THE EFFECT OF WATER DEFICIT ON REPRODUCTIVE GROWTH OF *ZEA MAYS* L.

by

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Dedicated to my parents -

the late Mr. W.C.H. Damptey

and

Mrs. Matilda Damptey

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ABBREVIATIONS

ABA	Abscisic acid
atms	Atmospheres
BAP	Benzyl amino purine
BHT	Butylated hydroxy toluene
b.p.	Boiling point
CCC	2-(Chloroethyl)trimethyl ammonium chloride
cm	Centimetres
^{o}C	Degrees centigrade
EDTA	Ethylenediaminetetra acetic acid
GA (GA_3)	Gibberellic acid
GLC	Gas liquid chromatography
g	Gram
HMDS	Hexamethyldisilazane
hr	Hour
IAA	Indole acetic acid
in	Inches
MCW	Methanol:chloroform:water
µg	Microgram
µl	Microlitre
mg	Milligram
ml	Millilitre
min	Minutes
mm	Millimetre

N	Normal
ng	Nanogram
%	Percent
PEP	Phosphoenol pyruvate
pg	Picogram
ppm	Parts per million
R_f	Distance migrated relative to the solvent front
RNA	Ribonucleic acid
sec	Seconds
SADH	Succinic acid-2-2-dimethylhydrazide
TIBA	Triiodobenzoic acid
TONY	p-Tolysolphonylmethylnitrosoamide
2,4-D	2,4-Diphenoxy acetic acid
v/v	Volume/volume (concentration)
Ψ	Water potential
w/v	Weight/volume (concentration)
wt	Weight

SUMMARY

In a plant of Zea mays (cv. IO Chief) grown without exposure to water deficit, seven axillary inflorescences are initiated but only the uppermost develops to form a mature cob. A period of water deficit imposed during the initiation and early growth of the terminal male inflorescence results in a permanent inhibition of tassel growth but enhanced growth of the lower axillary inflorescences. In these circumstances the axillary inflorescence immediately below the uppermost, and occasionally also the second one down, develop to form mature cobs. Water deficit imposed at any other phase of growth does not result in this response.

As all six lower axillary inflorescences develop to a varying extent before their growth is inhibited, it appears probable that their further development is controlled by correlative inhibition from some other plant organ. Of the two probable candidates for the source of inhibition, the upper axillary inflorescence and the tassel, it was shown that the tassel was the more important. The mechanism of inhibition was investigated by applying various growth substances and mineral nutrients to intact and detasselled plants. Increasing the supply of mineral nutrients to intact plants led only to a general stimulus to growth, but 2,4-dichlorophenoxy acetic acid (2,4-D) alone or in combination with gibberellic acid (GA_3) prevented the growth of the lower axillary inflorescences

following removal of the tassel or a period of water deficit. 2-Chloroethylphosphonic acid (Ethrel) and a cytokinin (6-benzyl amino purine, BAP) were without effect and it was concluded that the most likely controlling factor in the correlative inhibition of the lower inflorescences was auxin, possibly together with gibberellic acid, originating in the tassel.

Of the treatments applied to intact, watered plants, only abscisic acid (ABA), tri-iodo benzoic acid (TIBA) and the growth retardant Succinic acid-2-2-dimethylhydrazide (SADH) selectively promoted the growth of the lower axillary inflorescences, thus mimicking the effects of water stress. Of these, the effect of TIBA was assumed to be due to its known ability to block auxin transport and hence this evidence was taken to support the concept of auxin being important in the control of axillary growth in this system. As ABA is known to accumulate rapidly in plants subjected to a water stress, this response of the axillary inflorescences to applied ABA was thought to be of probable significance in the interpretation of the similar response to water stress and was investigated further.

The endogenous ABA content of various organs of corn plants subjected to a water deficit was assessed. At the end of an eight-day period of water deficit, there was a ten-fold increase in the concentration of ABA in the leaf, a five-fold increase in the two axillary inflorescences below the uppermost axillary

inflorescence and a two-fold increase in the tassel, the uppermost inflorescence and the lowest inflorescences. A periodic measurement of ABA within a similar eight-day period of water deficit, however, showed that there was a very rapid increase in the concentration of ABA, up to ten-fold, in the tassel within the first two days of water deficit when the water potential of the third leaf was -7.7 bars. Thereafter the rate of increase in concentration of ABA in this organ was reduced whilst in the other organs there was a gradual increase in concentration of ABA up to the end of the period of water deficit, when the water potential had fallen to -17 bars. From this data, there appeared to be a relationship between the concentration of ABA in the plant, especially in the tassel, and the enhancement of growth of the lower axillary inflorescence. This was supported by the fact that the application of the growth retardant B995 to watered plants caused both an increase in the concentration of endogenous ABA in the plant and a promotion of the growth of the lower axillary inflorescences.

In conclusion, it is suggested that the exposure of corn plants (cv. IO Chief) to water stress at the stage of tassel initiation and early development leads to a rapid increase in the endogenous ABA content of the plant which in turn inhibits tassel growth and the production and possibly transport of auxin from the tassel. As a consequence of the lowered auxin supply from the

tassel, the lower axillary inflorescences are released from inhibition and grow.

STATEMENT

I hereby declare that the thesis here presented is my own work, that it contains no material previously published, except where due reference is made in the text, and that no part of it has been submitted for any other degree.

H.B. DAMPTEY.

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1. INTRODUCTION

Water in both the soil and the plant encounters forces due to the presence of solid phase, dissolved salts, external gas pressures and the gravitational field. These effects are expressed in the terms of the potential energy of water (Gardner 1965). The loss of water from the plant leaf by transpiration results in a lowering of the water content of the plant which consequently reduces the potential energy of the water in the leaf. The chemical potential in the plant or soil which is the difference between the partial specific Gibbs free energy of the system under consideration and of free pure water at the same temperature is referred to as the water potential (Slatyer and Taylor 1960).

Water movement along the soil-plant system occurs along a gradient of decreasing water potential. Water flow through the plant requires that root water potential be higher than leaf water potential. Any factor which reduces the soil water potential below zero tends to result in an internal water deficit in the leaves of the plant. The extent of this deficit will depend upon the soil water potential and the potential gradient to the external atmosphere.

The study of the effect of progressive water deficit on the plant has created the usage of several terminologies in an attempt

to describe the water status in the plant. These have included "water stress" and "moisture stress". Water stress has been defined by Milthorpe (1960) as that state when the conditions of water supply are unfavourable to optimum plant growth and the degree of water stress can be expressed in terms of the water potential of the plant. Moisture stress has been defined similarly (Shaw and Laing 1965). These terms do not define the physical status of water in the plant but rather the response of the plant to water deficit. It has been observed that the degree of water deficit that causes changes in plants differs from species to species (Slatyer 1957a). Thus the term water deficit appears to be the more appropriate.

As transpiration proceeds in a plant growing in an initially wet soil, the soil water content and the soil water potential are reduced. If this reduction in soil water continues without any replenishment of the soil water, the gradient of decreasing water potential through the pathway from soil to atmosphere which provides the force for water flow also declines. This results in a decline in plant water content and plant water potential and consequently causes an internal water deficit (Slatyer 1967). The response of a plant to a period of water deficit has been described by Gates (1968) as encompassing a wide range of physical, metabolic and subsequently morphogenetic effects. Thus as water deficit increases due to the depletion of water from the soil, the

physical status of water in the plant is affected. The sequence of events which occurs is that there is a progressive decrease in the soil water potential followed by a decrease in plant water potential to an extent which then induces stomatal closure. The gradient from soil to plant then becomes less steep until there is no longer a diurnal equilibrium between soil water potential and plant water potential. As leaf water content and leaf water potential decrease, leaf turgor potential (defined as the pressure potential identified with the instantaneous actual hydrostatic pressure exerted on the wall - Levitt 1974) also decreases. Depending upon the cell volume/turgor potential relationship and the structural characteristics of the leaves concerned, these may gradually droop and take on a progressively more wilted appearance. In leaves that exhibit complete wilting this occurs at a point of zero turgor potential when the leaf water potential is the same as the osmotic water potential (water potential resulting from solutes). It has been proposed that at this stage when leaf water potential, root water potential and soil water potential are all equal, the plant would be in a state of permanent wilting and the soil water content would be at the permanent wilting percentage (Slatyer 1957b). Some of the observed response of plants exposed to water deficit may be ascribed more or less directly to the physical status of the water in the plant tissue, thus reduced elongation whether of leaves, stem or roots, has been postulated by

Burstrom (1956) as being due to the reduction in cell turgidity. Other responses, for example the reduced growth rate, are more likely to be the secondary manifestation of the effects of the changed water status of the plant on various aspects of cellular metabolism.

Many aspects of plant growth and metabolism have been known to be affected by an episode of water deficit. There have been extensive and intensive studies of the phenomenon of water deficit which have resulted in the recent publication of several review articles (Vaadia et al. 1961; Henkel 1964; Kozlowski 1968, 1972; Hsiao 1973). This introduction will therefore be restricted to a brief discussion of the effects of water deficit on the general growth and metabolism of the plant, followed by a more detailed review of the effects on apical morphogenesis, reproduction and hormone physiology as well as related areas of nitrogen metabolism.

1.1. WATER DEFICIT AND GENERAL GROWTH AND METABOLISM

The relationship between the availability of water in the soil and plant growth was at first controversial. Veihmeyer and Hendrickson (1927, 1933, 1936) reported that water is equally available to the plant over the range from field capacity to permanent wilting. However Magstad et al. (1943), Hayward and Spurr (1944) and Gaugh and Wadleigh (1945) all reported a continuous relationship between soil water content and plant growth over this

whole range. Gates (1964), in commenting on this dispute, observed that studies of the effects of water deficit on growth has been biased towards marketable organs of the plant and this has been the source of controversy in these studies. For instance Veihmeyer and Hendrickson (1942) based their growth index in pear on fruit size and in their studies of cotton, the effect was assessed from boll growth (Adams et al. 1942). It is currently accepted that where plant growth in total is considered this is affected by soil water deficit over the whole range from field capacity down.

Although total plant growth is so affected, not all parts of the plant demonstrate an equal sensitivity. It has been repeatedly observed that the organs which are rapidly growing are the most sensitive to water stress (William and Shapter 1955; Aspinall et al. 1964). In this connection plant apices and other meristematic regions are frequently the organs most affected by an episode of water deficit.

Early attempts to quantify the effects of water deficit on growth were complicated by the fact that the measure of water status adopted concerned only the soil whereas the plant presumably responds to its own tissue water status. Carolous et al. (1965) went part-way towards solving this problem when they found that tomato growth and development were related both to relative soil water content and to atmospheric conditions. More recently however, water status has been employed, for instance leaf growth in

sunflower has been reported to occur only when the water potential of the leaf was above -3.5 bars (Boyer 1970b). The growth rate of aspen, pine, birch and spruce is directly proportional to the water potential, with aspen being the most affected and spruce the least (Jarvis and Jarvis 1963).

The effect of a water deficit on the growth rate of plants may be attributed to its effect on various metabolic processes. The most obvious process, photosynthesis, is known to decrease with decreasing water potential. It has been repeatedly reported that water deficit reduces photosynthesis (Brix 1962; Todd and Webster 1965). More recent work has shown that in corn, there was a reduction of photosynthesis whenever the leaf water potential fell below -4 to -5 bars and in soy beans when leaf water potential fell below -11 bars (Boyer 1970a). Photosynthesis depends on three main groups of processes each of which is affected by water deficit. These include the supply of CO_2 to the photosynthetic sites, which is dependent on stomatal aperture, photochemical processes, and the "dark" chemical process associated with the reduction of CO_2. These are complicated by the fact that the net assimilation rate which is an index of plant growth is determined not by photosynthesis alone but by respiration which may be influenced differently by water deficit (Slatyer 1967).

It is known that much of the reduction of photosynthesis in light caused by water deficit is through the closure of stomata

which thus limits the supply of CO_2. There have been reports of a close correlation between CO_2 assimilation and stomatal closure during water deficit (Brix 1962; El-Sharkaway et al. 1964; Troughton and Slatyer 1969). Nevertheless other reports suggest that the effect of water deficit on photosynthesis could be non-stomatal. Troughton (1969) and Troughton and Slatyer (1969) observed that net photosynthesis in cotton leaves declined at 50% relative water content partly as a result of non-stomatal factors. This has been confirmed in tomato at a relative water content of 80% (Duniway and Slatyer 1971).

As with CO_2 assimilation, the photochemical reaction has been found to be affected by a water deficit. A loss of Hill reaction in chloroplasts isolated from Beta vulgaris leaves exposed to water deficit has been reported. At a relative water content of 50%, photophosphorylation and photoreduction by the chloroplasts were almost completely abolished (Nir and Poljakoff-Mayber 1967). A reduction in Hill reaction has been reported with a less severe water deficit, with inhibition occurring at a water potential of -12 bars in pea and -8 bars in sunflower (Boyer and Bowden 1970).

Some enzymes of the "dark" chemical reaction, however, are not very sensitive to water deficit. The activities of ribulose-1-5 diphosphate carboxylase and phosphoribulose kinase extracted from leaves of Hordeum vulgare exposed to a water deficit down to -11 bars or 95% relative water content, were not affected

(Huffaker et al. 1970). The activities of these enzymes extracted from spinach chloroplast were only affected by a water deficit in vitro at a very high concentration of the osmoticum and then only when the chloroplasts were assayed intact (Huffaker et al. 1970) suggesting possibly that the integrity of organelles is important in stress effects (Hsiao 1973).

Generally a reduction in apparent photosynthesis occurs at water potentials from -1 bars to -3 bars and declines more or less linearly with turgor potential to a value of zero when the turgor potential is zero, thereafter becoming negative as respiration exceeds photosynthesis (Schneider and Childers 1941; Bordeau 1954; Brix 1962). There are varietal differences, however, in this effect of water deficit on photosynthesis. For instance, there was no effect of water deficit on non-stomatal CO_2 assimilation in cotton until the relative water content of the leaves was reduced to 56% (Troughton 1969) and in tobacco until there had been a 40% loss of fresh weight (Graziani and Livne 1971). On the other hand, in tomato (Duniway and Slatyer 1971) and in beet leaves (Hansen 1971) the non-stomatal assimilation of CO_2 was affected when leaf relative water content had fallen to only 80%.

In a study of the effect of water content on the photosynthesis of several species of tropical plants Dastur (1925) and Dastur and Desai (1933) observed a direct correlation between the

net assimilation rate and the water content of the leaves. Such an effect could be due to either effects on photosynthesis as described or to effects on the translocation of assimilates. Direct evidence for effects of water deficit on translocation has been obtained. For instance, single leaves of sunflower (Wiebe and Wirheim 1962) or of some woody species (Roberts 1964) exposed to a water deficit and to $^{14}CO_2$ transported less labelled compounds to the rest of the plant than did similar leaves on plants not subjected to a water deficit. However, an actual increase in translocation due to water deficit has been reported by Plaut and Reinhold (1965) who measured the translocation of ^{14}C labelled sucrose applied to the primary leaf of bean plants. This measured stimulation may, in fact, have been due to a favourable change in the water status of the tissue due to the drop of solution applied. The evidence for an effect of water deficit on translocation suggests that some of the effects of water deficit on photosynthesis might be indirect. There is a growing body of evidence for the conclusion that the rate of photosynthesis in leaves may, in some circumstances, depend on how rapidly the photosynthates are used in growth or are translocated to storage tissues (Humphries 1963; Humphries and Thorne 1964; Nosberger and Humphries 1965). The reduction in photosynthesis caused by a water deficit may hence be due to an inhibition of translocation which leads to an accumulation of photosynthates which in turn inhibit photosynthesis even when other factors are not limiting (Hartt 1967).

Any effect of water deficit on respiration can also result in significant changes in the rates of apparent photosynthesis. Even without such changes, the relative significance of a constant respiratory rate increases rapidly as photosynthesis is reduced by water deficit. As water deficit is imposed an increase in respiration has been observed followed by a reduction in rate as the plant adapts to stress (Slatyer 1967). With Phalaris tuberosa and Lolium multiflorum, Petrie and Wood (1938) reported an increase in the respiration rate when the leaf water content fell from 90 to 80%, but there was a decline in the rate with any further reduction in leaf water content. A similar trend was found in apple leaves (Schneider and Childers 1941; Negisi and Satoo 1954) and in corn (Musgrave and Moss 1961). Two reasons could be adduced for the initial increase in respiration during a water deficit. Firstly it could be due to the decreased resistance to gaseous diffusion following increased opening of stomata due to mild water deficit (Stalfelt 1955) and to a significant lowering of resistance to gaseous diffusion due to a slight loss of total water content of the wet cells (Kaul 1965). Alternatively, as suggested by Iljin (1957), it could be due to an induced hydrolysis of starch to sugar. The decrease in respiration with further decrease in leaf water content may be due to a decrease in the pools of respiratory metabolites, such as malate, as well as stomatal closure (Kaul 1965).

1.2. WATER DEFICIT, APICAL MORPHOGENESIS AND REPRODUCTION

1.2.1. Apical Morphogenesis

It has been suggested that the organs growing most rapidly at the time of a water deficit are most sensitive to the effects of that water deficit (William and Shapter 1955; Aspinall et al. 1964). In many circumstances the most sensitive response to water deficit occurs in the apical meristems (Gates 1968; Husain and Aspinall 1970). Both the initiation of primordia in the apical meristems and the enlargement of the cells thus differentiated are very sensitive to a water deficit (Slatyer 1969). Gates (1968) demonstrated that growth in the apical region of the tomato shoot was arrested by water deficit where the relative water content was approximately 75% but correction of the deficit resulted in a rapid resumption of apical growth. Similar responses were observed by Husain and Aspinall (1970). A water deficit resulted in a rapid inhibition of primordium production on the apex, but the differentiation of lateral primordia was less sensitive, resulting in persistent effects on the structure of the inflorescence. Domanskii (1959) and Konovalov (1959) both reported that water deficit during spikelet formation reduced the number of spikelets in oats. Stress at a similar period resulted in a delay in spikelet development and a reduction in the number of fertilized flowers due to pollen sterility (Novikov 1952, 1954). In both these reported effects of water deficit on spikelet formation of

wheats and oats, however, the level of water deficit was not defined. In a similar study, Nicholls and May (1963) reported for barley that a gradual decrease in the soil water potential to -0.8 atmosphere resulted in an appreciable retardation in the differentiation of both leaf and spikelet primordia and that primordia formation ceased at a soil water potential between -2 and -2.5 atmospheres. Furthermore, two abnormalities were found to occur on the apex either separately or together. These were a failure in the formation of a primordium on the apex resulting in the absence of a spikelet in that position and/or a rotation in the plane of symmetry of the whole apex.

The slow growth and the drastic after-effects caused by the sensitivity of the apical meristem to a period of water deficit also causes a breakdown in any apical dominance exerted by the apex. An episode of water deficit imposed on barley during the reproductive phase caused increased tillering and the earlier the water deficit was applied the more profuse the tillering (Aspinall et al. 1964). A similar reduction in apical dominance caused by a water deficit was also observed in Pisum sativum by McIntyre (1971) who demonstrated that a water deficit caused a marked increase in the growth of the lateral buds.

1.2.2. Reproduction

Most studies of the effects of water deficit on reproduction have centered on yield. Henkel (1964) reported that

the reproductive organs are particularly sensitive to drought and moreover that the androecium is more sensitive than the gynaecium, resulting in poor pollination and fertilization.

The reproductive period of a plant can be divided into three main phases when considering the effects of water deficit. These stages are (a) the phase from flowering to fertilization, (b) fruit enlargement and seed development, (c) fruit ripening.

1.2.2.1. Flowering and Fruit Set (Fertilization)

It has been repeatedly reported that this is the most sensitive stage to water deficit (Henkel 1964; Aspinall 1965; Salter and Goode 1967). Water deficit reduced the number of flower buds of apricots and delayed the time of differentiation when imposed at the period of flowering (Brown 1953). Early and prolonged water deficit caused smaller flowers and slower floral development in apples (Modlibowska 1961) and similar effects of water deficit have been observed in tomatoes, snap beans and lime beans (Vitum et al. 1953).

The most crucial period appears to begin with the appearance of pollen mother cells and ends when the process of pollination is over. Water deficit imposed during the period of pollen formation caused the walls of the anthers to die, the tapetum to be restricted and the flow of nutrients to the developing grain to stop (Skazkin and Zavadskaya 1957; Skazkin and Lukomaskaya 1962).

Pollen from wheat plants exposed to water deficit gave only 40% fruit set whilst that from the control plants gave 77% and the pollen from plants subjected to water deficit caused partly filled or empty heads (Anikiev 1960). Water deficit at the pollen meiosis phase in wheat caused male sterility in the lower florets of each spikelet without affecting female fertility (Bingham 1966). It is sometimes suggested that water deficit at the period of pollen shedding also causes dehydration of pollen grains (Slatyer 1969). However it also seems probable that growth of the pollen tube from the stigma to the ovule may be impaired, this being more crucial in plants such as corn where the pollen tube has to grow through the whole length of the silk (Slatyer 1969).

The effect of water deficit at the period of flowering and fertilization also depends on the type of flowering of the species. In species with determinate flowering the effect of water deficit on reproduction can be marked. In plants such as corn and some other cereals there is a critical period. For instance, water deficit reduced grain size in barley when imposed soon after anthesis (Aspinall et al. 1964). A similar critical period was evident in oats at heading where water deficit caused low yields due primarily to barrenness of the inflorescence, whilst in wheat the period of rapid elongation prior to heading appears to be the critical period (Domanskii 1961). In crops of more indeterminate nature where the reproductive phase may occur over a long period, the existence of a

critical period may not be evident. In soybeans for instance, it has been shown that the flowering period is less sensitive to water deficit than the pod filling stage (Laing 1965).

The effect of water deficit on the initiation of flowers and in particular on the number of leaves formed prior to the commencement of floral initiation has not been extensively investigated. The most pronounced effect has been reported in tobacco where a water deficit delayed floral initiation which took place 5 nodes higher than in the control plants (Hopkinson 1968). In sorghum, when a water deficit was imposed close to the normal time of onset of flowering, the mean leaf number was reduced by three leaves (Whiteman and Wilson 1965). These workers considered that the leaf number at which the initiation of flower occurs depends on an interaction between the amount of vegetative development necessary to allow the formation of a floral stimulus and the extent to which a water deficit may suspend development. If water deficit is imposed well ahead of the normal time for the onset of flowering, leaf initiation was resumed. It has frequently been observed that a water deficit imposed during the preflowering stage causes a delay in the date of flowering although there are some reports of advanced flowering caused by water deficit (Salter and Goode 1967). In sorghum the delay is closely related to the period of water deficit but this relationship is not clear in other crops. For instance, in wheat, it has been observed that seven days of

water deficit imposed during the preflowering period delayed anthesis for 8 to 18 days (Chinoy 1960).

Another effect of water deficit imposed during the phase of flowering and fertilization is the abscission of flowers and young fruits. The maximum rate of abscission in orange flower buds occurred three days after a hot dry spring day (Erickson and Brannaman 1960). This response has been interpreted by Kaufman (1972) as being due to the low soil and root temperatures and a possible high resistance to water absorption in the root which resulted in a water deficit within the plant. A similar hot day in summer did not result in abscission however, but here the reproductive organs were at a more advanced stage of development. Water deficit resulting in severe wilting of cotton, caused severe shedding of the leaves and reduced boll retention (Grimes et al. 1970). In contrast, no increase in the rate of abscission was observed when bush beans were exposed to a soil water deficit of -8 bars before, during or after flowering (Dubetz and Mahalle 1969). This difference in response may be due either to species differences or to the degree of stress experienced by the plants.

Although water deficit coinciding with the period of floral initiation and fruit set has generally been observed to be detrimental to plant yield there have been occasional reports of favourable effects. For instance regularly irrigated coffee and bamboo plants did not flower, whilst irrigation after a period of

drought resulted in flowering. On this evidence it was suggested that a period of water deficit might be necessary for flowering (Alvim 1960, 1964). Similarly flowering of Litchi chinensis occurred on only 50% of the branches of well watered plants whereas flowering occurred on 70 to 95% of the branches of plants exposed to a period of water deficit (Nakata and Suehisa 1969). Furthermore, the seed set of two varieties of wheat was increased in plants where soil water potential was decreased to -15 bars as compared with those grown at a soil water potential of -1.4 bars (Campbell et al. 1969). These workers demonstrated that a low oxygen diffusion rate in the plants grown in soil water potential of -1.4 bars was primarily responsible for the poor seed set.

1.2.2.2. Fruit and Seed Enlargement and Development

The effects of water deficit on plants at this phase of reproductive growth have been studied mainly with fruit trees and there are only a few reports from other plants. With cereals, however, it has been reported that during the first 3 to 4 weeks after anther dehiscence there was no effect of water deficit on the increase in grain weight of some Indian wheat varieties (Asana and Saini 1958).

The accumulation of dry weight in the cereal ear is dependent upon photosynthesis in the ear and some translocation from other parts of the plant. Although assimilates may be translocated to these organs prior to anthesis, by far the greatest contributions

come from the ear itself, leaves and stem during the period of grain growth (Thorne 1963; Carr and Wardlaw 1965). Water deficit during grain filling therefore, by reducing photosynthesis, causes a reduction in yield. Although it has been shown that a water deficit may not affect translocation in the conducting tissues themselves it was pointed out that translocation from the leaves is slowed down and prolonged by water deficit (Wardlaw 1967; 1969). All these effects of water deficit therefore contribute to the reduction in grain yield. Grain filling is a relatively rapid process, and most of the increase in plant weight after anthesis involves grain development, so that a reduction in photosynthesis at any point of the post anthesis stage may reduce grain weight with little opportunity for compensatory activity at later stages of grain filling (Aspinall 1965). However, there is also evidence that there is a limit to grain size in any one phenotype and that in plants with an adequate supply of water surplus photosynthates may be available. For instance, there was no increase in the grain weight of remaining barley grains following removal of other grains (Buttrose and May 1959). This suggests that a reduction in photosynthesis caused by water deficit may not lead to reduced grain weight until any surplus capacity for photosynthesis is eliminated (Slatyer 1969). As an example, reduced photosynthesis in wheat caused by a water deficit early in the grain filling stage could be compensated by enhanced translocation from the stem (Asana and Basu 1963). Stress occurring later in the grain

filling stage could not be compensated for in this manner, however, due to the hastened senescence of the leaves of plants exposed to water deficit (Asana and Saini 1958).

Similar responses have been observed with other herbaceous crops. In cotton, fruit enlargement during dry weather caused a reduction in the size of the bolls partly due to the fact that the period of enlargement was reduced (Anderson and Kerr 1943). Such a period of water deficit also reduced the fibre lengths in the bolls (Sturkie 1934). The maximum growth and yield in tomato fruits occurred in plants grown under a supply of water near field capacity and there was a reduction in the yield when plants were exposed to any period of water deficit (Salter and Goode 1967). Exposing beans to a water deficit during the phase of pod development also reduced the pod number (Shaw and Laing 1965).

The increase in the volume of large fruits, such as oranges, is inhibited by a water deficit (Hales et al. 1968; Erickson and Richards 1955). Even the diurnal changes in plant water status have a reversible effect on fruit size in some species; for instance cotton (Anderson and Kerr 1943) and oranges (Elfving and Kaufman 1971) but this occurs only at certain stages in fruit growth. As an example, with Montmorency cherries both young and mature fruits were insensitive to diurnal water deficits but unripe fruits were sensitive (Chaney and Kozlowski 1969). In such cases, fruit diameter decreased during the day when the water

potential of the leaves was low. During the evening, however, closure of the stomata and a reduction in the vapour pressure deficit allowed recovery from this water deficit and brought about an increase in fruit diameter (Elfving and Kaufman 1971). The shrinkage of the fruit due to the diurnal water deficit is fully reversible (Kaufman 1972).

1.2.2.3. Fruit Ripening

A period of water deficit during the period of fruit ripening can either hasten or delay ripening and there are reports to substantiate both effects. Date fruits irrigated at a soil water potential of -0.8 bars ripened earlier than those irrigated at -0.1 to -0.2 bars (Reuther and Crawford 1945) and a similar response has been found with tomato plants (Alijbury and May 1970).

In contrast to these reports, water deficit has been observed to delay ripening and maturing of some fruits. In cotton it has been observed that a delay in irrigation until most of the water in the soil was depleted resulted in fewer mature cotton bolls at harvest than where irrigation was applied earlier (Stockton et al. 1967). Similarly, irrigated peaches ripened uniformly whilst those exposed to water deficit ripened so irregularly that they had to be harvested twice instead of once (Feldstein and Childers 1965). These effects on ripening of fruits are further complicated by the fact that abscission is accelerated by water deficit leading to

the premature dropping of some fruits (Kaufman 1971).

Seeds generally lose water during the process of maturation and harvesting of grain, which is normally carried out when the seeds are thoroughly dehydrated, is delayed during cool and humid weather. The sensitivity of seeds to water depends largely on the type of fruit structure. For instance barley and wheat seeds are exposed whilst corn seeds are covered by the husk and tomato seeds are embedded in a fleshy placenta. It has been observed that there was no effect of water on the rate of dehydration of poppy seeds, enclosed in a capsule, during the first eighteen days after flowering but during the next twenty days high temperature and low humidity accelerated the rate of dehydration whilst cool and moist conditions delayed it (Prokofev and Kholodova 1968).

The progressive change in seed water content in some plants is not affected by environmental effects. For instance, even although the fleshy pericarp of pepper fruits maintained the relative humidity of the air surrounding the growing seeds at a high level, the seeds lost water to the pericarp (Prokofev and Kholodova 1968).

1.3. WATER DEFICIT AND PLANT HORMONES

Plant growth is regulated by an intricate mechanism of control whose ultimate source is the genome. Growth substances (hormones) are known to be important in the mediation of this regulation. As

water deficit produces some considerable morphological responses it is possible that a water deficit may modify the hormonal control of development. A water deficit may produce this effect by affecting the synthesis, mechanism of action or translocation of growth substances.

1.3.1. Abscisic Acid

The most dramatic effect of water deficit on hormones so far recorded is the rapid accumulation of abscisic acid (ABA) during the period of water deficit. Initially, Wright (1969) reported an increase in the inhibitor β content of excised wheat leaves held in a wilted state. An increase in this inhibitor fraction was observed within 2 hrs following a 9% loss of fresh weight caused by wilting and as much as a 40-fold increase was observed in 4 hrs. Further loss of water had a negligible effect. Wright and Hiron (1969) identified this inhibitor as (+) abscisic acid using optical rotary dispersion analysis. This increase in ABA content was temperature dependent and no change in the level of ABA was discernible when wilted leaves were maintained at 2°C. The time lapse and the temperature dependence of accumulation led to Wright and Hiron (1972) concluding that the formation of ABA was probably due to enzymatic conversion of a precursor.

The accumulation of ABA during a period of water deficit has been shown to follow from de novo synthesis (Milborrow and Noddle 1972) rather than from release from a precursor or conjugate as

suggested by Wright and Hiron (1972). Milborrow et al. (1972) observed that more labelled mevalonate was incorporated into ABA by leaves that had been fed mevalonate and then wilted than leaves that were fed and then kept moist. Moreover it is more than likely that the necessary presence of a considerable pool of a precursor or conjugate able to release large quantities of ABA would have been detected by extraction and bioassay. Instead it has been found that the glucose ester of ABA, the most probable precursor, is rarely present in a concentration exceeding a third of the amount of free ABA present (Milborrow 1974).

Since these early reports of the accumulation of ABA during water deficit in excised leaves, many other workers have reported a similar accumulation in intact plants subjected to water deficit (Milborrow et al. 1972; Mizrahi et al. 1970; Most 1969; Zeevart 1971). Furthermore, some workers have reported the accumulation of ABA as a result of indirect water deficit on the plants. For instance, it has been demonstrated that the initial wilting of the upper leaves of tobacco plants inoculated with the wilt inducing bacterium Pseudomonas solanacearum was correlated with the presence of an inhibitor, apparently ABA (Steadman and Sequiera 1970), and the leaves of tobacco plants subjected to osmotic stress by the addition of 0.1 M NaCl or 0.17 M mannitol to the nutrient medium were found to contain large quantities of ABA (Mizrahi et al. 1970).

Three probable sequential mechanisms in this water deficit induced synthesis of ABA have been proposed by Milborrow (1974). Firstly, a rapid synthesis which is triggered by the water deficit, followed secondly by a stop message when a certain concentration of ABA has been formed. In support of this mechanism, it has been observed that exogenously applied ($\pm$) ABA suppressed wilt-induced biosynthesis of endogenous ABA (Milborrow 1974). Thirdly and finally, the commencement of net destruction of ABA when turgor is regained, this could merely be a cessation of rapid synthesis if stress was not severe or long enough for synthesis to have reached a maximum concentration.

By measuring both the incorporation of labelled mevalonate and the increase in ABA content Milborrow and Robinson (1973) investigated the sites of synthesis of ABA. The leaves of avocado pear increased in ABA content whilst the stem and roots showed only a very slight response to wilting. Moreover, the incorporation of labelled mevalonate into ABA and the usual rise in ABA content during fruit ripening were not affected by water loss from avocado fruit slices which contain large amounts of ABA and continue to rapidly synthesise the compound. This led to their conclusion that ABA biosynthesis is regulated differently in roots, stems and fruits as compared with leaves.

Although ABA has been detected in all parts of the plant the localisation of the hormone within the cell is virtually unknown.

Chloroplasts isolated from avocado leaves and fruits are able to convert DL-mevalonate into ABA although in a low yield (Milborrow 1973) and ABA has been found within pea chloroplasts (Railton et al. 1974). It is possible that ABA is located mainly in the chloroplast though the presence of ABA in nectar demonstrates that some ABA must also be present outside the chloroplast (Milborrow 1974).

The role of ABA in the metabolism of the plant has been studied extensively. One of the most important roles in relation to water deficit is the closure of stomata as a result of the application of ABA first reported by Mittelheuser and van Steveninck (1969). A correlation between the increase in endogenous ABA and stomatal closure in dwarf bean plants subjected to water deficit has been found and it has been suggested that these observations provide strong grounds for believing that the accumulation of ABA results in the closure of stomata (Wright and Hiron 1970). It has also been demonstrated that ABA applied to the surface of barley plants could effect stomatal closure (Jones and Mansfield 1972). Although the effects of exogenous ABA is undisputed, doubts have been expressed as to the probability of the wilt-induced synthesis of endogenous ABA being fast enough to be the agent controlling stomatal aperture (Hsiao 1973). The onset of closure of corn stomata following the application of ABA can be as rapid as three minutes, however, and very small amounts of ABA can bring about the closure of stomata (Kriedmann et al. 1972). In

addition to inducing closure, ABA prevents the opening of stomata (Horton 1971).

The mechanism by which ABA regulates stomatal aperture is the subject of speculation. ABA application has been shown to induce starch accumulation, a fall of osmotic pressure from 14.1 to 9.8 bars, and efflux of potassium ions from guard cells of Xanthium pennsylvanicum (Jones and Mansfield 1970; Mansfield and Jones 1971). The presence of chloroplasts, the possible site of ABA synthesis (Milborrow 1974), in guard cells may be significant as may be the ability of ABA to inhibit the influx of potassium ions into stomatal guard cells (Horton and Moran cited by Milborrow 1974).

Further support for the concept that ABA is involved in the normal control of stomatal movement comes from experiments with a tomato mutant (flacca). This mutant was found to contain one-tenth of the amount of ABA as in a normal plant and their stomata were permanently open (Tal and Imber 1970). Application of 1 to 10 μg (±) ABA caused a rapid and progressive reduction in the transpiration rate of the leaves and leaf discs of this tomato mutant plant, together with a closure of the stomata in the dark (Tal and Imber 1970, 1971; Imber and Tal 1970).

Despite the evidence for the role of abscisic acid alone in the control of stomatal aperture, it has also been postulated that stomatal closure during water deficit is due to an interaction of

the effects of lowered cytokinin activity and the increase in ABA (Livne and Vaadia 1972). A reduction in the cytokinin activity in wilted plants has been reported (Itai and Vaadia 1965) and applied kinetin can cause opening of stomata within a few hours of application (Livne and Vaadia 1972). However this hypothesis does not appear to be of general application. Firstly the opening effect of kinetin is only apparent following a lag of approximately an hour following application (Meidner 1967) which is too slow to account for the rate of closure observed with water deficit. Secondly, stomata of Vigna sinesis, Phaseolus lunatis, P. vulgaris, Glycine max, Helianthus annuus, Cucurbita pepo, Acer saccharum and Liquidambar styraciflura do not respond to cytokinin treatment (Luke and Freeman 1968), nor do young leaves of tobacco plants (Livne and Vaadia 1965). Furthermore, the closure of stomata by applied ABA cannot be reversed by the application of kinetin (Hsiao 1973).

ABA was first identified and isolated by following its abscission-accelerating activity in the petiolar stumps of cotton explants. Similarly a growth inhibitory factor believed to be responsible for the premature abscission of immature yellow lupin fruits was later identified as (±) ABA (Cornforth et al. 1967; Koshimizu et al. 1966). This suggests that this compound may be involved in the accelerated abscission evident during or following water stress. There have been many attempts to demonstrate an

involvement of ABA in leaf abscission (Cooper et al. 1968; Edgerton 1971 cited by Milborrow 1974; Larsen 1969) but most have not been convincing due to the use of abnormally high concentrations (Milborrow 1974). The evidence for a role of ABA in fruit abscission is more certain, however. Two peaks of concentration of ABA in cotton fruits were found in the period between anthesis and ripening. The first peak coincided with the period of self thinning ("June drop") and, moreover, the aborted fruitlets contained a higher concentration of ABA than those which were retained. The second peak was followed by fruit dehiscence. It could therefore be concluded that ABA is involved in the regulation of abscission of cotton fruits (Davis and Addicott 1972).

The increase in concentration of ABA due to water deficit could cause dormancy of buds. Indeed one of the studies that also led to the isolation of (+) ABA (known then as "dormin") in sycamore leaves was the change in an inhibitory activity in extracts of seedlings grown in long or short days and the increase in content of the inhibitory material in summer, and early autumn (Phillip et al. 1958, 1959). Also the formation of resting buds has been induced in Betula pubescens grown in long day by treating the plants with extracts of plants grown under short day (Eagles and Wareing 1964) and the experiment was able to be repeated with pure synthetic ABA and also to induce the formation of dormant bud in Acer pseudoplatanus and Ribes nigrum (El-Antably et al.

1967). These workers strongly supported the idea that endogenous ABA might be causing dormancy. Recently, however, attempts to repeat these experiments using gas chromatography (instead of the original bioassay methods) have failed to show differences in ABA in Betula pubescens, Acer saccharum and A. pseudoplatanus in relationship with daylength (Lenton et al. 1972). Indeed, an increased ABA content has been found when spinach plants were transferred from short to long days (Zeevart 1971). There is nevertheless a positive correlation of endogenous concentration of ABA with dormancy in onions where it was found that (+) ABA increased as the bud became dormant and formed bulbs (Tsukamoto et al. 1969).

In view of the general inhibitory effect of exogenously applied ABA on plant growth, it has been speculated that endogenous ABA might play a similar role, but this has not been conclusively proven. One of the main reasons is the fact that there is no known specific inhibitor of ABA biosynthesis (Milborrow 1974). Nevertheless it has been observed that exposure of Xanthium plants to red/far red light caused an increase in the concentration of abscisic acid-like material in the lateral buds and that those buds did not grow, whereas plants grown in ordinary light branched profusely and contained between 50 and 250 times less inhibitor (Tucker and Mansfield 1972).

Many growth processes have been shown to be influenced by exogenously applied ABA including the arrest of growth of plant parts and the formation of resting organs (van Overbeek 1969; Bleichert and Steward 1970; Menon and Lal 1974; Altman and Goren 1971). A stimulation of growth by exogenously applied ABA has also been reported in some circumstances; parthenocarpic fruit development in Rosa (Jackson and Blundell 1968), hypocotyl elongation in cucumber (Aspinall et al. 1967), a tenfold stimulation of rice mesocotyl elongation (Milborrow 1974), promotion of the formation of buds in Begonia leaves (Heide 1968), a stimulation of flowering of short day plants in continuous light (Krekule and Ullman 1971; Naqvi et al. 1974 and Basler 1974) and a promotion of femaleness in cucumber plants (Rudich and Halevy 1974). ABA also has a variety of metabolic effects including inhibition of the transport of K and Cl and the flow of water through barley and maize roots (Cram and Pitman 1972); an inhibition of the basipetal transport of auxin (Pilet 1971) and inhibition of the synthesis of α-amylase and ribonuclease (Chrispeels and Varner 1967; Barton et al. 1973). ABA has been reported to promote the activity of RNAase (Leshem 1971), PEP carboxylase and malic enzymes (Sakhloo and Huber 1974) and to inhibit nucleic acid synthesis (Stewart and Smith 1972; Mondal and Biswas 1972, and Villiers 1968). These metabolic responses follow the application of exogenous ABA and it is not known if endogenous ABA controls all these facets of metabolism. In any case Milborrow (1974) has rightly warned that

when ABA is exogenously applied to a plant it may possibly enter certain parts of the cell where endogenous ABA does not normally penetrate and accumulates to a supranormal concentration which induces changes which might not be natural functions.

1.3.2. Cytokinins

One of the characteristic effects of a period of water deficit is an enhanced rate of ageing of the leaves (Livne and Vaadia 1972). The protein content of the mature leaves of normal plants decreases with age (Shah and Loomis 1965) and a decline in the protein content and an increase in protein degradation of plants under water deficit has been observed (Vaadia et al. 1961; Barnett and Naylor 1966). When detached leaves are treated with kinetin this ageing process is retarded (Richmond and Lang 1957; Mothes et al. 1959; Osborne and McCalla 1961; Leopold and Kawase 1964) and, with tobacco, a crude xylem exudate will also delay senescence (Kulaeva 1962). Since the presence of cytokinin in this xylem exudate was demonstrated (Kende 1965) it has been suggested that changes in metabolism and the accelerated ageing of shoots of plant exposed to water deficit might be due to a reduction in the supply of cytokinins from the roots (Itai and Vaadia 1965). Reduced cytokinin activity in root exudates from plants subjected to water deficit was indeed found (Itai and Vaadia 1965 and Itai et al. 1968). The reduced cytokinin activity of the xylem exudate of plants exposed to water

deficit increased to the level of the control plants two days after re-watering (Itai and Vaadia 1965; Vaadia and Itai 1968). Apart from this effect of water deficit on cytokinin content, salinity also affected the content, gradual addition of NaCl up to 0.1 M and mannitol up to 0.16 M to the nutrient medium of sunflower resulted in a decreased concentration of cytokinin in the xylem exudate. These same treatments, when applied to tobacco plants reduced the incorporation of ^{14}C-leucine into leaf disc protein whilst kinetin treatment prior to incubation with leucine partially restored the incorporation (Ben-Zioni et al. 1967). The reduction in the nucleic acid and protein content of plants exposed to a water deficit was partially counteracted by the application of benzyladenine to sugar beet leaves (Shah and Loomis 1965).

