the first mature leaf of pea plants taken during the stress cycle at the flat pod stage. Shaded areas significant at P = 0.01.

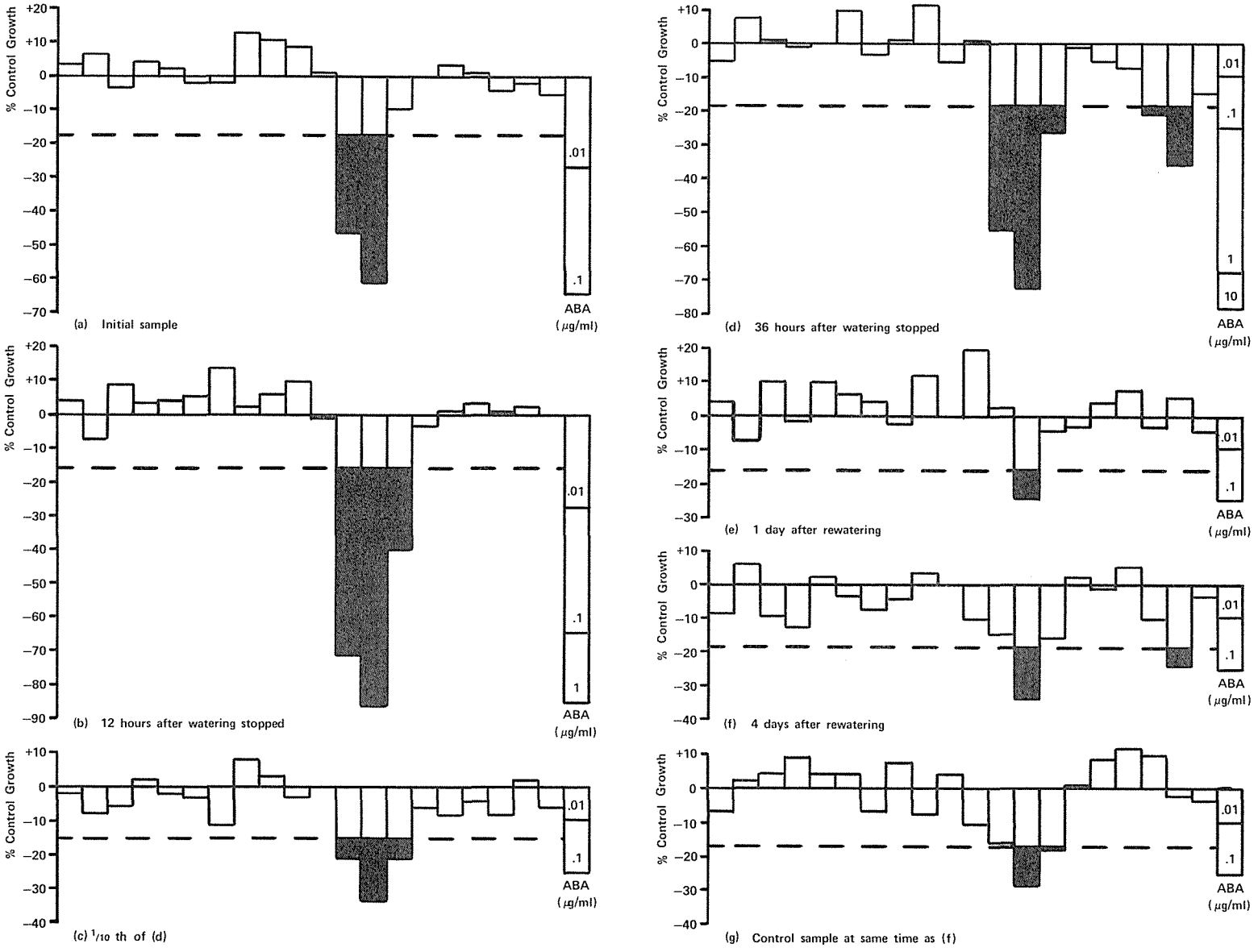


FIGURE 25 Growth response of wheat coleoptiles to chromatograph sections of the acidic ether fractions from extracts of the first mature leaf of pea plants, taken during the stress cycle at the pod swell stage. Shaded areas significant at P = 0.01.