The postulated regulatory function of cytokinin plants subjected to a water deficit has been criticised on two main grounds (Hsiao 1973). Firstly, the reported reduction in cytokinin activity was not pronounced and secondly the degree of water deficit was not quantitatively assessed. Furthermore, it is probable that the concentration of NaCl applied was toxic to the plants. Further doubt is cast on the role of cytokinin during a water deficit by the contradictory observations made by Mizrahi et al. (1971, cited by Livne and Vaadia 1972) using the same tobacco species as the previous workers. Here no reduction in cytokinin activity followed a day of wilting in an atmosphere of

low humidity nor was any reduction observed with salinity stress despite the fact that leaf transpiration was depressed and leaf ABA content was increased. This contrasts with the recent report by Vaadia (1971) where less than 30 minutes of wilting resulted in a substantial decrease in the cytokinin activity in root exudate following re-watering. A similar reduction was obtained with leaf discs which were wilted to 75% of the initial weight following 30 minutes of drying. The loss in cytokinin activity was partially recovered 180 hrs after rehydration. As stress appeared to act rapidly Itai and Vaadia (1965) suggested that the loss of activity was as a result of inactivation.

Endogenous cytokinin content is also affected by other forms of stress that affect growth. For instance, flooding caused a reduced cytokinin activity in the xylem exudate from sunflower plants (Burrows and Carr 1969). Similarly, a lowering of the pH in the root medium of maize from 7 to 4 caused a reduction in cytokinin activity (Andreenko et al. 1964). Furthermore, a marked decrease in the cytokinin content was observed before the appearance of symptoms of wilt disease in tomato plants infested with Verticillium dahliae (Krikon et al. 1970). In all of these cases, it is conceivable that the water status of the plant was affected and the changes in cytokinin activity may have been in response to this change. The effect of water deficit on cytokinin activity requires more precise work, particularly with regard to the degree

of water deficit applied before the importance of the effect can be reliably assessed.

1.3.3. Ethylene

The consideration of ethylene as a plant growth regulator was aided by the advent of gas chromatography which made it possible to measure small quantities of substances. Ethylene has long been known to induce abscission (Pratt et al. 1969). It had also been reported that the developing flowers and fruit of cotton abscise after re-watering plants that had been exposed to water deficit (Stockton et al. 1961). Abscission of cotton bolls or leaves appears to be related to the minimum water potential reached before dawn and when water potential at that time drops to -8 bars abscission of bolls and leaves is accentuated after re-watering (McMichael 1972). This abscission is regulated by ethylene as severe water deficit induced a sharp increase in ethylene production which declined quickly on re-watering (McMichael 1972). Despite this apparent relationship between ethylene production and abscission there was no clear linear relationship between the magnitude of water deficit and induced ethylene production and abscission. It has also been concluded that ethylene is one of the regulators of young fruit abscission (Lipe and Morgan 1972).

Further evidence for a stimulation of ethylene production by water deficit is the report of Ben-Yeshoshua and Aloni (1974) who

observed that Valencia orange plants maintained in a 50% relative humidity atmosphere released more ethylene and contained more ethylene within the tissues than similar plants maintained in water saturated air. Furthermore water deficit induced defoliation from detached branches. Relieving the water deficit of such leaves by transferring them to a mist chamber resulted in a lowering of the rate of ethylene released to the level occurring in leaves maintained continuously in a mist chamber. This ability to recover from water deficit was evident following only 10 to 20 hrs of water deficit when the relative turgidity of the leaves was 50% to 60%. With further water deficit the release of ethylene was not lowered when the plant was rehydrated in a mist chamber. Ethylene release was directly proportional to the relative turgidity of the detached leaves. However, there was no direct relationship between ethylene production and abscission (McMichael et al. 1972).

It is clear that water deficit induced enhanced ethylene production in at least some plant species. There is reliable evidence that ethylene in physiological concentrations affects a number of plant processes (Pratt et al. 1969) and although the relationship between water deficit, ethylene production, and abscission is not clear it is likely that some of the effects of water deficit are mediated through enhanced ethylene production.

1.3.4. Other Hormones

Reports of effects of water deficit on gibberellic acid and auxin are scarce. It has been reported that water deficit increases the activity of IAA-oxidase (Darbyshire 1971a). Since an inverse relationship between IAA-oxidase and the concentration of endogenous IAA has been demonstrated (Jain et al. 1969; Shaw and Hawkins 1958), Darbyshire concluded that water deficit would reduce endogenous auxin concentration through enzymatic degradation in the stressed plants and this would lead to a retardation of growth during water deficit. This hypothesis has been criticised on the grounds that cell extension appears to be much more sensitive to water stress than does the activity of IAA-oxidase and that it would be difficult to see how Darbyshire's hypothesis could apply in a straightforward way (Hsiao 1973). It has also been suggested that there is a diurnal rhythm of auxin concentration in the plant due to the periodic changes of water status of the plant (Tal and Imber 1971) but in this work direct measurements of either auxin concentration or water status in the plant were not made and conclusions are therefore indirect. Similarly Moss and Downey (1971) attributed a change in the sex ratio of corn produced by water deficit to a reduction in internal auxin concentration.

1.4. ENZYME ACTIVITY AND WATER DEFICIT

The growth and development of a plant depends on the metabolic processes occurring within the cells of that plant and these in turn depend upon the presence and concentrations of the reactants and enzymes. The activity of the enzymes will depend not only on the local environment of hydration, ions, temperature, cofactors inhibitors etc., but in addition, as many enzymes function as part of a complex structure, the association with other enzymes, lipids and other macromolecules will be important (Todd 1972). As a water deficit would appear to have a direct effect on many of the conditions influencing enzyme reactions it is conceivable that the rates of many enzyme reactions within the cell will be affected directly or indirectly by water deficit. This in turn would affect the rates and direction of metabolic reactions and consequently the general growth of the plant. The most obvious possibility would be a direct effect of dehydration on the enzymes themselves. It has been suggested that dehydration of a tissue would cause dramatic shifts in overall metabolism leading to degradation or inactivation of particular enzymes (Todd 1972). This however, might not occur in plants since the extent of dehydration at which this might happen would not be reached by a normally wilted plant.

The study of the effect of water deficit on enzyme activity is very difficult because enzyme studies involve homogenisation

which has two serious inherent problems. Firstly the act of homogenisation itself exposes the enzymes to other enzymes and other conditions which are completely different from the situation in the cell. Enzymes which are associated with specific organelles due to compartmentation are exposed to other organelles to which they are never exposed *in situ*. Secondly the enzymes are homogenised in a certain medium which therefore changes the degree of water potential in which the enzyme activity is being tested. Even if the grinding medium is kept at the desired water potential the points discussed above still make it invalid. Thus results of studies of effect of water potential on enzyme activity should be taken with caution since they might not relate to what exists *in situ*.

The recorded effects of water deficit on enzyme activity can however, be classified into two main categories. Some enzymes show a decrease in activity during water deficit whilst others show an increase. It has been concluded that severe stress lowers the activity of all enzymes, although moderate water deficit frequently raises the activity of the hydrolytic enzymes (Todd 1972). Among the enzymes whose activities are readily reduced by water deficit are nitrate reductase and phenylalanine ammonia lyase. In the first report of a decrease in activity of nitrate reductase caused by water deficit (Mattas and Pauli 1965) the effect was confounded by an increase in temperature during the experiment, and it was

pointed out that the increased temperature could have caused the decrease in activity (Hsiao 1973). In a subsequent report, however, the decrease in activity of nitrate reductase and phenylalanine ammonia lyase with water deficit alone was substantiated in young maize plants (Bardzik et al. 1971). The activities of the two enzymes decreased markedly with a drop in relative water content of 10% to 20% but the activities did not decline to zero even when the relative water content was 50% below that of control plants. Instead, enzyme activity appeared to approach a new steady state. On rehydration partial to complete recovery of enzyme activity occurred within 24 hrs. This decrease in nitrate reductase activity resulting from water deficit has been reported to be due to a reduction in enzyme synthesis at low water potentials (Morilla et al. 1973). In view of the general inhibition of protein synthesis by water deficit (Naylor 1972) it may be suspected that enzymes with a short half life such as nitrate reductase and phenylalanine ammonia lyase will be reduced in activity due to this suppression of protein synthesis. There is also a similar decline in the activities of lactic, malic, and glucose-6-phosphate dehydrogenase with progressive dehydration (Stutte and Todd 1969; Tsai and Todd 1969).

Hydrolytic and degradative enzymes in general tend to increase in activity during a moderate to severe water deficit. α-Amylase and ribonuclease activity have been observed to increase

during water deficit (Takaoki 1968; Genkel et al. 1967) although the functional significance of these increases in activity is unknown. Since there is usually a decrease in the starch content of plants exposed to water deficit and an increase in sugar content one might infer that water deficit increases the activity of amylolytic enzymes. On the other hand the observed decrease in starch content may be due to a decrease in photosynthesis rather than an increase in hydrolysis (Hsiao 1973). Indeed, it has been reported that sucrose accumulates in plants exposed to a water deficit but not glucose as would be the case if the underlying process were an enhancement of α-amylase activity (Stewart 1971). In an analogous situation it has been demonstrated that the observed reduction in ribosomal RNA during water deficit is not associated with an increase in ribonuclease activity (Morrila et al. 1973). It has been suggested that the hydrolytic enzymes which increase in activity during water deficit are probably separated from their substrate due to compartmentation in the cell. Homogenisation during extraction leads to their being mixed with the substrate in the cell giving rise to misleading results (Hsiao 1973).

In contrast to the apparent increase in the activity of α-amylase in leaves induced by a water deficit, α-amylase formation in germinating seeds is depressed by water deficit. Lowering of the incubating medium by a few bars with mannitol (0.2 M or more) led to a substantial inhibition of α-amylase production by

isolated barley aleurone layers (Jones 1969).

The effect of water deficit on enzymes that are present in more than one form has not been very extensively studied. It has been demonstrated that succinic and glucose-6-phosphate dehydrogenase changed quantitatively whereas malic dehydrogenase changed both quantitatively and qualitatively after severe water deficit (Stutte and Todd 1969). Also glycolytic activity was suppressed almost completely in droughted corn leaves whilst, at the same time, the rate of oxidation via the pentose phosphate pathway was doubled (Abrarov 1969). This shift was apparently a consequence of the synthesis of new enzymes since the increase in pentose phosphate pathway activity was not observed when azauracil was supplied during drought.

Certain enzymes may be active at low water contents when others have been denatured. This might cause a change in the balance of metabolic processes in the plant and could be one of the causes of the lethal effects of severe drought.

1.5. WATER DEFICIT AND NITROGEN METABOLISM

1.5.1. Protein Synthesis

Reports of the effect of water deficit on net protein synthesis are limited. Since it was first claimed that water deficit results in an enhanced hydrolysis of protein (Mothes 1931)

other workers (MacPherson 1952; Kemble and MacPherson 1952) have substantiated this claim but all have used excised, wilted plants. Mothes' claim has been criticised on the grounds that his experimental plants were starved (Petri and Wood 1938) but nevertheless these same workers also reported hydrolysis of the protein of tobacco leaves on intact plants during drought. It has been suggested that many of the claims of enhanced hydrolysis of protein resulting from drought must be re-interpreted (Gates 1964). Firstly, protein synthesis may be interrupted in plants subjected to a water deficit giving a false impression of increased hydrolysis. Secondly the pooling of protein measurements from young and old leaves is misleading as Dove (1964) found that old tomato leaves lost protein during a period of water deficit whereas young ones did not. It has been observed that there was a net reduction in both the soluble and total protein fractions in sugar beet leaves exposed to water deficit (Shah and Loomis 1965), but the soluble protein fraction was either not affected or reduced when relative water content was reduced to about 60% in wheat plants (Todd 1972; Todd and Basler 1965).

Although the evidence presented clearly favours a net reduction in protein content during water deficit, this decrease in protein content may be due either to an acceleration of protein degradation or an inhibition of synthesis. Some workers have attempted to resolve this problem by examining the incorporation

of labelled amino acids into protein. A technical difficulty with such studies is that the tissue was generally floated in a solution of labelled amino acid after the plants had been exposed to water deficit and re-watered. In such cases the measurements can only be interpreted as a result of an after effect of water deficit. Using such methods, however, Ben-Zioni et al. (1967) reported a reduction by half in the incorporation of labelled leucine into protein of tobacco leaves following mild to moderate water deficit, complete recovery of the rate of incorporation requiring three to four days. Similarly, using root tips of maize and rehydrating the tissue before measuring amino acid incorporation, it was found that an inhibition of incorporation occurred when such tissues were dried to 30% of the original fresh weight. If the tissue was not rehydrated before incorporation was measured, inhibition occurred when the tissue was dried to only 11% of the original fresh weight (Nir et al. 1970). This suggested that protein synthesis is inhibited by a water deficit of mild or moderate intensity but that continuation of the effect following re-watering required a more severe water deficit.

Due to problems in such incorporation studies Hsiao (1973) indirectly assessed effects on protein synthesis by measuring the polysome profile in plants subjected to water deficit. This was on the supposition that the more protein synthesis was occurring in the cell the greater would be the proportion of the polysomes to monosomes and the larger would be the polymers present.

Water deficit caused a shift from polymeric to monomeric form in rapidly growing meristem tissue, noticeable 30 minutes after the initiation of water deficit. The shift from the polymeric to the monomeric form appeared to be proportional to the intensity of water deficit in green shoots of maize. Similarly a loss of dimers and trimers following the loss of more than 50% of the water from root apices of Zea mays has been reported (Nir et al. 1970).

The stage of the process of protein synthesis which is most sensitive to water deficit has been controversial. Although it has been postulated that water deficit affects protein synthesis at transcriptional level (Nir et al. 1970), some evidence suggests that the effect of water deficit is at the translational level. For instance, the application of cycloheximide prevented the change in the distribution of polysomes induced by water deficit (Hsiao 1970). Furthermore, re-watering maize seedlings subjected to water deficit caused a rapid regeneration of polymeric ribosomes, although there was a lag period that apparently depended on the duration and degree of water deficit (Hsiao 1970). These observations, coupled with the rapidity of response to water deficit and quick reversibility following re-watering led to the conclusion that water deficit inhibited the elongation of peptide chains through an effect at the translational level but not at the transcriptional level (Hsiao 1970).

The shift from polymeric to monomeric ribosomal RNA induced by water deficit may be due to the reported increase in RNAase activity (Tvorus 1970) but it has been found that the decline in polysome level preceded any noticeable increase in RNAase activity during the period of water deficit (Hsiao 1970). Moreover there was no apparent correlation between the shift from polymeric to monomeric ribosome and RNAase activity. Similarly, it has been reported that the reduction in polyribosome content of corn exposed to water deficit was not associated with ribonuclease activity (Morilla et al. 1973). Considering this evidence, it does not appear likely that the early shift of ribosomes from a polymeric to a monomeric form can be mediated through an increase in RNAase activity.

Since it has been claimed that a water deficit causes a reduction in cytokinin activity (Itai and Vaadia 1965; Itai et al. 1968) in the plant and also considering the fact that the application of benzyladenine alleviates the water deficit induced inhibition of amino acid incorporation into protein (Shah and Loomis 1965) it has been suggested that protein synthesis is governed by cytokinin activity (Itai et al. 1965). However, apart from the inconsistency in observations of alleviation of water deficit effect with cytokinin the reversible shift of ribosomal forms appears to occur too rapidly for it to be controlled by changes in cytokinins.

1.5.2. Amino Acid Accumulation

One common reaction of plants to water deficit is an accumulation of free amino acids. It has been suggested that this is as a result of the protein hydrolysis brought about by the water deficit (Mothes 1931). This view has been refuted on the grounds that the composition and proportion of the amino acids accumulated does not reflect the result expected from the hydrolysis of "average" cell protein (Naylor 1972). Water deficit usually causes a marked accumulation of free proline in many plants (Kemble and McPherson 1954; Mothes 1956; Prusakova 1960; Chen et al. 1964; Barnett and Naylor 1966; Singh et al. 1973). An increase in some amides due probably to the deamination of free amino acids has also been reported (Mothes 1966). Initial incorporation and retention of ^{14}C into free amino acid in Bermuda grass was found to be the highest in plants subjected to moderate water deficit (-15 bars) followed by plants subjected to severe water deficit (-30 bars) and then the control (Barnett and Naylor 1966). The incorporation of amino acid into protein, however, was highest in the control plants, followed by those subjected to moderate water deficit (20% of the control) whilst very little label was incorporated into protein of plants subjected to a severe water deficit. These data showed that although free amino acids were synthesised during the water deficit, the incorporation of the synthesised amino acid into protein was

poor (Barnett and Naylor 1966). Free proline has been found to be the most common amino acid that accumulates in plants subjected to water deficit. This accumulation is readily reversed by re-watering (Routley 1966). An increase of 10 to 125 times in the concentration of proline accompanied by a decrease in glutamic acid and alanine has been found in plants subjected to water deficit (Barnett and Naylor 1966). This accumulation of amino acids may be at the expense of carbohydrate as added sugars increase the accumulation of amino acids (Routley 1966). By varying the amount of carbohydrate and the addition of inhibitors of the enzymes of the tricarboxylic acid cycle, it was demonstrated that most of the accumulated proline was newly formed.

This increase in the free amino acid pools might be a mechanism for preventing an accumulation of NH_4^+ in the cell (Henkel 1964) but many speculative roles have been ascribed to the accumulation of proline. For instance, it has been suggested to act in a protective role against desiccation since application of exogenous DL-proline promoted the recovery of wheat plants from water deficit (Tyankova 1967). It may be significant in this connection that Singh et al. (1973) found a relationship between proline accumulation potential and varietal drought resistance. Alternatively, it could be a form of energy storage which is released following re-watering since proline could be metabolised to glutamic acid which could also be metabolised into α-keto-glutarate which could then be incorporated into the Krebs cycle.

1.5.3. Nucleic Acids

Although there have been a few reports of changes in both the composition and content of the nucleic acid pools in plants following water deficit (Kessler and Frank-Tischel 1962; Gates and Bonner 1959; Genkel et al. 1967) it has recently been suggested that these reports should be extensively re-evaluated as the techniques employed were questionable (Hsiao 1973). As many of the reports are, in fact, conflicting, no clear picture emerges.

1.6. WATER DEFICIT AND REPRODUCTION IN ZEA MAYS

Any of the metabolic processes discussed above which are modified as a result of water deficit may be the main causes of the morphological changes observed during and following an episode of water deficit. There has been little attempt so far to link metabolic studies with morphological changes and, indeed, the morphological changes are also not well understood.

Most of the recorded morphological changes caused by an episode of water deficit have been observed with grasses, especially cereals. For instance, the responses of the shoot apex to a period of water deficit have been demonstrated with the terminal inflorescences of perfect flowers of grasses, particularly with cereals (Nicholls and May 1964). It is probable that some of the details of the response in floral development could be better

observed with a monoecious grass such as Zea mays where the formation of male and female flowers is separated both in position and, to a limited extent, in time (Bonnett 1948). Such a plant also offers an opportunity to observe the influence of water deficit on axillary inflorescences.

There have been a number of observations of the effects of water deficit on development, morphogenesis and sex determination in Zea mays. Development of the reproductive organs has been observed to be sensitive to a period of water deficit. A period of water deficit retards the developmental events of corn (Seimer et al. 1969). Water deficit imposed at the period before tasselling delayed both tasselling and silking by 4 or 5 days (Robins and Domingo 1953) and water deficit imposed later in development delayed silking (Moss and Downey 1971; Barnes and Woolley 1969). Not one of these studies involved the dissection and observation of the developing inflorescences, however, so the effects of water stress on contemporary development are not known.

The effect of water deficit on reproductive growth of plants whose flowering is determinate is quite marked. Since flowering of corn is determinate water deficit has quite drastic effects on its reproductive growth (Shaw and Laing 1965). In such determinate species there is usually a marked critical period when a period of water deficit has a deleterious effect on yield. It has been repeatedly reported that the period between tasselling and silking

is the most critical period when water deficit causes the highest reduction in yield. The depletion of soil moisture to wilting percentage for one or two days during tasselling caused as much as 22% reduction in grain yield whilst depletion to the wilting point for 6 to 8 days during the same period caused 50% reduction in yield (Robins and Domingo 1953). When water deficit was imposed in the field at the stage of tasselling to silking by imposing 2 or 3 wetting and drying cycles equivalent to 7 days of water deficit, a 51% reduction in yield was realised (Denmead and Shaw 1960). The reduction in yield resulting from water deficit has been attributed to a direct reduction of leaf area, as well as to a reduction in photosynthesis. It has been observed that inhibition of leaf enlargement occurs in corn at a water potential below -4 bars and that leaf enlargement is more sensitive to water deficit than photosynthesis which declines more gradually during water deficit (Boyer 1970a). This is explained as probably being due to the different roles of water in the different processes. Reduction in the rate of photosynthesis is thought to be brought about partly by closure of stomata whilst changes in cell enlargement is due directly to the water status of the plant (Boyer 1970a).

Although there is a reduction of yield when corn is exposed to a water deficit at certain stages, the extent of reduction and the period of highest sensitivity seem to differ between cultivars.

For instance Barnes and Woolley (1969) reported that imposing a period of water deficit on multi-ear and single eared varieties until the relative turgidity of the 5th leaf in each variety was 75% at the period of tassel emergence caused a reduction of yield of 7.5% in the multi-eared variety and 6.8% in the single eared variety. However, exposing the plant to similar degree of water deficit at the period of pollination caused a reduction in yield of 14% in the multi-eared variety but a reduction in yield of 73% in the single eared variety. A similar treatment at ear initiation caused 22% reduction in yield in the multi-eared variety and 48% in the single eared variety. Thus for the multi-eared variety the most sensitive period to water deficit is during ear initiation whilst in the single-eared variety (noted for its high sensitivity to water deficit) the most sensitive period is during pollination. These workers claimed that the relatively high yield of the multi-eared variety when water deficit is imposed during pollination is largely due to a high number of second ears which developed whilst the relatively low yield when stressed at ear initiation is due to the very few second ears that developed.

The period of grain filling is also known to be sensitive to water deficit. It was found that when plants were exposed to water deficit for 2 or 3 cycles, each cycle being 2 or 3 days at the end of which the soil moisture availability was 50%, there

was a 21% reduction in yield (Denmead and Shaw 1960). The reduction in yield at this stage was attributed to an effect of water deficit on the translocation of photosynthates to the grain since it has been reported that when $^{14}CO_2$ was fed to the leaves of corn plants droughted to an average leaf potential of -20.2 bars during grain filling, more radioactivity was retained in both fed and non-fed portions of leaf than plants which were irrigated and whose average leaf potential was -15 bars (Brevdan and Hodges 1973).

Water deficit is also known to affect the sex ratio of corn. Exposure of corn plants to a period of 5 days of water deficit 59 days after germination led to an increase in male flower formation and an inhibition of the formation of female flowers - this could even extend to the development of male flowers in the axillary inflorescence (Moss and Downey 1971). This was suggested to be due to a reduction in auxin content caused by the water deficit since Heslop-Harrison (1960) found that auxin applied to corn plants resulted in an increase in female expression as indicated by male sterility and female florets being formed in the tassel. Moreover, Sladky (1969) found that in corn a low internal concentration of auxin and a high concentration of gibberellin-like substances accompanied differentiation of the tassel and an increment in auxin concentration was associated with ear differentiation.

The fact that most studies of effect of water deficit on reproduction has been done with grasses which have terminal inflorescences, the retardation of developmental events caused by a period of water deficit, the marked critical period, the difference between cultivars in the effect of water deficit, together with the accounts of floral morphogenesis in corn grown without water deficit (e.g. Bonnett 1948), suggested that Zea mays would be an interesting subject for an investigation of the effects of water deficit on meristem growth and differentiation as well as on the reproductive organs themselves.

2. MATERIALS AND METHODS

2.1. MATERIALS

2.1.1. Plant Material

Throughout the project all plants were raised from corn seeds of cultivar IO Chief which is a one-eared hybrid developed in the U.S.A. The seeds were purchased from Rumsey Seeds Pty. Ltd. of Australia. Seeds of 1 lb net in tins were purchased from time to time and kept in tin boxes in the seed store of the Department of Plant Physiology of Waite Institute of University of Adelaide. Each tin of seeds was kept in the seed store for a maximum period of three months.

2.1.2. Chemicals and Reagents

The chemicals and reagents used in this project were of analytical grade. The sources of the chemicals are listed below:-

(±) Abscisic acid (racemic mixture): Sigma Chemical Co., Mo., U.S.A.

Anakrom ABS: Analabs Inc., Connecticut, U.S.A.

Benzylaminopurine (Benzyl adenine): International Chemical Corporation, New York.

Butylated hydroxy toluene: Calbiochem, California, U.S.A.

Cellulose powder MN 300: Macharey Nagel and Co., Duren, Germany.

2-Chloroethylphosphonic acid (Ethrel): Amchem Products Inc., PA., U.S.A.

2-(Chloroethyl)trimethyl ammonium chloride (CCC): Amchem Products Inc., PA., U.S.A.

2,4-Dichlorophenoxy acetic acid (2,4-D): British Drug House Ltd., England.

Dowex 50W-X8 (200 to 400 mesh): Bio-Rad Laboratories, California, U.S.A.

Ethylenediaminetetra acetic acid: BDH Chemicals Ltd., England.

Gaschrom Q (100 to 120 mesh): Applied Science Lab., Pennsylvania, U.S.A.

Gibberellic acid (97.4%): Merck and Co., New Jersey, U.S.A.

Hexamethyldisilazane: Applied Science Lab., Pennsylvania, U.S.A.

OV 17: Applied Science Lab., Pennsylvania, U.S.A.

*Phaseic Acid: Dr. Brian Loveys, C.S.I.R.O. Division of Horticultural Research, Glen Osmond.

*QF1 + DC 200 1% on Varaport 30 (100 to 120 mesh): Customs Department, South Australia.

Silyl-8: Pierce Chemical Co., Illinois, U.S.A.

Succinic acid-2-2-dimethylhydrazide (SADH): Naugatuk Chemical Division, Conn., U.S.A.

p-Tolysulphonylmethylnitrosoamide: Koch-Light Laboratories, England.

2,3,5-Triiodobenzoic acid: Koch-Light Laboratories, England.

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* Gifts of these compounds are gratefully acknowledged.

2.1.3. Solvents

All solvents used for the extraction of abscisic acid, phaseic acid and amino acids and in chromatography were distilled in a glass fractionating column before use.

Petroleum spirit (b.p. 60° to 80°C) was dehydrated by distilling and storing over calcium chloride for two weeks after which it was stored over freshly extruded sodium wire and kept in a tightly capped container according to the method described by Vogel (1956).

2.2. METHODS

2.2.1. Cultural Practices

All experimental plants were raised by sowing six seeds in a 9 litre plastic bucket filled with John Innes compost. These were thinned to one seedling per bucket usually seven days after germination. In experiments where plants were treated approximately a week after germination, thinning was carried out earlier. The plants were graded according to size and equal-sized plants were retained for experimentation. The plants retained following thinning were allowed to grow to maturity in the same bucket.

All experiments were performed in one of two glass houses which were heated to 22° $\pm$ 2°C when necessary. The heating was

turned on usually between May and mid-June. No plants were raised during the month of June through to late August since the natural light intensity was too low to permit rapid growth. Except during the period when water deficit was imposed, plants were watered copiously to run-off once daily.

In the initial stages of this project 500 ml of Hoagland solution was added to each pot during the period of silking. This was prepared with the following salts, with the concentration in ppm shown in brackets: $Ca(NO_3)_2.4H_2O$(1653), KNO_3(505), KH_2PO_4(348), $MgSO_4.7H_2O$(493), Fe.EDTA(1.0 Fe), $MnSO_4$(0.25 Mn), H_3BO_3(0.25 Bo), $ZnSO_4$(0.25 Zn), $CuSO_4$(0.02 Cu), and Na_2MoO_4(0.02 Mo). Later, when plants were harvested during the silking period, it was not found necessary to add the Hoagland solution since the plants did not show any sign of lack of mineral nutrition by that period.

2.2.2. Method of Imposition of Water Deficit* and Measurement of Ψ

Water deficit was imposed on the plants by withholding water from those plants until persistent rolling of the leaves was observed for two consecutive days whereafter plants were re-watered. On the average stress periods lasted for 10 days. This empirical criterion was selected rather than an objective

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* Throughout the text the terms "water deficit" and "water stress" are used synonymously.

measurement of plant water status because insufficient plants were available for repeated destructive sampling. However, the water potential (Ψ) of the third leaf of both the drought stress plants and the watered plants were measured at the end of the period of stress. Ψ was measured with the Spanner thermocouple psychrometer (Barrs, 1968) as follows:

The thermocouple chambers were cleaned and dried just prior to measurement. Ten leaf discs of the third leaf were quickly cut from the central portion of the leaf (5 discs from each side of the midrib) with a cork borer of 1.2 cm diameter and placed in the chambers of the thermocouples which were then quickly stoppered. Each batch of ten discs from each plant was placed in a separate chamber. All the chambers with their respective thermocouples were then immersed in a water bath whose temperature was thermostatically controlled at $25^{\circ}C \pm 0.1^{\circ}C$ and equilibrated for 2 hrs before taking the reading of each sample. Each measurement was made in triplicate.

The thermocouple measurements were converted to water potential using a standard curve obtained with a range of NaCl solutions of known Ψ.

2.2.3. Measurement of Morphological Parameters

The total heights of the plants were measured from soil level to the base of the lowest lateral axis of the tassel. Tassel length was measured from the base of the lowest lateral axis

to the tip of the central axis of the tassel. The length and breadth of the axillary shoot and the axillary bud sizes were measured. The axillary inflorescence consisted of the axillary shoot and the axillary bud. The axillary shoot is here defined as the whole axillary structure including the prophyllum, whilst the axillary bud is considered to be the organ which is situated within the axillary shoot, covered by the prophyllum and which at maturity bears seeds. The developmental stages of axillary buds were assessed using a scoring system adopted and adapted from reports of Bonnett (1940) and Hanway (1963) as explained in Experiment 3.1.1. (Table 3.1.1.).

2.2.4. Excision of Plant Parts

The developing tassel within the enfolding leaves was located by holding the base of the stem of the plant between the forefinger and the thumb and moving these fingers acropetally with a little pressure till the tip of the stem was felt. A longitudinal slit was made through the foliage at this point with a scalpel and the leaves were gently pushed to each side till part of the tassel was seen. The slit was then continued down the plant to reveal the stem below the tassel. A section of the stem, approximately 1 cm long, was then excised and the tassel was gently pushed up to create a gap so as to prevent any physical contact between the tassel and the cut surface of the stem. The foliage at the sides of the slit was gently pressed together and

the plant was left to grow until the folded foliage matured and opened out and thus extruded the dead tassel. The tassel was not removed completely from the plant at the time of excision as to do so would have caused considerable damage to the foliage.

The uppermost axillary shoot was located by feeling the tip of the stem as described above followed by cutting longitudinally through the abaxial side of the midrib of the nearest outermost leaf to the tip of the stem. This cut was carefully deepened until the axillary shoot was seen. In order to confirm that this was the uppermost axillary shoot, a similar cut was made at the opposite side of the plant at the next higher node. Absence of an axillary shoot at this node confirmed that the axillary shoot at the lower node was the uppermost axillary branch. This was then removed by enlarging the longitudinal cut to reveal the base of the axillary branch and severing it with a cut made at the point of attachment to the stem. The detached axillary shoot was then removed completely with as little damage to the foliage as possible.

2.2.5. Application of Growth Substances

Growth substances were applied to the plants by a wick method. The bases of 1 ml stoppered plastic centrifuge tubes were cut off and an unwaxed cotton thread was put through the cut end and then through the stopper such that 3 cm of the cotton protruded from the base and about 15 cm from the stopper. The cut

base was then quickly dipped in molten wax and cooled, sealing up the base and anchoring the cotton firmly in the tube. The capacity of the tube so prepared was about 0.7 ml.

Following the preparation of the tubes, the desired quantity of the growth substance solution, usually 600 µl, was placed in the tube which was then stoppered. The loose end of the thread from the top of the tube was threaded to a needle 5 cm long and 1 mm at its broadest point. Using the needle the whole apparatus was threaded into the appropriate part of the plant, the needle being pushed completely through the plant. The thread was then disengaged from the needle, leaving the thread embedded in the plant and the tube suspended at the side of the plant. The thread then acted as a wick and the growth substance was absorbed into the plant by capillarity, usually within 24 hours.

The efficiency of this method and the distribution of the solution absorbed was examined by supplying a solution of eosin through a thread embedded in the plant just below the tassel as described above. It was found that within ten hours not only had the solution been completely absorbed but the whole aerial portion of the plant was coloured orange and the eosin could be detected without sectioning the plant. A longitudinal section through the entire aerial portion of the plant showed an extensive distribution of the eosin to all portions of the plant. Where growth substances were applied to detasselled plants, the tube with the

appropriate growth substance was threaded in a similar way, as described above, to a point 1 cm below the cut surface of the stem.

2.2.6. Method for Abscisic Acid (ABA) Assay

All measurement of endogenous ABA was done with a Gas Liquid Chromatograph (GLC) of a Varian aerograph series 1400 fitted with tritium foil electron capture detector.

2.2.6.1. Preparation for Assay

2.2.6.1.1. Washing of Chromatography Papers

All papers used for chromatography were Whatmann No. 3 MM. These were washed before use by sequential elution with 0.1 M EDTA, distilled deionised water, 2 N acetic acid, distilled deionised water and distilled methanol. The paper was then air dried before use.

2.2.6.1.2. Preparation of Column Packing

In the early measurements of ABA the packing used in the GLC column was 3% OV 17 on Gaschrom Q (mesh size 100 to 120). The preparation of this packing was as follows:-

30 g of Gaschrom Q was silanised by treatment with distilled benzene containing 0.5 hexamethyldisilazane (HMDS) to just cover the Gaschrom in a litre round bottom flask. The benzene and excess HMDS were removed under reduced pressure at 55° to 60°C on a rotary evaporator and then heated to 60°C for about 30 mins.

This procedure was then repeated twice.

The silanised Gaschrom Q was then suspended in methylene chloride until the liquid was 6 mm above the solid. To this, 30 ml of anhydrous methylene chloride containing 1 gm of OV 17 was added. The slurry was thoroughly mixed by swirling, and the methylene chloride was removed under reduced pressure at 55° to 60°C on a rotary evaporator as before. The OV 17 coated support was then dried in an oven at 60°C.

The 3% OV 17 on Gaschrom Q thus prepared was used until it was established that this column packing did not separate cis-trans phaseic acid from trans-trans ABA. The column packing was then changed to 0.8% QF1 + 0.2% DC 200 on Varaport 30 (mesh size 100 to 120) as used by Coombe (1972). This was able to separate phaseic acid and trans-trans ABA. All results of ABA and phaseic acid reported in this project were therefore obtained using this column packing (see Appendix 2 for phaseic acid report).

This packing was obtained from the Australian Customs Department, South Australia. The packing was prepared by a bulk method where the liquid phases, 1% QF1 and 1% DC 200 were separately dissolved in anhydrous methylene chloride and poured into a Buchner funnel containing the solid support of Varaport 30. The slurries were then dried overnight under vacuum and finally mixed to provide suitable chromatographic separation.

2.2.6.1.3. Silanisation of Column

The method of silanisation of the column was developed by Firn (1969). The glass column was cleaned by sucking through sequentially acetone and anhydrous benzene using a water pump. After drying in an oven the column was rinsed three times with 3% HMDS in benzene, and dried at 60°C for 15 mins after each rinsing.

2.2.6.1.4. Packing of Column

The column was packed by placing 12 mm of glass wool in one end and packing the straight section with the appropriate solid support and the coiled section with the appropriate column packing. The procedure was repeated at the other end all under vacuum using the suction pump.

The column was then installed in the GLC and conditioned at 270°C for 24 hrs before use.

2.2.6.2. Extraction Purification and Measurement of Free ABA

The method for ABA assay was an adaptation of that of Coombe (1972). The material to be extracted was harvested and quickly frozen in liquid nitrogen and stored at -20°C until use. It was found by experiment that the endogenous ABA content of material harvested and stored in this way did not change within at least 3 months and all estimations were carried out within that period. Sub-sampling for the ABA assay was carried out as described

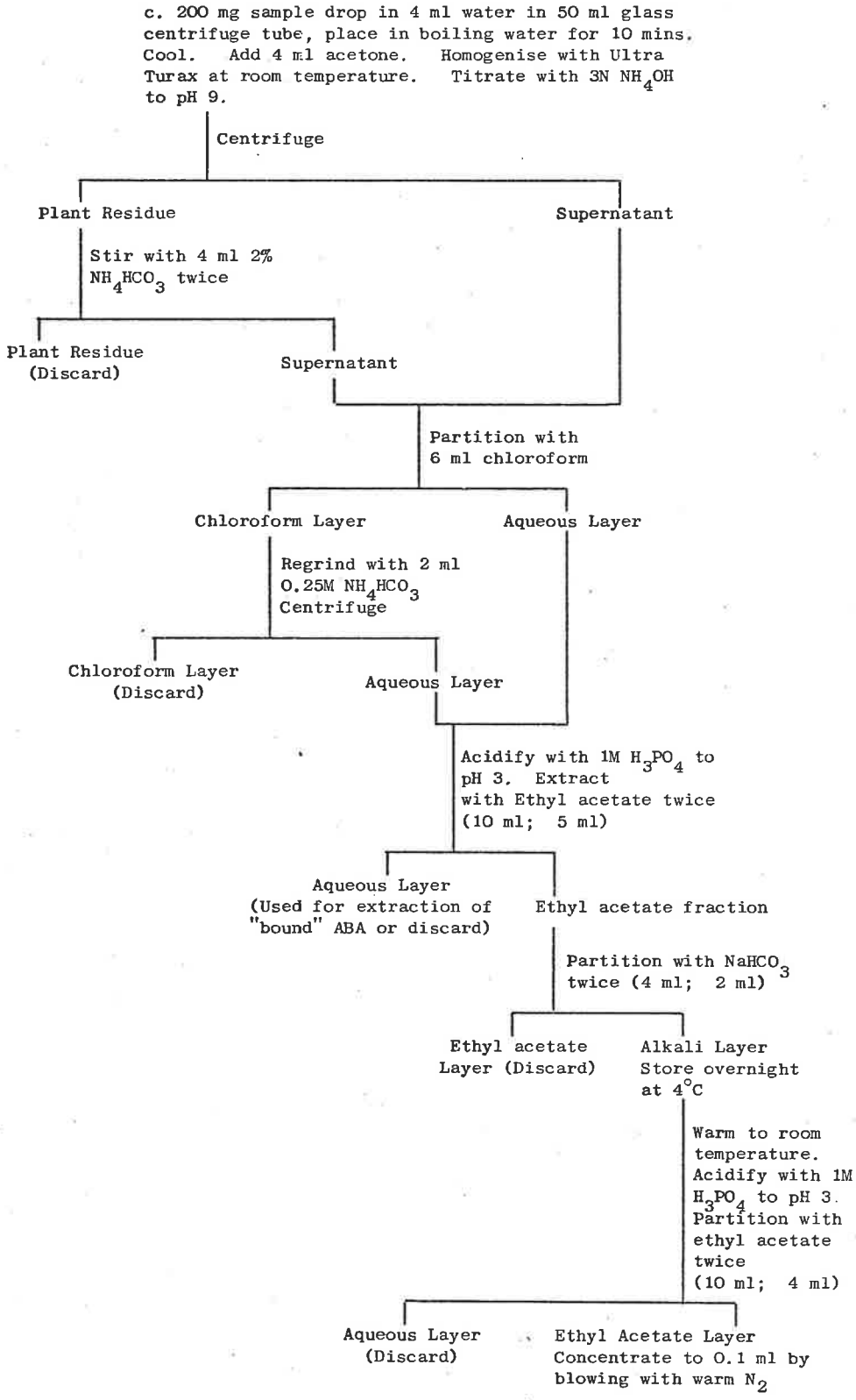
under the appropriate experiments.

Extraction: The flow diagram shows the extraction procedure adopted (Fig. 2.1.). The reason for choosing this method of extraction is that, since acetone/water is used for homogenising, there is no need for evaporation which should be done if methanol or ethanol was used. Since 24 samples were handled at every extraction this would have been a bottleneck in the extraction procedure. The efficiency of extraction of acetone/water was found to be comparable with methanol or ethanol. $NaHCO_3$ was used instead of NH_4HCO_3 at the overnight storage stage because it was found by GLC that a more purified sample was obtained.

Purification: The concentrated ethyl acetate was chromatographed as follows: The acid-washed 3 MM paper (see 2.2.6.1.1.) was loaded with about 0.2 ml per spot of ethyl acetate in which 1 mg/ml butylated hydroxy toluene (BHT) had been dissolved to reduce oxidation of the crude extract. The concentrated extract was then streaked (3.5 cm) on the paper at the spots which were loaded with ethyl acetate in which BHT was dissolved. 5 μg of ABA was spotted at each side of the paper as markers. The paper was run by descending chromatography in isopropanol:ammonia solution: water (10:1:1 v/v) for 4 hrs. It was then dried for 15 mins. The tracer markers were found by exposure of the region of the markers to U.V. light when ABA was identified as a fluorescent spot. Care was taken not to expose the extract to the U.V. light by

FIGURE 2.1.

Flow Diagram of Extraction of ABA



covering the rest of the chromatographic paper with Aluminium foil. The located ABA spots were marked and the zone of the extract with equivalent R_f was cut out and eluted overnight with 20% methanol. The eluate was evaporated to dryness under warm nitrogen and then rinsed twice with anhydrous petroleum spirit (see 2.1.3.) and evaporated.

GLC: The ABA in the extract was further separated, identified and quantified by gas liquid chromatography. The dry extract was dissolved in 0.2 ml of methanol:ethyl acetate (1:1 v/v) and methylated with diazomethane by the method of Powell (1964) as modified by Firn (1968). Diazomethane was prepared by reacting 2 ml carbitol, 2 ml of 60% (w/v) potassium hydroxide and about 100 mg p-tolysulphonylmethylnitrosoamide (TONY). The resulting diazomethane was carried in a stream of dry nitrogen saturated with methanol:ethyl acetate, through small bore "Teflon" tubing and bubbled through the extract. Bubbling was stopped after the solution had turned yellow, indicating the presence of excess diazomethane, and the extract was then dried completely under nitrogen.