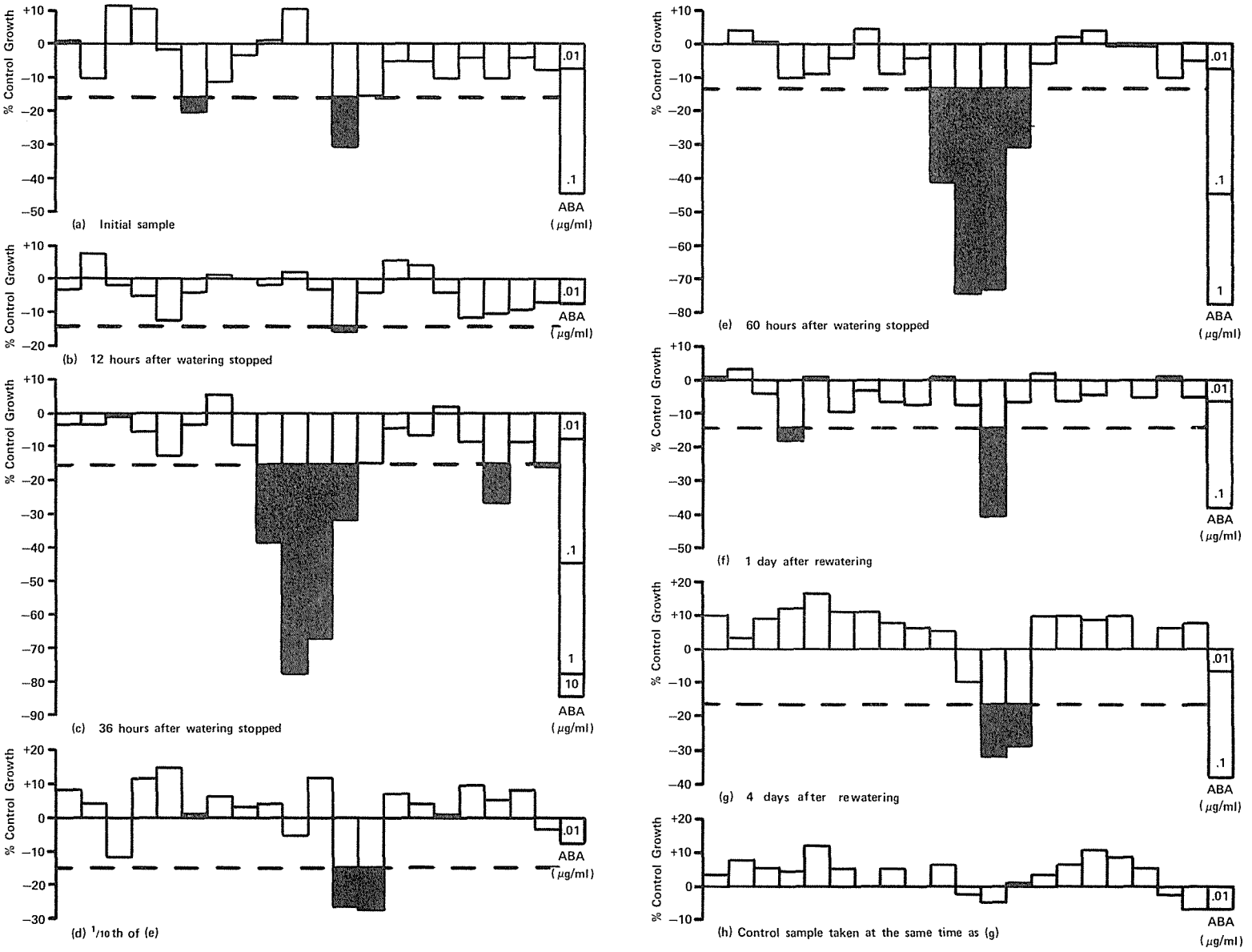


FIGURE 26 Growth response of wheat coleoptiles to chromatograph sections of the basic ether fractions from extracts of the first mature leaf of pea plants taken during the pre-flowering stress cycle. Shaded areas significant at P - 0.01.

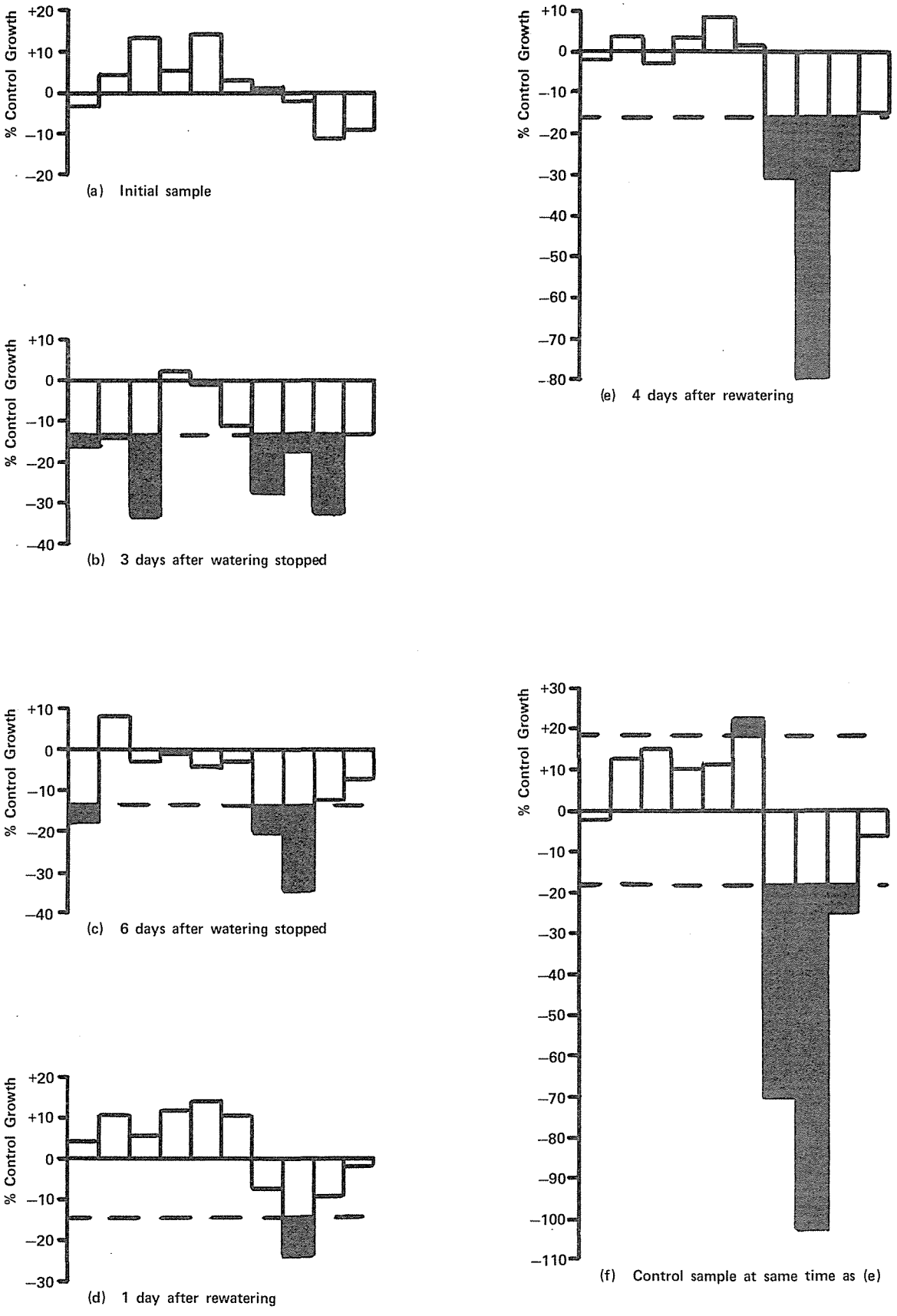
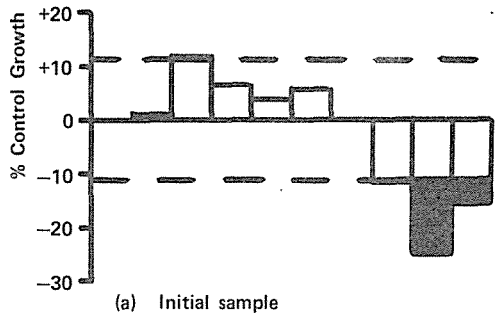
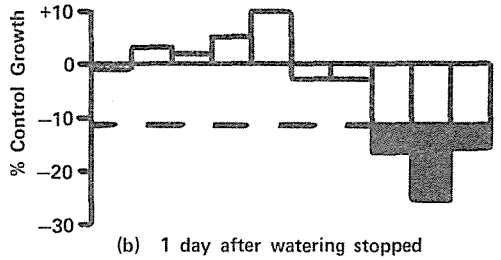


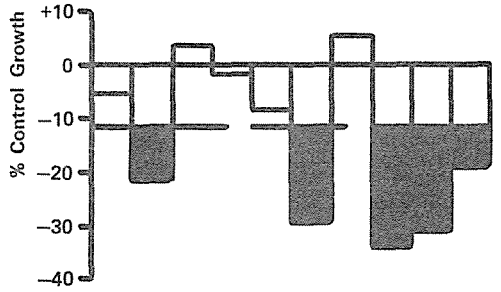
FIGURE 27 Growth response of wheat coleoptiles to chromatograph sections of the basic ether fractions from extracts of the first mature leaf of pea plants taken during the early flowering stress cycle. Shaded areas significant at P = 0.01.



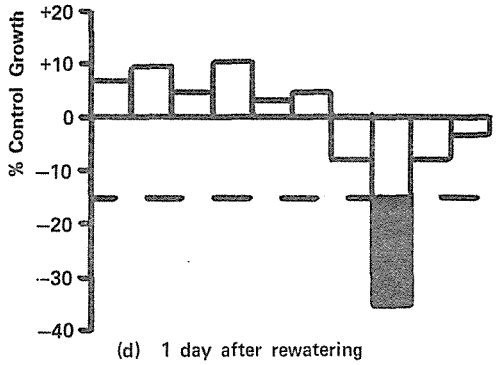
(a) Initial sample



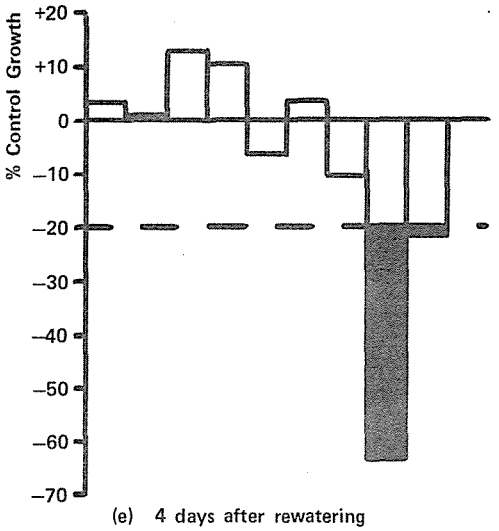
(b) 1 day after watering stopped



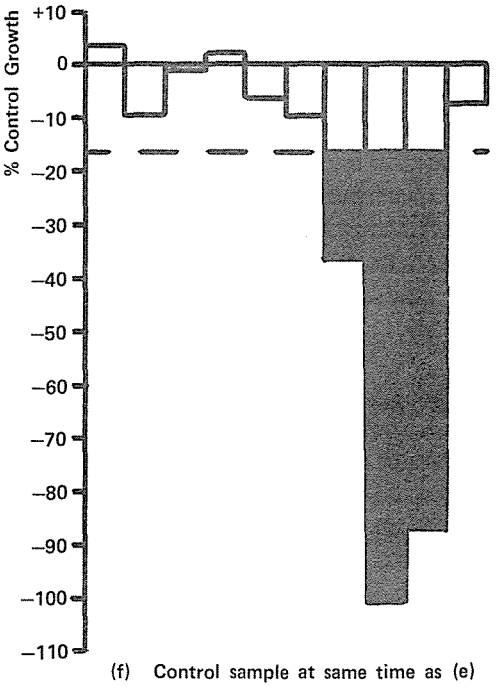
(c) 2 days after watering stopped



(d) 1 day after rewatering



(e) 4 days after rewatering



(f) Control sample at same time as (e)

FIGURE 28 Growth response of wheat coleoptiles to chromatograph sections of the basic ether fractions from extracts of the first mature leaf of pea plants taken during the flat pod stress cycle. Shaded areas significant at P = 0.01.