The method of measurement on the GLC was essentially that of Coombe (1972). The GLC used was a Varian aerograph Series 1400 fitted with glass column and tritium foil electron capture. The column was packed with 0.8% QF1 + 0.2% DC 200 on Varaport 30 of 100 to 120 mesh (see 2.2.6.1.2.). Routinely, the injector

temperature was maintained at 240°C, the column at 180°C and the detector at 280°C. The flow rate of dry nitrogen gas through the column was 26 ml/hr.

The amount of ABA in the extracts was measured by comparison of peak height with a standard curve obtained from a series of known ABA concentrations between 50 and 500 pg of methyl cis-trans ABA dissolved in 2 µl ethyl acetate. The retention time of the standards was approximately 4 mins for the cis-trans and 6 mins for the trans-trans ABA. The extract was treated similarly by dissolving the dried methylated extract in 200 µl ethyl acetate and injecting 2 µl of this on to the column. The peak height of the tracing whose retention time coincided with those of the standards injected immediately before the extract was measured. The peak heights of the cis-trans and the trans-trans ABA were pooled and calculated as the total ABA in the extract.

Routinely, 24 samples could be handled in each extraction run and, to allow for varying sensitivity of the E.C. detector, the standard curve was re-drawn after every two measurements of the extracts. Before measuring each batch of samples, the GLC column was conditioned by injecting 10 µl of Silyl-8 followed by 2 µl of ethyl acetate containing 1 µg of methyl cis-trans ABA. From time to time, when the sensitivity of the GLC had declined, the tritium foil was removed and sonicated in 5% KOH in methanol, and rinsed six times in methanol. Alternatively, the foil was

mechanically washed in 5% KOH in methanol and again rinsed six times in methanol. In either case the tritium foil was dried for 10 minutes before re-installing in the GLC.

All solvents used for the whole procedure of extraction, purification, and measurement had the following concentrations of BHT added as an anti-oxidant.

Solvent	Concentration of BHT (μg/ml)
Acetone	10
NH_4OH	5
NH_4HCO_3	5
Chloroform	1
H_3PO_4	10
Ethyl acetate	10
20% Methanol	20
Methanol:ethyl acetate (1:1 v/v)	20

In all measurements of ABA in this project, the recovery percentage was assessed and allowed for in each extraction lot. Each sample was divided equally into two 200 mg sub-samples. 40 μg of ABA was then added to one sample before extraction. Since the two samples were of the same tissue it is assumed that the concentration of endogenous ABA should be the same and at the end of measurement the difference in ABA content between the two samples should represent the added ABA. Since the quantity added was known the

percentage recovery could be calculated, with 40 μg as 100% recovery. Recoveries of 40 to 70% were found and were applied to the measurements of endogenous ABA to calculate the actual ABA content of the sample. Every reported concentration of ABA in this project is an average of three replicates. On the whole there were very few interfering compounds by the stage the extracts were being measured as can be seen in Fig. A.2.2. (see Appendix).

2.2.7. Extraction Purification and Measurement of Bound (Conjugated) ABA

Bound ABA (probably abscisal β-D-glucopyranoside) was extracted by hydrolysing the non-aqueous fraction remaining following the initial extraction with ethyl acetate (see page 66). The method of hydrolysis is essentially those of Hiron and Wright (1973) and Davison and Young (1974). The hydrolysis was carried out by titrating the aqueous layer to pH 11 with 3 N NaOH in a centrifuge tube. The tube was then placed in a water bath at a temperature of 60°C for 45 minutes. The hydrolysed extract was cooled to room temperature and acidified with H_3PO_4 to pH 3. It was then partitioned twice with ethyl acetate (10 ml and 5 ml) and the pooled ethyl acetate fractions were evaporated to about 0.1 ml under nitrogen. Finally the sides of the vial were washed with 0.1 ml ethyl acetate.

Following hydrolysis, further purification and measurement was the same as that of free ABA described above.

3. RESULTS AND DISCUSSIONS

3.1. THE EFFECT OF WATER DEFICIT ON THE GROWTH AND DEVELOPMENT OF THE REPRODUCTIVE ORGANS

3.1.1. Morphological Development in the Absence of Water Deficit

Introduction: The sequences of events in floral differentiation in the male and female inflorescences of Zea mays cv. Golden Bantam have been described by Bonnett (1940) and Hanway (1963). These descriptions were not completely adequate for the present purposes as neither report covered the entire sequence of development from initiation to maturity and both were based on a different cultivar. As a detailed knowledge of the morphological development of the floral organs was essential in interpreting the effects of water stress on flower formation, the development of the inflorescences in the cultivar IO Chief was examined in plants grown without water deficit.

Method: Plants were grown as described in Section 2.2.1. Commencing 7 days after germination random samples of five plants were harvested every 3 or 4 days. The terminal apices of the harvested plants and the axillary branches were dissected out and observed under a binocular dissecting microscope, or, after 30 days growth, with the naked eye. The stage of development of the terminal apices and of each of the axillary branches were recorded

for each sample. Sampling was discontinued when the husks of the cobs showed signs of senescence.

Results: The cultivar IO Chief was found to follow an essentially similar sequence of morphological development of the reproductive organs as did Golden Bantam but with some differences in detail (Table 3.1.1.).

Initiation of reproductive development commenced approximately 20 days after germination. Initiation of the terminal male inflorescence preceded floral development in the axillary branches by approximately seven days. The first sign of initiation of the terminal male inflorescence was elongation of the domed vegetative apex, followed by the initiation of branch primorida in acropetal succession on the elongated apex. A proportion of the more basal branch primordia elongated to form lateral axial branches bearing further primordia. These, together with the more terminal primordia on the main axis, then differentiated to form spikelets, each of which contained two staminate flowers.

The axillary female inflorescences of corn pass through a series of developmental stages which have been described by Bonnett (1940) and Hanway (1963). As stated earlier, the numerical scales of development proposed by these authors were not completely satisfactory for the present purpose as neither covered the complete sequence of development. Accordingly, a scale was devised for the cultivar IO Chief incorporating features of both the previous

TABLE 3.1.1.

The Sequence of Development in the Uppermost Axillary Inflorescence and the Terminal Male Inflorescence in the Cultivar IO Chief

[Adapted from Bonnett (1940) and Hanway (1963)]

Approximate time after germination (days)	Axillary Inflorescence at Node 7		Terminal Inflorescence
	Score	Stage	Stage
18	0	Vegetative, dome short	Dome elongating
20			Branches differentiating
23			Basal branches elongate
25			Spikelet initiation
27	1	Dome elongating	Spikelet and empty glume on central axis differentiate
28	2	Spikelet branches differentiate	
29	3	Unequal division of branch initials	
30	4	Paired rows of spikelets on basal two-thirds of inflorescence	Inflorescence fully differentiated

33	5	Silk primordia present on florets of basal spikelets	
37	6	Silks at base visible to eye	
39	7	All silks visible	Inflorescence full size but covered by foliage
42	8	Basal silks grown to tip of enclosing prophyllum	
44	9	Two-thirds of silks to tip of prophyllum	
45	10	All silks to tip of prophyllum	
48			Tassel emerges from foliage
53			Pollen shed
56	11	Silks withering	
58	12	Grains at "blister" stage	
60	13	Embryo growth rapid	
65	14	Grains commence to dent	
70	15	All grains dented	
76	16	Husk begins to senesce	

proposals but allowing a detailed description of the development of the axillary inflorescence from initiation to senescence of the husk around the mature cob (Table 3.1.1.).

The order of development for the inflorescences at different nodes was identical but the rate at which development progressed varied considerably. The cultivar IO Chief was found to produce almost invariably 12 nodes below the male inflorescence. The lower seven of these nodes were the sites of visible axillary branches but none were found at higher nodes. Only the uppermost of the axillary branches, that at node 7, developed into a mature ear in normal circumstances and IO Chief is thus classified as a single-ear cultivar. With the exception of the lower two axillary branches all the other axillary branches initiated an inflorescence and progressed through a certain degree of floral differentiation. All development in the axillary branches below node 7 was inhibited after approximately six weeks growth and at this time the branches had reached various stages of development, depending on their position on the plant (Table 3.1.2.).

During development the gradient of axillary shoot growth rate from node to node on the main stem was reversed (Figure 3.1.1.). The branches were initiated in acropetal succession with the initiation and growth of the corresponding leaves from the terminal apex. The size distribution of the vegetative branches prior to any floral development reflected this sequence of initiation in

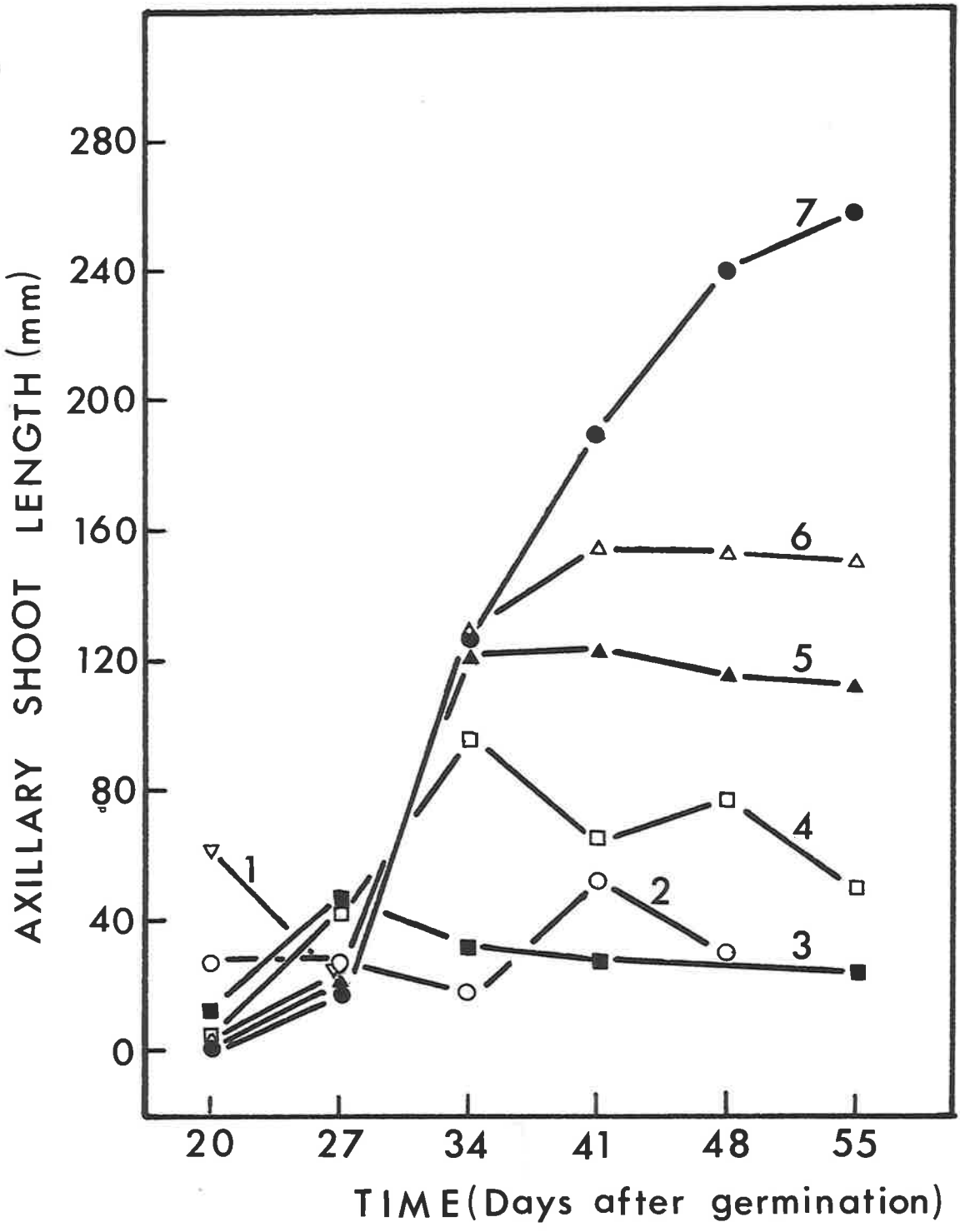
TABLE 3.1.2.

Stage of Inflorescence Development (Table 3.1.1.)
at Each Node When Growth is Arrested at All but Node 7
(Plants Grown Without Water Deficit)

	Base					Uppermost	
Node	1	2	3	4	5	6	7
Stage	Abort.	Abort.	2	3	5	7	10

FIG. 3.1.1.

Growth in length of the axillary shoots of plants grown without encountering any period of water deficit. The numbers on the graph refer to the node at which the axillary shoot is growing, node 1 being the lowest node bearing a leaf.


AXILLARY SHOOT LENGTH (mm)
280
240
200
160
120
80
40
0
20
27
34
41
48
55
TIME (Days after germination)
1
2
3
4
5
6
7

that the lower axillary branches were larger than the higher ones. Flowers were initiated first on the branch at node 7 (the uppermost node) however and thereafter the gradient of both floral differentiation and of branch growth rate was reversed with the uppermost branches growing most rapidly. The lower two axillary branches ceased growth and eventually aborted (the branch at node 1 at 3 weeks, that at node 2 at 6 weeks). The remaining branches (at nodes 3 to 6) commenced floral differentiation and persisted on the plant to maturity but their growth was eventually completely inhibited. Growth at all nodes, except node 7, ceased more or less simultaneously but at the time of growth cessation the lower branches were less advanced in both floral differentiation and physical size than those further up the plant (Fig. 3.1.1.).

3.1.2. The Effect of Water Deficit Imposed Immediately After the Time of Initiation of the Tassel

Method: Plants were grown as described previously (Section 2.2.1.). Five randomly selected plants were harvested 14, 17 and 20 days after germination and the mean stage of floral development was ascertained by dissection. At 20 days it was observed that tassel initiation had occurred and that branch differentiation on the male inflorescence had commenced.

Water was withheld from 50 plants 20 days after germination whilst a further 50 plants were watered daily for comparison. The

stress was continued until all the leaves on the plants had curled inwards on two consecutive days. When this occurred, 10 days after commencing the water stress, the plants were all re-watered. Immediately before re-watering ten plants were selected at random from each treatment and the water potential (Ψ) of the third leaf (counting from the apex) was measured using a Spanner thermocouple psychrometer (Barrs 1965) together with total height, tassel length, axillary shoot size and the axillary bud size. At the same time the stages of development of the axillary shoots were assessed using the score system of Table 3.1.1. Further samples were selected weekly from the remaining plants for the following 4 weeks and all these parameters with the exception of leaf water potential were assessed. The plants were maintained with abundant water during this period.

Results: At the time the plants were re-watered the water potential of the stressed plants was -11.5 bars whilst that of those not subjected to stress was -4.1 bars.

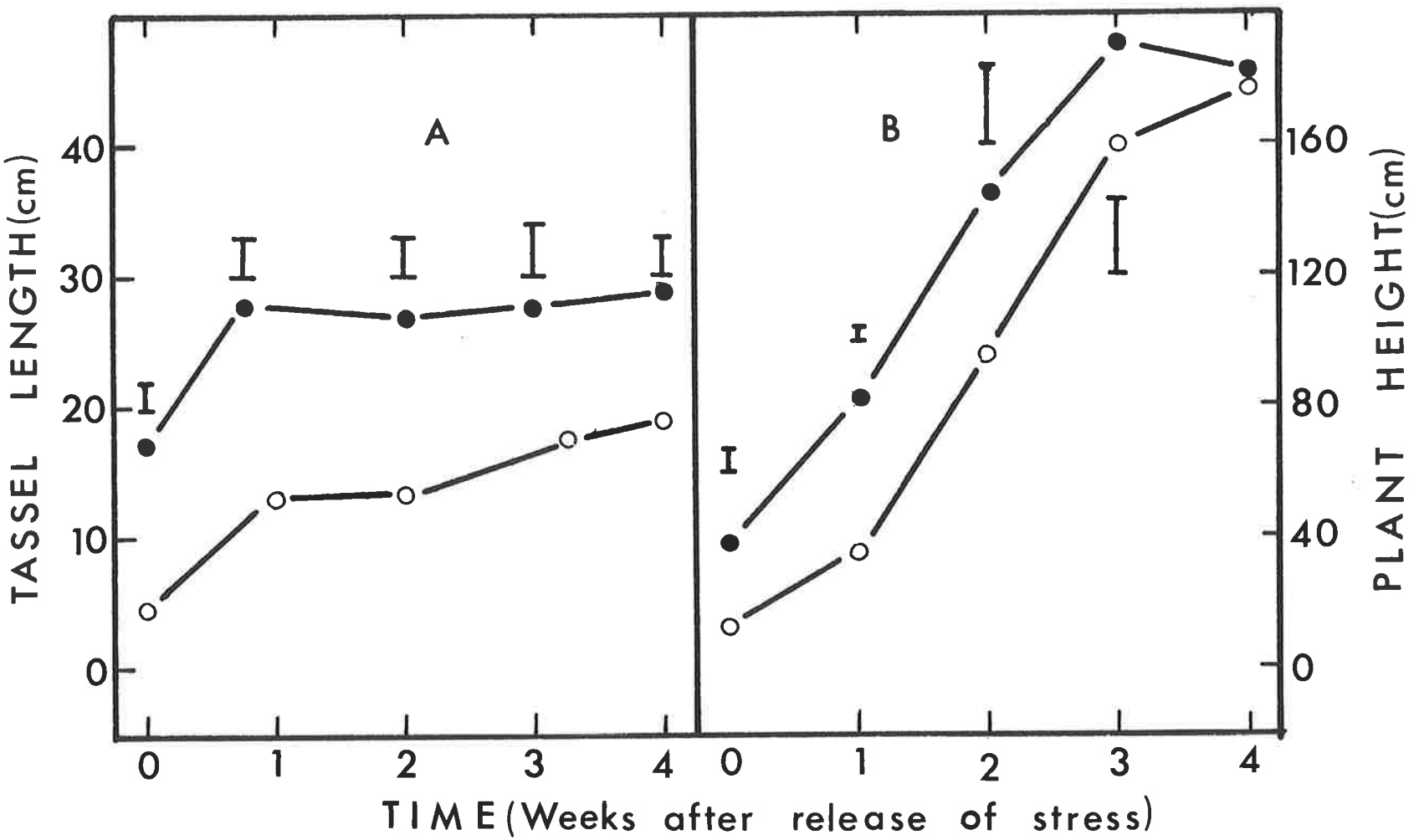
Water deficit inhibited growth in plant height during the period of stress but the watered plants ceased to elongate approximately three weeks after the stressed plants were re-watered, whereas the stressed plants continued to grow for a further week and reached the same final height (Fig. 3.1.2.).

The water deficit also inhibited the growth of the developing tassel and at the end of the period of stress the tassels of the

FIG. 3.1.2.

Effect of a period of water deficit during the period of tassel differentiation (20 to 30 days after germination) on (A) tassel length, (B) plant height.

●-● Control ○-○ Water deficit


A
B
TASSEL LENGTH(cm)
PLANT HEIGHT(cm)
0
10
20
30
40
0
40
80
120
160
0
1
2
3
4
TIME (Weeks after release of stress)

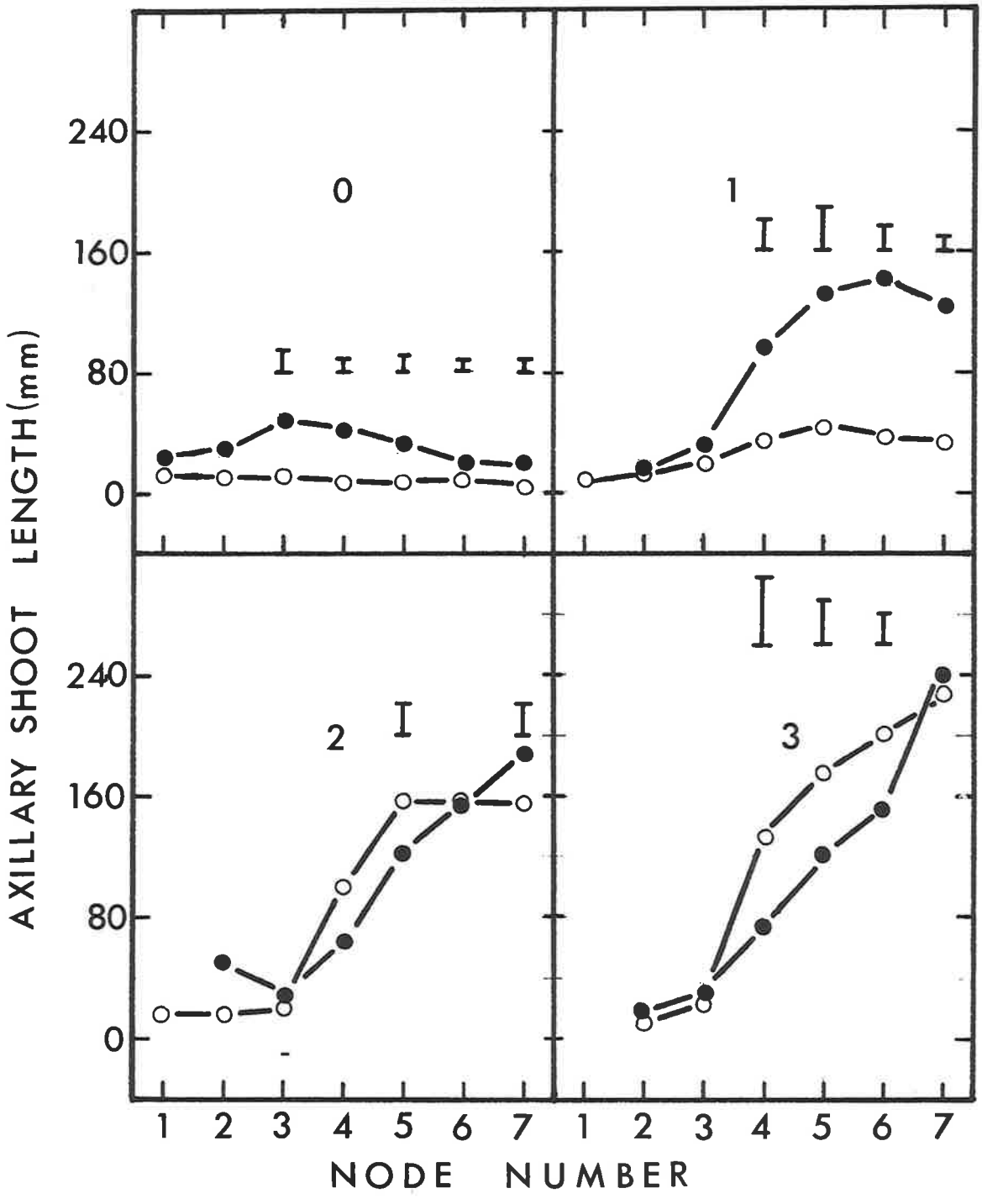
stressed plants were markedly shorter than those of the plants that were watered throughout (Fig. 3.1.2.). Although tassel growth was renewed after re-watering, it also continued in the watered plants and tassel growth ceased in both sets of plants at the same time, one week after re-watering. The resulting inhibition of tassel length in the plants subjected to water deficit was accompanied by a marked degree of sterility in the spikelet on the lateral branches, although spikelets on the main branches were generally fertile. In contrast to the effects of the water deficit on overall plant height, this response was maintained.

A further morphological effect of the water deficit concerned the growth of the axillary female inflorescences. At the end of the period of water deficit, the growth of all these axillary branches was observed to have been inhibited (Fig. 3.1.3.). Following re-watering the axillary branches and their corresponding buds resumed growth and within two weeks had grown to the same size as the corresponding branches on plants watered throughout. Thereafter all the branches apart from the uppermost (at node 7) on the watered plants ceased further growth. On the plants which had previously been subjected to an episode of water deficit, however, all axillary branches continued to grow. Thus following a further week, not only was the inflorescence at node 7 the same size in both treated and untreated plants, but the lower branches on the stressed plants had grown larger than the corresponding branches on the watered plants.

FIG. 3.1.3.

Effect of a period of water deficit during the period of tassel differentiation (20 to 30 days after germination) on axillary shoot length. 0-3 refers to the time at which the measurements were made, in weeks following re-watering of plants subjected to the deficit. Node bearing axillary shoots are numbered acropetally.

●-●Control ○-○Water deficit


AXILLARY SHOOT LENGTH (mm)
240
160
80
0
0
1
2
3
1 2 3 4 5 6 7
NODE NUMBER

The effect of an episode of water deficit on the development of the apices in the axillary branches closely followed the effect on the overall growth of the branches (Fig. 3.1.4.). The morphological development of the axillary buds was retarded during the period of water deficit in parallel with their growth. At the time of re-watering, the uppermost five axillary buds on the watered plants had developed at least as far as the first stage of floral morphogenesis (Table 3.1.3.). These buds on the watered plants continued to develop slowly for a further two weeks when morphogenesis was arrested in all but the inflorescence at node 7, confirming the observations in the previous experiment (Table 3.1.2.).

In comparison with these buds on the watered plants, the corresponding buds on the water-stressed plants were each retarded by at least one developmental stage by the end of the period of water deficit (Table 3.1.3.). Following re-watering, however, the upper six buds continued to develop for at least three weeks, the development of buds at nodes 5 to 7 being particularly rapid. Within these three weeks the bud at node 7 reached the same stage of development as the equivalent bud on the watered plants. Moreover the buds at nodes 5 and 6 were further developed than those at equivalent positions on the watered plants. With further growth, the plants subjected to a period of water deficit produced mature cobs at nodes 6, 7, and sometimes 5, whereas watered plants produced only a single cob (at node 7).

FIG. 3.1.4.

The effect of a period of water deficit during the period of tassel differentiation (20 to 30 days after germination) on the growth and development of axillary inflorescences 4 weeks after re-watering. On the right is a watered plant and on the left is a plant exposed to water deficit. Note the comparative size of the tassels and also that the axillary inflorescence at node 6 of the plant exposed to water deficit has developed into a cob and that at node 5 is considerably enlarged when compared with the equivalent axillary inflorescence on the watered plant.

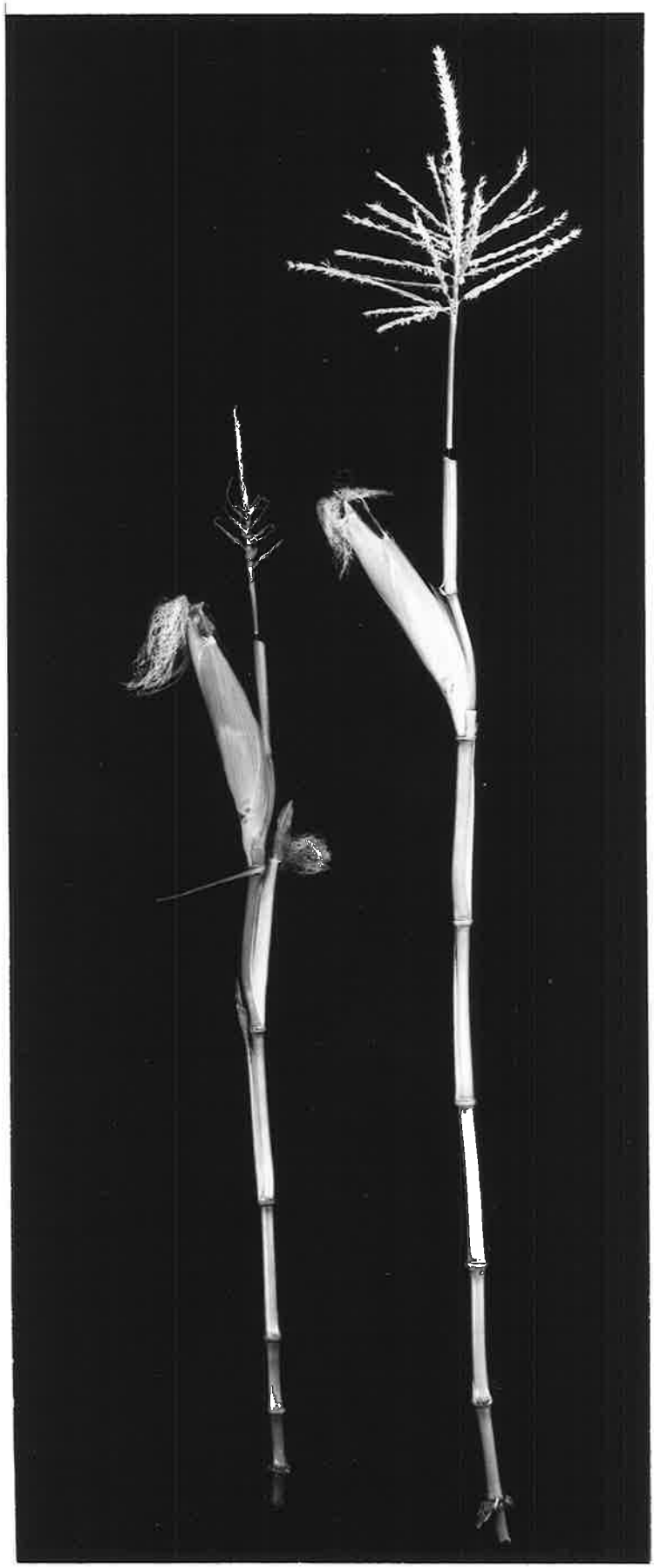


TABLE 3.1.3.

Inflorescence Development (Table 3.1.1.) of Axillary Shoots on Plants Subjected to a Water Deficit During the Period of Tassel Differentiation 20 to 30 Days After Germination

Node	Treatment	Time (Days from Re-watering Plants) 0	7	14	21
		Stage of Development			
1	Watered throughout	Abort.	-	-	-
	Water deficit	0	0	Abort.	-
2	Watered	0	1	Abort.	-
	Water deficit	0	0	0	2
3	Watered	2*	2	2	3
	Water deficit	0*	1	2	3
4	Watered	3*	3	3	3
	Water deficit	1*	2	3	4
5	Watered	3	5	5	5*
	Water deficit	2	3	6	7*
6	Watered	4	6	7	7*
	Water deficit	3	4	7	10*
7	Watered	4	7*	10*	11
	Water deficit	3	4*	8*	11

Pairs of value marked * are significantly different (P = 0.05).

Data analysed using Kruskal-Wallis One Way Analysis of Variance.

3.1.3. Water Deficit Before the Onset of Growth of the Reproductive Organs

3.1.3.1. The Effect of Water Deficit Imposed Before Tassel Initiation - Water Deficit Commencing 7 Days After Germination

Introduction: In order to explore the lower limit to the period of plant development in which a water deficit would induce an alteration in axillary inflorescence growth, a water deficit was imposed on a group of plants soon after germination before any evidence of initiation of the male inflorescence.

Method: Plants were grown as before (Section 2.2.1.) for seven days when the inflorescence development of a random sample of 5 plants was ascertained. On this same day, water was withheld from half of the remaining plants whilst the others were watered daily as before. At this stage all leaves had been initiated but only four had emerged. Water was withheld from the plants for a period of ten days and at the end of this period, ten plants from each treatment were randomly selected and their total heights, the size of axillary shoots, and the water potential of the third leaf were measured. The developmental stages of the axillary branches and the terminal inflorescence were also assessed. All the plants were thereafter watered. Five weeks after re-watering the remaining plants were harvested and the same parameters were measured.

Results: At the end of the period of water deficit there had been no initiation of tassels in the watered or the stressed plants nor had there been initiation of female inflorescences in any of the axillary branches of any plant. The water potential (Ψ) of the watered plants was found to be -3.7 bars and that of the stressed plants -10.7 bars.

At the end of the period of water deficit only the axillary branches at nodes 1 and 2 of the watered plants were larger than the equivalent branches on the stressed plants (Fig. 3.1.5.). These branches later aborted on both sets of plants and five weeks after the plants were re-watered there was no influence of the period of water deficit on branch size at any node. In this experiment and the subsequent ones the data for the axillary bud size are not presented since they followed very closely those of the corresponding axillary branch sizes and therefore do not give any further information.

The terminal male inflorescence was also unaffected by the water deficit, the average size of the tassels being 27.5 cm in both watered and drought stressed plants.

Similarly there was no difference in the developmental stages of the axillary buds at corresponding nodes in both watered and water stressed plants (Table 3.1.4.).

Thus water deficit imposed during the period before initiation did not result in any stimulation to the enlargement of the

FIG. 3.1.5.

Effect of a period of water deficit during the period of tassel differentiation (20 to 30 days after germination) on axillary bud length. O-3 refers to the time at which the measurements were made, in weeks following re-watering of plants subjected to the deficit. Node bearing axillary buds are numbered acropetally.

●-● Control O-O Water deficit

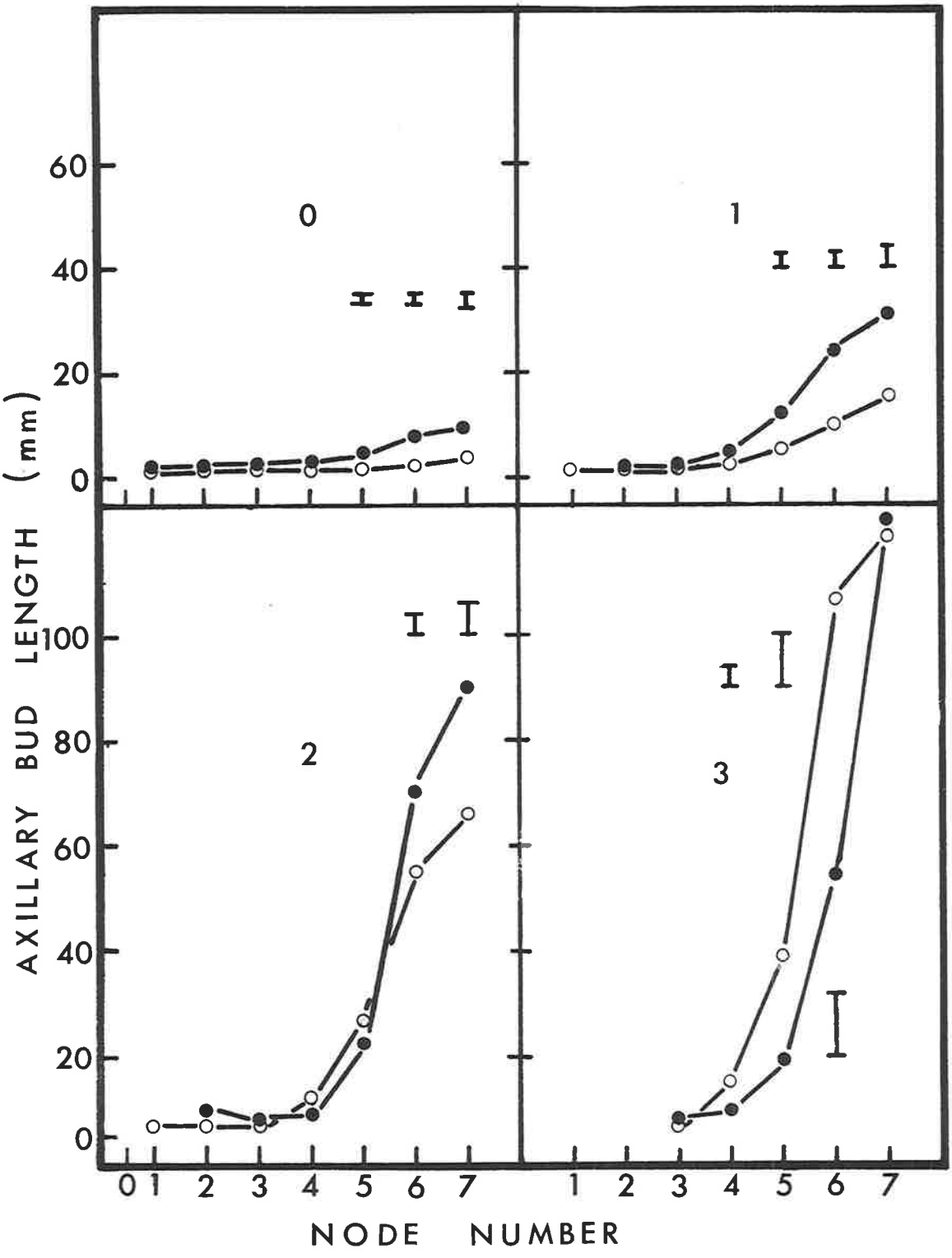

AXILLARY BUD LENGTH (mm)
0
20
40
60
0
20
40
60
80
100
0
1
2
3
4
5
6
7
NODE NUMBER
0
1
2
3
4
5
6
7
1
2
3

TABLE 3.1.4.

The Stage of Inflorescence Development (Table 3.1.1.) of Axillary Buds Five Weeks After Re-watering Plants Subjected to a Water Deficit Before Tassel Initiation (7 to 17 Days After Germination)

Node	Watered throughout	Water deficit
	Stage of Development	
1	Aborted	Aborted
2	Aborted	Aborted
3	2	2
4	3	3
5	6	5
6	7	7
7	12	12

There was no significant difference between the two treatments in the development of the inflorescences at any one node.

axillary branches nor did it influence axillary bud development.

3.1.3.2. The Effect of Water Deficit Imposed Before Tassel Initiation - Water Deficit Commencing 13 Days After Germination

Introduction: The observation that a period of water deficit commencing seven days after germination was without effect on the growth and development of the axillary inflorescences is consistent with the hypothesis that the growth and development of these reproductive organs is only influenced by water deficit occurring during the period of floral initiation. As floral initiation in this variety of sweet corn commenced approximately 20 days after germination it was decided to further delineate the sensitive phase by subjecting plants which were older than 7 days but which had not as yet entered the period of floral initiation to a period of water deficit.

Method: Plants were grown as before for 13 days after germination when a randomly selected sample of 5 plants were dissected to ascertain the stage of inflorescence development. Water was withheld from half of the remaining plants, commencing on that day and continuing for a period of 10 days. The control plants were watered daily throughout. At the end of this period, ten plants were randomly selected from each treatment and the water potential of the third leaf was measured as in previous experiments;

the total height of the plants, tassel lengths and the axillary shoot sizes were also measured and the developmental stages of the various axillary buds were assessed. All the remaining plants were watered daily for a further 4 weeks when all plants were harvested.

Results: At the end of the period of water deficit the water potential of the third leaves of the watered plants was -3.7 bars whilst that of the drought stressed plants was -11.4 bars. The plants had not entered into the reproductive phase at the commencement of the period of water deficit but the tassels were initiated during the period of water deficit. At the end of the period of stress, which was 23 days after germination, the average tassel length in the watered plants was 3.2 mm whilst that of the stressed plants was 2.1 mm. At this time, as in the previous experiment, only the growth of the axillary branches at nodes 1 and 2 had been inhibited by the water deficit. Following four weeks further growth these axillary branches had aborted and no other axillary branch was affected by the episode of water deficit either in growth (Fig. 3.1.6.) or in development (Table 3.1.5.). Again, tassel length was not affected, the early difference having disappeared to leave an average tassel length of 26.5 cm in both sets of plants.

It may be concluded that the subsequent growth and development of the axillary inflorescences is affected by a period of water

FIG. 3.1.6.

The response of axillary shoot growth (in length) to an episode of water deficit occurring before tassel initiation (7 to 17 days from germination). 0 and 5 refer to the times at which the measurements were made, in weeks following re-watering of the plants subjected to an episode of water deficit.

●-● Control ○-○ Water deficit

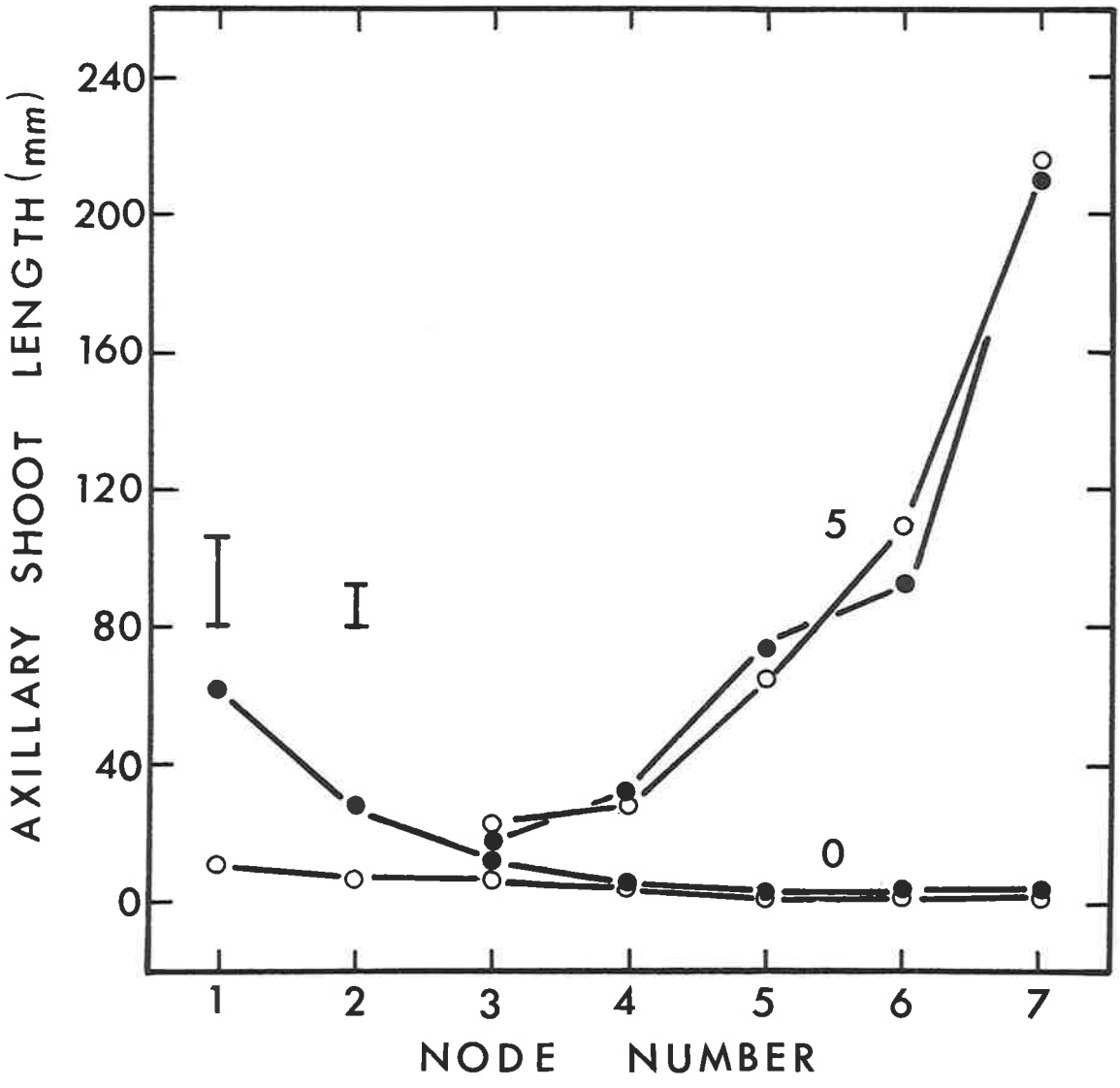

AXILLARY SHOOT LENGTH (mm)
240
200
160
120
80
40
0
1
2
3
4
5
6
7
NODE NUMBER
5
0

TABLE 3.1.5.