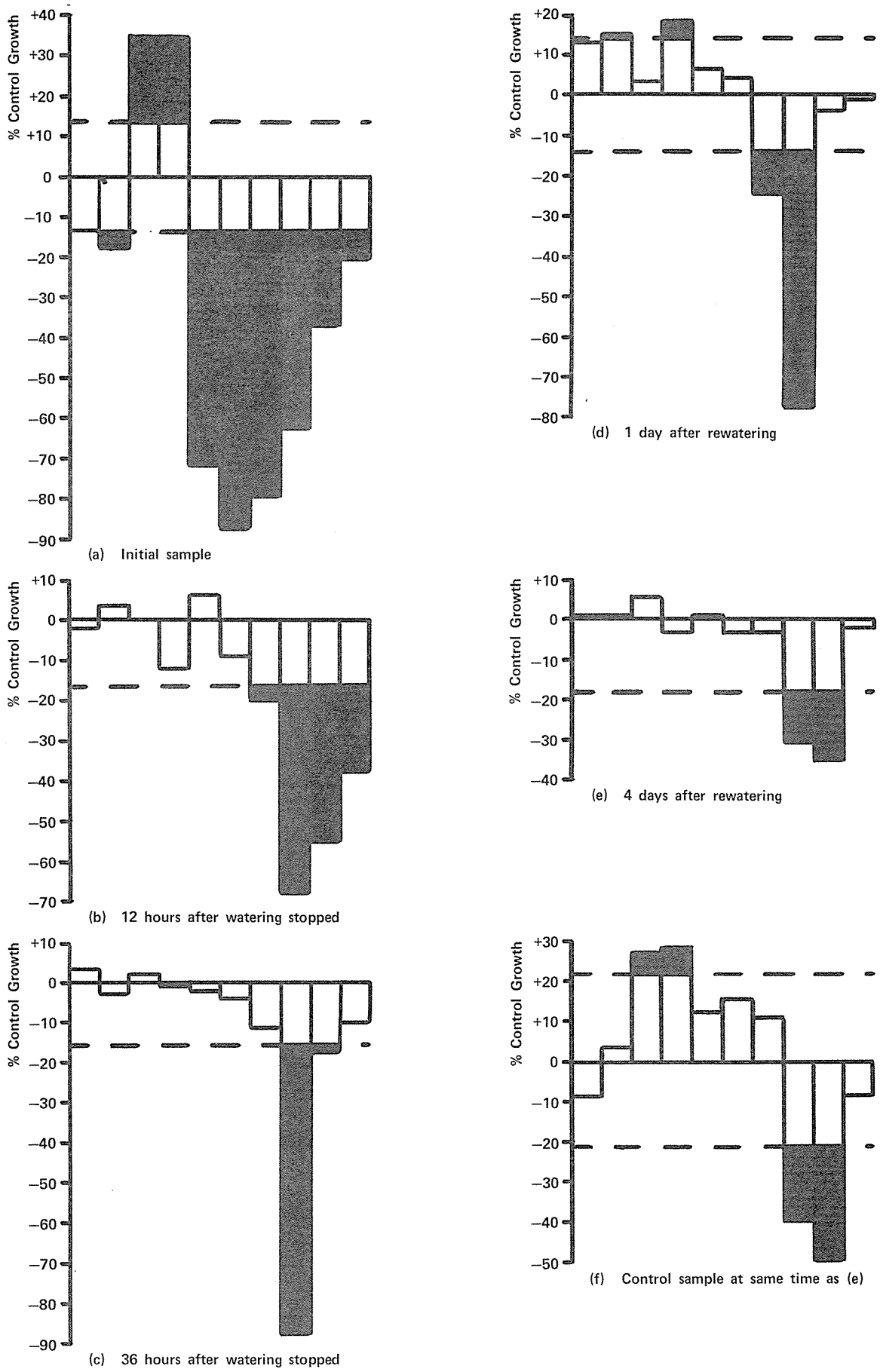


FIGURE 29 Growth response of wheat coleoptiles to chromatograph sections of the basic ether fractions from extracts of the first mature