The Stage of Inflorescence Development (Table 3.1.1.) of Axillary Buds 28 Days After Re-watering Plants Subjected to a Water Deficit (Between 13 to 23 Days After Germination)

Node	Watered throughout	Water deficit
	Stage of development	
1	Aborted	Aborted
2	Aborted	Aborted
3	3	3
4	4	4
5	6	6
6	7	8
7	13	13

There was no significant difference between the two treatments in the development of the inflorescences at any one node.

deficit coinciding with tassel morphogenesis but not by a water deficit occurring either before tassel initiation or during the earliest stages of tassel initiation.

3.1.4. The Effect of Water Deficit Imposed After Tassel Emergence

Introduction: The previous experiments served to delineate the commencement of axillary shoot sensitivity to the effects of water deficit. They suggest that this sensitivity does not commence before the initiation of the terminal male inflorescence or even until after initiation. The present experiment was designed to explore the later limit of this sensitivity and whether it continued to later stages of inflorescence development.

Method: Plants were grown for 40 days when 500 ml of full strength Hoagland solution was applied to the soil of each plant to promote growth. 50 days after germination, when the tassels of all plants had emerged and all silks of the inflorescence of node 7 had elongated beyond the prophyllum (Stage 10) water was withheld from 20 plants. At that stage the node to node acropetal gradient of axillary inflorescence development evident in the mature plants was established. Water deficit was relieved after 9 days when the water potential of ten randomly selected plants from both treatments were measured. At this time total height, tassel length, and axillary shoot size were also measured and the develop-

mental stages of the axillary buds were assessed. Thereafter the remaining plants were watered daily for 3 weeks when the remaining plants were harvested. At the time this experiment was conducted the temperature external to the glass house was low so the minimum temperature in the experimental area was maintained at 22°C $\pm$ 2°C throughout the period of the experiment.

Results: On the day the plants were re-watered, the leaf water potential of the watered plants was -4.7 bars and that of those subjected to water deficit was -13 bars. The period of water deficit resulted only in a significant inhibition of the growth of the female inflorescence at node 7 and even this response was rapidly lost following re-watering (Fig. 3.1.7.). Both watered and stressed plants produced only a single mature cob of similar size at node 7, although grain filling in the cob of the drought stressed plants was very poor. Similarly axillary bud development was little affected by the stress. By the time the final assessment was made, three weeks after re-watering, it was only at node 7 that the axillary buds were retarded in comparison with the corresponding buds on the watered plants (Table 3.1.6.) but even this response was not statistically significant.

Clearly, the differential effect of water deficit on the growth of inflorescences at lower nodes was lost when the deficit occurred at this later stage of growth (Fig. 3.1.8.).

FIG. 3.1.7.

The response of axillary shoot growth (in length) to an episode of water deficit occurring before tassel initiation (13 to 23 days from germination), measured 4 weeks after re-watering.

●-● Control O-O Water deficit

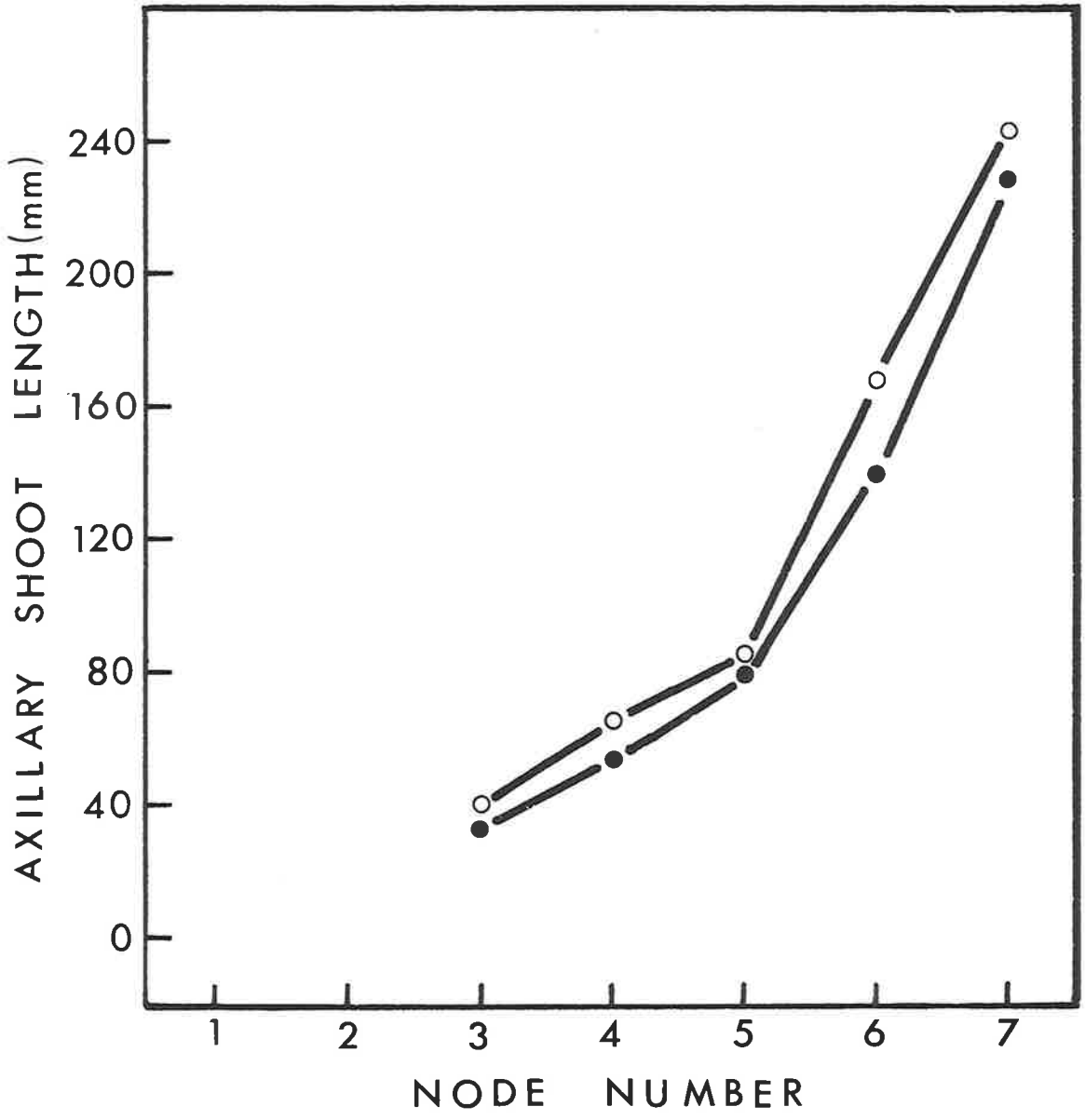

AXILLARY SHOOT LENGTH (mm)
240
200
160
120
80
40
0
1
2
3
4
5
6
7
NODE NUMBER

TABLE 3.1.6.

The Stage of Inflorescence Development (Table 3.1.1.) of Axillary Buds 21 Days After Re-watering Plants Subjected to a Water Deficit After Tassel Emergence (50 to 59 Days After Germination)

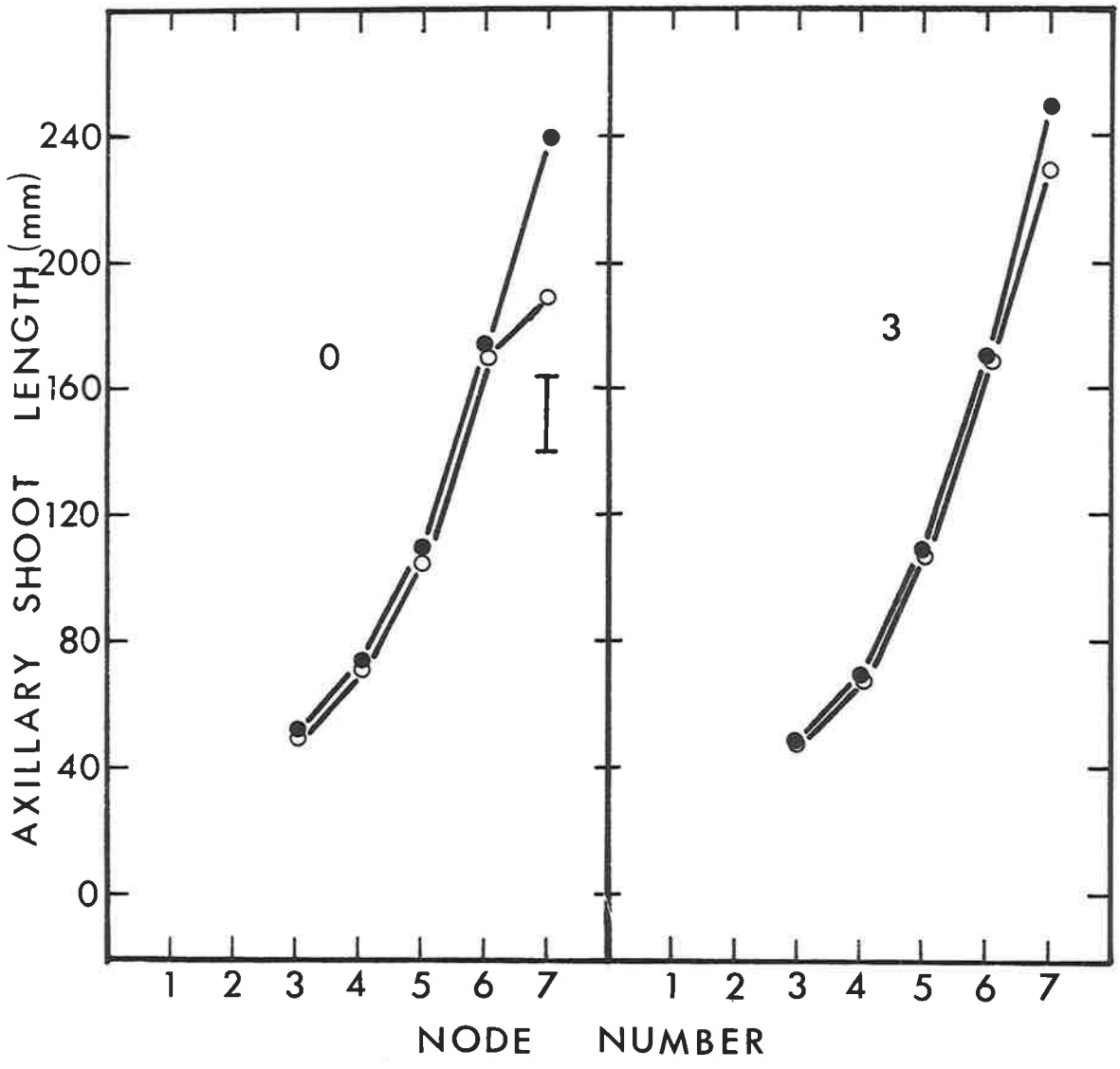
Node	Watered throughout	Water deficit
	Stage of Development	
1	Aborted	Aborted
2	Aborted	Aborted
3	2	2
4	4	3
5	5	5
6	8	8
7	16	14

There was no significant difference between the two treatments in the development of the inflorescences at any one node.

FIG. 3.1.8.

The response of axillary shoot growth (in length) to an episode of water deficit occurring at silking (50 to 59 days after germination). 0 and 3 refer to the times at which the measurements were made, in weeks following re-watering of the plants subjected to an episode of water deficit.

●-●Control ○-○Water deficit


AXILLARY SHOOT LENGTH (mm)
240
200
160
120
80
40
0
0
3
1 2 3 4 5 6 7
1 2 3 4 5 6 7
NODE NUMBER

3.1.5. The Effect of Repeated Episodes of Water Deficit on Growth and Development of the Axillary Inflorescences

Introduction: The results of previous experiments have shown that the growth and development of the axillary inflorescences is enhanced when a water deficit is imposed during the period of growth marked by tassel initiation and early development. Only a single episode of water deficit was imposed within that period and it was of interest to ascertain whether the response could be enhanced by further exposure to water deficit.

Method: Plants were grown for 21 days after germination when five plants were randomly selected and dissected. As tassel initiation was found to have commenced, a random sample of 30 plants were then subjected to water deficit. These plants were not watered for the following ten days whilst the remaining plants were watered daily. At the end of ten days the leaf water potential of ten randomly selected plants from each treatment was measured. All the remaining plants were then watered for 14 days. By this time the equivalent axillary branches of the plants in the two treatments were expected to be again identical although tassel growth in the stressed plants was inhibited as shown in Experiment 3.1.2. (Fig. 3.1.3.).

Ten of the plants previously stressed were then subjected to a further water deficit for 9 days i.e. from 45 to 54 days after

which all the plants were watered daily. Ten plants were thus watered throughout the experiment, a further ten were subjected to one period of water deficit and the third batch of ten plants were subjected to two such periods.

Results: At the end of the first period of water deficit, Ψ of the watered plants was -3.6 bars whilst that of the stressed plants was -11.8 bars. At the end of the second stressing Ψ of the watered plants was -4.1 bars whilst that of the stressed plants was -13.8 bars.

Subjecting the plants to two periods of water deficit did not cause any enhancement of axillary branch growth at nodes 5 and 6 (Table 3.1.7.). Indeed the growth of the inflorescences at nodes 6 and 7 was inhibited to the extent that three weeks after the release of the second water deficit, both were significantly smaller than the equivalent inflorescence on plants watered throughout. Similarly total plant height was significantly reduced by the second episode of water deficit.

Despite this inhibitory effect of the second episode of water deficit on the growth of axillary inflorescences, there was no effect on their development (Table 3.1.8.). At each node the stage of development of the axillary female inflorescence on the plants subjected to two episodes of water deficit was the same as that of the equivalent inflorescence on plants subjected to only one such episode. In the case of the inflorescences at nodes 5

TABLE 3.1.7.

The Effect of Two Episodes of Water Deficit During the Period of Tassel and Axillary Inflorescence Initiation and Development (21 to 31 and 45 to 54 Days From Germination) on the Growth of the Inflorescences, Measured 75 Days After Germination

Treatment	Plant Height (cm)	Tassel Length (cm)	Length x Breadth of Axillary Branches at Nodes: 1 (mm)	2 (mm)	3 (mm)	4 (mm)	5 (mm)	6 (mm)	7 (mm)
Watered throughout	185	25	Abort.	Abort.	35 x 5	57 x 8	75 x 11	153 x 17	243 x 43
Water deficit Days 21 to 31 only	180	10	Abort.	27 x 6	55 x 7	130 x 10	160 x 15	193 x 25	260 x 46
Water deficit Days 21 to 31 and 45 to 54	126	8	Abort.	26 x 6	27 x 8	58 x 8	81 x 13	117 x 24	183 x 41
Significant difference (P = 0.05)	11	2	-	-	10 x 1	26 x 1	31 x -	25 x 3	25 x 3

TABLE 3.1.8.

The Effect of Two Episodes of Water Deficit During the Period of Tassel and Axillary Inflorescence Initiation and Development (21 to 31 and 45 to 54 Days After Germination) on the Development of the Axillary Inflorescences Determined 75 Days After Germination

Treatment	Stage of Development of Buds (Table 3.1.1.) at Nodes:						
	1	2	3	4	5	6	7
Watered throughout	Abort.	Abort.	2	4_a	6_a	8_a	15
Water deficit Days 21 to 31 only	Abort.	2	4	6_b	8_b	11_b	15
Water deficit Days 21 to 31 and 45 to 54	Abort.	2	2	6_b	9_b	11_b	14

Figures with different letters within columns are significantly different. Statistical analysis based on Kruskal-Wallis One Way Analysis of Variance.

and 6, floral development was in advance of that of equivalent inflorescences on the watered plants. Tassel length was only marginally more strongly inhibited by the two episodes of water deficit than by the single episode (Table 3.1.7.). Hence the major effect of the second period of water deficit was to reduce the growth in length of the axillary inflorescences without inhibiting their rate of floral development.

3.1.6. DISCUSSION

The pattern of growth and development of the axillary inflorescences reported for this cultivar conforms generally to the patterns previously described for single-cob corn cultivars (Sass 1960; Collins 1963; Collins and Russell 1965). Of particular interest, in view of the response to water deficit, is the change in the gradient of size of branches at successive nodes which appears to be correlated with floral initiation in these structures (Fig. 3.1.1., Table 3.1.1.). This change has been linked with a supposed inhibitory effect of the growing tassel, growth at the upper nodes accelerating once tassel growth is completed (Collins 1963). This was not confirmed with this cultivar as axillary shoots which did not abort early in development showed no evidence of a period of growth inhibition. Moreover rapid growth of the upper axillary inflorescences preceded cessation of tassel growth. Nevertheless, the main period of terminal male inflorescence

growth preceded that of the axillary female inflorescences.

Following upon inflorescence initiation in the axillary shoots, the growth of the uppermost axillary inflorescence outstrips that of those further down the main axis. The lower axillary shoots are eventually arrested in development and the lower two senesce and abort (Table 3.1.1.). This developmental pattern is analogous, in a general way, to the apical dominance phenomenon in many vegetative plants. Indeed, the system in corn has been ascribed to dominance of the terminal male inflorescence (Collins 1963) or to dominance of the uppermost axillary female inflorescence (Bonnett 1940; Sass 1960). Sass (1960) reported that removal of the uppermost female inflorescence at silk emergence from a single cob cultivar resulted in the maturation of the next lower inflorescence. It must be noted that this pattern of axillary inflorescence growth and response to excision of the putative inhibiting structure differs from the more familiar apical dominance response in vegetative plants in that the highest bud responds rather than the lowest bud.

When a water deficit is imposed upon this developmental sequence the immediate response is growth inhibition no matter when the deficit occurs or what organ is considered, although the extent of inhibition varies with the stage of development. Clearly, however, the growth of the plant organs subsequent to termination of a period of water deficit is dependent upon both the phase of

development and the organ considered. For example growth of the tassel was unaffected by a water deficit occurring prior to tassel initiation (day 7 to 17) or after tassel emergence (day 50 to 59), was temporarily inhibited by a water deficit occurring at tassel initiation (day 13 to 23), this inhibition disappearing following re-watering, but was permanently inhibited by a water deficit during tassel elongation (day 20 to 30). This permanent inhibition of elongation was accompanied by a degree of floret sterility.

A marked sensitivity of primordium production at the terminal apex to the effects of water deficit has been described for barley (Aspinall and Husain 1970) and a similar response may exist with corn. The virtual cessation of tassel growth in both watered and previously droughted plants at the same time, despite major differences in size (Fig. 3.1.2.) suggests that some factor independent of tassel size may be concerned in tassel elongation. Such a system could be similar to that suggested for barley where floret development was proposed to be concerned in the termination of primordium production in the apex (Aspinall and Husain 1970).

The growth of the uppermost axillary inflorescence was inhibited during any period of water deficit coinciding with rapid growth but this inhibition was compensated by rapid growth following the water deficit. The only exception was where two episodes of water deficit were imposed during the period of rapid growth (Table 3.1.7.). In this case the second episode of water deficit

caused a permanent decrease in inflorescence size but had less effect on floral development, suggesting that growth in size is more sensitive to water deficit than morphological development (Aspinall and Husain 1970).

The more basal axillary inflorescences, particularly those at nodes 5 and 6, were also inhibited by water deficit during the rapid growth phase but this inhibition was over-compensated by rapid growth following re-watering (Fig. 3.1.3.). If one accepts that the growth of these lower axillary inflorescences is normally inhibited by a process analogous to apical dominance, then it follows that water deficit must weaken this control to allow growth. This suggestion is not without precedence as McIntyre (1971) has demonstrated that apical dominance in Pisum is modified by a water deficit. Further, termination of water deficit during vegetative growth in barley led to profuse growth of previously inhibited axillary branches (Aspinall et al. 1964). It is proposed, therefore, that the period of water deficit permanently affected the growth or metabolic potential of some normally dominant plant organ so that the balance of correlative inhibition within the plant was affected. The results of this change will only be displayed when renewed growth was made possible by an improved water supply.

Although it is conceivable that the inhibitory potential of a meristem could be reduced without any visible signs of change in

that meristem, it would appear more likely that such a change would follow from a reduction in the growth of the meristem. Sachs et al. (1967), for instance observed a reduction in the growth rate of the apical bud in Pisum resulting in the loss of correlative inhibition due to that bud.

In the present case, structures most likely to be exerting dominance are the tassel and the uppermost female inflorescence. On the present evidence the tassel would appear the most likely candidate as inhibition of its growth is correlated with promotion of the growth of the lower axillary inflorescences. Treatments which stimulated the growth of the uppermost axillary inflorescence in the period following a water deficit but resulting in an inhibition of the growth of the tassel also led to promotion of the growth of the inflorescences at nodes 5 and 6.

It is therefore concluded that axillary shoot growth at nodes 5 and 6 is subject to correlative inhibition originating probably in the tassel and that this inhibition is modified by water deficit.

3.2. INVESTIGATION OF THE ORGAN EXERTING DOMINANCE

3.2.1. Introduction

It has been shown in the previous chapter that in a plant grown without any episode of water deficit there are seven axillary shoots all but the lowest two of which initiate female inflorescence primorida. These develop to various stages before growth of all but the uppermost axillary inflorescence is arrested (Table 3.1.2.).

An episode of water deficit commencing approximately 20 days after germination and continued for 20 days resulted in a partial release of this inhibition of axillary growth so that the inflorescences at nodes 5 and 6 grew and developed to a significantly greater extent than corresponding inflorescences on plants which had not been exposed to any period of water deficit.

The tassel is the apical meristem of the shoot system and, by analogy with vegetative systems of apical dominance, is a likely candidate as the source of dominance controlling the growth of the axillary inflorescences. The growth potential of the tassel is permanently reduced by an episode of water deficit at that phase of development, which correlates with the response of the axillary shoots (Fig. 3.1.2.). It is thus logical to hypothesise that the growth of the axillary inflorescences, particularly at the lower node, is controlled by correlative inhibition originating in the terminal male inflorescence. Collins (1963) attributed the

suppression of growth of the axillary inflorescences during early development to tassel growth and claimed that the growth of all the axillary inflorescences was inhibited until tassel growth was essentially complete. This did not occur with the present cultivar, IO Chief, but nevertheless apical dominance exerted by that meristem is possible.

Alternatively the uppermost axillary inflorescence may act in this role. It is the only axillary inflorescence that reaches maturity and grows rapidly while the lower inflorescences are inhibited. In his study of the morphogenesis of the floral parts of maize, Bonnett (1940) suggested that the uppermost axillary shoot is the source of dominance resulting in the inhibition of the lower axillary inflorescences in single eared plants. Similarly Bauman (1960) reported that this upper axillary inflorescence exerted dominance over the lower axillary inflorescences. Neither of these reports included experimental verification of the suggestion, however, but Sass (1960) reported, without presenting data, that removal of the uppermost axillary inflorescence induced growth and maturation of the next lower axillary inflorescence. In contrast to the effect of a period of water deficit on the tassel, the growth of the uppermost axillary inflorescence is only inhibited during the period of water deficit, compensatory growth after re-watering results in this inflorescence reaching the same mature size as that on control plants.

These studies suggest that inhibition of the growth of the lower axillary inflorescences could arise either from the terminal male inflorescence, the uppermost female inflorescence or possibly a combination of both sources. There is little or no experimental evidence to discriminate between these possibilities and a resolution of this problem is essential to an understanding of the effects of a period of water deficit. The present experiments were carried out in an attempt to clarify this situation.

3.2.2. The Effect of Excision of the Terminal Male Inflorescence and Excision of the Most Distal Female Inflorescence on the Growth and Development of the Lower Axillary Inflorescences

Method: The aim of this experiment was to investigate the role of the tassel and the uppermost axillary inflorescence during the period when water deficit resulted in an induction of growth of the lower axillary inflorescence. Ideally, the treatments imposed (organ excision) should have coincided with the period when the maximum influence of water deficit was obtained i.e. commencing 20 days after germination (Fig. 3.1.1.). At that time, however, the tassel and axillary shoot primordia were small and inaccessible beneath a number of developing leaves. Removal of the meristems at this time would have necessarily involved considerable damage to the plant foliage, so the excision treatments were not imposed until 27 days after germination when they could be performed with a minimum

of damage.

The tassels of six plants, the inflorescences at node 7 of six plants and the tassels and the inflorescences at node 7 of a further six plants were removed at that time. These, together with a further six intact plants were watered copiously each day throughout the experiment. The size and development of each of the axillary inflorescences were measured three weeks after imposition of the treatments.

Results: Removing the tassels had no effect on the growth or the development of the axillary inflorescences at node 7 (Table 3.2.1.), the inflorescence which normally produces a mature cob. The growth of the axillary inflorescences at nodes 3, 4, 5 and 6 was stimulated by tassel removal, however, the stimulation being greater the higher the inflorescence was situated on the plant. This stimulation was marked by an increase in both the length and the breadth of the axillary structures. At nodes 5 and 6 the stimulation was considerable, the inflorescences on treated plants being three to four times larger than those on intact plants.

Removing the uppermost axillary inflorescence also stimulated growth at the lower nodes, but here the stimulation was not as great nor did it extend as far down the plant. Only the shoots at nodes 5 and 6 were affected, and these were not as large as the equivalent shoots on plants with tassels removed.

TABLE 3.2.1.

Influence of the Tassel and the Uppermost Axillary Inflorescence on Shoot Growth at the Lower Nodes

Treatment	Length x Breadth (mm) 3 Weeks After Treatment of Shoots at Nodes:						
	1	2	3	4	5	6	7
Control	Abort.	Abort.	17 x 3	22 x 4	30 x 5	60 x 8	202 x 34
Tassel Removed	Abort.	Abort.	22 x 4	57 x 7	139 x 11	154 x 18	235 x 40
Uppermost Axillary Shoot Removed	Abort.	Abort.	14 x 2	19 x 4	76 x 9	120 x 25	-
Tassel and Uppermost Axillary Shoot Removed	Abort.	Abort.	23 x 4	97 x 9	187 x 18	230 x 41	-
L.S.D. (P = 0.05)			3 x 1	17 x 1	37 x 5	41 x 7	-

Where both of the presumably dominant meristems were excised, growth of all the remaining axillary shoots was again stimulated. This stimulation was significantly greater at all nodes than that due to removal of the upper axillary shoot alone, and greater at all but node 3 than that due to removal of the tassel alone. The stimulation in growth produced by each of the excision treatments, alone or in combination, was largely paralleled by a stimulation in inflorescence development (Table 3.2.2.).

One may conclude from the results of this experiment, therefore, that the presence of both the axillary shoot at node 7 and of the terminal male inflorescence inhibits the growth of the lower axillary shoots. It would appear that the presence of the male inflorescence is more inhibitory than the presence of the shoot at node 7, as its removal results in a greater stimulation of growth.

3.2.3. The Interaction of Excision of the Suspected Meristems and Water Deficit on the Growth and Development of the Lower Axillary Inflorescences

3.2.3.1. The Effect of Excision of the Tassel on the Growth and Development of Axillary Inflorescences of Plants Exposed to Water Deficit

Introduction: It has been shown that removal of the tassel results in the release of the inhibition of the growth and development of the lower axillary inflorescences (Table 3.2.1.). Moreover

TABLE 3.2.2.

Influence of the Tassel and of the Uppermost Axillary Inflorescence on the Development of the Lower Axillary Buds

Treatment	Stage of Development (Table 3.1.1.) 3 Weeks After Treatment of Buds at Nodes:						
	1	2	3	4	5	6	7
Control	Abort.	Abort.	2	2_a	4_a	6_a	11
Tassel Removed	Abort.	Abort.	3	4_b	6_a	9_b	12
Uppermost Axillary Shoot Removed			2	2_a	6_a	11_{bc}	-
Tassel and Uppermost Axillary Shoot Removed			3	5_b	9_b	12_c	-

Figures with similar letters in each column are not significantly different. Statistical analysis based on Kruskal-Wallis One Way Analysis of Variance.

a period of water deficit during the initiation and rapid growth of the tassel not only has a similar effect on the growth of the lower axillary inflorescences (Fig. 3.1.3.) but also results in a permanent inhibition of tassel growth (Fig. 3.1.2.).

These results suggest that the effect of the water deficit on the growth of the axillary shoots may be indirect and mediated through the effect on the terminal male inflorescence. If, as appears likely, the effect of water stress on axillary shoot growth is correlated with the impaired development of the terminal male inflorescence, then removal of the terminal male inflorescence, by removing the point of action of the water deficit, should abolish the response of the axillary shoots to a water deficit.

Method: The tassels of 12 plants were removed 28 days from germination. Half of these were water stressed and the other half were watered normally. Six intact plants were water stressed at the same time as those described above and six other intact plants were watered throughout. At this period of growth the axillary shoots had been shown previously to be sensitive to both water deficit (Fig. 3.1.3.) and to removal of tassel (Table 3.2.2.).

The water deficit was imposed for 9 days whereafter all the plants were watered daily. The sizes of axillary shoots and the developmental stages were measured and assessed three weeks after the release of stress.

Results: Neither removing the tassel, subjecting the plants to a water deficit or a combination of both treatments had any significant effect on the ultimate size of the inflorescence at node 7 (Table 3.2.3.). As in previous experiments (3.2.2.) this axillary shoot appears to be unaffected by such treatments.

As before, subjecting the intact plants to a period of water stress during the early development of the tassel resulted in an eventual significant enlargement of the axillary shoots at nodes 5 and 6. In this case the shoots at still lower nodes (3 and 4) were not affected. Removing the tassel, without any period of water deficit, had a similar although slightly greater effect, the axillary shoot at node 6 being larger in this treatment than with a water deficit.

When the treatments of tassel removal and water deficit were combined, the response was identical to the water stress alone treatment with the exception of the axillary shoot at node 6 which was significantly broader although not longer. In comparison with the watered plants in which the tassel was removed, these plants subjected to the combined treatment had smaller axillary shoots at all nodes, the difference being significant at node 6. These data lead to the conclusion that both water deficit and tassel removal produce the same response in the axillary shoots, and that a combination of the two factors does not produce any further response.

TABLE 3.2.3.

Effect of Water Deficit on Axillary Shoot Growth of Detasselled Plants

Treatment	Length x Breadth (mm) 3 Weeks After Treatment of Shoots at Nodes:						
	1	2	3	4	5	6	7
Control	Abort.	Abort.	14 x 4	19 x 4	31 x 6	70 x 11	209 x 31
Whole Plants Stressed	Abort.	Abort.	12 x 4	27 x 5	73 x 9	131 x 15	216 x 34
Unstressed Detasselled Plants	Abort.	Abort.	17 x 5	29 x 6	88 x 9	185 x 21	232 x 41
Stressed Detasselled Plants	Abort.	Abort.	15 x 3	22 x 6	73 x 10	146 x 19	190 x 38
L.S.D. (P = 0.05)			N.S.	N.S.	23 x 2	31 x 3	N.S.

TABLE 3.2.4.

Effect of Water Deficit on the Development of the Axillary Buds of Detasselled Plants

Treatment	Stage of Development (Table 3.1.1.) 3 Weeks After Release of Stress of Buds at Nodes:						
	1	2	3	4	5	6	7
Control	Abort.	Abort.	2	3	4	8_a	11
Whole Plants Stressed	Abort.	Abort.	2	3	6	9_{ab}	11
Unstressed Detasselled Plants	Abort.	Abort.	2	4	6	10_b	12
Stressed Detasselled Plants	Abort.	Abort.	2	3	6	10_b	11

Figures with similar letters in each column are not significantly different. Statistical analysis based on Kruskal-Wallis One Way Analysis of Variance.

The rate of inflorescence development at each node (Table 3.2.4.) as distinct from increase in physical size was also influenced by the treatments. In general, the responses paralleled those of growth in size. Thus there was no effect of either treatment on the development of the inflorescence at node 7, and both treatments, alone or in combination, promoted the development of the inflorescences at nodes 6 and 5. The only major difference was that with the inflorescence at node 6, the effect of a water deficit in reducing growth in the plants with the tassels excised was not paralleled by altered development of that inflorescence.

These data support the view that the terminal male inflorescence is involved in the inhibition of the growth and development of the axillary shoots at nodes 6 and below. They also strongly suggest that the effect of water deficit on these axillary shoots is mediated through an effect on the terminal inflorescence, as removal of that organ abolishes the response to water deficit.

3.2.3.2. The Influence of the Inflorescence at Node 7 on the Response of the Axillary Shoots at Lower Nodes to Water Deficit

Introduction: In Experiment 3.2.1. removal of the uppermost axillary inflorescence resulted in an increase in the growth of the shoots at nodes 5 and 6. This response occurred even when the tassel was also removed, suggesting that this axillary shoot also participates in the regulation of the growth of lower shoots. The

previous experiment (Experiment 3.2.3.1.) showed that removal of the tassel essentially removed the response of the lower axillary shoots to water deficit. This could be interpreted as indicating that the upper axillary shoot was not involved in this response to water deficit. It was considered, however, that this suggestion should be tested more directly.

Method: The uppermost axillary shoots (at node 7) of 12 plants were removed 28 days after germination. Half of these were stressed and half watered. Water stress was imposed on 6 intact plants at the same time and a further six intact plants were watered throughout. The period of water stress was 9 days. Three weeks after re-watering the sizes of all axillary shoots were measured and their development were also assessed.

Results: It can be seen from Table 3.2.5. that the effect of water stress on the intact plants was similar to that reported before in that the growth of the axillary shoots at nodes 5 and 6 was significantly promoted. Removing the upper axillary inflorescence also resulted in a large promotion of the growth of the axillary shoot at node 6, and a lesser response at node 5. Where plants in which the upper axillary shoot was excised were subjected to a water deficit, the growth of the shoot at node 6 was slightly reduced, but that of the shoots at nodes 4 and 5 was promoted.

Similar results were found with development of shoots except that the inflorescence at node 5 was not significantly more

TABLE 3.2.5.

Effect of Water Deficit on Growth of Plants Whose Uppermost Axillary Shoots Have Been Removed

Treatment	Length x Breadth (mm) 3 Weeks After Release of Stress of Shoots at Nodes:						
	1	2	3	4	5	6	7
Control	-	-	15 x 3	25 x 5	37 x 7	58 x 12	185 x 26
Whole Plants Stressed	-	-	19 x 3	25 x 5	63 x 13	158 x 16	200 x 26
Uppermost Bud Removed and Unstressed	-	-	16 x 4	29 x 5	67 x 9	194 x 25	-
Uppermost Bud Removed and Stressed	-	-	19 x 5	58 x 7	97 x 15	176 x 30	-
L.S.D. (P = 0.05)			-	8 x 1	26 x 2	31 x 4	N.S.

TABLE 3.2.6.

Effect of Water Deficit on Development

of Plants Whose Uppermost Axillary Buds Have Been Removed

Treatment	Stage of Development (Table 3.1.1.) 3 Weeks After Release of Stress of Buds at Nodes:						
	1	2	3	4	5	6	7
Control	-	-	2	3	4_a	6_a	11
Whole Plants Stressed	-	-	2	3	6_{ab}	9_b	11
Uppermost Bud Removed and Unstressed	-	-	2	3	6_{ab}	11_b	
Uppermost Bud Removed and Stressed	-	-	2	5	7_b	11_b	

Figures with similar letters in each column are not significantly different. Statistical analysis based on Kruskal-Wallis One Way Analysis of Variance.

developed in stressed than unstressed plants whose uppermost axillary shoots were removed (Table 3.2.6.). Thus it can be concluded that, unlike the removal of the tassel, removal of the uppermost axillary shoot does not prevent the lower axillary shoots from responding to a period of water deficit by increased growth and development.

3.2.3.3. The Effect of Water Deficit on the Growth and Development of the Lower Axillary Inflorescences of Plants Whose Male and/or Uppermost Axillary Inflorescences Have Been Excised

Introduction: In the previous experiments it was found that excision of either the tassel or, to a lesser extent, the uppermost axillary inflorescence resulted in a stimulation to the growth of the lower axillary inflorescences. Water stress also stimulated the growth of these inflorescences, and this water stress induced stimulation was abolished by tassel removal but not by removal of the upper axillary inflorescence. It was concluded that whilst both tassel and upper axillary inflorescence may be involved in the correlative inhibition of the lower inflorescences, only the influence of the tassel is modified by water stress. These conclusions were drawn from separate experiments, however, and the present experiment has been designed to repeat these treatments in more comparable conditions.

Method: Plants were raised as described under General Methods. 28 days after germination they were grouped into 4 sets of 12. The tassels of the first set were excised as described previously. The uppermost axillary inflorescences were excised as described under General Methods. Both the tassels and the uppermost axillary inflorescence were excised from the third set and no portion of the fourth set was excised. Water was withheld from half of each set whilst the other half was watered daily to run off. The plants were stressed for seven days and were then re-watered and treated in the same manner as the control plants thereafter. Three weeks after re-watering, the sizes of the axillary shoots were measured and the stages of development of the axillary buds were assessed.

Results: The effect of excision of the tassel on the response in growth of the lower axillary shoot to water stress was essentially similar to that in Experiment 3.2.3.1. That is, excision of the tassel abolished the enhanced growth of shoots of plants exposed to water deficit (Table 3.2.7.). Similarly, the response in growth of the lower axillary shoots to a period of water deficit of plants whose uppermost axillary inflorescences had been excised was, to a large extent, similar to that of Experiment 3.2.3.2. There were, however, some differences in detail. In this experiment, excision of the uppermost axillary inflorescence did not stimulate the growth of the axillary shoots at nodes 5 or 6

TABLE 3.2.7.

The Effect of Water Deficit on the Growth of Axillary Inflorescences of Plants Whose Male and/or Uppermost Axillary Inflorescences Have Been Excised

Treatment	Length x Breadth (mm) 3 Weeks After Release of Stress of Shoots at Nodes:						
	1	2	3	4	5	6	7
Control	Abort.	Abort.	30 x 5	52 x 7	100 x 10	150 x 19	226 x 35
Water Stressed	Abort.	Abort.	29 x 6	112 x 13	120 x 13	175 x 21	225 x 37
Tassel Excised	Abort.	Abort.	77 x 8	103 x 10	159 x 16	207 x 14	249 x 40
Tassel Excised and Stressed	Abort.	Abort.	49 x 8	112 x 10	141 x 15	194 x 25	223 x 38

Uppermost Shoot Excised	Abort.	Abort.	38 x 7	99 x 10	114 x 19	136 x 38	-	-
Uppermost Shoot Excised and Stressed	Abort.	Abort.	103 x 14	141 x 20	135 x 20	188 x 32	-	-
Tassel and Uppermost Shoot Excised	Abort.	Abort.	95 x 10	146 x 20	193 x 24	182 x 35	-	-
Tassel and Uppermost Shoot Excised and Stressed	Abort.	Abort.	91 x 9	137 x 18	190 x 24	177 x 35	-	-
L.S.D. (P = 0.05)			16 x 2	17 x 3	21 x 2	15 x 6	N.S.	

in unstressed plants. Furthermore, unlike the previous experiment, the growth of the inflorescence at node 6 was enhanced by water stress, not inhibited. Thus, in this experiment, the effect of water stress on the growth of the lower axillary shoots of plants whose uppermost axillary shoots had been removed was identical to the effect of water stress on a whole plant.

The effect of water stress on plants whose terminal male inflorescence (tassel) as well as the uppermost axillary inflorescence had been excised was similar to that on plants whose tassels alone were removed. That is, a period of water stress did not cause any enhancement of the growth of the lower axillary shoots of the stressed plants when compared with the unstressed plants.

The influence of these treatments on the development of the axillary buds paralleled that on the growth of these same axillary shoots as described above (Table 3.2.8.). Here again the effect of water stress on the development of the axillary buds of plants whose uppermost axillary shoots were removed was similar to that of whole plants (3.2.3.2.).

These data, therefore, confirmed the previous conclusion that excision of the tassel abolished the further promotive effect of water stress on growth of the lower axillary inflorescences, whilst excision of the uppermost axillary inflorescences did not. It is apparent, therefore, that the terminal male inflorescence is the main organ involved in the inhibition of the growth and development

TABLE 3.2.8.

The Effect of Water Deficit on the Development of Axillary Inflorescences of Plants Whose Male and/or Uppermost Axillary Inflorescences Have Been Excised

Treatment	Stage of Development (Table 3.1.1.) 3 Weeks After Release of Stress of Buds at Nodes: 1	2	3	4	5	6	7
Control	Abort.	Abort.	2_a	3_a	6_a	9	11
Water Stressed	Abort.	Abort.	2_a	6_b	7_{ab}	10	11
Tassel Excised	Abort.	Abort.	3_a	5_{ab}	7_{ab}	10	11
Tassel Excised and Stressed	Abort.	Abort.	3_a	5_{ab}	7_{ab}	11	11
Uppermost Shoot Excised	Abort.	Abort.	4_a	6_b	9_{bc}	11	-
Uppermost Shoot Excised and Stressed	Abort.	Abort.	7_b	9_c	10_{bc}	11	-
Tassel and Uppermost Shoot Excised	Abort.	Abort.	8_b	8_{abc}	11_{bc}	11	-
Tassel and Uppermost Shoot Excised and Stressed	Abort.	Abort.	8_b	8_{bc}	11_{bc}	11	-

Figures with similar letters in each column are not significantly different. Statistical analysis based on Kruskal-Wallis One Way Analysis of Variance.

of the axillary shoot at nodes 6 and below. Furthermore, the effect of water deficit on the growth of and development of these axillary inflorescences appears to be mediated through an effect on this male terminal inflorescence.

3.2.4. DISCUSSION

In a normal plant of this cultivar, although seven axillary inflorescence are initiated, only the uppermost develops to form a mature cob. The remaining six lower axillary inflorescences develop to various stages but are thereafter inhibited from further growth. The lowest two senesce and eventually abort. Whilst this occurs in plants plentifully supplied with water, a period of water deficit results in enhanced growth of all the lower axillary inflorescences with those at node 6 and occasionally at 5 growing to maturity as well as that at node 7. The most likely explanation of this phenomenon is that some organ of the plant inhibits the growth of the lower axillary inflorescences but that this correlative inhibition is removed by a period of water deficit. The tassel is the apical meristem of the shoot system and could, by analogy with other shoot systems, be the source of dominance. However, the uppermost axillary inflorescence could also be the source of dominance as it is actively growing during the period considered and occupies a more acropetal position than the inhibited axillary inflorescences. As the excision of both the tassel and

the axillary inflorescence at node 7 resulted in the greatest stimulation to the growth of the lower axillary shoots, it appears that both organs are involved in the control of the growth of the lower axillary inflorescences. This conclusion is supported by Collins (1963) on the one hand, who found that the growth and development of the tassel resulted in inhibition of the growth of the axillary inflorescences, and by Bonnett (1940) and Bauman (1960), on the other, who concluded that the uppermost axillary inflorescence was the source of dominance.

Excision of the terminal male inflorescence (the tassel) resulted in the growth of enlarged axillary inflorescences at almost all the lower nodes. Similarly, a period of water deficit imposed on intact plants resulted in enlarged axillary inflorescences at the lower nodes but, significantly, a period of water deficit imposed on detasselled plants did not promote any further enlargement of these axillary inflorescences but rather tended to inhibit their growth, particularly at node 6 (Table 3.2.3.). In contrast, although excision of the axillary inflorescence at node 7 resulted in enhanced growth of lower axillary inflorescences, this occurred only at a limited number of sites (Tables 3.2.5. and 3.2.7.) and was not as pronounced as where tassels were removed. Moreover unlike the detasselled plants, excision of the uppermost axillary inflorescence did not abolish the promotion of the growth of the lower axillary inflorescences caused by exposure of the plants to a

period of water deficit (Tables 3.2.5. and 3.2.7.). These considerations led to the conclusion that whilst both the tassel and the developing inflorescence at node 7 are concerned in the inhibition of the growth of the lower inflorescences which occurs in the intact plants, the tassel is the major source of this inhibition and is the organ involved in the response to a water deficit.

This hypothesis is supported by the fact that water stress inhibited the growth of all organs during the period of deficit but only the tassel failed to show enhanced growth when the plants were re-watered. It would seem that a period of water deficit at this time permanently damages the tassel to such an extent that the normal growth rate is not recovered after re-watering. This permanent damage to the tassel is comparable in its effect on the growth of the lower axillary inflorescences with complete excision of the tassel since both result in a stimulation to the growth of those meristems.

Whereas removal of the tassel influences the growth of all the axillary inflorescences, removal of the uppermost axillary inflorescence appears to have a more localised effect (Tables 3.2.5. and 3.2.7.). In most cases the response was confined to nodes 5 and 6, immediately below the excised inflorescence. This is comparable to the correlative inhibition relationships between lateral buds reported by Sachs (1966) for pea and McIntyre (1968)

for flax. This correlative inhibition did not appear to be altered by a period of water deficit and it is perhaps significant that, in control plants with the tassel intact, the growth of the inflorescence at node 7 was not permanently inhibited by the period of water deficit but was actively renewed when the plant was re-watered.

3.3. INVESTIGATION OF THE MODE OF ACTION OF THE ORGAN EXERTING DOMINANCE

3.3.1. Introduction

It has been concluded (3.2.4.) that the developing tassel is involved in the control of the growth and development of the lower axillary inflorescences. Since the tassel is the terminal apical bud of the shoot system this phenomenon could be described as one of apical dominance. Although the present case concerns the apparent dominance of one floral meristem over others, reports pertaining to the more fully investigated apical dominance of vegetative apices would appear to be pertinent. It is clear that despite intensive study of the problem of apical dominance the phenomenon is as yet not completely understood. The subject has been extensively reviewed (Gregory and Veale 1957; Phillips 1959 and Cutter 1972) and only a brief account of the hypotheses advanced to account for the phenomenon will be discussed here.

As early as the turn of the century, Goebel (1900) postulated that dominance in plants is due to diversion of nutrients to the apical bud or the dominating organ thus preventing nutrients from reaching the lateral buds and consequently inhibiting their growth. Although this hypothesis was put forward by early workers, some modern support for the theory has come from the investigations of McIntyre (1964) and Husain and Link (1966) who reported that ^{32}P was translocated preferentially to the most actively growing part

of Pisum sativum. In such studies, however, it is difficult to delineate cause and effect. More convincingly McIntyre (1969) reported that isolated lateral buds of Agropyron repens provided with 2% sucrose solution grew whereas similar buds on intact plants did not. Furthermore, there was a marked increase in the carbohydrate content of buds 48 hours after isolation from the rhizome apex of the same plant. Later, McIntyre (1971) demonstrated with Pisum sativum that axillary buds were limited in their growth by either nitrogen or carbohydrate which could determine the degree of apical inhibition. Finally Wakhloo (1970) reported that the application of potassium in high concentration brought about a release from complete inhibition of the lower axillary buds of Solanum sisymbrifolium. Despite the fact that these and other reports suggest that nutrition is a limiting factor in apical dominance there is contrary evidence. For instance, Phillips (1968) found that the total nitrogen, phosphorus and potassium content per unit dry weight of the apical 5 mm of axillary buds of Pisum sativum was higher in those inhibited by the apical bud than in those growing actively.

As an alternative explanation of the phenomenon, many reports support the view that apical dominance is mediated through plant hormones. Again the hypothesis is of respectable antiquity as Errera suggested in 1904 that apical dominance is caused by "internal secretions". It was later reported by Snow (1925) that

a diffusible substance from the apex caused the inhibition. When auxin (IAA) was recognised as a growth substance, Thimann and Skoog (1934) demonstrated that the application of IAA to decapitated Vicia faba plants maintained the inhibition of the axillary buds. Thimann (1937) followed up this investigation by showing that the concentration of auxin for maximum growth varied from organ to organ the sensitivity increasing in the order stem, bud, root. He accordingly proposed that auxin was translocated from the apex to the lateral buds where it accumulated in supra-optimal concentration. Although stems could grow at this concentration, lateral bud growth was inhibited. This hypothesis, the direct auxin theory, therefore assumes synthesis of auxin at the apical bud and translocation to the lower buds to effect inhibition of growth.

One of the early difficulties in acceptance of the direct auxin theory was that auxin transport was thought to be strictly basipetal thus it was difficult to envisage how auxin could be transported acropetally from the node to the lateral bud to effect an inhibition of growth. Nevertheless from the studies of Wickson and Thimann (1960) it has been shown that though IAA transport is mostly basipetal, there is some acropetal movement in both stem tissue and young lateral buds. They therefore postulated that the prime causal factor in maintenance of apical dominance is transport of auxin down the stem and up into the buds.

Although acropetal transport of auxin apparently occurs other considerations appear to make the direct auxin theory untenable. It has been repeatedly demonstrated that the auxin content of inhibited buds or shoots is lower than that of actively growing parts (van Overbeek 1938; Libbert 1954; Thomas 1972). Moreover, Allsopp (1956) reported that lateral buds of decapitated Marsilea plants grew even at high auxin concentrations in culture medium whilst in the correlative inhibition within intact plants there was a complete arrest of lateral bud development at an appreciably lower internal auxin concentration. Jacobs et al. (1965) have also demonstrated that apical dominance in Coleus blumei does not appear to be controlled by auxin from the apex.

Although some reports appear to support the direct auxin theory (Wardlaw 1946; Naylor 1958), in general in these studies the auxin has usually been applied in lanolin paste at concentrations higher than those likely to occur in the plants. Moreover, the cells in contact with the paste are exposed to high auxin concentrations whilst the internal cells receive a low concentration, which, together with other possible effects on the underlying cells makes comparison between plants treated in this way and intact plants difficult.

Attempts have been made to explain the undoubted effects of nutrients and auxin in a single hypothesis. Went (1939) concluded that a high auxin concentration in the shoot tip diverted nutrients

to that area, and postulated that the role of auxin in apical dominance is to direct nutrient material to the shoot tip or to prevent them reaching the lateral buds. In support of this general hypothesis, Davies and Wareing (1965) demonstrated that ^{32}P accumulated at the decapitated tip of the plant only when auxin was applied and concluded that in some unknown manner basipetal transport of auxin affected solute transport along the whole length of the translocating system. This view has been questioned by Sebanek (1967) who found that treatment of the decapitated stump of pea seedlings with IAA in lanolin resulted in axillary bud inhibition only when the IAA concentration was greater than 0.25%. Such concentrations, however, had little more effect on the accumulation of ^{32}P than did a concentration of 0.007% IAA, which was without effect on lateral bud growth. Sebanek concluded that the high concentration of auxin inhibited lateral growth not by a diversion of nutrients but by phytotoxicity. This report emphasises a problem in the interpretation of experiments in which lateral bud growth has been inhibited by auxin applied to the decapitated stem. With the exception of a report by Libbert (1964), all reports on the application of IAA to decapitated stems have described lateral bud inhibition only at auxin concentrations at least ten-fold higher than could be obtained by diffusion from the apical bud of the plant.

A further explanation of auxin-nutrient interactions is that auxin inhibits the differentiation of vascular tissue between the axillary bud and the main stem (Gregory and Veale 1957; Sorokin and Thimann 1964; Panigrahi and Audus 1966; Sachs 1970). Although such inhibition of the development of a vascular connection between the bud and the main stem would limit further growth of the lateral shoot (Cutter 1972), a vascular connection is neither necessary for the release of dominance of a bud nor is it necessary for translocation of carbohydrates (Wardlaw and Mortimer 1970). Moreover, as Cutter (1972) points out, the main shoot apex itself and many of the developing leaf primordia around it are devoid of vascular tissue but nevertheless undergo meristematic growth. Conversely, Peter and Fletcher (1973) found functional mature sieve tubes connected to the phloem system located in inhibited cotyledonary and first node buds of soybean plants. Thus inhibition of bud growth in soybeans cannot be attributed to an incomplete or non-functional vascular system.

The hypotheses discussed so far suggest a direct involvement of auxins in apical dominance. Snow (1937) proposed rather that auxin plays an indirect role by stimulating the production of an inhibitory substance in the stem which is then translocated to the buds and brings about inhibition. This supposed inhibitor has not been isolated or identified.

Growth substances other than auxin have also been reported to influence apical dominance. Kinetin applied directly to inhibited lateral buds of Pisum allows their growth (Wickson and Thimann 1958) and Maltzahn (1959) found that kinetin applied simultaneously with IAA in agar gel to the top of a decapitated Splanchnum ampullaceum plant reversed the inhibition of lateral buds caused by the application of IAA alone. Similar effects of IAA and cytokinins were reported by Sachs and Thimann (1964) with Helianthus annuus and Helianthus tuberosus as well as by Panigrahi and Audus (1964) with Vicia faba.

In contradiction to these reports Davies, Seth and Wareing (1966) found that the inhibition of lateral buds of bean plant by auxin was enhanced by the addition of kinetin with the applied auxin. They further demonstrated that the uptake of ^{14}C IAA by decapitated beans was increased in the presence of kinetin (and leads to extensive transport of ^{14}C IAA in the stem) and concluded that the increased bud inhibition resulting when auxin and kinetin are applied together may be due either to greater amounts of auxins reaching the bud or due to the redirection of metabolites from the buds to the point of hormone application. Although the role of cytokinin in apical dominance is not clear, due possibly to differences between investigations in the experimental material and hormone concentrations employed, it would appear that the hormone may be implicated in the endogenous response.

Gibberellic acid also influences apical dominance, generally stimulating the growth of inhibited buds (Kato 1953; Brian et al. 1955). Phillips (1969) has also reported an antagonistic effect of GA_3 on IAA induced inhibition of the bud growth of both pea and bean and Sebanek (1965) found that the release of the cotyledonary bud from inhibition resulted in a measurable rise in the gibberellin content of the bud 48 hrs before visible morphological development.

In contrast to these reports Jacob and Case (1965) and Scott et al. (1967) found that substitution of a mixture of IAA and GA for the shoot apex of Pisum restored apical dominance more effectively than IAA alone. Similarly, Brian et al. (1959), Stoddart (1959) and Bradley and Crane (1960) reported an enhancement of apical dominance in intact plants supplied with exogenous GA. Phillips' (1969) interpretation of these conflicting results is that exogenous GA may enhance the growth of lateral shoots which have already begun to grow but reinforces the inhibition of shoots that are still inhibited. He attributed this inhibitory aspect of the role of gibberellin to an increase in the auxin level within the plant, for which there is evidence.

Current examinations of apical dominance have emphasised the importance of an interplay of several factors. For instance, Shein and Jackson (1971, 1972) have suggested that the balance of hormones at different phases of growth of the plant, control apical dominance and Thimann et al. (1971) have proposed a similar hormone

interaction with the addition of light, nitrogen and phosphorus. Similar comprehensive hypotheses have been advanced by Jackson and Field (1972) and Woolley and Wareing (1972).

Despite the lack of agreement on the basic mechanism of apical dominance, it is apparent that hormone content and nutritional supply (both mineral and photosynthetic) are implicated. As the data in the previous chapter strongly suggest that axillary inflorescence development is controlled primarily by the terminal tassel, it is logical to enquire whether this control is exercised through the action of a hormone, through competition for a limiting nutrient, or through a combination of both.

It has been demonstrated in the previous two chapters that the growth patterns of the axillary inflorescences are changed by an episode of water deficit imposed at the period of initiation and growth of the terminal tassel. Thus the effect of water deficit brings about a modification of the dominance effect. The experiments to be described investigate the effect of various growth substances on whole plants to explore whether any of them could mimic, enhance, or reverse the effect of water deficit. Experiments were also performed with decapitated plants to find out if any of these growth substances could substitute for the tassel in the control of the growth of the axillary inflorescences.

The growth substances used comprised abscisic acid (ABA), 2,4-dichlorophenoxy acetic acid (2,4-D), 2,3,5-triiodobenzoic acid

(TIBA), benzyl aminopurine (benzyl adenine or BAP), gibberellic acid (GA_3), 2-chloroethylphosphonic acid (Ethrel) and the growth retardant (2-chloroethyl)trimethyl ammonium chloride (CCC). These growth substances were chosen for a variety of reasons. Plants which have been subjected to water stress are known to accumulate abscisic acid (Hiron and Wright 1969, 1973; Most 1971; Zeevart 1971) and it was considered that the effect of water stress on axillary shoot growth could be mediated through an accumulation of ABA during the period of water stress. If this were so, then application of ABA might mimic or enhance the effect of water stress when applied at the appropriate time. Auxins have long been implicated in apical dominance although their role has not been elucidated. A synthetic auxin, 2,4-D, was used in this study rather than indole-acetic acid (IAA), due to problems with the purity and stability of IAA. TIBA was also used to explore the possible role of auxin as TIBA has been reported to block auxin transport (Panigrahi and Audus 1966). The responses to a cytokinin (BAP) and a gibberellin (GA_3) were also explored because of the reports of the involvement of these hormones in apical dominance. Finally it has been reported that water deficit causes synthesis of ethylene and it has also been reported that ethylene breaks down apical dominance (Hall and Morgan 1963, cited by Burg and Burg 1968), whilst Burg and Burg (1968) reported an inhibition of bud growth by ethylene. It is possible, therefore, that ethylene is involved

in the water stress mediated inhibition of tassel growth and it was decided to examine the effect of Ethrel in this investigation. Finally, it has been frequently reported that growth retardants, including CCC, reduce the effects of water stress on plant growth (Halevy 1967). Although the effects of the growth retardants described have been concerned with the general growth of the plant, it was decided to include CCC in the present survey to see whether it influenced the specific response under investigation.

Together with this extensive examination of the role of growth substances in this response, the effect of the level of mineral nutrition was also examined. As the phenomenon under investigation appeared to be one of apical dominance, and mineral nutrient supply exerts a considerable although incompletely understood role in apical dominance, it was considered that an examination of the phenomenon would be incomplete without an exploration of the effects of mineral nutrition. No serious examination of the response to light intensity (and hence of carbohydrate supply) was attempted. Plants were grown throughout the year, except in the months from May to August when light intensity was particularly low, and no systematic seasonal variations in response were found.

3.3.2. The Effect of Growth Substances on Growth and Development of the Axillary Inflorescences - Application to Intact Plants

Introduction: The phenomenon under study has been shown to be essentially that of apical dominance which is modified by an episode of water stress. One way in which water stress could produce such a modification is through a change in the balance of growth substances in the plant. In order to explore this possibility, a range of growth substances were applied to intact, non-stressed plants and their effects were compared with the effects of water stress on axillary inflorescence growth. Growth substances were chosen which were known to be involved in apical dominance relationships in other situations (auxin, cytokinin, gibberellin), were known to be modified by water stress (abscisic acid, ethylene) or were known to vary endogenous concentrations of hormones involved in apical dominance (growth retardants, TIBA).

Two experiments are reported. In the first the growth substances used were: ABA, 2,4-D, Ethrel, BAP and the growth retardant CCC. The concentrations employed were selected based on various reports in the literature. Where possible, the criterion upon which concentrations were based were those reported as being extracted from Zea mays; failing this, concentrations found in other plants were used as a guide. Some of the major reports on which decisions were based are as follows: ABA (Hiron and Wright

1969; Higham and Smith 1969); 2,4-D (Heslop-Harrison 1960; Thimann et al. 1971); Ethrel (Burg and Burg 1968; Weaver 1972); BAP (Fisher 1971; Weaver 1972); CCC (Weaver 1972; Cathey 1964).

In the second experiment Ethrel was omitted because it did not appear to have any effect in the first experiment. CCC was also omitted because it was decided to concentrate the investigation at this stage on growth hormones. Consequently gibberellic acid was used as well as TIBA. The reason for applying TIBA was to find the effect of interruption of auxin translocation on the growth pattern of the axillary inflorescences. The major reports on which the concentrations of GA were based are: Jones and Phillips (1964), Jacob and Case (1967) and Sladky (1969). Similarly that of TIBA was based on Gausman and Duncan (1953) and Panigrahi and Audus (1966).

Method: In the first experiment plants were raised until 23 days after germination when growth substances were applied as described under General Methods. The respective concentrations used are as shown below.

Growth Substance	Concentration (µg/ml)	Volume (µl)	Total Wt. Administered (µg)
ABA	260	600	156
2,4-D	20	600	12
Ethrel	300	600	180
BAP	100	600	60
CCC	1000	600	600

ABA and 2,4-D were dissolved in approximately 0.1 ml of distilled ethanol and made up to the required concentration with distilled deionised water. BAP was prepared by dissolving 100 mg in approximately 5 ml of 0.5 N NaOH heated to 60°C. The resulting solution was then made up to a litre with distilled deionised water. 3 N HCl was then titrated against the prepared solution to a pH of 7 before use. CCC was dissolved directly in distilled deionised water. Each growth substance was applied to six plants. At the same time water deficit was imposed on six other plants whilst yet six other plants were watered as control together with all the plants to which growth substance was applied. Empty tubes were threaded to all the six plants which were water stressed and 600 µl of distilled deionised water were also threaded to the control plants at similar positions as those of the plants to which growth substances were applied. It was observed that all of the growth substances solutions had been absorbed into the plants 24 hrs after application. Water deficit was relieved 12 days after imposition whereafter all plants were watered daily. Sixteen days after re-watering the growth in length of the axillary shoots was measured and the developmental stages of the axillary buds were assessed. The total height of the plants and the tassel length were also measured. In this first experiment, the measurements were made at an earlier stage of growth than in any other experiment reported. This was because the plants were growing in late autumn with the light intensity falling progressively and it was thought that this

might adversely affect further growth. Despite this early harvest the enlargement of axillary shoots of the water stressed plants could be seen before harvest.

The second experiment was carried out in October (i.e. during spring) and the method was the same as described in the previous experiment. The application of growth substances and water stress were imposed 25 days after germination and the plants were water stressed for 10 days. The concentrations used were as given below:

Growth Substance	Concentration (µg/ml)	Volume (µl)	Total Wt. Administered (µg)
ABA	260	600	156
2,4-D	20	600	12
BAP	100	600	60
GA	10	600	6
TIBA	100	600	60

GA and TIBA were each dissolved in 0.1 ml distilled ethanol and made to required concentrations with distilled deionised water.

Three weeks after re-watering the total heights of the plants, the tassel lengths and axillary shoot sizes were measured and the stages of axillary bud development assessed.

Results: There was no significant difference in the axillary shoot lengths at node 7 of all the plants in either experiment (Tables 3.3.1. and 3.3.3.). In the first experiment the axillary shoot length at nodes 4, 5 and 6 of plants exposed to water deficit

as well as those to which ABA and CCC were applied were all significantly longer than the control. Similarly in the second experiment, axillary shoots 4, 5 and 6 of plants exposed to water deficit, ABA and TIBA and, in addition, the axillary shoot at node 3 of the ABA and TIBA-treated plants, were all significantly longer than those of the control. TIBA treated plants retained axillary shoots at node 2 which usually abort by this stage of growth. Except the axillary shoot at node 5 of Ethrel and 2,4-D treated plants in the first experiment and those at nodes 5 and 6 of the GA treated plants in the second experiment, none of the other axillary shoots of plants to which Ethrel, 2,4-D, GA and BAP were treated in both experiments showed any significant enhancement of growth.

The effect of water deficit and these growth substances on the development of axillary buds paralleled those of the growth of the axillary shoot except that the development of the axillary bud at node 4 of water deficit, ABA, CCC and TIBA treated plants were not statistically significantly different from those of the control plants (Tables 3.3.2. and 3.3.4.). There was no significant difference between the development of the buds at all nodes of Ethrel, 2,4-D, GA and BAP treated plants and those of the control at any node.

The results of these experiments with intact watered plants demonstrate that the effects of water deficit on axillary

TABLE 3.3.1.

Effect of Growth Substance on Growth of Axillary Shoots on Intact Plants

Treatment	Total Height of Plant (cm)	Tassel Length (cm)	Length (mm) 51 Days After Germination, of Shoots at Nodes: 1	2	3	4	5	6	7
Control	69	27	Abort.	9	12	10	14	70	110
Water Stress (23 to 35 Days After Germination)	60	17	8	15	20	40	70	97	120
ABA	74	23	8	12	20	35	60	97	110
2,4-D	67	26	Abort.	10	13	20	50	80	115
Ethrel	64	28	Abort.	10	12	19	55	76	108
BAP	53	26	Abort.	10	19	20	35	71	100
CCC	67	20	Abort.	11	12	29	77	115	112
L.S.D. (P = 0.05)	5	3	N.S.	N.S.	N.S.	10	14	12	N.S.

TABLE 3.3.2.

Effect of Growth Substances on Development of Axillary Buds on Intact Plants

Treatment	Stage of Development (Table 3.1.1.) 2 Weeks After Release of Stress, of Buds at Nodes: 1	2	3	4	5	6	7
Control		1	1	2	3_a	4_a	8
Water Stress (25 to 35 Days After Germination)	1	1	2	3	5_b	7_b	8
ABA	1	1	2	3	5_b	7_b	8
2,4-D		1	1	2	4_{ab}	6_{ab}	8
Ethrel		1	1	2	3_a	5_{ab}	7
BAP		1	1	2	3_a	4_a	7
CCC		1	1	3	6_b	7_b	8

Figures with similar letters in each column are not significantly different. Statistical analysis based on Kruskal-Wallis One Way Analysis of Variance.

TABLE 3.3.3.

Effect of Growth Substances on the Growth of Axillary Shoots on Intact Plants

Treatment	Total Height of Plant (cm)	Tassel Length (cm)	Length x Breadth (mm) 56 Days After Germination of Shoots at Nodes: 1	2	3	4	5	6	7
Control	186	27	Abort.	Abort.	28 x 5	32 x 5	81 x 7	145 x 12	248 x 34
Water Stress (25 to 35 Days After Germination)	190	13	Abort.	Abort.	32 x 5	43 x 7	175 x 10	209 x 15	260 x 35
ABA	180	25	Abort.	Abort.	52 x 6	50 x 7	145 x 10	182 x 15	263 x 38
2,4-D	184	28	Abort.	Abort.	31 x 4	26 x 4	83 x 8	160 x 13	263 x 35
BAP	183	28	Abort.	Abort.	30 x 4	30 x 4	80 x 8	158 x 13	260 x 35
TIBA	182	27	Abort.	99 x 5	51 x 5	62 x 7	130 x 9	180 x 17	271 x 38
GA	183	29	Abort.	Abort.	29 x 5	45 x 6	120 x 9	158 x 15	260 x 38
L.S.D. (P = 0.05)	N.S.	4			8 x 2	12 x 1	32 x N.S.	25 x 3	N.S.

TABLE 3.3.4.

Effect of Growth Substances on Development of Axillary Buds on Intact Plants

Treatment	Stage of Development (Table 3.1.1.) 3 Weeks After Release of Stress, of Buds at Nodes: 1	2	3	4	5	6	7
Control	Abort.	Abort.	3	3	4_a	7_a	14
Water Stress (25 to 35 Days After Germination)	Abort.	Abort.	3	4	6_b	10_b	14
ABA	Abort.	Abort.	3	4	7_b	9_b	14
2,4-D	Abort.	Abort.	2	3	4_a	7_a	14
BAP	Abort.	Abort.	3	3	4_a	7_a	14
TIBA	Abort.	4	4	4	7_b	10_b	15
GA	Abort.	Abort.	2	3	5_a	8_{ab}	15

Figures with similar letters in each column are not significantly different. Statistical analysis based on Kruskal-Wallis One Way Analysis of Variance.

inflorescence growth and development can be duplicated by ABA but not by Ethrel: both ABA and ethylene have been shown to be produced by plants exposed to a period of water deficit. The response can also be reproduced by applied TIBA, which is thought to affect morphogenesis through inhibition of auxin transport. The large effect of CCC was not explored further as it was decided to concentrate further investigations on the involvement of hormones in the mode of action of the organ exerting dominance.

3.3.3. Studies on Substitution of Growth Substances for the Tassel

3.3.3.1. The Effect of Substitution of Growth Substances for the Tassel on Growth and Development of the Axillary Inflorescences - Application of Single Growth Substances

Introduction: The data discussed so far suggest that the tassel is the most important organ exerting dominance over the growth and development of the lower axillary inflorescences and that this dominance is modified by an episode of water deficit since relief of the stress brings about enhanced growth of the lower axillary shoots. In order to understand this modification of internal control by water deficit, the mechanism of apical dominance in the watered plants has been examined. The tassel is six nodes removed from the most proximal axillary inflorescence,

and the dominance of the tassel over the lower axillary inflorescences may be mediated through plant growth substances or through the preferential diversion of nutrients to the tassel and away from the axillary buds. Many workers have demonstrated that the substitution of growth substances for the excised apex of a plant can maintain the inhibition of the lateral buds. For instance, Thimann and Skoog (1934) demonstrated this by substitution of IAA for the apex of Vicia faba and Jacob and Case (1965), and Scott et al. (1967) maintained the inhibition of lateral buds of decapitated Pisum with a mixture of IAA and GA_3. In the light of these reports it was decided initially to investigate the effects of substituting single growth substances for the tassel on the growth of the lower axillary shoots and the development of the buds.

Auxins and gibberellic acid have both been previously substituted for the apex of various plants (Jacob and Case 1965), so 2,4-D and gibberellic acid were included in this study. Cytokinins have been shown to release buds from dominance (Wickson and Thimann 1958; Maltzahn 1959) and BAP was applied as an example. It has been suggested that auxin, particularly at high concentrations, promotes endogenous ethylene production which causes some of the recorded responses (Weaver 1972). Ethrel, which releases ethylene in plant tissues (Weaver 1972), was included to check this possibility. The concentrations and total quantities absorbed by the plant were maintained at the same rates as in the previous

experiments but as it had been shown that the effects of the treatments were realised within approximately three weeks, the applications were spread over 20 days such that by the end of this period the quantities used in the previous experiments had been absorbed by the plants.

Method: The tassels of 30 plants were removed as described under General Methods, 30 days after germination. The following growth substances were then applied:

Growth Substance	Concentration (µg/ml)	Dose/day (µl)	Total Wt. Administered in 20 Days (µg)
2,4-D	2	300	12
GA	1	300	6
Ethrel	30	300	180
BAP	10	300	60

All growth substances were prepared as described previously. Each growth substance was applied to six plants. The tube with the appropriate growth substance was threaded to the stem 1 cm below the point at which the tassel was excised. To another batch of six plants 300 µl of double distilled water was similarly threaded to each plant. Six other plants were not detasselled and were used as control plants. All the plants were watered daily to run off. The same quantity and type of growth substance was put into each tube every morning for 20 days. After every two days, within the period of application of growth substances, each tube was removed and threaded further up the stem so that it was 1 cm below the tip

of the stem. At the end of the period of application of the growth substances (i.e. 20 days) the growth and development of the axillary inflorescences were measured and assessed.

Results: Plant height was reduced by removing the tassel (Table 3.3.5.) but applying GA_3 to detasselled plants partially restored this elongation whereas Ethrel inhibited elongation; 2,4-D and BAP were without effect.

Elongation of the axillary inflorescence at node 7 was unaffected by detasselling or any of the growth substances, but the width of this inflorescence was considerably increased by removal of the tassel. This effect was influenced only by 2,4-D which slightly inhibited the enlargement.

Excision of the tassel promoted the growth of the lower axillary shoots at nodes 5 and 6 and the various applied growth substances had only minor effects on this promotion. GA, 2,4-D and BAP marginally reduced the length of the axillary shoot at node 6 and with GA in particular this inhibition extended to nodes 5 and 4 (Table 3.3.5.). In all experiments recorded so far, the data for the axillary shoots, including the bracts has been presented. Although measured, data for the enclosed axillary buds, excluding the bracts, has not been presented as it has followed that of the axillary shoots closely. In the present experiment, however, the axillary shoot at node 6 on detasselled plants treated with GA was significantly longer than that on intact control plants but the

TABLE 3.3.5.

Effect of Growth Substances on the Growth of the Axillary Shoots of Detasselled Plants

Treatment	Total Height (cm)	Length x Breadth (mm) 20 Days From the Commencement of Application of Growth Substances of Axillary Shoots at Nodes: 1	2	3	4	5	6	7
Control	160	Abort.	Abort.	13 x 3	25 x 5	48 x 9	114 x 14	219 x 31
Detasselled Without Growth Substance	122	Abort.	Abort.	14 x 4	28 x 6	87 x 10	173 x 21	242 x 41
Detasselled + 2,4-D	108	Abort.	Abort.	14 x 4	30 x 6	68 x 9	145 x 18	241 x 38
Detasselled + GA	136	Abort.	Abort.	12 x 4	16 x 4	49 x 8	146 x 17	234 x 39
Detasselled + Ethrel	81	Abort.	Abort.	15 x 4	25 x 6	113 x 11	176 x 21	237 x 41
Detasselled + BAP	103	Abort.	Abort.	15 x 4	35 x 6	74 x 11	145 x 19	206 x 43
L.S.D. (P = 0.05)	26	-	-	N.S.	10 x N.S.	39 x N.S.	27 x 5	N.S. x 3

TABLE 3.3.6.

Effect of Growth Substances on the Growth of Axillary Buds* at Node 6 of Detasselled Plants - 20 Days From Commencement of Application of Growth Substance

Control	Detasselled Without Growth Substance	Detasselled + 2,4-D	Detasselled + GA	Detasselled + Ethrel	Detasselled + BAP	L.S.D. (P = 0.05)
52 mm	71 mm	78 mm	60 mm	74 mm	73 mm	13 mm

* minus bracts (prophyllum)

TABLE 3.3.7.

Effect of Growth Substances on Development of Axillary Buds of Detasselled Plants

Treatment	Stage of Development (Table 3.1.1.) 20 Days From Commencement of Application of Growth Substances at Nodes:						
	1	2	3	4	5	6	7
Control	-	-	1	3	5	7_{a}	11
Detasselled without Growth Substance	-	-	3	4	6	10_{ab}	12
Detasselled + 2,4-D	-	-	2	4	6	10_{ab}	12
Detasselled + GA	-	-	2	2	4	9_{ab}	12
Detasselled + Ethrel	-	-	2	3	7	10_{b}	12
Detasselled + BAP	-	-	2	3	6	10_{b}	12

Figures with similar letters in each column are not significantly different. Statistical analysis based on Kruskal-Wallis One Way Analysis of Variance. Note that the Kruskal-Wallis analysis discriminates on range therefore Ethrel and BAP-applied plants are significant.

buds without bracts were not significantly different (Table 3.3.6.). This suggests that GA was more effective in suppressing axillary growth on detasselled plants than is evident from the axillary shoot data.

Removing the tassel tended to promote the floral development of the axillary inflorescences, particularly at node 6. This effect was not reversed by the applied growth substances, indeed it may have been slightly enhanced by Ethrel and BAP (Table 3.3.7.).

Although the effects of the applied growth substances in this experiment were not large, and in no case was complete substitution for the tassel achieved, both GA_3 and to a lesser extent 2,4-D, induced a partial inhibition of the growth of the lower axillary buds. There was little response to the other two growth substances.

3.3.3.2. The Effect of Substitution of Growth Substances for the Tassel on the Growth and Development of Axillary Inflorescence - Interactions of Growth Substances

Introduction: In the previous experiment GA_3 and 2,4-D could partially substitute for the tassel in the control of both stem elongation and axillary inflorescence size and development. It is probable, however, that removing the tassel would cause changes in the supply of more than one endogenous growth hormone,

and likely that the growth of the axillary inflorescence is influenced by a balance of endogenous hormones including components originating in the tassel. The substitution of a single growth substance for the tassel might be inadequate in re-imposing the apical dominance exerted by that organ. It has been demonstrated by Jacob and Case (1965) and Scott et al. (1967) that a mixture of IAA and GA were able to inhibit the growth of lateral buds of decapitated pea seedlings and it was decided to investigate the effect of various combinations of the effective growth substances used in the previous experiment as substitutes for the tassel.

As there was little response to Ethrel alone in this system, this growth substance was omitted and GA, 2,4-D and BAP were combined in all possible ways giving four combinations. The concentrations and dosages of the individual growth substances were maintained at the same levels as when applied alone except in the combination of all three substances where the dosage was reduced to allow for the capacity of the tubes.

Method: Five sets of 8 plants were detasselled as before, 30 days after germination. Four combinations of growth substances were made up as follows:

2,4-D:GA	(1:1 v/v)
2,4-D:BAP	(1:1 v/v)
GA:BAP	(1:1 v/v)
2,4-D:GA:BAP	(1:1:1 v/v)

The concentration of each growth substance was as in the previous experiment, viz:

2,4-D - 2 µg/ml
GA - 1 µg/ml
BAP - 10 µg/ml

600 µl of each combination was applied to 6 detasselled plants each day for 20 days. Thus the volume of each individual growth substance applied per day was 300 µl, except in the case of the combination of all three where only 200 µl of each growth substance was applied. The same quantity of distilled deionised water was applied in the same way to 6 further detasselled plants. Six other plants were not detasselled and were used as control. All plants were watered to run off daily. Following 20 days of application of the growth substances, the sizes and developmental stages of the axillary inflorescences were measured and assessed.

Results: All the treated plants were significantly shorter than the control plants; there was no evidence for any promotion of elongation by GA, as was observed when it was applied alone.

The growth of the axillary inflorescence at node 7 was unaffected by tassel removal or by the growth substance applications, but the growth of the inflorescences at nodes 3 to 6 were significantly stimulated by tassel removal. A mixture of 2,4-D and GA completely abolished this stimulation at all nodes, 2,4-D and BAP together and the combination of 2,4-D, GA and BAP was

TABLE 3.3.8.

Effect of Combinations of Growth Substances on the Growth of Axillary Shoots of Detasselled Plants

Treatment	Total Height (cm)	Length x Breadth (mm) 20 Days From the Commencement of Application of Growth Substances of Axillary Shoots at Nodes: 1	2	3	4	5	6	7
Control	139	Abort.	Abort.	18 x 5	27 x 5	29 x 6	58 x 13	217 x 28
Detasselled Without Growth Substance	122	Abort.	Abort.	36 x 6	53 x 8	86 x 13	129 x 16	230 x 33
Detasselled + 2,4-D:GA	116	Abort.	Abort.	17 x 3	24 x 5	36 x 7	63 x 13	236 x 36
Detasselled + 2,4-D:BAP	108	Abort.	Abort.	18 x 4	26 x 6	48 x 8	95 x 14	201 x 34
Detasselled + GA:BAP	108	Abort.	Abort.	62 x 6	72 x 9	101 x 12	125 x 12	205 x 36
Detasselled + 2,4-D:BAP:GA	95	Abort.	Abort.	18 x 3	19 x 5	34 x 9	99 x 17	210 x 36
L.S.D. (P = 0.05)				14 x 2	15 x 3	28 x 3	41 x N.S.	N.S.

TABLE 3.3.9.

Effect of Combinations of Growth Substances on the Development of the Axillary Buds of Detasselled Plants

Treatment	Stage of Development (Table 3.1.1.) 20 Days From Commencement of Application of Growth Substances of Shoots at Nodes:						
	1	2	3	4	5	6	7
Control	-	-	2	3	4_a	7_a	11
Detasselled Without Growth Substances	-	-	3	5	7_{ab}	8_{ab}	11
Detasselled + 2,4-D:GA	-	-	2	3	4_a	8_{ab}	11
Detasselled + 2,4-D:BAP	-	-	2	3	4_a	8_{ab}	11
Detasselled + GA:BAP	-	-	3	5	7_b	9_{ab}	11
Detasselled + 2,4-D:GA:BAP	-	-	2	3	5_{ab}	9_b	11

Figures with similar letters in each column are not significantly different. Statistical analysis based on Kruskal-Wallis One Way Analysis of Variance.

slightly less effective having rather less effect at node 6 (Table 3.3.8.). In contrast, the combination of GA with BAP had no effect on the growth of the inflorescences at nodes 5 and 6 but significantly promoted the growth of those at nodes 3 and 4.

Similar responses were found with inflorescence development (Table 3.3.9.) except that many of the differences did not reach the level of statistical significance.

In the previous experiment it was found that either GA or 2,4-D could substitute, to some extent, for the terminal tassel. Here it has been found that only combinations of hormones which included 2,4-D were effective in that role. These two experiments suggest that auxins supplied by the developing tassel play a major role in the growth and development of the axillary inflorescences in this cultivar of corn.

3.3.4. The Interaction Between Growth Substances and Water Deficit in the Growth and Development of Axillary Inflorescences

3.3.4.1. Effect of Growth Substances on the Growth and Development of Axillary Inflorescences of Plants Exposed to Water Deficit - Application at the Beginning of a Period of Stress

Introduction: It has been demonstrated that ABA, TIBA and CCC applied to intact watered plants could mimic to varying extents

the effects of water stress on the growth and development of the axillary inflorescences. Furthermore, in watered plants, the controlling influence of the tassel on axillary inflorescence development could be substituted by applied 2,4-D either alone, or in combination with GA and possibly BAP. It is possible, therefore that ABA, TIBA and CCC act through the tassel to modify the supply of auxin or other hormones and that water stress acts in a similar manner. The dominance relationship might therefore be a result of a hormone balance which is maintained by the tassel. Since water stress causes permanent damage to the tassel (Experiment 3.1.2.) it would, on that assumption, naturally modify this balance. With this in view, it was decided to explore whether application of some growth substances at the beginning of a period of water stress could correct the probable imbalance caused by the water stress and thus prevent the enlargement of the axillary inflorescences.

The growth substances used were 2,4-D, GA, BAP and Ethrel. The first three substances inhibited axillary growth to varying extents when applied to detasselled plants. Although Ethrel was without effect in the detasselled system, it was included in the present experiment as it has been shown that ethylene is released in some water-stressed plants (McMichael 1972). The concentration of each growth substance was maintained at the same level as in the previous experiments.

Method: Growth substances were applied to a number of plants 24 days after germination as described in the previous experiments. The concentrations and dosage of growth substances were as shown below:

Growth Substance	Concentration (μg/ml)	Volume (μl)	Total Wt. Administered to plant (μg)
2,4-D	20	600	12
GA	10	600	6
BAP	100	600	60
Ethrel	300	600	180

Water stress was imposed on all plants supplied with growth substances together with six further plants not supplied with growth substance commencing on the day the growth substances were applied. Another six plants were watered throughout as control. Empty tubes were threaded to the water stressed plants not supplied with growth substances and the control plants were threaded to tubes containing 600 μl of double distilled water. The duration of the period of water stress was 8 days and thereafter all plants were watered daily for a further three weeks when the sizes of axillary shoots were measured and the development of axillary buds assessed.

Results: Neither water deficit nor the additional effect of any of the growth substances significantly reduced plant height (Table 3.3.10.). All the plants subjected to water deficit had shorter tassels than those of the watered plants. GA significantly

TABLE 3.3.10.

The Influence of Growth Substance Applied at the Beginning of Stress on Growth of Axillary Shoots

Treatment	Total Height (cm)	Tassel Length (cm)	Length x Breadth (mm) 3 Weeks After Release of Stress of Shoots at Nodes: 1	2	3	4	5	6	7
Control	155	26	Abort.	Abort.	40 x 6	60 x 8	90 x 10	126 x 13	176 x 19
Water Stress	140	11	20 x 4	20 x 5	22 x 6	94 x 8	121 x 10	158 x 18	173 x 21
Water Stress + 2,4-D	148	14	Abort.	Abort.	25 x 5	55 x 8	85 x 8	130 x 13	173 x 20
Water Stress + Ethrel	142	13	Abort.	Abort.	15 x 4	25 x 5	56 x 8	138 x 13	163 x 19
Water Stress + GA	145	9	Abort.	17 x 4	17 x 4	92 x 9	119 x 11	169 x 18	165 x 19
Water Stress + BAP	138	12	27 x 7	33 x 9	50 x 9	96 x 10	158 x 14	181 x 17	175 x 17
L.S.D. (P = 0.05)	N.S.	4	-	-	11 x 1	19 x 2	22 x 2	25 x 3	N.S.

increased this effect of water deficit, the tassels of these plants being shorter than all others.

The axillary shoot at node 7 was the same size in all treatments, being unaffected by water stress or the growth substances. The axillary shoots at nodes 4, 5 and 6 were larger on the plants subjected to water stress than on the control plants and the shoots at nodes 1 and 2 persisted on these but not the control plants. GA had no additional effect on axillary shoot growth but the cytokinin BAP produced some additional promotion of growth of the axillary shoots, at nodes 5 and 3. In contrast, applied 2,4-D completely reversed the effects of water stress on axillary shoot growth: in plants supplied with 2,4-D the axillary shoots at nodes 4, 5 and 6 were identical to those in watered plants. Ethrel had a similar effect, particularly on the lower nodes: the shoots at nodes 3, 4 and 5 were even smaller than those on watered plants.

The general trend of the results of the development of the axillary buds follows that of growth in size of the shoots (Table 3.3.11.). Although the axillary bud at node 6 of water stressed plants and those to which GA and BAP were additionally applied were more developed than those of the control plants this statistically significant difference was lost in buds at nodes 4 and 5. The development of all the axillary buds on water stressed plants supplied with 2,4-D or Ethrel was similar to that of the buds on the watered plants.

TABLE 3.3.11.

The Influence of Growth Substances Applied at the Beginning of Stress on the Development of Axillary Buds

Treatment	Stage of Development (Table 3.1.1.) 3 Weeks After Release of Stress of Buds at Nodes:						
	1	2	3	4	5	6	7
Control	-	-	2	4	5_a	7_a	11
Water Stress	0	1	2	5	6_{ab}	10_b	11
Water Stress + 2,4-D	-	-	2	3	5_{ab}	8_{ab}	11
Water Stress + Ethrel	-	-	1	3	4_a	8_{ab}	11
Water Stress + GA	-	1	1	4	6_{ab}	10_b	11
Water Stress + BAP	2	2	3	5	7_b	11_b	10

Figures with similar letters in each column are not significantly different. Statistical analysis based on Kruskal-Wallis One Way Analysis of Variance.