leaf of pea plants taken during the pod swell stress cycle. Shaded areas significant at P = 0.01.

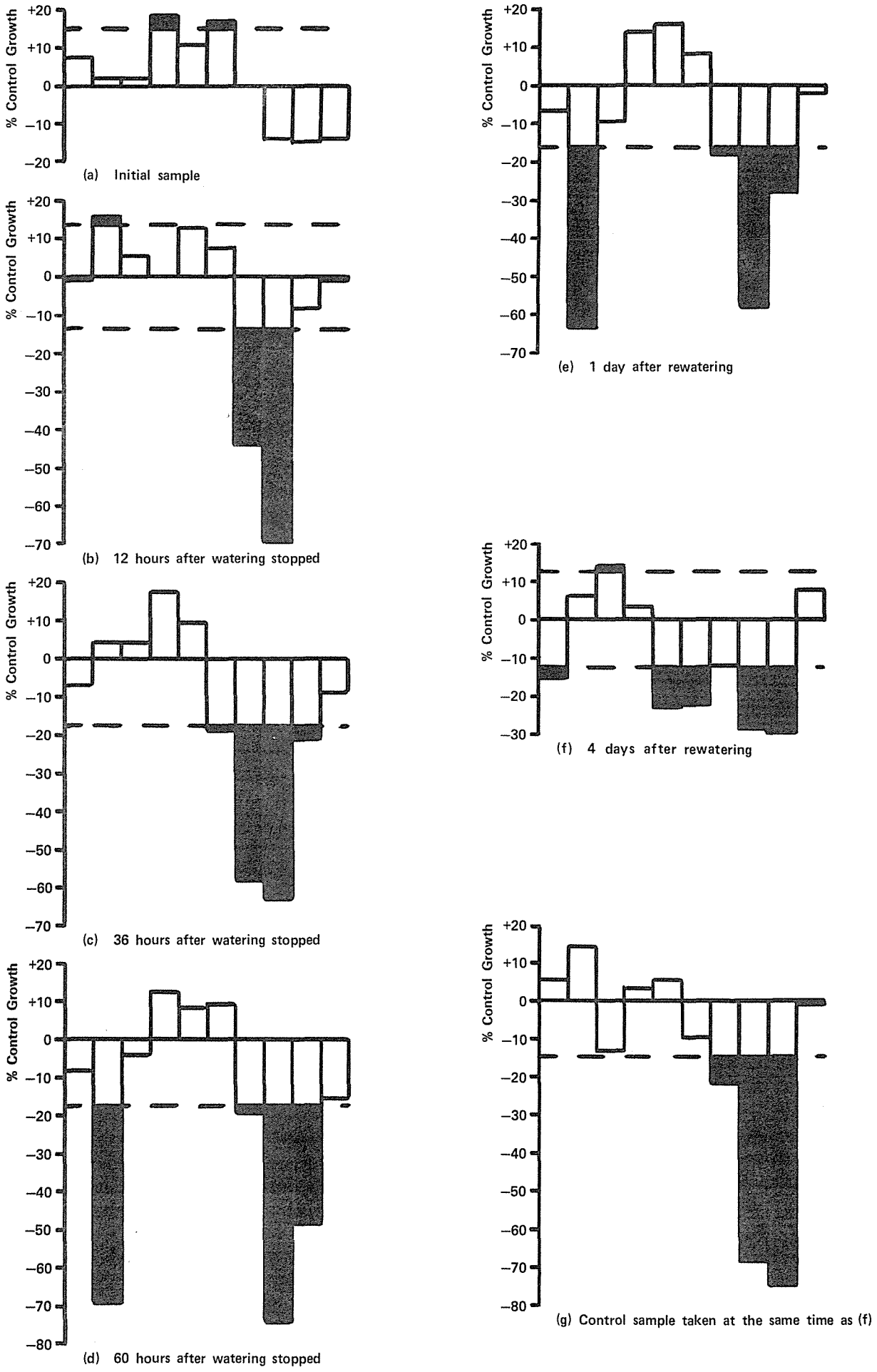
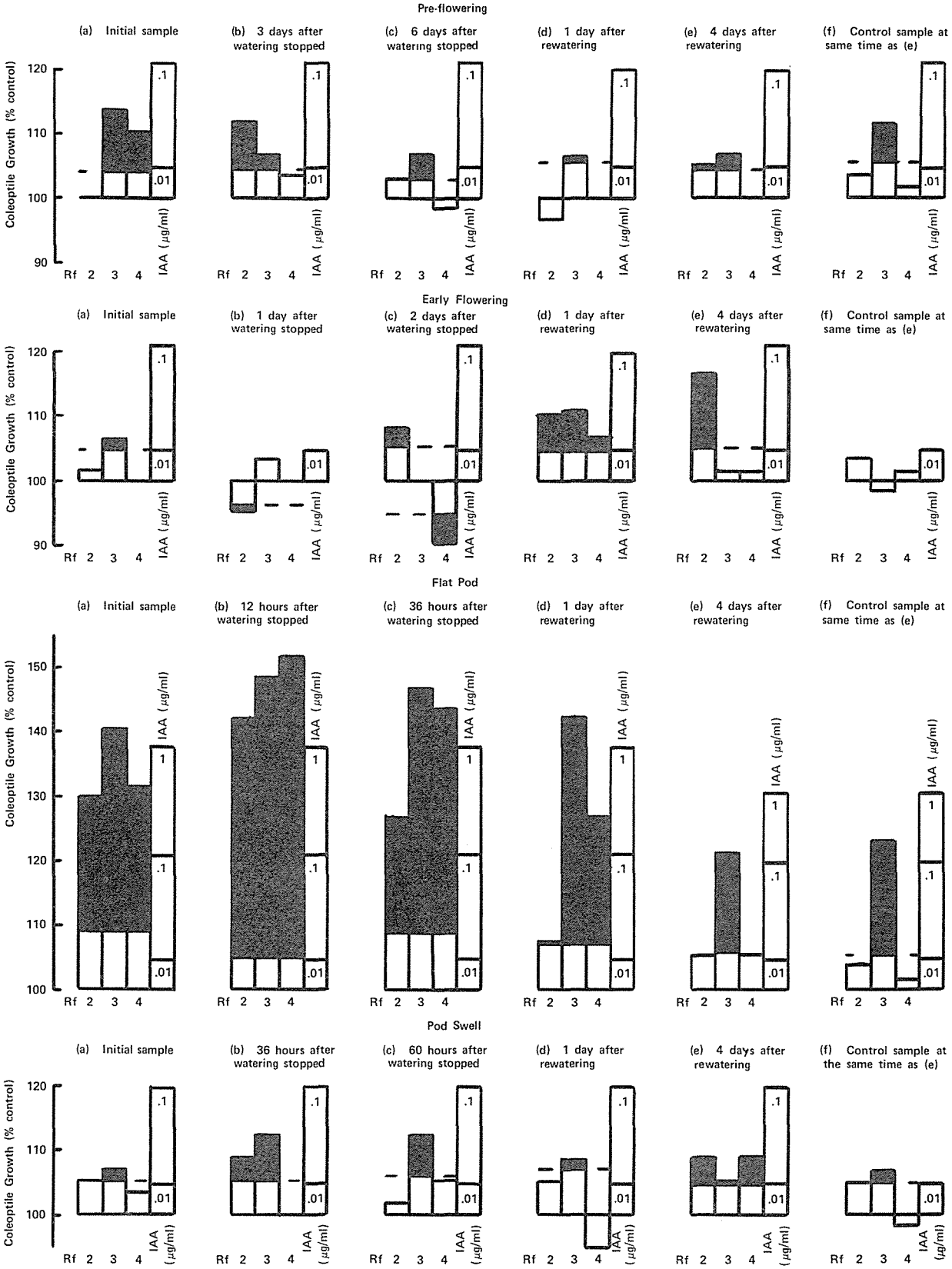