This experiment shows that 2,4-D and Ethrel prevent the promotion of growth of the axillary inflorescence of plants due to exposure to a period of water deficit when they are applied at the beginning of the water deficit.

Certain other noteworthy observations were made in this experiment. Firstly, apart from the fact that the tassels of the GA-treated plants were significantly shorter than those on other plants, they were completely sterile, whilst on all other plants subjected to water stress the florets on the lateral branches alone were sterile. The laterals of these GA treated plants were devoid of florets and the central axis which bore fertile florets in the other treated plants developed only the pedicel of the florets. Secondly, the BAP treated plants developed an abundant coating of epidermal hairs, especially on the stem and the axillary shoots.

3.3.4.2. Effect of Growth Substances on Growth and Development of Plants Exposed to Water Deficit - Application at the End of a Period of Stress

Introduction: It has been shown in the previous experiment that some growth substances were able to prevent the enlargement of the lower axillary inflorescences evident in plants subjected to water deficit when applied at the commencement of the period of water deficit. Although water deficit at the time of tassel enlargement promotes lower axillary inflorescence growth, the effect is not apparent until after the stress has been relieved as the

immediate effect of the stress is to inhibit growth at all locations. The applied growth substances may have reversed the response either by counteracting some change in the plants during the period of stress or by persisting in the plant through the stress period and producing an effect in the period following rewatering. To check on this second possibility, growth substances were applied to plants immediately following an episode of water deficit, to ascertain whether similar effects could be produced.

The growth substances and their concentrations were as in the previous experiment with the exception of ABA which was included in the growth substances used in this experiment to see if it would further enhance the promotion of the growth of the lower axillary inflorescences caused by the episode of water deficit.

Method: Water stress was imposed on 36 plants, 22 days after germination, for nine days. Six other plants were watered as control. On the day the water deficit was relieved the following growth substances were applied to each of 6 plants as described in the previous experiments:

Growth Substance	Concentration (μg/ml)	Volume (μl)	Total Wt. Administered to Plant (μg)
ABA	240	600	144
GA	10	600	6
2,4-D	20	600	12
BAP	100	600	60
Ethrel	300	600	180

Empty tubes were threaded to a further six water stressed plants and tubes containing 600 µl of double distilled water were threaded to the control plants. All plants were watered after the application of growth substances. From this day onwards all plants were watered to run-off daily for three weeks when the axillary shoot sizes were measured and the development of the axillary buds assessed.

Results: As in the previous experiment there was no significant difference in total height between any of the plants, although all plants subjected to water stress were shorter than the controls (Table 3.3.12.). The tassels of all the treated plants were significantly shorter than those of the control plants and tassel length was unaffected by any of the growth substance treatments. Evidently the effect of the episode of water stress on tassel growth was not reversed by growth substances applied either before or after the episode of stress (Tables 3.3.10. and 3.3.12.). In this experiment also there was no significant difference in axillary shoot size at node 7 between any of the treatments.

Water stress again promoted axillary shoot growth at the lower nodes (5 and 6 only in this case - Table 3.3.12.). This promotion was unaffected by ABA, GA_3 or Ethrel, where the axillary shoots were of an identical size to those on plants subjected to water stress alone.

TABLE 3.3.12.

Influence of Growth Substances Applied at the End of a Period of Stress on Growth of Axillary Shoots

Treatment	Total Height (cm)	Tassel Length (cm)	Length x Breadth (mm) 3 Weeks After Release of Stress of Shoots at Nodes: 1	2	3	4	5	6	7
Control	160	27	Abort.	Abort.	20 x 4	27 x 4	30 x 5	88 x 9	211 x 35
Water Stress	148	17	Abort.	Abort.	25 x 4	25 x 4	70 x 10	129 x 17	195 x 38
Water Stress + 2,4-D	158	16	Abort.	Abort.	15 x 4	17 x 4	25 x 5	86 x 10	200 x 36
Water Stress + Ethrel	149	16	Abort.	Abort.	19 x 4	26 x 4	68 x 10	130 x 16	200 x 38
Water Stress + GA	152	14	Abort.	Abort.	17 x 4	27 x 5	70 x 8	128 x 16	195 x 40
Water Stress + BAP	155	17	Abort.	Abort.	20 x 4	38 x 7	65 x 10	80 x 13	215 x 38
Water Stress + ABA	157	15	Abort.	Abort.	15 x 4	25 x 4	65 x 8	135 x 15	205 x 37
L.S.D. (P = 0.05)	N.S.	4	Abort.	Abort.	N.S.	8 x 1	19 x 2	27 x 4	N.S.

When cytokinin (BAP) was applied to plants at the commencement of an episode of water stress there was a significant promotion of axillary shoot growth at some lower nodes (Table 3.3.10.) but here, where BAP was applied at the end of the period of stress, this response was lost. Although enlargement of the axillary shoot at node 4 was promoted, that at node 6 was significantly inhibited and others were unaffected.

As with the earlier application, however, application of 2,4-D reversed the effect of the episode of water stress on axillary shoot growth and there was no significant difference between the axillary shoot sizes at any of the nodes and the equivalent shoots on the watered plants.

The effects of the treatments on the development of the axillary buds was generally similar to that on axillary shoot growth (Table 3.3.13.). There was no significant difference in axillary bud development at nodes 3, 4 and 7 between the treatments. As with axillary shoot size, only 2,4-D of the applied growth substances reversed the effect of prior water stress on axillary shoot development.

The most significant observation in this experiment was that 2,4-D applied at the end of an episode of water stress could reverse the effects of that stress on axillary shoot growth and development in the same fashion as 2,4-D applied at the beginning of the stress. In contrast, Ethrel applied at the end of the

TABLE 3.3.13.

The Influence of Growth Substances Applied at the End of a Period of Stress on the Development of Axillary Buds

Treatment	Stage of Development (Table 3.1.1.) 3 Weeks After Release of Stress of Buds at Nodes: 1	2	3	4	5	6	7
Control	-	-	2	2	3_a	6_a	11
Water Stress	-	-	2	2	7_b	10_c	12
Water Stress + 2,4-D	-	-	2	2	3_a	7_{ab}	11
Water Stress + Ethrel	-	-	2	2	6_{ab}	9_{bc}	12
Water Stress + GA	-	-	2	2	7_b	10_c	12
Water Stress + BAP	-	-	2	2	8_b	8_{abc}	12
Water Stress + ABA	-	-	2	2	5_{ab}	9_{bc}	12

Figures with similar letters in each column are not significantly different. Statistical analysis based on Kruskal-Wallis One Way Analysis of Variance.

period of stress rather than at the beginning showed no ability to reverse the effects of the water stress.

From these experiments it could be concluded that, apart from 2,4-D, the effects of all the other growth substances on water-stressed plants seem to depend on the period of application. It may be that, with the exception of 2,4-D, these hormones act to modify the response of the tassel to the episode of water deficit such that its ability to control axillary shoot growth after the stress, possibly through an auxin system, is changed. In contrast, 2,4-D may act more directly in the period following water stress, the effect of the earlier application being due to persistence of the compound in the plant through to the post-stress period.

3.3.5. Effect of Mineral Nutrition on the Growth and Development of the Axillary Inflorescences of Plants Exposed to Water Deficit

Introduction: Nutrition, both mineral and photosynthetic, has been frequently reported to be directly or indirectly involved in the phenomenon of apical dominance. Although the experiments described so far in this chapter show that hormones are probably involved in the dominance relationships in this plant under investigation, it is also possible that competition for nutrition might be a further factor determining the growth of the axillary inflorescences. This possibility was explored by providing a liberal supply of mineral nutrients to plants in the expectation that this

might vary the effect of water stress.

Method: Two sets of plants of different nutritional status were obtained. Plants were sown in potting compost as before but one week after germination 500 ml of half strength Hoagland solution was applied to one batch of 12 plants whilst the other batch was watered to run-off. Thereafter 500 ml of full strength Hoagland solution was applied once a week to the high nutrient status plants. The low nutrient status plants were supplied with water only. 22 days after germination, 6 plants of each of the two nutrient status classes were water stressed for 10 days and thereafter all plants were watered daily to run-off. During the period of stress, the application of nutrient solution was suspended, but the 6 plants that were not water stressed were watered normally. Three days after relieving the plants from water stress, application of Hoagland solution to all 12 plants was resumed as before. All plants were watered daily to run-off except when Hoagland solution was applied.

Three weeks after relieving the plants from water stress the sizes of the axillary shoots and the development of the buds were measured and assessed.

Results: If the hypothesis that the apical dominance system under consideration is controlled by a competition for mineral nutrients between the tassel and the axillary inflorescence, and that water stress decreases the competitive ability of the tassel is

correct, then increasing the mineral nutrient supply should have a number of characteristic effects. Firstly, in plants not subjected to water stress, increasing the mineral nutrient supply should preferentially promote the growth of the lower axillary inflorescences. As can be seen (Table 3.3.14.), additional mineral nutrition had no effect on tassel growth, as measured by length, nor any significant effect on the uppermost axillary inflorescence. The axillary inflorescence at nodes 5 and 6, however were markedly promoted in growth, that at node 6 being double the size of that on the low nutrient status plants. This first prediction of the hypothesis is therefore met.

Secondly, in plants subjected to water stress where growth of the tassel is inhibited and presumably the competition of that organ for mineral nutrients reduced, one might not expect a differential effect of additional mineral nutrition on the lower axillary inflorescences, but rather an overall promotion of all organs (apart from the tassel). This prediction is also fulfilled as the axillary inflorescences at nodes 7, 6, 5 and 4 are all larger on water stressed plants given additional nutrients than on low nutrient-status water stressed plants.

A third prediction of the hypothesis may also be made; if the tassel is the main organ competing for mineral nutrients and its growth is restricted by water stress, then one might reasonably expect that water stress would have a greater effect on the

TABLE 3.3.14.

Effect of Mineral Nutrition and Water Deficit on the Growth of Axillary Shoots

Treatment	Total Height (cm)	Tassel Length (cm)	Length x Breadth (mm) 3 Weeks After Release of Stress of Shoots at Nodes: 1	2	3	4	5	6	7
Control	149	28	-	-	13 x 4	23 x 6	40 x 7	64 x 13	196 x 31
Water Stress	118	22	-	-	15 x 4	33 x 6	69 x 9	107 x 15	194 x 29
High Nutrition No Water Stress	136	27	-	-	15 x 4	28 x 6	65 x 8	129 x 14	213 x 30
High Nutrition and Water Stress	114	21	-	-	19 x 4	44 x 8	97 x 10	176 x 18	252 x 40
L.S.D. (P = 0.05)	13	2	-	-	N.S.	8 x -	14 x 2	27 x 3	25 x 6

axillary inflorescences of plants on a low nutrient status than those on a high. This prediction is not fulfilled, water stress having approximately the same promotive effect on the growth of the inflorescences at nodes 4, 5 and 6 at both a high and a low nutrient status.

These three conclusions are also substantiated by the data on axillary inflorescence development (Table 3.3.15.) although here differences between treatments do not reach statistical significance in as many comparisons. It would appear, particularly from the first prediction, that mineral nutrition is at least involved in this apical dominance system. The interaction with water stress is unclear, however, as the promotive effect of water stress on axillary inflorescence development is not lessened by an augmented supply of mineral nutrients although the relative responsiveness of the inflorescences at the individual nodes is changed.

3.3.6. The Effect of 2,4-D and Mineral Nutrition on the Growth and Development of Plants Exposed to Water Deficit

Introduction: The data from the previous experiment indicates that the growth of axillary shoots is affected by the level of mineral nutrition supplied. Moreover this effect of mineral nutrition interacts with the effect of water stress under investigation. Undoubtedly, however, hormone control of axillary growth is also involved and the earlier experiments suggest that auxin

TABLE 3.3.15.

Effect of Mineral Nutrition and Water Deficit on the Development of Axillary Buds

Treatment	Stage of Development (Table 3.1.1.) 3 Weeks After Release of Stress of Buds at Nodes: 1	2	3	4	5	6	7
Control	-	-	2	3	4_{a}	7_{a}	11
Water Stress	-	-	2	3	5_{ab}	9_{ab}	11
High Nutrition No Water Stress	-	-	2	3	6_{ab}	9_{ab}	11
High Nutrition and Water Stress	-	-	2	3	7_{b}	10_{b}	11

Figures with similar letters in each column are not significantly different. Statistical analysis based on Kruskal-Wallis One Way Analysis of Variance.

originating in the developing tassel may be the controlling factor. The present experiment was designed to examine any possible interaction between these two proposed control systems, by observing whether applied 2,4-D could inhibit the promotion of axillary shoot growth produced by water stress and an enhanced mineral nutrient supply.

Method: Two sets of plants of different nutritional status were raised as in the previous experiment. 25 days after germination both sets of plants were treated as follows: A number of plants from each set were water stressed. 600 µl of 2,4-D at a concentration of 20 µg/ml was applied to 6 plants from each set. Empty tubes were attached to another 6 plants from each set of water stressed plants. To the last group of 6 water stressed plants from each set, 2,4-D of the same concentration and dosage as above was applied at the end of the period of stressing. 2,4-D at the same concentration was applied to 6 plants from each set of the unstressed plants whilst double distilled water was applied to the last 6 plants from each set of unstressed plants. In all treatments the tube containing the appropriate substance, was threaded to the base of the tassel (see 2.2.5.). All the unstressed plants were watered to run-off daily. Water was withheld from the stressed plants for 7 days and thereafter all plants were watered to run-off daily. The axillary shoot size and the stages of development of the axillary bud were measured and assessed three

weeks after re-watering.

Results: As in all previous experiments, the episode of water stress significantly enhanced the growth of the lower axillary shoots (Table 3.3.16.) but not that of the uppermost axillary shoot. In fact, in this experiment, not one of the treatments, nutritional, hormonal or stress significantly affected the growth of this shoot. The response to additional mineral nutrition was much less pronounced in this experiment than in the previous, the stimulation to the growth of the lower axillary shoots being marginally significant with or without concomitant water stress. As growth of these shoots was undoubtedly suppressed in this experiment compared with the previous, this finding casts doubt on the supposition that availability of mineral nutrients is of importance in determining the growth of the lower shoots.

2,4-D was without measurable effect on the growth of intact, unstressed plants of high or of low nutritional status. Where 2,4-D was applied at the beginning or the end of a period of stress, however, there was a tendency for the growth substance treatment to inhibit the growth of the lower axillary shoots such that they resembled more closely those on plants not subjected to stress. This effect was not large, and was not uniformly affected by the time at which 2,4-D was applied nor by the nutritional status of the plant.

TABLE 3.3.16.

The Effect of 2,4-D and Mineral Nutrition on the Growth of Axillary Inflorescence of Plants Exposed to Water Deficit

Treatment	Length x Breadth (mm) 3 Weeks After Release of Stress of Shoots at Nodes:						
	1	2	3	4	5	6	7
Control	Abort.	Abort.	30 x 5	52 x 7	100 x 10	150 x 19	226 x 35
2,4-D	Abort.	Abort.	32 x 6	59 x 8	94 x 11	160 x 19	230 x 40
Water Stress	Abort.	Abort.	29 x 6	112 x 13	120 x 13	175 x 21	225 x 37
2,4-D at Beginning of Stress	Abort.	Abort.	33 x 7	66 x 9	117 x 12	167 x 22	238 x 38
2,4-D at End of Stress	Abort.	Abort.	30 x 6	51 x 8	115 x 12	168 x 22	228 x 41
High Nutrition	Abort.	Abort.	36 x 6	53 x 7	105 x 12	160 x 20	240 x 39

High Nutrition and 2,4-D			37 x 6	50 x 8	109 x 12	167 x 19	240 x 37
High Nutrition and Water Stress	Abort.	Abort.	30 x 7	91 x 10	126 x 15	175 x 23	238 x 36
High Nutrition and 2,4-D at Beginning of Stress	Abort.	Abort.	30 x 6	50 x 7	122 x 13	174 x 20	241 x 40
High Nutrition and 2,4-D at End of Stress	Abort.	Abort.	32 x 6	66 x 9	123 x 11	155 x 19	229 x 40
L.S.D. (P = 0.05)			N.S.	12 x 1	11 x 1	8 x 2	N.S.

TABLE 3.3.17.

The Effect of 2,4-D and Mineral Nutrition on the Development of Axillary Inflorescence of Plants Exposed to Water Deficit

Treatment	Stage of Development (Table 3.1.1.) 3 Weeks After Release of Stress of Shoots at Nodes: 1	2	3	4	5	6	7
Control	Abort.	Abort.	2	3_a	6	9	11
2,4-D	Abort.	Abort.	2	4_{ab}	6	9	12
Water Stress	Abort.	Abort.	2	6_b	7	10	11
2,4-D at Beginning of Stress	Abort.	Abort.	2	4_{ab}	7	10	11
2,4-D at End of Stress	Abort.	Abort.	2	4_{ab}	7	10	11
High Nutrition	Abort.	Abort.	2	4_{ab}	7	10	11
High Nutrition and 2,4-D	Abort.	Abort.	2	4_{ab}	7	9	11
High Nutrition and Water Stress	Abort.	Abort.	2	5_{ab}	7	10	11
High Nutrition and 2,4-D at Beginning of Stress	Abort.	Abort.	2	4_{ab}	7	9	11
High Nutrition and 2,4-D at End of Stress	Abort.	Abort.	2	4_{ab}	6	9	11

Figures with similar letters in each column are not significantly different. Statistical analysis based on Kruskal-Wallis One Way Analysis of Variance.

The stage of development of the lower axillary inflorescences was little affected by any of the treatments applied (Table 3.3.17.), such variations as did occur, however, support the general conclusions drawn from the growth in size of the shoots.

Whilst the results of this experiment provide further support for the hypothesis that the effects of water stress on axillary shoot development may be mediated through changes in auxin within the plant, they do not support the suggestion that this control system acts through modification of the nutrient supply to the shoot.

3.3.7. DISCUSSION

It has been demonstrated earlier that the tassel is the major source of the correlative inhibition of the growth of the lower axillary inflorescences. This inhibition, by analogy with other apical dominance systems, may be due to hormones produced in the tassel, to competition for nutrition between the organs, or to an interaction of the two mechanisms. Support for the suggestion that hormones produced within the tassel are involved is provided here by the finding that application of certain growth substances to plants from which the tassel has been excised can restore the inhibition of the growth of the lower axillary inflorescences. When single growth substances were substituted for the tassel, the synthetic analogue of auxin, 2,4-D, was able to inhibit the growth

of the axillary shoots to some extent (Table 3.3.5.). Moreover, where mixtures of growth substances were applied to detasselled plants, all the mixtures that contained 2,4-D brought about inhibition of growth of these lower axillary inflorescences (Table 3.3.8.). Additionally, the application of TIBA to watered, intact plants promoted the growth of the lower axillary inflorescences. This also supports the view that tassel produced auxin is involved in the inhibition as TIBA has been reported to inhibit the basipetal transport of IAA in the plant (Panigrahi and Audus 1961).

Apart from these effects of 2,4-D, however, gibberellic acid alone applied to detasselled plants also produced an inhibition of the lower axillary inflorescences and moreover the mixture of 2,4-D and GA applied to detasselled plants was most effective in restoring the inhibited growth of the lower axillary inflorescences. Thus GA appears to be involved in the dominance mechanism. A direct inhibition of growth of the axillary inflorescences by GA seems unlikely. More probably, GA might promote the synthesis of auxin elsewhere, probably in the axillary inflorescence at node 7 which is translocated to the lower axillary inflorescences to inhibit their growth. It has been reported that IAA biosynthesis from tryptophan in bean hypocotyl was increased by application of GA (Varga 1968, cited by Varga 1973) and also GA increased the conversion of tryptophan to IAA in a cell free enzyme preparation from apical tissue of bean shoots (Varga 1973). Alternatively GA

could promote the translocation of auxin from the axillary inflorescences at node 7. This is not without precedence since it has been demonstrated that gibberellic acid caused an increase in both uptake and movement of auxin applied to donor blocks on cut apical surfaces of Lens stem (Pilet 1965). Thus it appears that auxin, in combination with GA, effects the inhibition of growth of the lower axillary inflorescences.

Water deficit modifies the correlative inhibition exerted by the tassel on the growth of the lower axillary inflorescences and allows their further growth. This effect is accompanied by a pronounced inhibition of the growth of the tassel itself. Within the context of the previous discussion, the inhibition of the growth of the tassel would most likely diminish auxin production by the organ and this alone may account for the reduction. It has been reported that water stress causes an increase in activity of IAA-oxidase in pea and in tomato leaves (Darbyshire 1971a) and it has also been shown that certain peroxidases disappear whilst new ones appear in wheat leaves with water stress (Stutte and Todd 1969). In addition water deficit may also decrease the translocation of auxin from the tassel to the axillary shoots since Basler et al. (1961) demonstrated that a decrease in relative water content below 80% in bean caused a drastic reduction in the translocation of 2,4-D. Caution must be employed in interpreting the effects of water deficit in these ways, however, as the evidence

for the involvement of IAA and possibly GA in the growth pattern of the axillary inflorescences is entirely circumstantial since all the experiments carried out have concerned the exogenous application of growth substances and the endogenous hormone concentrations are unknown.

Moreover, though it appears circumstantially that auxin is involved and that a period of water deficit might probably cause its degradation by increasing the activity of IAA-oxidase, this reported effect has been questioned by Mills and Todd (1969) who found that there is a marked decline of IAA-oxidase activity with increasing water stress in wheat leaves and also a direct reduction in the endogenous concentration of auxin due to water deficit has not been reported.

The hypothesis suggested so far to account for the enhanced growth of the lower axillary inflorescences following an episode of water deficit involve the reduction in the supply or transport of a hormone from the tassel. A well established effect of water stress, however, is a dramatic increase in the concentration of the hormone abscisic acid in plant tissues. The concentration of ABA has been shown to increase dramatically in a number of species (including Zea mays) during water deficit (Hiron and Wright 1969; Most 1969; Zeevart 1971; Gilles et al. 1974) and this increase has been shown to be due to enhanced synthesis (Milborrow et al. 1973). This increase in the concentration of ABA in the tissues

may conceivably play a role in the phenomenon under discussion and it is of particular significance that ABA applied to intact, non-stressed plants also stimulates growth of the lower axillary inflorescences (Tables 3.3.1. and 3.3.3.). This effect of applied ABA (and possibly of enhanced concentrations of endogenous ABA) could be due to a direct stimulating effect of the hormone on the growth of the axillary inflorescences which had been arrested. More probably, however, the mode of action might be indirect, through the effect of a high concentration of ABA on the metabolic processes occurring in the tassel. It has been repeatedly reported that ABA inhibits RNA synthesis when added to RNA polymerase by lowering DNA template activity (van Overbeek *et al*. 1968; Monod *et al*. 1970; Mondal and Biswas 1972). Synthesis of protein might have thus been lowered which would affect *de novo* synthesis of enzymes in turn affecting many other processes including hormone synthesis. This would eventually modify the mechanism of inhibition of growth of the lower axillary inflorescences. It has also been observed that metabolic inhibitors of DNA inhibit the effect of auxins and gibberellins (Kamisaka *et al*. 1970). Thus if ABA inhibited DNA template activity, both auxins and gibberellins could be affected which would in turn affect the dominance relationship in the plant.

Another possible effect of ABA is on the translocation of auxins. ABA has been reported to inhibit the transport of IAA-2-^{14}C (Pilet 1971; Kaldeway 1971; Naqvi *et al*. 1974) and a

high concentration of ABA induced by water deficit could impair the translocation of auxin from the tassel.

These considerations led to the conclusion that a role for IAA (and possibly GA) in the control of axillary inflorescence growth need not necessarily conflict with a role for ABA in determining the response to water deficit. In summary it is suggested that auxin, probably together with GA, is translocated from the tassel to the axillary inflorescences and causes an inhibition of growth of the axillary inflorescences. Water deficit stimulates the synthesis of ABA which affects the supply of these hormones or their translocation to the axillary sites, and this stimulates the growth of the axillary inflorescences.

Other hypotheses to explain the control of axillary inflorescence growth and the effect of water deficit have also been examined. Cytokinins have been thought to be involved in the maintenance of dominance over the lateral buds. Substitution of a combination of 2,4-D and BAP for the tassel affected the inhibition of the growth of the lower axillary inflorescences better than the individual substitution of these substances. This appears to support the report that the inhibition of lateral buds on decapitated bean plants by auxin was enhanced by the addition of kinetin (Seth et al. 1966). Nevertheless the substitution of BAP alone did not prevent the enlargement of the axillary inflorescences of detasselled plants. Thus, though cytokinin might play a role in

the dominance relationship in the plant, this is not a clear association.

It has been reported that an episode of water deficit can cause a stimulation of the synthesis of ethylene in the petioles of cotton (McMichael et al. 1972) and in leaves of Valencia orange (Ben-Yehoshua and Aloni 1974). If this is a general phenomenon, like the stimulation in ABA production, it is possible that ethylene was here produced during the period of water deficit and caused a breakdown of the dominance relationship. Hall and Morgan (1963, cited by Burg and Burg 1968) reported such an effect. Further, ethylene inhibited the basipetal transport of auxin in cotton petioles (Morgan and Gausman 1966; Beyer and Morgan 1969) and in pea (Pratt and Goeschl 1969). However the application of Ethrel as a source of ethylene to plants at the beginning of water deficit prevented the enhanced growth of the lower axillary inflorescences whilst the same concentration of Ethrel applied at the end of the water deficit had no effect. There is no evidence therefore that Ethrel (or ethylene) stimulates the growth of axillary inflorescences in this variety of corn in the same way as water deficit or ABA.

Mineral nutritients have been repeatedly reported to cause a breakdown in apical dominance when liberally applied but to enhance apical dominance when limiting. For instance, it has been reported that an increased supply of mineral nutrients caused a breakdown in

apical dominance in Pisum sativum (McIntyre 1964; Husain and Linck 1966), in Agropyron repens (McIntyre 1969) and in Solanum sisymbrifolium (Wakhloo 1970). Similarly Aspinall (1961) and Fletcher et al. (1974) have reported profuse growth of tillers on application of mineral nutrients to barley plants. This effect of mineral nutrition in differentially stimulating the growth of axillary buds did not occur in this cultivar of Zea mays. Rather, an increase in the supply of mineral nutrients resulted in a general stimulation of growth including the uppermost axillary inflorescences (Table 3.3.13.). This is in contrast to the effect of tassel removal or water deficit where the lower axillary inflorescences were preferentially stimulated. Probably in this case mineral nutrition was limiting growth in general and its application resulted in an increase in the growth of axillary inflorescences. This effect was not realised when the experiment was repeated (Table 3.3.16.) and it appears unlikely that mineral nutrition is involved in the stimulation of growth of the lower axillary inflorescences caused by an episode of water deficit.

In summary therefore, the evidence from these experiments in which growth substances were applied to plants does not support the hypothesis that either ethylene or cytokinin are involved in the apical dominance or its modification by water deficit. However the data are consistent with the tassel acting as a source of auxin and possibly gibberellin which together control the growth and development of the lower axillary inflorescences. In addition the

effects of water deficit on apical dominance were mimicked by the supply of ABA to intact watered plants suggesting that water deficit induced increases in the concentration of endogenous ABA and that these might be important in the phenomenon considered. Clearly, a complete elucidation of the inter-relationships between the effects of the various hormones during and following water stress on the phenomenon under study would require a knowledge of the endogenous concentrations of auxin, gibberellins and abscisic acid in the various organs throughout this period. Such a complete study was beyond the scope of the present investigation, and a choice of the further work to be attempted had to be made. It was decided to assess changes in endogenous ABA rather than in auxin (or gibberellins) for several reasons. Firstly a reliable, accurate and sensitive physical method for the determination of ABA is available whereas the bioassay procedures available for auxin determination are less reliable. Secondly, there is abundant evidence for changes in abscisic acid concentration with water stress in a variety of plants whereas such changes in endogenous auxin content have not been clearly demonstrated. Thirdly, and most significantly, in the hypothesis advanced, an increase in endogenous abscisic acid concentration upon stress is postulated as a primary event from which secondary manifestations, including reduced auxin supply, derive. It was considered more important therefore to investigate this primary event at this stage, rather

than to be concerned with secondary effects, even though these are of crucial importance in the overall response.

3.4. INVESTIGATIONS OF THE ENDOGENOUS LEVELS OF ABSCISIC ACID (ABA) AND ITS PROBABLE MODE OF ACTION IN WATER DEFICIT PLANTS

3.4.1. The Effect of Water Deficit on Endogenous ABA

Introduction: It has been demonstrated that the tassel is the main source of dominance over the lower axillary inflorescences and in view of the fact that the tassel is six nodes removed from the most proximal lower axillary inflorescence the effect is most probably mediated through hormones. Evidence was presented that auxin, together possibly with gibberellin, is the most likely hormone to be involved. An episode of water deficit causes permanent damage to the tassel and results in the growth of enlarged lower axillary inflorescences. This effect is mimicked by the application of ABA to intact, watered plants. It has been repeatedly reported that water stress induces a marked and rapid increase in the concentration of ABA in many plants (Wright 1969; Wright and Hiron 1969; Mizrahi 1970; Most 1971) including corn (Gilles et al. 1974). It is logical to suggest, therefore, that the effect of water stress in promoting the growth of the lower axillary inflorescences is mediated through a concomitant increase in endogenous ABA content. Since the response to water stress in this corn cultivar on ABA concentration was unknown, it was decided to investigate the levels of endogenous ABA of both stressed and unstressed plants.

In order to ascertain the concentrations of ABA within the whole aerial parts of the plants, measurements were taken in the various parts of the plant as follows: the tassel, the third leaf, the uppermost axillary inflorescences (i.e. at node 7), the axillary inflorescence at nodes 5 and 6, and the axillary inflorescences at nodes 3 and 4. The tassel was investigated separately because it was thought that the effect of ABA could be due to an inhibition of auxin production or translocation in that organ as mentioned under Section 3.3.7. (see page 189). The uppermost axillary inflorescence is relatively unaffected by either water stress or the presence of the tassel and is usually the only one that grows to maturity. The axillary inflorescences at nodes 5 and 6 were considered together as they demonstrate the largest effect of water stress i.e. they rarely form mature cobs in watered plants but frequently do so following a period of water stress. The inflorescences at nodes 3 and 4, whilst showing some response to water stress, do not form mature cobs. Finally, a leaf sample was included to allow comparison with other studies of ABA concentration in leaves. Leaf 3 was chosen as it was the leaf on which water potential measurements were made.

Method: Plants were raised as described under General Methods. 22 days after germination, 12 plants were water stressed by withholding water and 12 plants were watered to run-off daily. Water was withheld for 8 days and the plants were then harvested and separated into the tassel, the leaves, the axillary inflores-

cences at nodes 5 and 6 and the axillary inflorescences at nodes 3 and 4. Leaf samples were harvested by cutting about 30 discs from the central portion of the 3rd leaf of each plant with a cork borer of 1.2 cm diameter. The samples were then frozen in liquid nitrogen and stored as described previously (Section 2.2.6.2.) for a maximum period of two months, the first extractions being started after one week of storage. Just prior to extraction, three replicates for each organ in each treatment were randomly chosen. Since the sample weight for the extraction was restricted to 200 mg (it was established that this weight gave consistent results with this method of extraction) it was necessary to sub-sample in the case of the tassel, the uppermost axillary inflorescence and the axillary inflorescence at nodes 5 and 6. With the axillary inflorescences, each was cut into two halves longitudinally with a scalpel and one-half was used for the assay. The tassel was cut longitudinally into two halves and the central axis, one lateral branch at the mid section and the lowest section of the stalk from one half were used as the sub-sample for the assay. The sub-samples thus prepared were quickly weighed, placed in 100 ml centrifuge tubes and 4 ml double distilled water was added. The samples were placed in a boiling water bath for 10 minutes and were then cooled. 4 ml of acetone was added to each sample and the extraction, purification and measurement of ABA carried out as described under General Methods (Section 2.2.6.2.).

TABLE 3.4.1.

The Effect of Water Stress on the Endogenous Concentration of ABA

Plant Organ	ng/g Fresh Wt.		Log_{10} ng/g Fresh Wt.		
	Control	Water Stress	Control	Water Stress	L.S.D. (P = 0.05)
Tassel	203	587	2.31	2.77	0.20
Leaf	81	820	1.90	2.91	0.32
Axillary Inflorescence at Node 7	162	290	2.21	2.46	N.S.
Axillary Inflorescence at Nodes 5 and 6	230	1050	2.36	3.02	0.41
Axillary Inflorescence at Nodes 3 and 4	290	400	2.46	2.60	N.S.

Results: Water stress caused an increase in the concentration of extractable ABA in all organs of the plant (Table 3.4.1.). Nevertheless the increase in concentration following water stress was less than what has been reported for other plants (Hiron and Wright 1969; Gilles et al. 1974). The concentration varied considerably between different organs of the plant. In watered plants, the leaf appeared to contain the lowest concentration of ABA with the axillary inflorescence at node 7 approximately double the concentration and the other organs still higher. Water stress caused a ten-fold increase in the concentration of ABA in the leaf, 4 to 5-fold in the axillary inflorescences at nodes 5 and 6, and a 2 or less-fold increase in the concentration in other organs. As a result of these changes, the highest concentration of ABA in stressed plants was to be found in the axillary inflorescences at nodes 5 and 6, followed by the leaf and tassel. The concentration in the inflorescence at node 7 remained low.

It appears, therefore, that although a period of water deficit results in an increase in the concentration of ABA in all parts of the plant, there are marked differences in the distribution of this increase within the plant. It is of considerable interest that the axillary inflorescences at nodes 5 and 6 which exhibit the largest growth response to water stress and applied ABA also showed the greatest change in endogenous ABA following water stress, and the inflorescence at node 7 which produces the

least change in growth response had the least change in ABA content.

These data were obtained after a prolonged (8 day) exposure to water stress and it is possible that the ABA concentration varied with time during the period of exposure to stress. This possibility was examined in the next experiment.

3.4.2. The Effect of Varying Periods of Water Deficit on the Concentration of ABA in the Plant and the Subsequent Growth and Development of the Axillary Inflorescences

Introduction: It has been demonstrated that water stress imposed on this variety of corn causes a general increase in the concentration of ABA in the plant. Since the application of ABA to intact watered plants causes a similar effect to that of water stress it appears that ABA is probably involved in the phenomenon of the enhanced growth of the lower axillary inflorescences following water stress. The increases in ABA concentration found following eight days of water deficit were not large, however, and it was thought possible that considerable further metabolism of the ABA synthesised during this extended period of water deficit could have occurred. This could, moreover, affect the relative accumulation of ABA in the various plant organs.

In order to investigate this possibility water stress was imposed on the plants for varying periods of time and both the

free and conjugated ABA were measured at each period of stress and related to the subsequent development of the axillary inflorescences on similarly-treated plants. The conjugated form of ABA was measured because it has been reported that of the three main compounds to which ABA is metabolised the major one is the glucose ester (Milborrow 1970) which has been identified as abscisyl-β-D glucopyranoside.

Method: Plants grown as before (2.2.1.) were watered to run-off daily for 24 days after germination when half the number of plants were water-stressed by withholding water. Two days after commencement of water stress the leaf water potentials of 10 of each of the stressed and control plants were measured. At the same time these plants were harvested and the various organs were stored for ABA assay as described previously. A further six stressed plants were re-watered at this time and grown on to assess axillary inflorescence development. This procedure of measuring the water potential of the third leaf, harvesting and storing the various organs and re-watering 6 stressed plants was repeated on the 4th, 6th and 8th day after stressing. Thereafter all remaining plants were watered daily to run off for three weeks when the height of the plant, the total length, number and average lengths of laterals of the tassels, and the size of the axillary inflorescence were measured and the developmental stages of each axillary inflorescence assessed.

Extraction of ABA from the stored samples was started one week after the last harvesting. All measurement of ABA from these stored samples was completed within six weeks following the last harvesting. Samples from the same day of harvesting were extracted at the same time.

Results: The water potential of the stressed plants decreased rapidly at first until the sixth day, after which there was little further decrease (Table 3.4.2.). The total height of the plants was unaffected by the various periods of water stress but tassel growth was reduced (Table 3.4.3.). Both total tassel length and number of laterals were reduced by as little as two days exposure to water stress. More prolonged stress had more severe effects including inhibition of tassel branch elongation. It is apparent that the growth of the tassel is very sensitive to the effects of water stress.

As in previous experiment, there was no significant difference between the growth of the uppermost axillary inflorescences in any of the treatments though the actual differences were quite large in this experiment. The size of the axillary inflorescences at nodes 4, 5 and 6, as measured by breadth, length or both, was increased following as little as 2 days of water stress. Moreover, subjecting the plants to any longer period of water stress had no significantly greater effect on the growth of these axillary inflorescences. It can be concluded that the growth of

TABLE 3.4.2.

The Effect of Varying Periods of Deficit on Leaf Water Potential

Period of Stress	Water Potential	
	Control	Stress
2 days	-3.7 bars	-7.7 bars
4 days	-5.3 bars	-10.2 bars
6 days	-5.1 bars	-17.2 bars
8 days	-5.2 bars	-17.7 bars

TABLE 3.4.3.

The Effect of Varying Periods of Deficit on the Growth of the Tassel and Axillary Inflorescences

Treatment	Height (cm)	Total Tassel Length (cm)	No. of Laterals	Average Length of Lateral (cm)	Length x Breadth (mm) 3 Weeks After Release of Stress of Shoots at Nodes: 1	2	3	4	5	6	7
Control	178	27	19	10.4	Abort.	Abort.	19 x 5	53 x 8	102 x 12	169 x 19	218 x 32
2 Days of Stress	162	21	16	10.1	Abort.	Abort.	30 x 6	86 x 11	143 x 16	185 x 23	228 x 34
4 Days of Stress	178	21	17	7.3	Abort.	Abort.	27 x 6	79 x 9	167 x 16	203 x 25	250 x 40
6 Days of Stress	173	14	17	3.4	Abort.	Abort.	27 x 5	66 x 8	134 x 14	188 x 25	225 x 40
8 Days of Stress	169	11	15	2.2	Abort.	Abort.	22 x 5	96 x 10	148 x 16	184 x 24	225 x 39
L.S.D. (P = 0.05)	N.S.	3	3	1.3			N.S.	32 x 2	41 x 3	30 x 4	N.S.

TABLE 3.4.4.

The Effect of Varying Periods of Deficit on the Development of the Axillary Inflorescences

Treatment	Stage of Development (Table 3.1.1.) 3 Weeks After Release of Stress of Buds at Nodes: 1	2	3	4	5	6	7
Control	Abort.	Abort.	3	4	6_a	9_a	11
2 Days of Stress	Abort.	Abort.	3	6	7_{ab}	11_b	12
4 Days of Stress			3	5	8_{ab}	11_b	12
6 Days of Stress			3	4	7_{ab}	11_b	12
8 Days of Stress			3	5	9_b	11_b	12

Figures with similar letters in each column are not statistically different. Statistical analysis based on Kruskal-Wallis One Way Analysis of Variance.

these lower axillary inflorescences is as sensitive to water stress as is the growth of the tassel. The effect of varying period of stress on development of the axillary buds was less pronounced than the changes in size but followed the same general pattern (Table 3.4.4.).

The concentrations of free ABA in all parts of the watered, control plants at the end of the eight day period (Figs. 3.4.1. to 3.4.5.) were similar in every respect to those found in the previous experiment (Table 3.4.1.): the concentrations in the meristematic regions, tassel and axillary buds, were 3 to 4-fold higher than the concentration in the leaf lamina. This difference became established between days 2 and 4 in the present experiment when the concentration in the meristematic regions increased rapidly, and may have been associated with the 1 to 2 bar drop in water potential recorded for these plants at this time.

The free ABA concentrations in the water-stressed plants were somewhat higher at the 8-day sample than in the previous experiment but the relative differences between organs were preserved.

There were also some differences in the timing of this increase in ABA concentration with water stress in the various parts of the plant. The largest initial response was in the tassel: following two days of stress when the leaf water potential had fallen only to -7.7 bars, there had been a more than ten-fold increase in the concentration of ABA in the tassel (Fig. 3.4.1.).

FIG. 3.4.1.

The effect of varying periods of water deficit on the concentration of endogenous free and "bound" (conjugate) ABA in the tassel.

●-● Free ABA in tassel of control plants

○-○ Free ABA in tassel of stressed plants

■-■ "Bound" ABA in tassel of control plants

□-□ "Bound" ABA in tassel of stressed plants

See Appendix A.3.1. for Statistical Analysis (page 267).

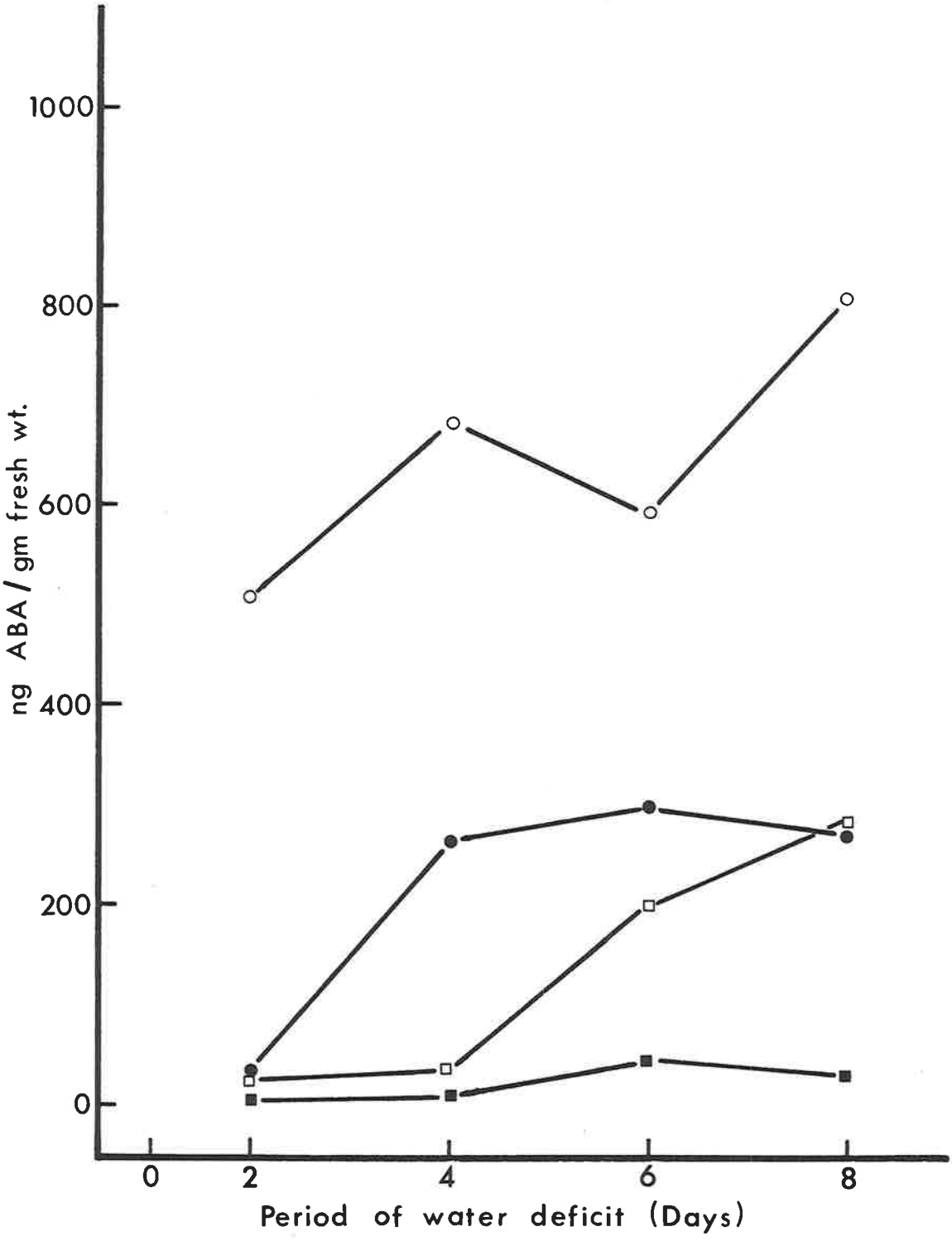

1000
800
600
400
200
0
ng ABA / gm fresh wt.
0
2
4
6
8
Period of water deficit (Days)

FIG. 3.4.2.

The effect of varying periods of water deficit on the concentration of endogenous free and "bound" (conjugate) ABA in the third leaf.

●-● Free ABA in the leaf of control plants

○-○ Free ABA in the leaf of stressed plants

■-■ "Bound" ABA in the leaf of control plants

□-□ "Bound" ABA in the leaf of stressed plants

See Appendix A.3.2. for Statistical Analysis (page 268).

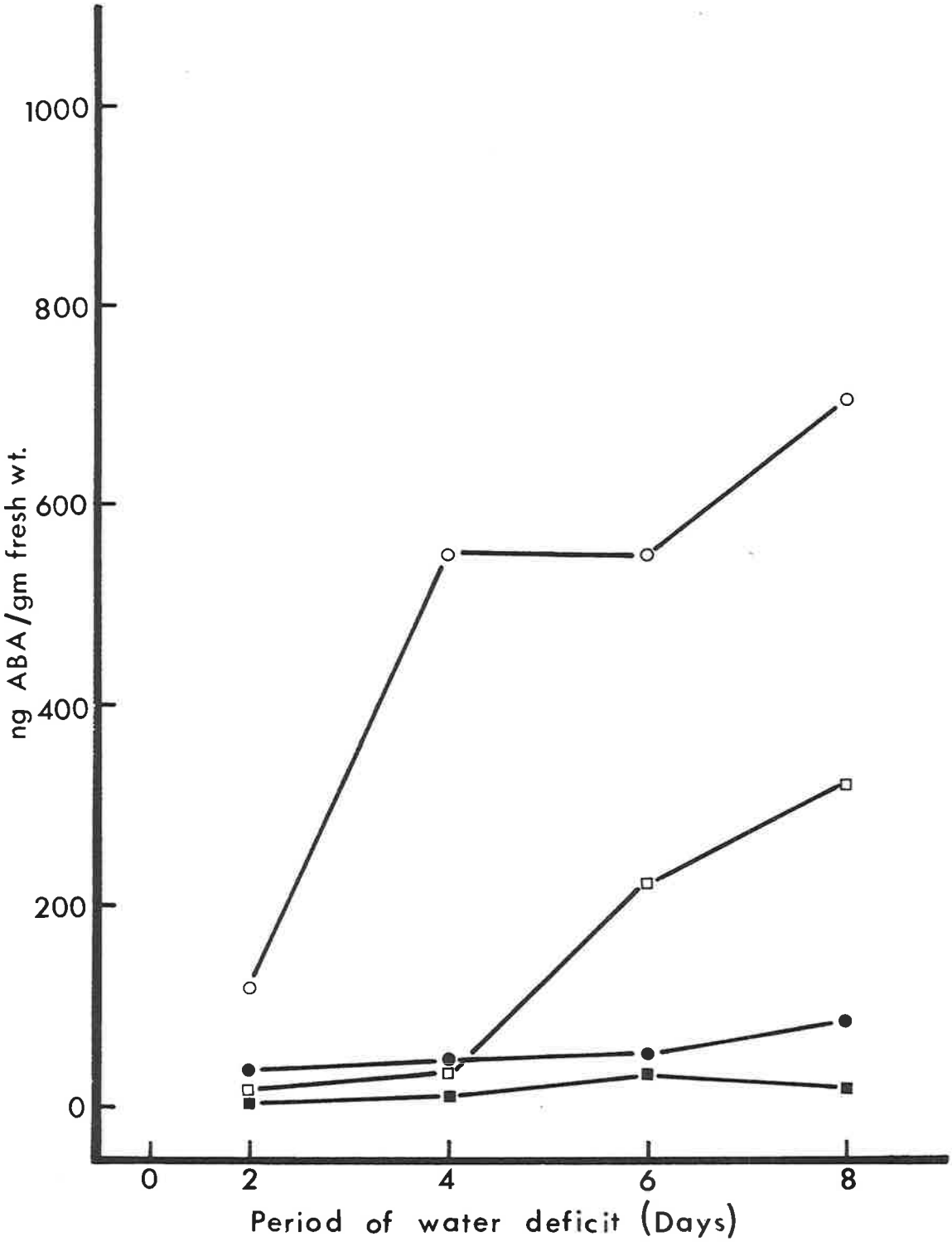

1000
800
600
400
200
0
ng ABA /gm fresh wt.
0
2
4
6
8
Period of water deficit (Days)

FIG. 3.4.3.

The effect of varying periods of water deficit on the concentration of endogenous free and "bound" (conjugate) ABA in the axillary inflorescence at node 7.

●-● Free ABA in the axillary inflorescence at node 7 of control plants

○-○ Free ABA in the axillary inflorescence at node 7 of stressed plants

▪-▪ "Bound" ABA in the axillary inflorescence at node 7 of control plants

□-□ "Bound" ABA in the axillary inflorescence at node 7 of stressed plants

See Appendix A.3.3. for Statistical Analysis (page 269).

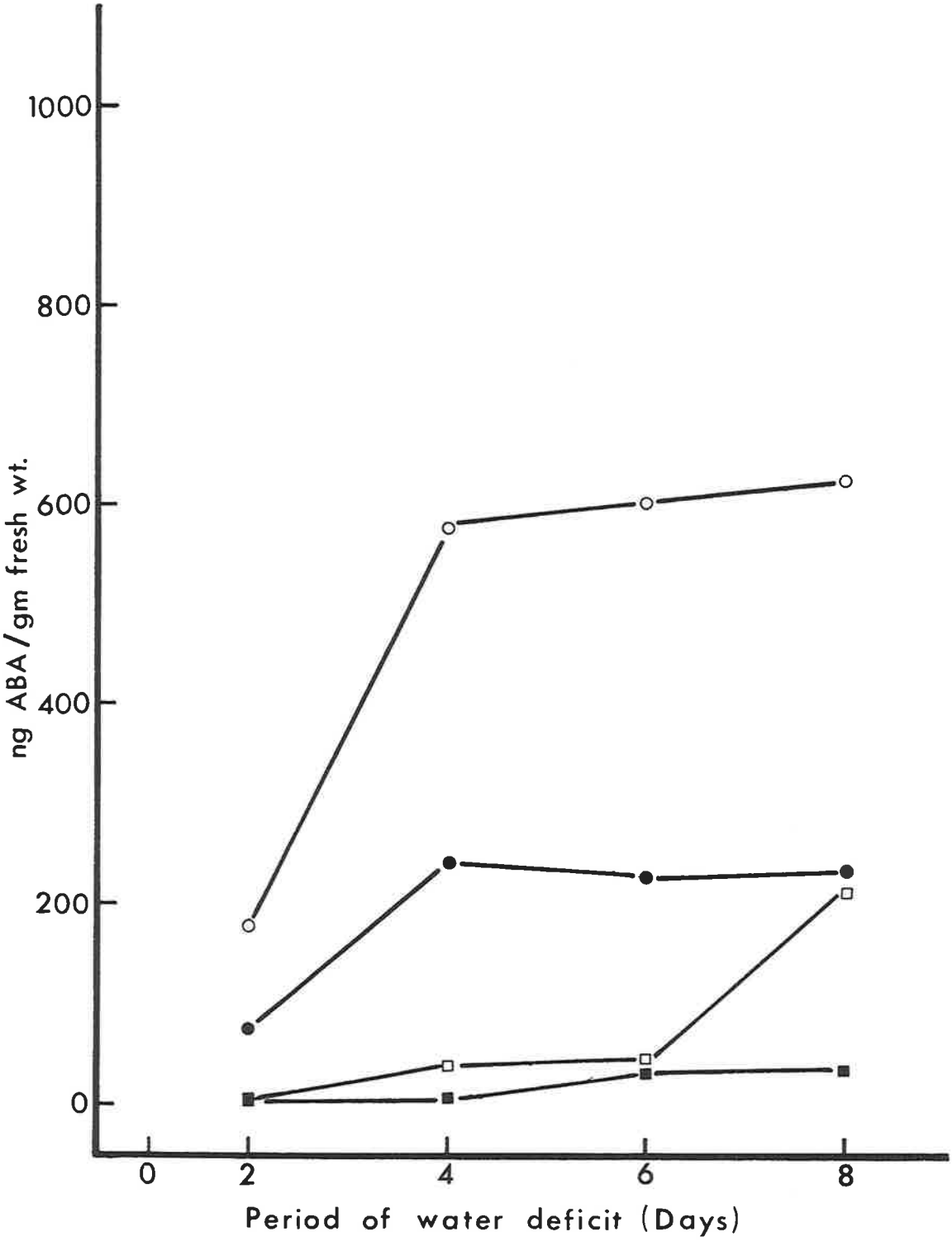

1000
800
600
400
200
0
ng ABA / gm fresh wt.
0
2
4
6
8
Period of water deficit (Days)

FIG. 3.4.4.

The effect of varying periods of water deficit on the concentration of endogenous free and "bound" (conjugate) ABA in the axillary inflorescences at nodes 5 and 6.

●-● Free ABA in the axillary inflorescence of the control plants

○-○ Free ABA in the axillary inflorescence of the stressed plants

■-■ "Bound" ABA in the axillary inflorescence of the control plants

□-□ "Bound" ABA in the axillary inflorescence of the stressed plants

See Appendix A.3.4. for Statistical Analysis (page 270).

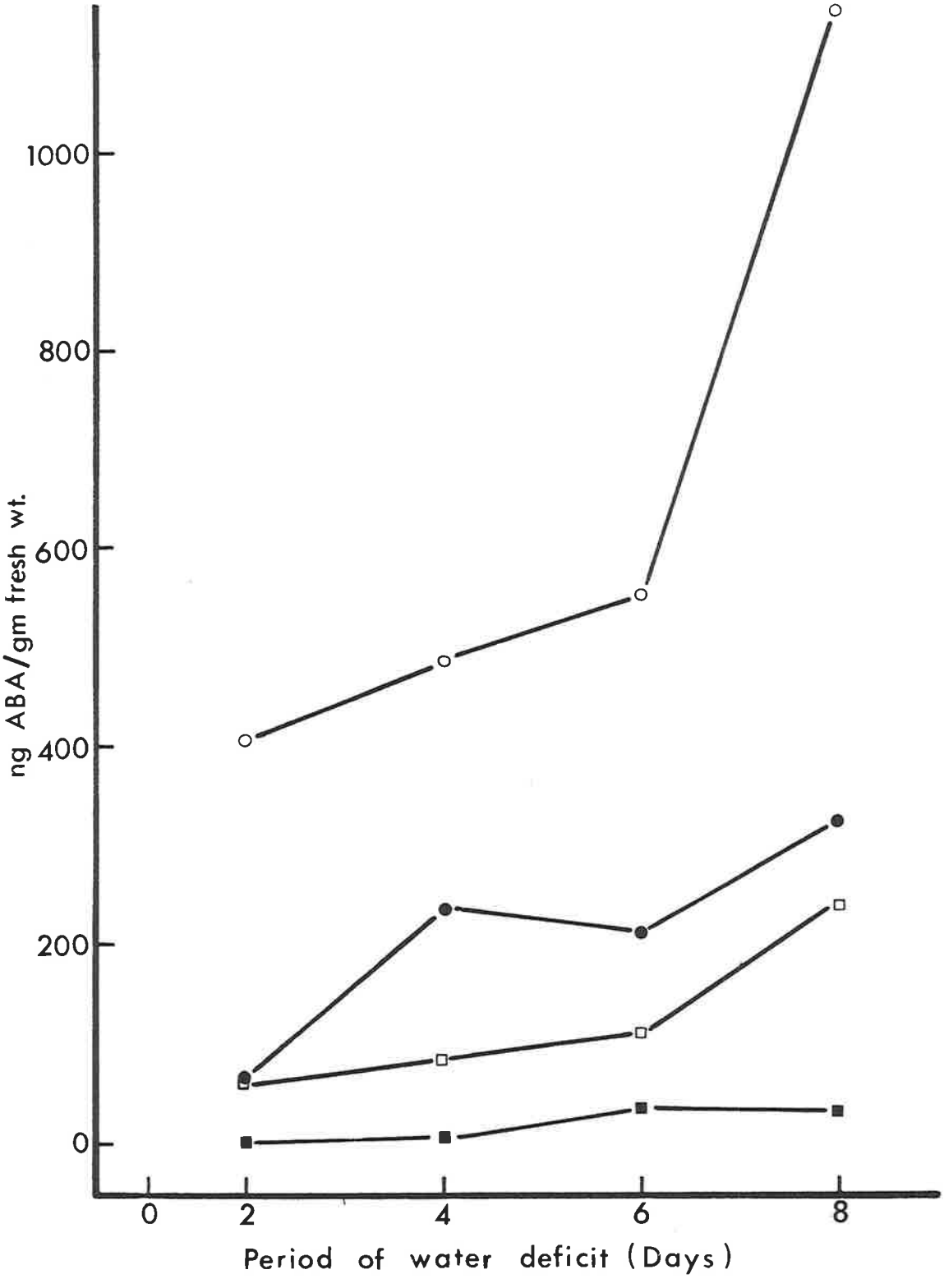

1000
800
600
400
200
0
ng ABA/gm fresh wt.
0
2
4
6
8
Period of water deficit (Days)

FIG. 3.4.5.

The effect of varying periods of water deficit on the concentration of endogenous free and "bound" ABA in the axillary inflorescence at nodes 3 and 4.

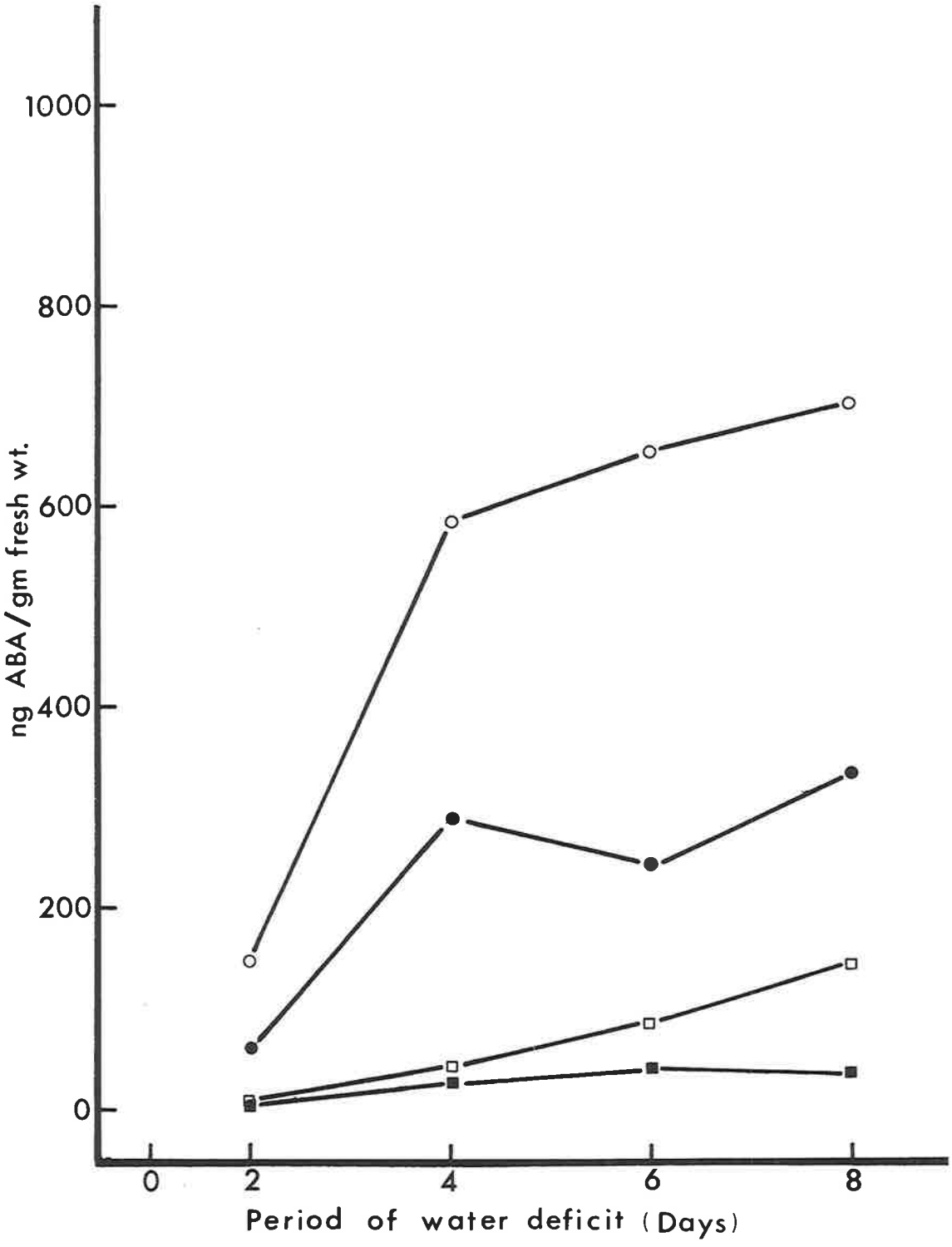
●-● Free ABA in the axillary inflorescence of the control plants

○-○ Free ABA in the axillary inflorescence of the stressed plants

■-■ "Bound" ABA in the axillary inflorescence of the control plants

□-□ "Bound" ABA in the axillary inflorescence of the stressed plants

See Appendix A.3.5. for Statistical Analysis (page 271).


1000
800
600
400
200
0
ng ABA/gm fresh wt.
0
2
4
6
8
Period of water deficit (Days)

Although there had been some responses in the other organs at this time, these were smaller; the greatest increase in ABA concentration in these parts of the plant occurred between days 2 and 4 when the water potential had fallen further. Following the second day of stress there was little further increase in the concentration of ABA in the tassel until after the 6th day when the water potential had fallen to -17 bars and appeared to be stable. The initial increase in concentration of ABA in the axillary inflorescence at nodes 5 and 6 was similar to that in the tassel in that, by the second day, there had been a six-fold increase in concentration in the stressed plants (Fig. 3.4.4.). However, unlike the tassel, this accumulation continued until the eighth day of stress when the concentration reached the highest level measured in the whole plant. The increases in concentration in the leaf (Fig. 3.4.2.), the uppermost axillary inflorescence (Fig. 3.4.3.) and the lowest axillary inflorescence (Fig. 3.4.5.) were similar. There was a gradual increase in the concentration of ABA in the leaves with time and as the rate of accumulation did not fall as drastically after day 4 as in the other organs, the concentration in the leaves approached that in the axillary inflorescence at nodes 5 and 6 by day 8.

The concentration of conjugated ABA in the plant tissues was always less than that of the free compound (Figs. 3.4.1. to 3.4.5. inclusive) and in the watered plants was always less than 100 mg/g fresh wt. although there was a tendency for the concentration to

rise with time, particularly in the leaf. In the water-stressed plants, the rapid increase in concentration of free ABA induced by the water deficit was in each case followed some 2 to 4 days later by an accumulation of "bound" ABA reaching a level ranging between 10 and 40% of free ABA. This increase in the "bound" form tended to occur earlier in the leaf and tassel than in the axillary inflorescences. It would appear that the accumulation of free ABA may have itself promoted the synthesis of the "bound" form.

3.4.3. A Study of the Mode of Action of Exogenously Applied (±) ABA and the Growth Retardant SADH (succinic acid-2-2-dimethylhydrazide)

Introduction: Abscisic acid applied to intact watered plants can mimic the effect of water deficit on the growth and development of the lower axillary inflorescences (Tables 3.3.1. and 3.3.3.). Moreover, it has been shown that water stress induces an increase in the concentration of endogenous ABA in the plant, this increase being most rapid in the developing tassel. As it has been suggested that the ABA, either endogenous or exogenous, may produce its effect on the axillary inflorescences through action in the tissues of the tassel, it is of importance to determine whether the application of ABA to the plant results in an increase in the free ABA recoverable from the tassel.

A further test of the hypothesis that ABA is involved in the regulation of axillary inflorescence growth was derived from the

fact that growth retardants, in particular CCC (Table 3.3.1.) and SADH (Jaffe et al. 1963, cited by Cathey 1965), promote the growth of the lower inflorescences. It was reasoned that if this effect is due to similar processes to those that occur during water stress, then the application of a growth retardant to the plant should result in an increase in the endogenous concentration of ABA in the plant tissues. SADH was chosen as the growth retardant for testing because it was of interest to test if this growth retardant would promote enhanced growth of the axillary inflorescences in this cultivar as reported by Jaffe et al. (1963); the concentration employed was based on reports by Cathey (1965) and Weaver (1972).

Method: Plants were raised with watering to run-off daily until 24 days after germination when 600 µl of ABA at a concentration of 264 µg/ml was applied to each of 18 plants, 600 µl of a solution containing 1000 µg/ml of SADH was applied to a further 12 plants and 600 µl of double distilled water was applied to another set of 12 plants. All applications were made to the shoot below the tassel (Section 2.2.5.). Two days after application of the growth substance, 6 plants of those to which ABA was applied were harvested and the tassels excised, frozen and stored as before. Five days later 6 plants from each treatment were harvested, the tassels were removed from the ABA treated plants and the third leaves and tassels from the SADH treated and control plants. These

samples were frozen and stored as before for ABA assay. Throughout this experiment all plants were watered daily to run-off. The plants which were not harvested at these times were allowed to grow for a further 3 weeks when they were also harvested and their total height, tassel lengths, and axillary shoot sizes were measured and the development of the axillary buds was assessed. The free and "bound" ABA in the stored plant material was extracted, purified, and measured one week after the second harvest. The extraction procedure was modified in this experiment from that previously described (2.2.6.2.) in that 4 ml water was added to each of the weighed samples in a centrifuge tube. These were then placed in a boiling water bath for 10 minutes, cooled, and 4 ml of acetone was added. The samples were then covered with aluminium foil and kept in the darkness at a temperature of 20°C for 16 hours. The pH of the samples was then raised to 9 by titrating with NH_4OH. From this point onwards the extraction procedure was the same as before (2.2.6.2.). Adoption of this modified procedure obviated the necessity for the laborious homogenisation step included previously. The efficiency of extraction achieved was similar to that obtained with the previous procedure.

Results: The application of the growth substances did not significantly affect the total height of the plants. However, the tassels of the plants which were treated with ABA were significantly shorter than those of the control plants; those treated with

SADH were the same as the control plants (Table 3.4.5.). Application of either ABA or SADH did not affect the size of the uppermost axillary shoot (node 7) but lower axillary inflorescences (nodes 4, 5 and 6) of plants treated with ABA or SADH were significantly longer than those of the control with the exception of the inflorescence at node 4 in plants which were treated with SADH. SADH also increased the breadth of the inflorescences at nodes 4 and 5.

Although the effects on the floral development of the axillary buds followed closely the effects on the growth of the axillary shoot, these were not statistically significant (Table 3.4.6.). However, the application of either ABA or SADH caused enhancement of the growth and development of the lower axillary inflorescences and SADH promoted the growth of an increased number of ears as reported for the cultivar Golden Bantam (Jaffe et al. 1963, cited by Cathey 1965).

Seven days after application of ABA the concentration of free ABA in the tassels of the treated plants was significantly higher than that in the tassels of the control plants (Table 3.4.7.) On the second day after ABA application, the amount of free ABA in the tassels of the treated plants was intermediate between the treated and control plants at seven days. No untreated plants were sampled on day 2, however, so this value cannot be commented on further.

TABLE 3.4.5.

The Influence of ABA and SADH on the Growth of Corn and its Reproductive Organs

Treatment	Total Height (cm)	Tassel Length (cm)	Length x Breadth (mm) 3 Weeks After Application of the Growth Substances, of the Axillary Shoots at Nodes: 1	2	3	4	5	6	7
Control	131	26	Abort.	Abort.	35 x 7	65 x 8	92 x 11	138 x 18	233 x 35
Applied ABA	122	22	Abort.	Abort.	64 x 7	109 x 8	133 x 12	175 x 20	248 x 37
Applied SADH	128	25	Abort.	Abort.	54 x 7	78 x 9	127 x 13	166 x 19	237 x 35
L.S.D. (P = 0.05)	N.S.	2			19 x -	31 x 1	26 x 2	27 x 3	N.S.

TABLE 3.4.6.

The Influence of ABA and SADH on the Development of Axillary Buds

Treatment	Stage of Development (Table 3.1.1.) 3 Weeks After Application of Growth Substances of Buds at Nodes:						
	1	2	3	4	5	6	7
Control	Abort.	Abort.	2	4	6	8	11
Applied ABA	Abort.	Abort.	3	5	7	9	12
Applied SADH	Abort.	Abort.	2	4	7	10	11

TABLE 3.4.7.

The Influence of Exogenously Applied ABA and SADH on the Concentration of Endogenous Free ABA in the Tassel and Leaf

Treatment	Tassel (ng/g fresh wt.)	Leaf (ng/g fresh wt.)
Control (Day 7)	92	200
Applied ABA (2 Days After Application)	121	-
Applied ABA (7 Days After Application)	176	-
Applied SADH	105	350
L.S.D. (P = 0.05)	35	80

TABLE 3.4.8.

The Influence of Exogenously Applied ABA and SADH on the Concentration of "Bound" ABA in the Tassel and Leaf

Treatment	Tassel (ng/g fresh wt.)	Leaf (ng/g fresh wt.)
Control	85	52
Applied ABA (2 Days After Application)	132	-
Applied ABA (7 Days After Application)	104	-
Applied SADH	33	99
L.S.D. (P = 0.05)	51	42

The concentration of free ABA in the tassels of plants which had been treated with SADH was not different from that in the tassels of the control plants, though the concentration of free ABA in the leaves of those treated plants was considerably higher than that in the leaves of the control plants. With the exception of the tassels of plants treated with SADH, the content of "bound" ABA increased wherever the free ABA concentration rose following ABA or SADH application (Table 3.4.8.).

3.4.4. An Investigation of the Relationship Between the Concentration of Free ABA in the Tassel and That in the Axillary Inflorescence at Nodes 5 and 6

Introduction: In Experiment 3.4.1., it was found that the organs which accumulated ABA rapidly, and to the greatest degree, following a period of water deficit were the tassel and the axillary inflorescences at nodes 5 and 6 which are the inflorescences most commonly responding to water stress by a subsequent promotion of growth. The accumulation of ABA occurred most rapidly in the tassel and there was a lag of approximately 2 days before a comparable rate of accumulation occurred in other organs. As removal of the tassel promotes the growth of the lower inflorescences and the effect of water stress on axillary inflorescence growth appears to be mediated through the tassel, it was decided to ascertain whether tassel removal would also influence ABA accumulation in these axillary inflorescences during water stress.

Method: Plants were raised as described before and were watered daily to run-off till 25 days after germination. The tassels of six plants were then excised and the detasselled plants were water stressed. At the same time six whole plants were water stressed. All plants had been water stressed for 7 days when the axillary inflorescence at nodes 5 and 6 were harvested and stored. Although the water potentials of these plants were not measured, both the detasselled and the intact plants showed signs of stress (as defined under 2.2.2.) at the same time. After a week of storage, the axillary inflorescences from both treatments were extracted, purified and the free ABA content was measured as described in the previous experiment.

Results: The free ABA in the axillary inflorescences at nodes 5 and 6 of whole plants exposed to a period of water deficit was significantly higher than that of the same inflorescences from plants whose tassels had been excised before the period of water stress (Table 3.4.9.).

3.4.5. A Test of the Proposed Role of the Tassel and/or ABA in the Control of the Growth of the Lower Axillary Inflorescences of Plants Exposed to a Period of Water Deficit

Introduction: The tassel has been demonstrated to be the main source of dominance over the lower axillary inflorescences and

TABLE 3.4.9.

The Effect of Detasselling on the Concentration of Endogenous Free ABA in the Axillary Inflorescences at Nodes 5 and 6 of Plants Exposed to Water Deficit

Treatment	ng ABA/g fresh wt.
Intact water-stressed plants	844
Detasselled water-stressed plants	457
L.S.D. (P = 0.05)	311

it has also been shown that an episode of water deficit inhibits this dominance effect. Excision of the tassel or subjecting plants to an episode of water stress results in enhanced growth of the lower axillary inflorescences. Removing the tassel and stressing the same plant simultaneously, however, does not result in any further enhanced growth of these inflorescences when compared with plants which have either been detasselled or stressed. This led to the conclusion that the effect of water stress was mediated through the tassel, but whether the tassel response occurred during or following the episode of water stress was unknown as detasselling was always performed at the beginning of the period of stress. In order to understand more clearly the dominance effect of the tassel and its interaction with an episode of water deficit it was decided to investigate the effect of detasselling plants at the end or at the beginning of a period of water deficit.

Application of ABA to intact plants mimicks the effect of an episode of water deficit. It has also been demonstrated that an episode of water deficit causes an increase in the concentration of endogenous ABA throughout the plant. This has led to the suggestion that the stimulation of the growth of the lower axillary inflorescences of plants which have been exposed to a period of water deficit is probably caused by the effect of the increased ABA on the metabolic processes of the tassel which causes a breakdown of the dominance exerted by the tassel. If this hypothesis is

correct, then simultaneous excision of the tassel and application of ABA should not result in any further enhancement of axillary inflorescence growth above that caused by the application of ABA or by detasselling the plant. On the other hand, combination of ABA treatment followed by excision of the tassel could result in an enhancement of the growth of those inflorescences as compared with application of ABA to intact plants. This was also investigated.

Method: Plants were raised as described previously and were watered to run-off daily until 27 days after germination. 18 plants were then subjected to water stress and six of these were detasselled at this time (T_5). 600 µl of ($\pm$) ABA at a concentration of 264 µg/ml was applied to each of another set of 18 plants and 6 of these were also detasselled (T_8). 600 µl of double distilled water was threaded to 6 other plants (T_1) and these and all the ABA treated plants as well as yet another set of 6 plants which were detasselled on that day (T_2), were watered to run-off daily. The plants subjected to water stress were not watered for 8 days and at the day of re-watering, 6 of the stressed plants, 6 of the ABA treated plants, and 6 plants that were watered throughout, were also detasselled. These are referred to as T_6, T_9 and T_3 respectively. All the plants were then watered daily to run-off. The intact plants which were stressed are referred to as T_4 and those to which ABA was added were also T_7. Three weeks after re-watering, the sizes of the axillary shoots were measured and the

developmental stages of the axillary buds were assessed. The experimental design is summarised as follows:

Treatment No.	Stress	ABA Applied at Day 27	Tassel
T_1	No	No	Present
T_2	No	No	Excised at day 27
T_3	No	No	Excised at day 35
T_4	Yes	No	Present
T_5	Yes	No	Excised at day 27
T_6	Yes	No	Excised at day 35
T_7	No	Yes	Present
T_8	No	Yes	Excised at day 27
T_9	No	Yes	Excised at day 35

Results: A period of water deficit and application of ABA caused a significant reduction in growth in length of the tassel. None of the treatments had any significant effect on the growth of the uppermost axillary inflorescence (Table 3.4.10.). As expected, all the treatments resulted in a significant enhancement of the growth of the lower axillary inflorescences when compared with the intact watered plants.

If one compares the intact plants that were watered throughout (T_1 and T_7), with those plants that were detasselled 27 days after germination (T_2, T_5, T_8), and those that were detasselled 35

TABLE 3.4.10.

The Influence of Detasselling, Application of ABA and Water Deficit on the Growth of Axillary Inflorescences

No.	Treatment		Tassel	Tassel Length (cm)	Length x Breadth (mm) 3 Weeks After Release of Stress of Shoots at Nodes						
	Stress	ABA Applied at Day 27			1	2	3	4	5	6	7
T_1	No	No	Present	28	Abort.	Abort.	42 x 6	72 x 9	98 x 12	147 x 20	233 x 36
T_2	No	No	Excised at day 27	-	Abort.	Abort.	69 x 8	133 x 10	160 x 14	195 x 24	254 x 40
T_3	No	No	Excised at day 35	-	Abort.	Abort.	40 x 7	100 x 10	155 x 13	186 x 22	254 x 44
T_4	Yes	No	Present	23	Abort.	Abort.	60 x 7	112 x 13	130 x 13	175 x 21	235 x 37
T_5	Yes	No	Excised at day 27	-	Abort.	Abort.	43 x 7	85 x 10	108 x 13	157 x 20	233 x 40
T_6	Yes	No	Excised at day 35	-	Abort.	Abort.	26 x 6	55 x 9	113 x 12	168 x 23	252 x 39
T_7	No	Yes	Present	25	Abort.	Abort.	48 x 6	79 x 8	139 x 12	166 x 17	238 x 40
T_8	No	Yes	Excised at day 27	-	Abort.	Abort.	75 x 7	123 x 11	153 x 15	178 x 24	245 x 43
T_9	No	Yes	Excised at day 35	-	Abort.	Abort.	102 x 9	120 x 14	161 x 16	190 x 22	238 x 43
L.S.D. (P = 0.05)				2			10 x 1	14 x 1	13 x 1	8 x 1	N.S.

days after germination (T_3, T_6, T_9), one can conclude that removal of the tassel earlier causes more growth of the axillary inflorescences at nodes 3 and 4 compared with later removal but there is no further effect on the enhancement of growth of the axillary inflorescences at nodes 5 and 6.

A period of water deficit alone does not have as great an effect as removing the tassel on day 27 but water deficit alone has a larger effect on the growth of the axillary inflorescence at node 3 than detasselling on day 35.

A comparison between detasselling and water deficit also shows that detasselling, either on day 27 at the beginning of stress, or day 35 at the end of stress, reduces the response to water deficit. Similarly the combination of detasselling and water deficit always reduces the response to detasselling alone and this is consistent with a previous finding (Tables 3.2.3. and 3.2.7.).

Thus it appears that the growth of axillary inflorescences at nodes 3 and 4 is probably sensitive to a period of water deficit earlier than the growth of those at nodes 5 and 6. The effect of water deficit on the tassel during the period of stress probably results in a disturbance of the tassel control of the growth of axillary inflorescences after re-watering. Water deficit itself appears to inhibit axillary inflorescence growth.

It has again been demonstrated that application of ABA to intact plants significantly increases the growth of the lower

axillary inflorescences. Nevertheless the effect of ABA alone was not as great as detasselling at either period.

Comparison between plants that were detasselled on day 27 and to which ABA was applied (T_8), with those similarly treated but to which no ABA was applied (T_2), shows that the axillary inflorescences of the ABA treated and detasselled plants were smaller than those that were detasselled alone. Thus it appears that ABA inhibits the growth of the axillary inflorescences when it is acting directly. Nevertheless, the axillary inflorescence of the ABA treated plants which were detasselled on day 35 (T_9) were all larger than those untreated plants that were detasselled on day 35 (T_3), this being significant at nodes 3 and 4. Between days 27 and 35, ABA appears to have reduced the tassel effect, and in particular promoted the growth of axillary inflorescences at nodes 3 and 4. This is consistent with the deduction that the growth of axillary inflorescences at nodes 3 and 4 is sensitive earlier than the growth of those at nodes 5 and 6. The effect of the various treatments on development parallelled that of growth as described above (Table 3.4.11.).

This data support the view that the ABA effect in promoting the growth of axillary inflorescences is mediated through its effect on the tassel and is not a direct effect on stimulation of the growth of the axillary inflorescences themselves. Indeed, ABA appears to have a direct inhibitory effect on the growth of axillary inflorescences

TABLE 3.4.11.

The Influence of Detasselling, Application of ABA and Water Deficit on the Development of Axillary Inflorescences

No.	Treatment: Stress	Treatment: ABA Applied at Day 27	Tassel	Stage of Development (Table 3.1.1.) 3 Weeks After Release of Stress of Buds at Nodes: 1	2	3	4	5	6	7
T_1	No	No	Present	Abort.	Abort.	2	4_a	7	9	11
T_2	No	No	Excised at day 27	Abort.	Abort.	4	6_{ab}	7	10	11
T_3	No	No	Excised at day 35	Abort.	Abort.	3	5_{ab}	7	10	11
T_4	Yes	No	Present	Abort.	Abort.	3	5_{ab}	7	10	11
T_5	Yes	No	Excised at day 27	Abort.	Abort.	3	4_a	8	10	11
T_6	Yes	No	Excised at day 35	Abort.	Abort.	2	3_a	6	10	11
T_7	No	Yes	Present	Abort.	Abort.	3	4_a	7	9	11
T_8	No	Yes	Excised at day 27	Abort.	Abort.	4	6_{ab}	8	10	11
T_9	No	Yes	Excised at day 35	Abort.	Abort.	4	7_b	8	10	11

Figures with similar letters in each column are not significantly different. Statistical analysis based on Kruskal-Wallis One Way Analysis of Variance.

3.4.6. DISCUSSION

It was concluded from the results of the application of abscisic acid to intact, watered plants, that the promotion of lower axillary inflorescence growth produced by an episode of water stress was probably due to the increase in endogenous ABA concentration within the plant during water stress. Furthermore, it was argued that the action of ABA in this response is probably indirect and mediated through an effect of the inhibitor on the growth of the tassel. In order to test these hypotheses, information was required on the concentration of endogenous ABA in the plant and its distribution during water stress and on the response of the plant to applied ABA in the presence or absence of the tassel.

It was indeed found that the endogenous abscisic acid content of the plant rose rapidly and extensively upon the induction of water stress. The increase in concentration of ABA caused by exposure of plants to a period of water deficit has been reported in other plants. A large increase in concentration of ABA was reported in wheat leaves (Wright 1969; Wright and Hiron 1969), sugar cane plants (Most 1971), Xanthium (Zeevart 1971) and Zea mays leaves (Gilles et al. 1974; Larque-Saavedra and Wain 1974); these authors report increases of up to forty-fold. In the present work the maximum increases realised were up to ten-fold. The increase in the concentration of ABA appears to depend on the water potential of the plant. It has been reported that in

Ambrosia artemisifolia and A. trifida there is a critical water potential of range -10 atms to -12 atms at which the accumulation of ABA starts, suggesting a threshhold water potential that stimulates ABA synthesis (Zabadal 1974). The data for Zea leaves (Fig. 3.4.2.) is not inconsistent with this generalisation.

In view of the increase in the ABA content of the axillary inflorescences at nodes 5 and 6 following water stress, the question as to whether ABA acts directly or indirectly through the tassel on the growth of these inflorescences needs to be reviewed. In all experiments it was found that the increase in the concentration of ABA was greatest in these axillary inflorescences and these are the structures whose growth is usually most stimulated by such a period of water deficit. It might be suggested that the increased concentration of ABA in the inflorescences could act directly to stimulate their growth. Such a direct effect of ABA in stimulating growth seems to be at variance with most of the reported effects of ABA. Application of ABA to plants is known to cause cessation of bud growth in Betula pubescens (Eagles and Wareing 1964), cessation of extension growth in Betula pubescens and Acer pseudoplatanus (El-Antably et al. 1967; van Overbeek et al. 1968) and the formation of resting organs in Betula pubescens, Acer pseudoplatanus and Ribes nigrum (El-Antably et al. 1966; van Overbeek et al. 1969). However, a few reports have also shown stimulatory effects of the application of ABA on growth processes. For instance, the application of ($\pm$) ABA to Begonia leaves stimulated adventitious

bud formation (Heide 1968), brought about parthenocarpic fruit set in Rosa sherardii (Jackson and Blundell 1966), promoted growth of grape berries (Hale and Coombe 1974), and resulted in a ten-fold stimulation of rice mesocotyl growth in the darkness (Milborrow 1974). It is conceivable, therefore, that the presence of a high concentration of ABA in the axillary inflorescences per se might stimulate their growth.

The responses of plants to ABA applied in the presence or absence of the tassel is not consistent with this explanation, however (Table 3.4.10.). When plants from which the tassels were removed are compared with those from which the tassels were removed on the same day but were also treated with ABA it is apparent that the applied ABA slightly inhibits the growth of the lower axillary inflorescences; there is no evidence for direct promotion. The more plausible explanation is that the water deficit leads to a high concentration of ABA which affects the growth and metabolism of the tassel resulting indirectly in the enhanced growth of the lower axillary inflorescences. Water stress caused an increase in the concentration of endogenous ABA in the tassel and also a permanent reduction in the growth of that organ, whilst all other aerial organs regained their normal growth rate following re-watering. Moreover, the application of ABA to watered plants, which also caused enhanced growth of the lower axillary inflorescences, also inhibited the growth of the tassels (Tables 3.4.5. and 3.4.10.). It would appear, therefore, that an increase in

the concentration of ABA in the plant does appear to affect the growth of the tassel. Since it has been demonstrated that the tassel is the main source of correlative inhibition of the lower axillary inflorescences, it appears that the mode of action of ABA might be through this inhibition of growth of the tassel. High concentrations of ABA are known to affect some metabolic processes. These include effects on RNA synthesis as well as subsequent effects on auxin and gibberellin production (Section 3.3.7.). Thus ABA might cause a release of the lower axillary inflorescence through its effect on the metabolic processes of the tassel. These conclusions are supported by the finding that the effect of applied ABA on axillary inflorescence growth is dependent on the presence of the tassels (Table 3.4.10.).