FIGURE 30 Growth response of *Avena* coleoptiles to chromatograph sections of the acidic ether fractions from extracts of the first mature leaf of pea plants. Shaded areas significant at P = 0.01.



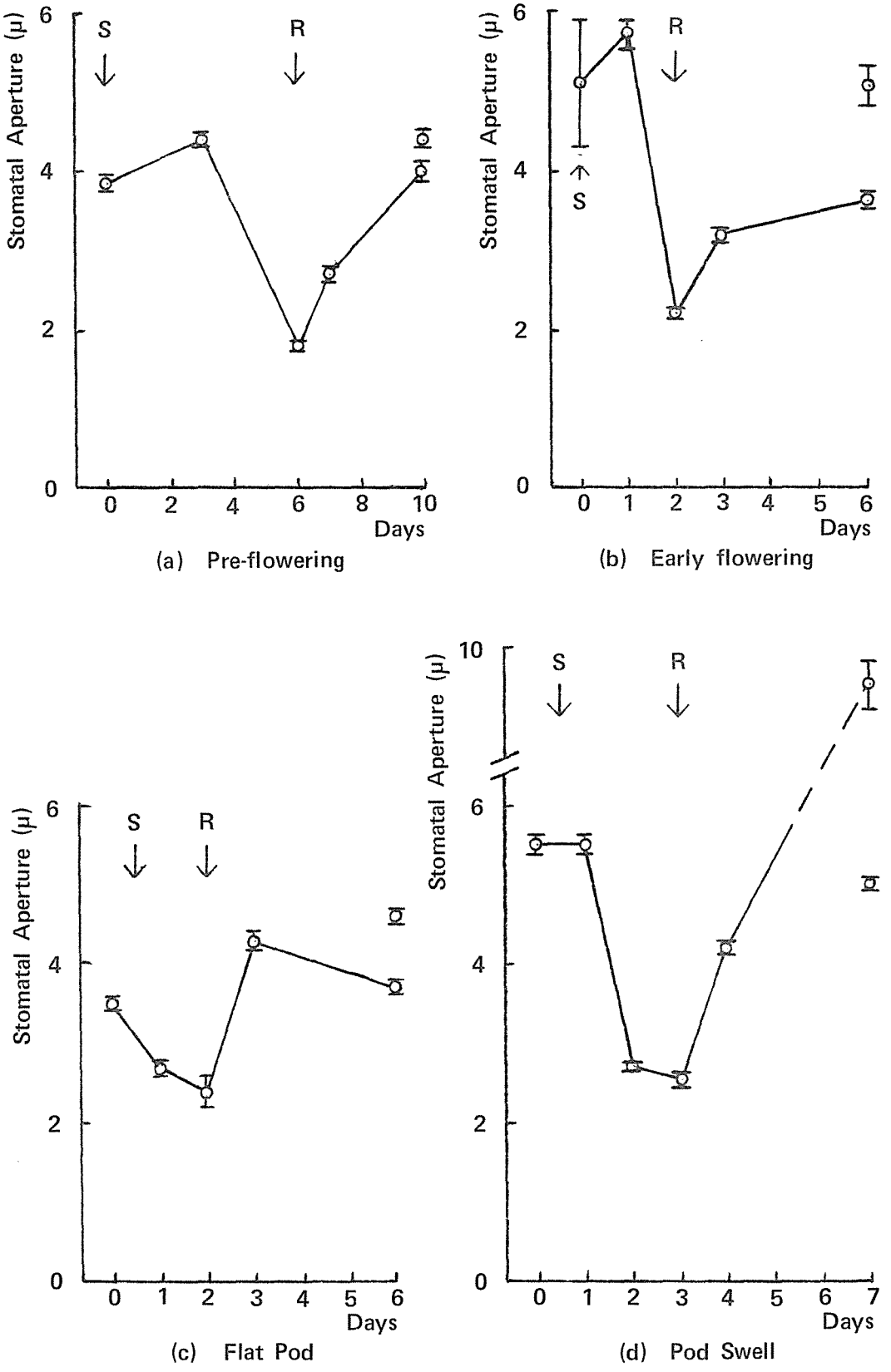


FIGURE 31 Response of stomata on *Commelina communis* epidermal strips to eluates of Rf 5 – 7 from chromatographs of the acidic ether fraction from extracts of the first mature leaf of pea plants taken during water stress cycles. Standard errors shown.

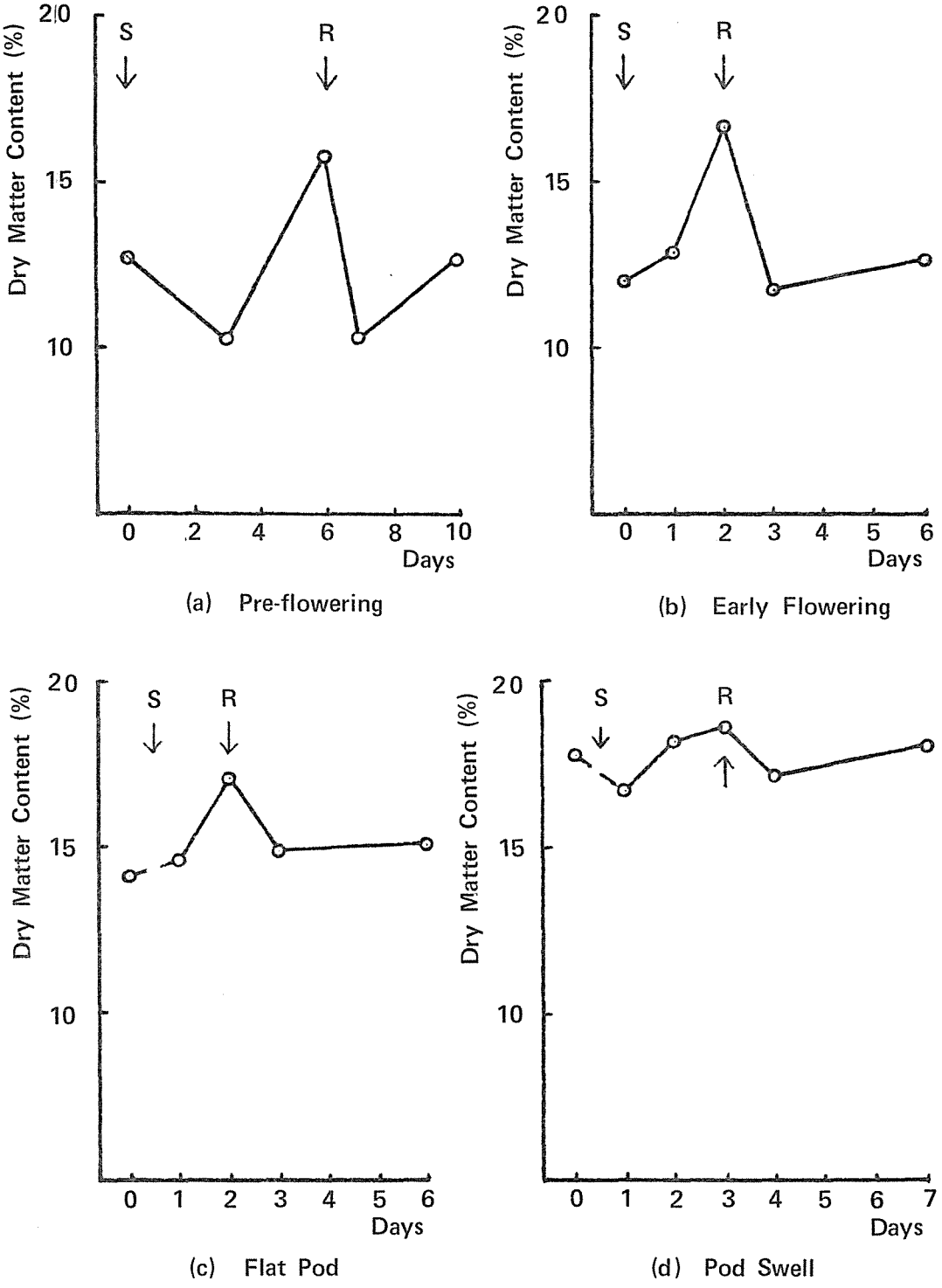


FIGURE 32 Trends in the dry matter content of the first mature pea leaf during wilting cycles.

TABLE V. Final yield data (not shown in Table IV) for pea plants stressed at different stages of growth.