It is consistent with this hypothesis that when plants were exposed to water stress, the most rapid increase in endogenous ABA concentration occurred in the tassel (Fig. 3.4.1.). The concentration in this organ increased ten-fold over that in watered plants within 2 days of inducing water stress when the leaf water potential was only -7.7 bars. Similar concentrations in other organs were reached after a further 2 days stress. With this in mind, it is of considerable significance that the effect of water stress on axillary inflorescence growth was almost as great when plants were re-watered after 2 days exposure to stress as when they were re-watered after 8 days stress (Table 3.4.3.). This suggests

strongly that it is the action of ABA in the tassel which is important in determining the growth of the axillary branches and that the ABA concentration in other organs, including the axillary inflorescences themselves is of much less relevance. It has indeed been suggested (Experiment 3.4.5.) that an increase in the ABA concentration of the axillary inflorescences probably inhibits their growth. This need not be inconsistent with the finding that water stress leads to a promotion of the growth of these organs. It has been shown firstly that during the actual period of stress, the growth of all organs is inhibited (Section 3.1.); secondly it has been shown that the effect of tassel on the growth of the inflorescences at nodes 5 and 6 occurs after the period of water stress. It is known from other systems that re-watering leads to a rapid drop in endogenous ABA content (Hiron and Wright 1973) and it is suggested that this also occurs in corn, causing no lasting inhibition in the axillary inflorescences but a persistent effect in the tassel.

The source of the ABA accumulated in the tassel and elsewhere is unknown. The ABA may be synthesised *in situ* in the tassel, or translocated thence from the leaves which are usually considered the primary site of synthesis (Railton *et al.* 1974). In this connection it is interesting that the endogenous ABA concentration rose more rapidly upon stress in the tassel than in the tested leaf, although the younger leaves may have shown a different response. The effect of removing the tassel on the concentration

of ABA in the axillary inflorescences strongly suggests, however, that the tassel has a role in determining either the synthesis or distribution of ABA during water stress.

The hypothesis that ABA is concerned in the control of axillary growth is strengthened by the finding that applied SADH caused an increase in ABA concentration in the leaves of watered plants together with a subsequent enlargement of the lower axillary inflorescences. Although the concentration of ABA measured in the tassel seven days after application of SADH was not significantly higher than the concentration in the tassel of the control plants, that in the leaves was higher. It might be that the synthesised ABA was translocated to the tassel later than seven days after application. It must be mentioned that even when (±) ABA was directly applied to the plants it was not until seven days that its concentration in the tassel became significantly higher than that in the tassel of the control plants. The synthesis of ABA in plants to which a growth retardant has been applied is not without precedent, since Considine and Coombe (1972) showed that application of CCC caused a net increase in the endogenous ABA concentration in berries.

4. GENERAL DISCUSSION

The growth pattern of a well watered plant of the cultivar IO Chief of Zea mays is similar to the single-eared corn cultivar described by Sass (1960), Collins (1963) and Collins and Russell (1965). In this cultivar there are usually seven axillary inflorescences initiated but only the uppermost (node 7) develops to form a mature cob. A period of water deficit imposed during initiation and early growth of the terminal male inflorescence (the tassel), which occurs approximately 20 to 40 days after germination, results in a permanent inhibition of tassel growth and enhanced growth of the lower axillary inflorescences such that the axillary inflorescence at node 6, and occasionally at node 5 also, develops to form a cob. Water deficit imposed at any other phase does not result in this response. It is difficult to compare this response with other reports of the effects of water deficit on other cultivars of Zea mays (Denmead and Shaw 1960; Moss and Downey 1971) because in those studies water deficit was generally imposed at a later stage in the growth of the plant. As the effect of the water deficit depends largely upon the phase of growth at which it occurs comparisons between cultivars which differ in rates of development is also hazardous. For instance, in the unnamed cultivar used by Hanway (1963) there were 126 days between germination and physiological maturity while commencement of grain denting

occurred at day 102 after germination; with IO Chief, however, these events occur at 76 and 65 days respectively (Table 3.1.1.). It has been reported that a period of water deficit to the permanent wilting stage imposed on corn before maturity, at approximately 30 days after germination, had the major effect of reducing the size of the cobs harvested (Denmead and Shaw 1960). As no increase in cob numbers was reported, it may be surmised that this period of water stress was imposed at a phase of growth other than that investigated here. Although there do not appear to be any other reports of promotion of axillary inflorescence growth in corn by a water deficit, there have been several reports of a comparable effect on apical dominance in other plants. For instance, in barley a period of water deficit imposed during vegetative development induced more tiller buds to elongate following re-watering, the tendency being greater the earlier the stress was applied (Aspinall et al. 1964).

This phenomenon in corn is also seen essentially as a reduction in the correlative inhibition within the plant. The main source of this inhibition appears to be the tassel. Since the tassel is the terminal meristem of the plant the inhibition which it imposes can be termed apical dominance and it is this apical dominance which is modified by a period of water deficit. Such a response has been reported previously: inhibition of the vegetative axillary buds in Pisum sativum is relieved by a period of water

deficit (McIntyre 1971). These examples, together with the response of vegetative barley plants described by Aspinall et al. (1964) suggest that this may be a phenomenon of general occurrence. The uppermost axillary inflorescence also exerts some control over the growth of the lower two axillary inflorescences (i.e. at nodes 5 and 6). This latter response resembles the correlative inhibition of lateral bud growth by another lateral bud reported for pea by Sachs (1966) and flax by McIntyre (1968).

By analogy with other systems of apical dominance (Jacob and Case 1965) it is most likely that the inhibition of axillary inflorescence growth exerted by the tassel is due to growth hormones transported over the six-node distance separating the tassel from the nearest lower axillary inflorescence. This hypothesis is supported by the inhibition of the growth of the lower axillary inflorescences of detasselled plants by either 2,4-D alone or mixed with GA (Tables 3.3.5. and 3.3.8.). These data suggest that the hormone involved is auxin. Further support for this hypothesis was obtained from the response of intact, watered plants to TIBA (Table 3.3.3.). TIBA is known to block auxin transport (Panigrahi and Audus 1961) and here it caused a stimulation of the growth of the lower axillary inflorescences. Auxin alone may not be the sole agent controlling the inhibition as GA alone or in combination with 2,4-D also inhibited axillary inflorescence growth. These deductions can only be made with caution. Firstly,

the concentrations of the various growth substances applied were selected from published reports (Section 3.3.1.) but could be either supraoptimal or suboptimal for this cultivar of Zea mays. Secondly, even if the concentrations applied were appropriate, the concentration at the site of action was unknown and may have been inappropriate. Thirdly, a hormone applied exogenously may be distributed within the cell or the plant in a different manner to the endogenous hormone. This could modify the response to the hormone, resulting in morphological changes due to the abnormal distribution of the hormone. With these qualifications in mind, it would appear that the basis for the present suggestion that auxin and gibberellin are involved in this particular example of apical dominance is not strong. Nevertheless, these were the only substances, out of a number tested, which duplicated the effect of the terminal male inflorescence. A similar restoration of apical dominance following the application of a mixture of IAA and GA to a decapitated Pisum plant has been demonstrated (Jacob and Case 1965; Scott et al. 1968).

Other growth substances, reported to be involved in the control of apical dominance, such as cytokinins (Wickson and Thimann 1958; Maltzahn 1959; Panigrahi and Audus 1964) and ethylene (Hall and Morgan 1963, cited by Burg and Burg 1968) did not appear to be involved in this response in the cultivar of corn used in this study. Moreover, the level of mineral nutrition also did

not appear to be directly involved in the control of apical dominance here, an increase in supply only causing a general growth stimulation. This is at variance with numerous reports of an involvement of mineral nutrition in apical dominance, such as in Pisum sativum (McIntyre 1964; Husain and Link 1966), Agropyron repens (McIntyre 1969), Solanum sisymbrifolium (Wakhloo 1970) and in barley (Aspinall 1961; Fletcher 1974).

The reduction in the dominance exerted by the tassel and stimulation of growth of the lower axillary inflorescences caused by a period of water deficit during tassel initiation was accompanied by permanent damage to the tassel. This permanent damage in itself could account for the breakdown of the dominance or alternatively the effect on auxin metabolism may be more direct. It has been shown that floating excised wheat leaves on mannitol of a water potential of approximately -10 bars for 6 hrs stimulated the activity of IAA-oxidase (Darbyshire 1971a). Thus water deficit imposed on the plants could result in a decrease in endogenous auxin concentration. Furthermore it has been suggested that water deficit reduces the translocation of auxin (Basler et al. 1969).

These effects of water deficit on auxin metabolism and growth of the tassel may be direct, in the sense that they are caused by a reduction in the water potential of the tassel itself, or may be due to other changes in the plant as a whole. An increase in the concentration of endogenous ABA in plants exposed to a period of

water deficit was found in this cultivar and there is a considerable increase in the concentration of endogenous ABA in wheat (Hiron and Wright 1969), sugar cane (Most 1969), Xanthium (Zeevart 1971) and maize (Gilles et al. 1974) amongst other plants. This was associated with the enhanced growth of the lower axillary inflorescences. Moreover, application of exogenous ABA to normally watered intact plants resulted in a stimulation of the growth of the lower axillary inflorescences, comparable to the effect of a water deficit. Further evidence suggesting a role for ABA in this phenomenon comes from the finding that SADH applied to normally watered plants also resulted in both an increase in the concentration of endogenous ABA and an enhancement of the growth of the lower axillary inflorescences. All this evidence supports the suggestion that an increase in the concentration of ABA in the plant is responsible for the stimulation of the growth of the lower axillary inflorescences.

It is possible, despite the evidence for a role of the tassel in this phenomenon, that the increased concentration of ABA in the plants stimulates the growth of the lower axillary inflorescences directly. Stimulatory effects of ABA have been reported (Jackson and Blundell 1968; Aspinall et al. 1967; Milborrow 1974; Heide 1968) but this would appear to be an unlikely explanation for the present phenomenon. The early increase in endogenous ABA concentration in the tassel upon water stress (Fig. 3.4.1., Experiment

3.4.2.), the effect on axillary growth of applying ABA to intact or detasselled plants (Table 3.4.10.) and the increase in the concentration of ABA in plants to which SADH was applied, all suggest that the effect of the increased concentration of ABA is through an inhibition of the growth of the tassel. This results eventually in a stimulation of the growth of the lower axillary inflorescences.

Such a response may occur in two possible ways, firstly ABA could indirectly affect IAA production in the tassel through a general inhibition of the growth of that organ. Exogenous ABA is known to have such an inhibitory effect on the growth of plants (Section 1.3.1.) although the role of endogenous ABA in growth control is in dispute (Milborrow 1974). Alternatively ABA could have a more direct effect on some important metabolic process within the tassel which affects IAA metabolism. The data from this project do not distinguish clearly between these two possible effects of ABA on the tassel. Application of ABA (Experiment 3.3.1., Table 3.3.1.) or of SADH (Table 3.4.5.) to intact plants caused an enhanced growth of the lower axillary inflorescences without reducing the length of the tassel. This suggests that a direct effect of the increased ABA concentration on hormone metabolism might be the more probable.

The evidence discussed is in general agreement with the hypothesis which has been advanced, but there are some significant

gaps in our knowledge of the system. The evidence on endogenous abscisic acid concentrations within the plant has been confined to the period during which the plant was exposed to a water deficit. Conclusions were drawn from the fact that the tassel content of ABA had risen markedly after only two days exposure to water stress, at which stage the effect displayed as an ultimate stimulation of axillary growth was already latent within the plant. In contrast, the endogenous ABA content did not rise as rapidly in other parts of the plant and in particular in the axillary inflorescences involved in the response. However, during the period when the plant is deprived of water, the growth of all organs is inhibited and the ABA content of all organs increases; it is only after the plant is re-watered that the inhibition of the growth of the tassel on the one hand and the stimulation of the growth of the axillary inflorescences on the other is displayed. It follows that if, as seems likely, endogenous ABA is involved in the response then either the sensitivity of the two types of meristem to the hormone is different or the elevated ABA concentration persists in the tassel but not in the axillary inflorescences following re-watering. A knowledge of the ABA content of these meristems in the period immediately following water stress would assist in resolving this difficulty.

A further point of interest is the finding that the endogenous ABA concentration in the axillary inflorescences of detasselled stressed plants was less than that in intact stressed plants.

This factor is also difficult to accommodate in the overall hypothesis but may simply suggest either that the tassel exerts control over the translocation of ABA within the plant or that the change in axillary metabolism following removal of the dominant tassel does not favour ABA accumulation in that organ. Again, in this case, further information on ABA concentrations in other organs and in water non-stressed detasselled plants would be useful.

Although these experiments were conducted throughout with plants grown in pots in a glasshouse, some of the findings may be applied to the field situation. The described response of the plants to a period of water deficit imposed at silking supplements other reports of the response of Zea mays at this phase of growth (Denmead and Shaw 1960; Barnes and Woolley 1969; Moss and Downey 1971). It was found that a period of water deficit at this stage resulted in poor grain filling but not in significantly smaller cobs as reported by Denmead and Shaw (1960). The critical period where water deficit causes the greatest reduction in yield in this cultivar, as in others, undoubtedly is silking (see Section 3.1.4.).

Water deficit imposed at the period of initiation and early growth of the tassel resulted in the production of more cobs on the plant and an increase in the yield of these pot-grown plants. Caution must be exercised in applying this to field conditions, however, as it is difficult to compare the degree of water deficit attained in this project with those attained in the field.

Furthermore, there was a drastic effect on the growth of the tassels of the stressed plants (Fig. 3.1.2.) and the lateral branches on these tassels did not bear any florets. This suggests that pollen production by these plants would be severely limited. It has been reported that water deficit imposed on wheat at this period of growth resulted in sterile pollen (Anikiev 1960; Bingham 1966) which may also have been the case with corn. There was comparable seed set in the stressed and watered plants but this may have been due to the random distribution of watered and stressed plants where pollen from watered plants would have compensated for the lack of pollen from stressed plants. It is known that in Zea mays about 95% of ovules in a shoot are cross pollinated (Poehlman and Borthakur 1968). In the field, water stress at tassel initiation, although potentially increasing the number of cobs, could drastically reduce total yield due to the effect on the tassel.

Possibly the most important conclusion to be drawn from this project is that in maize, as in other cereals (Aspinall et al. 1964), the morphogenetic response of the plant to a period of water stress varies dramatically with the phase of development at which stress occurs. Consequently the evaluation of responses to stress in the field and of many previous reports of effects of stress on corn (Denmead and Shaw 1960; Moss and Downey 1971) is difficult without an appreciation of this basic fact.

APPENDIX

A.1. THE EFFECT OF WATER DEFICIT ON AMINO ACID CONTENT

Introduction: One of the most consistent effects of water deficit on plant metabolism is a pronounced accumulation of free amino acids. In particular, there is frequently a large increase in the concentration of free proline in many plants (Kemble and MacPherson 1954; Mothes 1956; Praskova 1960; Chen *et al.* 1964; Barnett and Naylor 1966; Singh *et al.* 1972). This accumulation of free proline may be associated with the increase in endogenous ABA concentration during water stress as it has been demonstrated that spraying ABA onto intact barley plants leads to an accumulation of free proline (Aspinall *et al.* 1972). As it has been shown that the concentration of endogenous ABA also increases in corn plants during water stress (Gilles *et al.* 1974, and present study), it was decided to find if there was also an accumulation of proline.

Method: Plants were raised as described (2.2.6.2.) and were watered to run-off until 21 days after germination. Half of the number of plants were then subjected to water stress and the other half were watered daily to run-off. The plants were water stressed for 10 days when the third leaves of both the water stressed plants and the watered plants were harvested for amino acid analysis. The procedure for harvesting was as follows: About 50 leaf discs

were cut with a cork borer of 1.2 cm diameter from the third leaf of each plant. The discs were divided into two groups which were quickly weighed. Each group weighed approximately 500 mg. One sample was frozen in liquid nitrogen and stored at -20°C. The other sample was put in an oven at 60°C for 48 hours and the dry weight calculated. The amino acid analyses of the stored sample were carried out after 3 days' storage. One sample each of the control and the stressed were handled during each assay. In all, 4 samples from each treatment were assayed and the results pooled. The procedure for the amino acid analysis was slightly modified from that of Hanworth and Heathcote (1969).

Washing of Cellulose Powder: The cellulose powder used was MN300. The powder was washed by slurrying 50 g of powder with 100 ml 80% distilled ethanol. The slurry was then poured into a Büchner funnel with a filter paper at the bottom and was filtered under reduced pressure. It was then washed with 50 ml of water. This was followed sequentially with the following solutions:

1. 300 ml solution comprising 180 ml isopropanol, 60 ml acetic acid and 60 ml water.
2. 200 ml of 25% distilled methanol.
3. 200 ml solution of 120 ml methanol and 80 ml HCl.
4. 200 ml distilled deionised water
5. 200 ml methanol.

The powder was then dried under vacuum for an hour, transferred to an oven and dried overnight at 60°C.

Preparation of Cellulose Layer Plates: 100 cc of water was slowly added to 17 g of washed cellulose powder and stirred slowly; this crude paste was then homogenised for 1 minute to form a slurry. 20 cm x 20 cm plates were poured using a Shandon Chromatogram spreader with slit width of 400 μ and dried overnight before use.

Preparation of Ion Exchange Resin: The resin used was Dowex 50W-8H (mesh size of 200 to 400). The heavy particles from the commercial resin were removed by suspending the resin in 2 volumes of distilled deionised water and stirring and decanting the water immediately. The resin was then suspended in 2 volumes of 2 N HCl. This was then heated to 100°C and allowed to cool for 30 min. The yellow supernatant liquid was decanted and the procedure was repeated until the resulting supernatant liquid was clear. The resin was heated to 80°C and washed once with ethanol and poured into a large glass column. To ensure the resin was in the H^+ form, 2 litres of 2 N HCl was run through the column. The resin was then washed with distilled deionised water until the pH of the eluate was neutral. It was then poured into a 500 ml conical flask and stored under a layer of distilled deionised water in the refrigerator until use.

Preparation of the Ion Exchange Resin Column: A glass column of internal diameter 1 cm x 13 cm with a basal sintered glass disc was filled with water. Glass wool was placed on top of the disc and the resin was poured into the column as a thick slurry. The column was then packed with a slight air pressure to give a column of 7 cm and the upper liquid was decanted. The walls of the column were washed with distilled deionised water to remove adhering resin. A layer of distilled deionised water was maintained above the resin at all times and the column was stoppered.

Extraction, Separation and Measurement

Extraction: A sample of approximately 0.5 g of leaves was weighed and homogenised with 10 ml methanol:chloroform:water (12:5:3 v/v) in a Douall conical glass homogeniser at room temperature. This was centrifuged and the supernatant collected. The residue was then shaken with a further 5 ml MCW, centrifuged and the supernatants pooled. To the pooled supernatant 5 ml chloroform and 7.5 ml water were added. The resulting two phase mixture was centrifuged and the top layer collected. This layer was evaporated to dryness in a rotary evaporator and the dry extract was then dissolved in 10 ml of distilled deionised water.

Separation: The plant extract was run through a Dowex W-8H$^+$ column. The non-adhering substances (neutral and acidic materials) were washed through the column with 50 ml distilled deionised water.

This was followed by 100 ml ethanol to remove lipids. The alcohol was then removed by washing with 25 ml distilled deionised water.

The acidic and neutral amino acids were eluted with 100 ml 2 N HCl and the basic amino acids were eluted with 10 ml 10 N HCl. The column was regenerated by washing with distilled deionised water until neutral.

The collected eluates were evaporated to dryness in a rotary evaporator. The dry extracts were then dissolved in 50 ml distilled deionised water and evaporated to dryness. This was repeated, usually twice, until no trace of HCl remained. The dry extract was then dissolved in 0.25 ml of distilled deionised water and spotted on a thin layer plate.

10 µl of the extract was spotted at a position 1.5 cm from the lower edge of the plate and 1.5 cm inside from the left hand edge of the plate using a 10 µl syringe and spotting 1 µl at a time. The spots were dried in a stream of warm air.

The plates were then run ascending, two-dimensionally. For the first dimension the plates were run with 100 ml of 2 propanol: butanone: 1 N HCl (60: 15: 25 v/v) for 2.5 hrs. They were then removed and dried in a stream of air for 15 mins and transferred to an oven with a temperature of 60°C for a further 15 min to remove the final traces of HCl.

For the second dimension, the plates were run at right angles to the first dimension with methyl propanol:butanone: propanone:methanol:water:ammonia (40:20:20:1:14:5 v/v) for 2.5 hrs. The solvents were then removed by drying the plates in an oven at 60°C for 15 mins.

The dry plates were dipped in ninhydrin cadmium acetate reagent and put in an oven at 60°C overnight to develop. The various amino acids were identified using plates of known amino acids which had been chromatographed as above.

Measurement: The plates were coated with a film of cellulose acetate solution comprising 6% cellulose acetate, 3% diethylene glycol and 2% camphor in acetone:propanol (3:1 v/v). A pool of cellulose acetate solution was poured onto one end of the plate and spread over the plate surface with a glass rod. After the cellulose acetate had dried completely at room temperature, each coloured spot was cut with a razor blade, lifted and placed in a 15 ml centrifuge tube with 3 ml eluting solvent comprising methanol:ethyl acetate:water (1:1:1 v/v) containing 1% acetic acid and 1% cadmium acetate.

The tubes were gently shaken for 10 mins using a glass marble as a stopper to prevent evaporation. They were then centrifuged at 1000 g for 5 mins and the optical densities of the coloured supernatant were measured at 505 mμ.

The quantity of each amino acid was calculated by reference to a standard curve drawn from known quantities of amino acids chromatographed as above.

Results and Conclusion: From Table A.1.1. it can be seen that the concentrations of most of the free amino acids in the stressed plants were higher than those in the control plants. The highest percentage increase in concentration of accumulated amino acids in stressed plants were glutamine, asparagine, leucine and serine in decreasing order. Proline increase due to water stress was found only to be two-fold. Tyrosine was the only amino acid which was found to decrease with an increase of water stress and isoleucine was not affected by water stress.

There was no apparent specific accumulation of proline in this cultivar of Zea mays although there was a general increase in the concentrations of most free amino acids. Evidently there is no universal relationship between an increase in the concentration of endogenous abscisic acid and an increase in the concentration of free proline.

TABLE A.1.1.

The Effect of Water Deficit on the Concentration of Amino Acids (μg/g dry wt.)

Amino Acid	Control	Stressed
Glycine	35	115**
Alanine	26	96**
Valine	4	19**
Leucine	4	29***
Isoleucine	4	5 N.S.
Proline	57	120**
Tyrosine	52	38
Serine	35	216***
Threonine	13	29*
Aspartic Acid	22	86**
Asparagine	4	38***
Glutamic Acid	109	134*
Glutamine	4	120***
Arginine	Trace	Trace
Phenylalanine	3	14**

*** Significant at 0.1% level of probability.

** Significant at 1% level of probability.

* Significant at 5% level of probability.

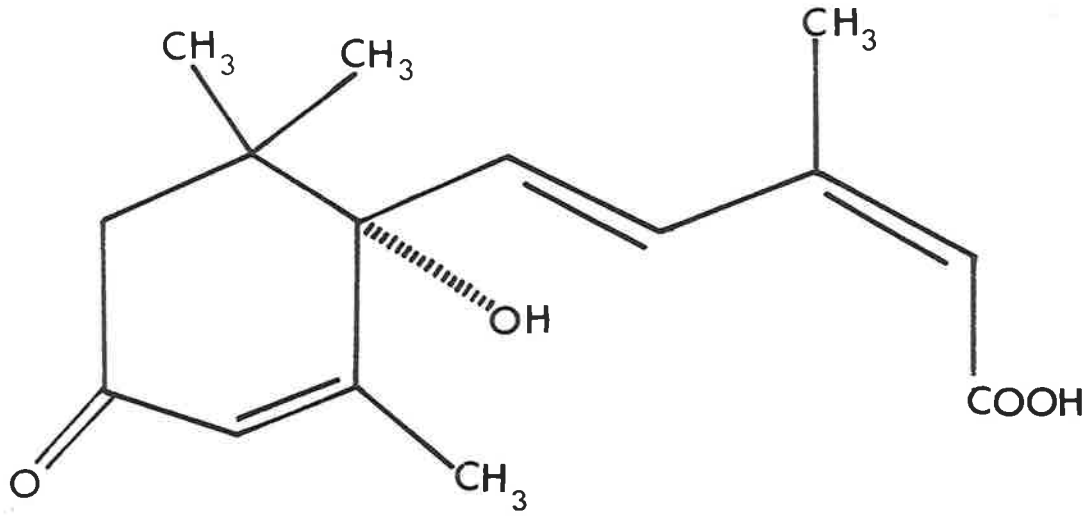
A2. DETECTION, EXTRACTION, PURIFICATION AND MEASUREMENT OF PHASEIC ACID IN CORN

Introduction: In the early measurements of endogenous free ABA it was realised that some unknown substance in the extract had the same retention time as trans-trans ABA. At that time the column packing was 3% OV 17 on silanised Gaschrom Q (100 to 200 mesh size), the temperature of the injection was 260°C, that of the column was 230°C and of the detector 280°C. An attempt was made to separate this unknown substance from trans-trans ABA by changing the temperatures in various ways but this was not successful. The unknown substance was suspected to be phaseic acid for two reasons. Firstly, it has a very similar molecular structure as ABA (Fig. A.2.1.) and secondly it is known that phaseic acid had the same retention time as trans-trans ABA under these conditions (Coombe, personal communication).

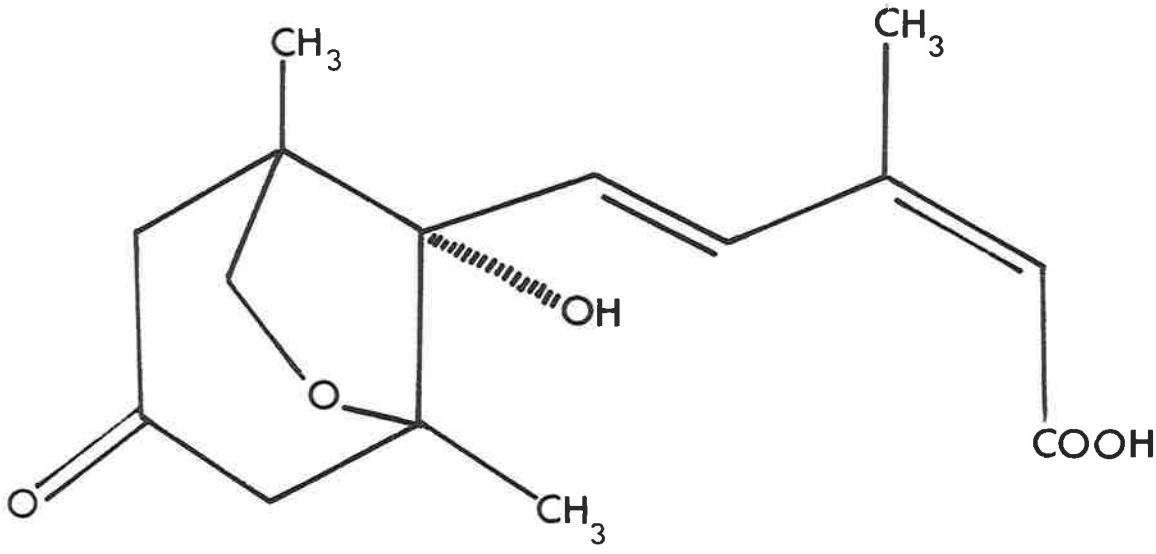
This was further explored by injecting samples containing the unknown substance alone or in mixture with ABA (from the standard) and phaseic acid (1:1 v/v) on to the column. From the GLC tracing it was observed that there was a marked increase in the peak associated with trans-trans ABA when the mixture was injected whilst the tracing of the ABA alone showed a lower peak of the trans-trans ABA. The peaks associated with cis-trans ABA were identical when either the mixture or the ABA alone were injected on to the column. Furthermore, when phaseic acid alone was injected

FIG. A.2.1.

Molecular structures of (+) abscisic acid and phaseic acid



(+)- ABSCISIC ACID



PHASEIC ACID

on to the column its retention time was found to be the same as that of trans-trans ABA and the peak height at that retention time was smaller than when it was included in a mixture with ABA. It was concluded that the peak observed at the retention time for trans-trans ABA when the extract was injected on the column resulted from a mixture of trans-trans ABA and phaseic acid.

In order to separate these two compounds, a new column was prepared packed with SE 30 on Anachrom ABS (80 to 100 mesh size) according to McNair and Bonelli (1969). Various combinations of temperatures of the injector and column were tried but no separation of trans-trans ABA and phaseic acid was achieved. The column packing was then changed to 0.8% QF1 + 0.2% DC 200 on Varaport 30 (Coombe 1972) and the temperature of the column was maintained at 180°C, the injector at 240°C and the detector at 280°C. Except for the detector temperature which was recommended by the makers, the other two temperatures were arrived at empirically. In these circumstances, the peaks of the suspected phaseic acid and the trans-trans ABA were distinctly separated (Fig. A.2.2.). When the extract was then put through this system it was observed that the unknown substance co-chromatographed with phaseic acid.

In further experiments in which endogenous ABA was measured, phaseic acid was also assessed to see if the various treatments of the plant also had an effect on the concentration of phaseic acid.

FIG. A.2.2.

GLC tracing showing the separation of phaseic acid from trans-trans abscisic acid.

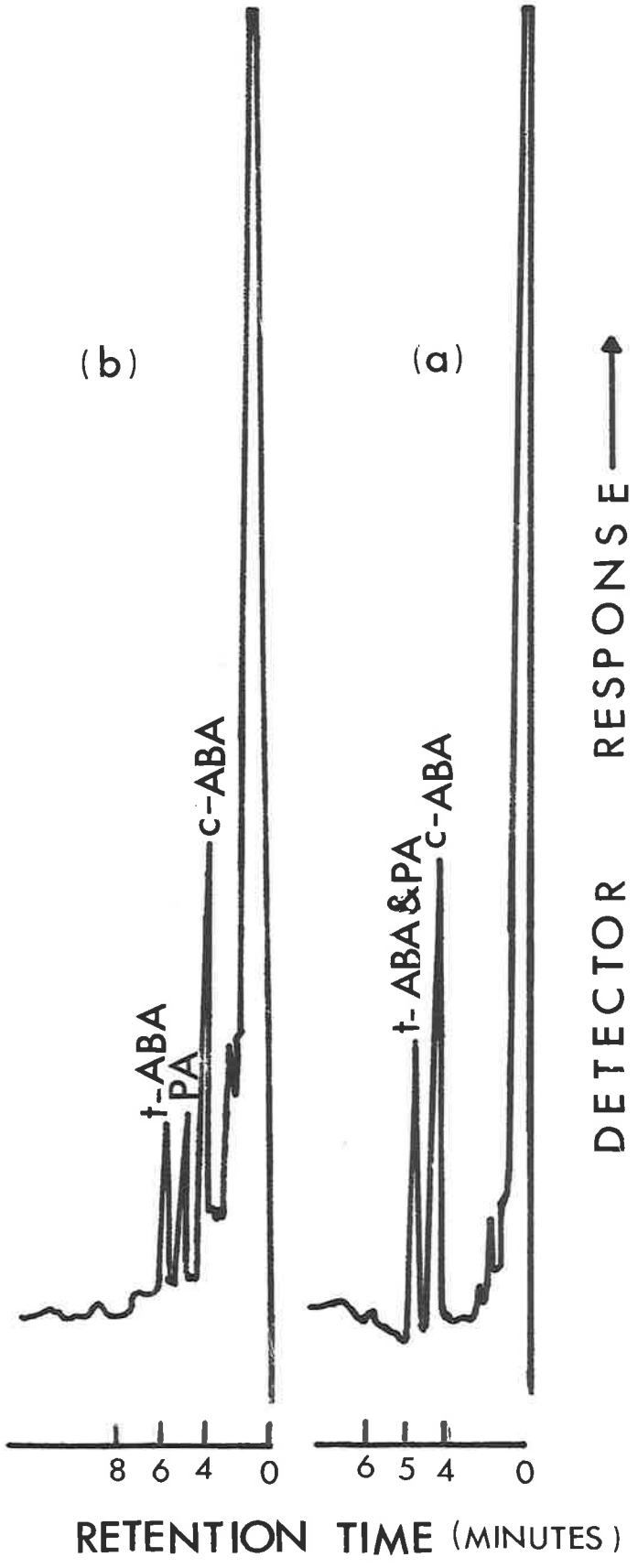
(a) A trace of the early measurements when column packing was 3% OV 17 on silanised Gaschrom Q (100 to 120 mesh).

Injector temperature:	260°C
Column temperature:	230°C
Detector temperature:	280°C

(b) A trace showing the separation of phaseic acid from trans-trans abscisic acid using column packing of 0.8% QF1 + 0.2% DC 200 on Varaport 30.

Injector temperature:	240°C
Column temperature:	180°C
Detector temperature:	280°C

c-ABA:	cis-trans abscisic acid
c-PA:	phaseic acid
t-ABA:	trans-trans abscisic acid


(b)
(a)
t-ABA
PA
c-ABA
t-ABA &PA
c-ABA
DETECTOR RESPONSE
8 6 4 0
6 5 4 0
RETENTION TIME (MINUTES)

Phaseic acid was measured on the same extracts used for determination of ABA in both free and bound forms. The concentration was also estimated in the same way. As insufficient authentic phaseic acid was available to produce a calibration curve, it was assumed that the molecule would have similar electron capture properties to ABA and the standard curve for ABA was used to quantify the phaseic acid. In each case, however, a single sample of phaseic acid was injected on the column as a check on the retention time. It was also assumed that the recovery percentage for phaseic acid was the same as that for ABA. These assumptions were based on the marked similarity of the two molecules (Fig. A.1.1.) but could not be tested directly.

Results and Conclusion: It was realised in Experiment 3.4.3. that the concentrations of phaseic acid in the extracts were so low as to be considered negligible. The results presented are therefore from Experiments 3.4.1. and 3.4.2. only.

Results from varying periods of water deficit on the concentration of free phaseic acid shows that there is an increase in the concentration of phaseic acid both in the control plants and in the stressed plants with time. In the axillary inflorescences of the stressed plants there also appears to be an increase in concentration at the end of the period of water deficit. As with ABA, the maximum increase in the concentration of phaseic acid was in the axillary inflorescences at nodes 5 and 6 of stressed plants.

It is of significance that the concentration of phaseic acid in both stressed and watered plants increased to the 6th day and then decreased in both plants by the 8th day. This is unlike bound ABA which increases with the increase in concentration of free ABA (see Figs. 3.4.1. to 3.4.5.).

The role of phaseic acid in plants is unknown but it is regarded as a natural metabolite of ABA (Milborrow 1974). If this is so and the phaseic acid measured here was derived entirely from ABA, the turnover and synthesis of ABA was more rapid than appears from the measured concentrations of ABA alone. Moreover, the results also support the conclusion that the maximum stress-induced increase in the concentration of ABA and related metabolites in the whole plant occurred in the axillary inflorescences at nodes 5 and 6.

TABLE A.2.1.

Effect of 8 Days of Water Deficit on the Concentration of Phaseic Acid in the Plant

Plant Organ	Free Phaseic Acid (ng/g fresh wt.)		L.S.D. (P = 0.05)
	Control	Water Deficit	
Tassel	52	68	N.S.
Leaf	48	56	N.S.
Axillary Inflorescence at Node 7	72	167	22
Axillary Inflorescences at Nodes 5 and 6	70	180	25
Axillary Inflorescences at Nodes 3 and 4	40	70	15

TABLE A.2.2.

Effect of Varying Periods of Water Deficit on the Concentration of Phaseic Acid in the Tassel

Duration of Water Deficit	Treatment	Free Phaseic Acid ng/g fresh wt.	Free Phaseic Acid L.S.D. P = 0.05	Bound Phaseic Acid ng/g fresh wt.	Bound Phaseic Acid L.S.D. P = 0.05
2 Days	Control	5	N.S.	Trace	
	Water Deficit	10		Trace	
4 Days	Control	2	-	4	N.S.
	Water Deficit	0.5		6	
6 Days	Control	16	12	16	N.S.
	Water Deficit	30		19	
8 Days	Control	48	N.S.	0.4	10
	Water Deficit	52		12	

TABLE A.2.3.

Effect of Varying Periods of Water Deficit on the Concentration of Phaseic Acid in the Leaf

Duration of Water Deficit	Treatment	Free Phaseic Acid ng/g fresh wt.	Free Phaseic Acid L.S.D. P = 0.05	Bound Phaseic Acid ng/g fresh wt.	Bound Phaseic Acid L.S.D. P = 0.05
2 Days	Control	2	N.S.	1	N.S.
	Water Deficit	3		1	
4 Days	Control	2	N.S.	25	N.S.
	Water Deficit	1		29	
6 Days	Control	14	N.S.	25	N.S.
	Water Deficit	19		38	
8 Days	Control	23	N.S.	14	N.S.
	Water Deficit	27		15	

TABLE A.2.4.

Effect of Varying Periods of Water Deficit on the Concentration of Phaseic Acid in the Axillary Inflorescence at Node 7

Duration of Water Deficit	Treatment	Free Phaseic Acid ng/g fresh wt.	Free Phaseic Acid L.S.D. P = 0.05	Bound Phaseic Acid ng/g fresh wt.	Bound Phaseic Acid L.S.D. P = 0.05
2 Days	Control	13	10	1	N.S.
	Water Deficit	1		2	
4 Days	Control	-	-	10	N.S.
	Water Deficit	-		10	
6 Days	Control	22	N.S.	3	15
	Water Deficit	32		30	
8 Days	Control	52	18	4	N.S.
	Water Deficit	159		7	

TABLE A.2.5.

Effect of Varying Periods of Water Deficit on the Concentration of Phaseic Acid in the Axillary Inflorescences at Nodes 5 and 6

Duration of Water Deficit	Treatment	Free Phaseic Acid ng/g fresh wt.	Free Phaseic Acid L.S.D. P = 0.05	Bound Phaseic Acid ng/g fresh wt.	Bound Phaseic Acid L.S.D. P = 0.05
2 Days	Control	15	12	4	N.S.
	Water Deficit	5		3	
4 Days	Control	-	-	10	N.S.
	Water Deficit	-		10	
6 Days	Control	36	N.S.	15	20
	Water Deficit	34		30	
8 Days	Control	40	23	5	N.S.
	Water Deficit	189		8	

TABLE A.2.6.

Effect of Varying Periods of Water Deficit on the Concentration of Phaseic Acid in the Axillary Inflorescences at Nodes 3 and 4

Duration of Water Deficit	Treatment	Free Phaseic Acid ng/g fresh wt.	Free Phaseic Acid L.S.D. P = 0.05	Bound Phaseic Acid ng/g fresh wt.	Bound Phaseic Acid L.S.D. P = 0.05
2 Days	Control	6	4	2	N.S.
	Water Deficit	1		2	
4 Days	Control	4	-	10	N.S.
	Water Deficit	Nil		15	
6 Days	Control	34	N.S.	23	30
	Water Deficit	32		57	
8 Days	Control	35	14	4	N.S.
	Water Deficit	87		3	

A.3. THE CONCENTRATIONS OF ENDOGENOUS FREE AND "BOUND" (CONJUGATE) ABA IN CONTROL PLANTS AND PLANTS EXPOSED TO VARYING PERIODS OF WATER DEFICIT

(See Figs. 3.4.1. to 3.4.5.)

TABLE A.3.1.

Effect of Varying Periods of Water Deficit on the Concentration of Endogenous Free and "Bound" (Conjugate) ABA in the Tassel

Duration of Water Deficit	Free ABA log ng/gm fresh wt.			"Bound" ABA log ng/gm fresh wt.		
	Control	Water Deficit	L.S.D. (P=0.05)	Control	Water Deficit	L.S.D. (P=0.05)
2 Days	1.63	2.71	0.28	0.70	1.48	0.34
4 Days	2.42	2.83	0.41	0.89	1.54	0.28
6 Days	2.46	2.76	0.22	1.63	2.30	0.28
8 Days	2.42	2.90	0.36	1.48	2.45	0.19

TABLE A.3.2.

Effect of Varying Periods of Water Deficit on the Concentration of Endogenous Free and "Bound" (Conjugate) ABA in the Leaf

Duration of Water Deficit	Free ABA log ng/gm fresh wt.			"Bound" ABA log ng/gm fresh wt.		
	Control	Water Deficit	L.S.D. (P=0.05)	Control	Water Deficit	L.S.D. (P=0.05)
2 Days	1.50	2.08	0.28	0.60	1.18	0.28
4 Days	1.68	2.75	0.53	1.00	1.54	0.28
6 Days	1.70	2.74	0.22	1.50	2.34	0.42
8 Days	1.89	2.84	0.72	1.23	2.50	0.22

TABLE A.3.3.

Effect of Varying Periods of Water Deficit on the Concentration of Endogenous Free and "Bound" (Conjugate) ABA in the Axillary Inflorescence at Node 7

Duration of Water Deficit	Free ABA log ng/gm fresh wt.			"Bound" ABA log ng/gm fresh wt.		
	Control	Water Deficit	L.S.D. (P=0.05)	Control	Water Deficit	L.S.D. (P=0.05)
2 Days	1.90	2.26	N.S.D.	-	0.70	-
4 Days	2.38	2.76	N.S.D.	0.70	1.60	N.S.D.
6 Days	2.34	2.78	0.28	1.50	1.58	N.S.D.
8 Days	2.37	2.80	N.S.D.	1.49	2.33	0.48

TABLE A.3.4.

Effect of Varying Periods of Water Deficit on the Concentration of Endogenous Free and "Bound" (Conjugate) ABA in the Axillary Inflorescence at Nodes 5 and 6

Duration of Water Deficit	Free ABA log ng/gm fresh wt.			"Bound" ABA log ng/gm fresh wt.		
	Control	Water Deficit	L.S.D. (P=0.05)	Control	Water Deficit	L.S.D. (P=0.05)
2 Days	1.83	2.61	0.69	0.30	1.78	0.72
4 Days	2.45	2.68	N.S.D.	0.70	1.94	0.70
6 Days	2.32	2.74	0.40	1.58	2.02	0.60
8 Days	2.51	3.06	0.40	1.51	2.39	0.48

TABLE A.3.5.

Effect of Varying Periods of Water Deficit on the Concentration of Endogenous Free and "Bound" (Conjugate) ABA in the Axillary Inflorescence at Nodes 3 and 4

Duration of Water Deficit	Free ABA log ng/gm fresh wt.			"Bound" ABA log ng/gm fresh wt.		
	Control	Water Deficit	L.S.D. (P=0.05)	Control	Water Deficit	L.S.D. (P=0.05)
2 Days	1.78	2.18	N.S.D.	0.30	1.0	N.S.D.
4 Days	2.45	2.76	0.30	1.40	1.63	N.S.D.
6 Days	2.38	2.81	0.40	1.69	1.93	0.20
8 Days	2.45	2.85	0.40	1.53	2.16	0.48

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