Series	Number of leaves		Pod weight (gms)		Leaf weight (gms)				Stem weight (gms)		Haulm fresh wt (gms)
					Alive		Dead				
	Alive	Dead	F.wt	D.wt	F.wt	D.wt	F.wt	D.wt	F.wt	D.wt	
Control	18.9	14.4	51.41	8.04	33.51	6.08	4.19	1.52	41.12	8.08	158.21
Stress preflowering	22.1	15.1	50.28	7.87	33.68	5.63	5.83	1.29	38.49	7.44	164.15
Stress flowering	24.1	26.2	59.55	8.38	41.67	5.87	7.47	2.70	55.07	9.48	205.00
Stress flat pod	17.2	17.5	42.28	5.97	24.65	3.69	4.12	1.66	29.26	5.08	128.52
Stress pod swell	12.0	18.9	35.79	4.62	15.93	2.59	5.21	2.65	42.77	5.71	111.10
Stress flowering and pod swell	16.5	27.9	41.77	5.56	22.71	3.47	7.11	3.01	38.75	7.18	142.58

APPENDIX III

APPENDIX III

STATISTICS

(A) STANDARD ERROR OF THE MEAN

For calculation of these values the following formula was used

$$S.E. = \frac{\sqrt{\frac{\Sigma (x - \bar{x})^2}{n-1}}}{\sqrt{n}} = \sqrt{\frac{\Sigma (x)^2 - \frac{(\Sigma x)^2}{n}}{n(n-1)}}$$

(B) LIMITS OF CONFIDENCE FOR HISTOGRAMS

Confidence limits were calculated using the short cut method of allowance (Link and Wallace, 1952; Tukey, 1953). The limits are calculated using the following formula.

$$L = \frac{\Sigma (\text{sample ranges}) \times \text{critical factor}}{\text{No of replicates per sample}}$$

This value is plotted above and below the control line and any peaks outside these limits are significant at P = 0.01. Critical values are shown in Table VI.

Example

To demonstrate the simplicity of this proceedure, the data for wheat coleoptile response to chromatograph sections of a neutral ether extract from mature pea leaves. The number of coleoptiles used to assay each section was ten. The range was determined by subtracting the shortest coleoptile from the longest in each case. The mean value for control coleoptile length in this case was 1.89cm.

	Chromatograph section Rf									
Control	1	2	3	4	5	6	7	8	9	10
Range	0.27	0.27	0.15	0.37	0.34	0.10	0.10	0.05	0.37	0.20	0.20

Sum of ranges = 2.42. Critical factor from Table VI for 11 samples, 10 replicates/sample is 0.51.

$$L = \frac{2.42 \times 0.51}{10} = 0.12$$

Any values outside of 1.89 ± 0.12 are significant at P = 0.01

TABLE VI CRITICAL FACTORS FOR ALLOWANCES FOR ONE-WAY (BALANCED) DIVISION INTO GROUPS 1% RISK
(Link and Wallace, 1952)

Replicates/treatment	Number of Groups								Number of Ranges							Number of Treatments			
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
2	7.92	4.42	2.96	2.06	1.69	1.39	1.20	1.03	.91	.82	.75	.68	.63	.59	.55	.51	.48	.46	.43
3	3.14	2.14	1.57	1.25	1.04	.89	.78	.69	.62	.57	.52	.48	.45	.42	.39	.37	.35	.34	.32
4	2.47	1.74	1.33	1.08	.91	.78	.69	.62	.56	.51	.47	.44	.41	.38	.36	.34	.32	.31	.29
5	2.24	1.60	1.24	1.02	.86	.75	.66	.59	.54	.49	.46	.42	.40	.37	.35	.33	.31	.30	.29
6	2.14	1.55	1.21	.99	.85	.74	.65	.59	.53	.49	.45	.42	.39	.37	.35	.33	.31	.30	.28
7	2.10	1.53	1.21	.99	.84	.74	.65	.59	.53	.49	.45	.42	.40	.37	.35	.33	.32	.30	.29
8	2.08	1.52	1.21	.99	.85	.74	.66	.59	.54	.50	.46	.43	.40	.37	.35	.33	.32	.30	.29
9	2.09	1.53	1.22	1.00	.85	.75	.66	.60	.54	.50	.46	.43	.40	.38	.36	.34	.32	.31	.29
10	2.10	1.55	1.23	1.01	.86	.75	.67	.61	.55	.51	.47	.44	.41	.38	.36	.34	.33	.32	.30
11	2.11	1.56	1.24	1.02	.88	.77	.68	.61	.56	.51	.48	.44	.42	.39	.37	.35	.33	.32	.30
12	2.13	1.58	1.25	1.03	.89	.78	.69	.62	.57	.52	.48	.45	.42	.40	.37	.35	.34	.32	.31
13	2.15	1.60	1.27	1.05	.90	.79	.70	.63	.58	.53	.49	.46	.43	.40	.38	.36	.34	.33	.31
14	2.18	1.62	1.28	1.06	.91	.80	.71	.64	.58	.54	.50	.46	.43	.41	.39	.37	.35	.33	.32
15	2.20	1.64	1.30	1.08	.92	.81	.72	.65	.59	.54	.50	.47	.44	.41	.39	.37	.35	.34	.32
16	2.22	1.65	1.31	1.09	.93	.82	.73	.66	.60	.55	.51	.48	.45	.42	.40	.38	.36	.34	.32
17	2.24	1.67	1.33	1.11	.95	.83	.74	.67	.61	.56	.52	.48	.45	.43	.40	.38	.36	.34	.33
18	2.27	1.69	1.34	1.12	.96	.84	.75	.68	.62	.57	.53	.49	.46	.43	.41	.39	.37	.35	.33
19	2.30	1.71	1.36	1.14	.97	.95	.76	.68	.62	.57	.53	.50	.46	.44	.41	.39	.37	.35	.34
20	2.32	1.73	1.38	1.15	.98	.86	.77	.69	.63	.58	.54	.50	.47	.44	.42	.40	.38	.36	.34

Entries are to be multiplied by the sum of the ranges within groups to obtain allowances for group totals

(C) ANALYSIS OF VARIANCE FOR FINAL YIELD DATA

Each set of data selected for Table IV was subjected to an analysis of variance using the F - test to see if any significance was present. If so, the means which were significantly different were determined using Duncan's Multiple Range Test.

Example

Data for % leaves alive

	Sample (i) Control series	(ii) Stress preflg.	(iii) Stress flg.	(iv) Stress flat pod	(v) Stress pod swell	(vi) Stress flg. & pod swell
Rep. 1	54.0	60.7	55.1	65.2	36.0	35.0
2	50.0	56.3	50.7	47.2	57.2	35.3
3	64.9	65.8	48.0	56.8	46.9	33.4
4	65.9	60.5	40.0	48.6	45.7	46.3
5	57.1	55.5	55.1	44.7	31.6	34.6
6	57.9	50.0	42.3	42.8	28.6	27.9
7	42.8	55.8	46.2	45.5	29.2	43.5
8	45.8	66.7	53.3	40.0	34.2	35.6
9	51.3	55.9	46.5	33.3	36.4	41.2
10	65.7	69.5	43.2	57.1	38.1	36.1
Sub-total	555.4	596.7	480.4	481.2	383.9	368.9
Mean	55.5	59.7	48.0	48.1	38.4	36.9
					Grand Total	2866.5

Sums of squares		d.f	variance	F	P=0.05
Between samples	141032.4 - 136947.0 = 4085.4	5	817.1	14.8	2.4
Within samples	144016.9 - 141032.4 = 2984.5	54	55.3		

$14.8 \gg 2.4$ therefore there are significant differences in the above figures.

To determine which means are significantly different they are first arranged in order of magnitude

(vi)	(v)	(iii)	(iv)	(i)	(ii)
36.9	38.4	48.0	48.1	55.5	59.7

The mean error is then determined

$$\text{Error} = \frac{55.3}{10} = 2.35$$

Duncan's values at P = 0.05

No of means	2	3	4	5	6
Value	2.83	2.98	3.08	3.14	3.20
Multiply by error factor	6.65	7.00	7.24	7.38	7.52

Now the ranked means are subtracted from each other and the differences compared with the corrected Duncan's values for significance.

(ii) - (vi) = 59.7 - 36.9 = 22.8 $22.8 > 7.52$ (sig. diff.)

(i) - (vi) = 55.5 - 36.9 = 18.6 $18.6 > 7.38$ (sig. diff.)

(iv) - (vi) = 48.1 - 36.9 = 11.2 $11.2 > 7.24$ (sig. diff.)

(iii)- (vi) = 48.0 - 36.9 = 11.1 $11.1 > 7.00$ (sig. diff.)

(v) - (vi) = 38.4 - 36.9 = 1.5 $1.5 < 6.65$ (not sig. diff.)

This is repeated for (ii) - (v), (i) - (v) etc, and then (ii) - (iii), etc, (ii) - (iv) etc and finally (ii) - (i), so that all means have been compared.

From these calculations it can determined which means differ significantly

(i)	(ii)	(iii)	(iv)	(v)	(vi)
55.5a	59.7a	48.0b	48.1b	38.4c	36.9c

Means followed by the same letter are not significantly different.

(D) TESTING SIGNIFICANCE OF MEANS

To test whether differences in water potential or stomatal aperture were significant, the t-test was used. To obtain the t value the standard error, of both means to be compared, was squared, summed and the square root of this value taken. The difference between the two means was then divided by this value thus giving the t value. The significance could then be determined from a table of t values.

(1) Significance of changes in leaf water potential

(a) Preflowering cycle

		Water pot. (±SE)	$(S.E.)^2$	$\Sigma(S.E.)^2$	$\sqrt{\ }$	t value	Significance
Sampling time	(1)	3.9 ± 0.38	0.14.4	0.161	0.401	$\frac{1.1}{0.401} = 2.74$	$P < 0.025$
	(2)	5.0 ± 0.13	0.017				
	(3)	11.4 ± 0.20					
	(4)	3.9 ± 0.18					
	(5)	4.2 ± 0.11					
	(5c)	4.1 ± 0.03					

(b) Flowering cycle

Sampling time	(1)	2.9 ± 0.15	0.0225	0.0840	0.29	$\frac{1.2}{0.29} = 4.14$	$P < 0.005$
	(2)	4.1 ± 0.25	0.0625				
	(3)	11.7 ± 0.58					
	(4)	4.2 ± 0.29					
	(5)	3.8 ± 0.14					
	(5c)	4.0 ± 0.31					

(c) Flat pod cycle

Sampling time	(1)	3.7 ± 0.08	0.0064	0.1434	0.384	$\frac{2.5}{0.384} = 6.5$	$P < 0.001$
	(2)	6.2 ± 0.37	0.1370				
	(3)	14.3 ± 0.63					
	(4)	4.4 ± 0.16					
	(5)	4.3 ± 0.11					
	(5c)	4.8 ± 0.14					

(d) Pod swell cycle

Sampling time	(1)	4.7 ± 0.23	0.0528	0.1898	0.435	$\frac{1.4}{0.435} = 3.22$	$P < 0.01$
	(2)	6.1 ± 0.37	0.1370				
	(3)	10.9 ± 0.34					
	(4)	11.5 ± 0.29					
	(5)	4.1 ± 0.13					
	(6)	4.3 ± 0.26					
	(6c)	4.1 ± 0.10					

(2) Significance of changes in stomatal aperture

(a) Preflowering cycle

Sampling time	(1)	6.0 ± 0.09	0.0081	0.0130	0.114	$\frac{0.8}{} = 7.02$	$P < 0.001$
	(2)	5.2 ± 0.07	0.0049				
	(3)	1.0 ± 0.03					
	(4)	2.3 ± 0.06					
	(5)	5.4 ± 0.08					
	(5c)	5.5 ± 0.09					

(b) Flowering cycle

Sampling time	(1) 6.5 ± 0.09	0.0081	0.0181	0.1348	$\frac{0.30}{0.135} = 2.22^{P}$	< 0.05
	(2) 6.2 ± 0.10	0.0100				
	(3) 1.0 ± 0.01					
	(4) 1.5 ± 0.03					
	(5) 6.1 ± 0.11					
	(5c) 6.4 ± 0.09					

(c) Flat pod cycle

Sampling time	(1) 6.1 ± 0.12	0.0144	0.0193	0.139	$\frac{3.00}{0.139} = 21.6^{P}$	< 0.001
	(2) 3.1 ± 0.07	0.0049				
	(3) 1.0 ± 0.02					
	(4) 1.5 ± 0.03					
	(5) 4.8 ± 0.09	0.0081	0.0162	0.127	$\frac{1.1}{0.127} = 8.64$	$P < 0.001$
	(5c) 5.9 ± 0.09	0.0081				

(d) Pod swell cycle

Sampling time	(1) 6.1 ± 0.09	0.0081	0.181	0.1348	$\frac{0.4}{0.135} = 2.96$	$P < 0.005$
	(2) 5.7 ± 0.10	0.100				
	(3) 0.9 ± 0.03					
	(4) 0.5 ± 0.04					
	(5) 1.5 ± 0.03					
	(6) 5.9 ± 0.10					
	(6c) 5.6 ± 0.07					

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