WATER STRESS AND APICAL MORPHOGENESIS IN BARLEY AND *LOLIUM TEMULENTUM* L.

by

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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.

ABSTRACT

The effects of water stress on apical morphogenesis in two long day plants, barley and Lolium temulentum were investigated. The water stress treatments consisted of short term and continuous progressive stress. The water status of the plants was assessed by relative turgidity measurements on the leaves.

The influence of pre-sowing hardening on the response of barley to short term water stress was investigated with two varieties, Prior 'A' and CI3576, following the published methods of Genkel (cited by May, Milthorpe and Milthorpe 1962). Hardening had no effect on shoot dry weight, primordium production or apical development either during water stress or in the recovery phase. Water stress in this experiment reduced the rate of primordium production and apical development, and these phenomena were further investigated in controlled environment conditions. Continuous progressive water stress imposed on plants growing in a long photoperiod retarded the development of the barley (c.v. Prior A) apex both in terms of the formation of primordia and the development of spikelets. Primordium production ceased when leaf relative turgidity fell below 90%. On the other hand, differentiation of the spikelets continued, albeit at a

reduced rate, even with severe stress when the leaf relative turgidity values had fallen to 43%. The response of the two parameters of apical growth was further investigated under different combinations of water stress and photoperiod. It was found that a water stress which resulted in cessation of primordium production did not inhibit spikelet differentiation which continued at a reduced rate. This reduced rate of spikelet development could be stimulated by an increase in photoperiod even during continuing water stress. Primordium formation, on the other hand, was only stimulated by an increase in the photoperiod when water stress was relieved. Changes in the photoperiod controlled the rate of spikelet development in a quantitative manner.

It is possible to interpret these effects of water stress on apical growth and morphogenesis in several ways. Firstly, water stress may have been interfering with the photoperiodic control of floral initiation; secondly, a direct effect on the water status of the apex may have been involved; and, thirdly, the effects of water stress on the apex may have been mediated indirectly through effects on translocation and assimilation.

The first of these suggestions was investigated by subjecting plants of Lolium temulentum to water stress at various periods before, during or following

photo-induction. Plants were grown in a modified sand culture and water stress was imposed by the addition of a polyethylene glycol solution to the rooting medium. Water stress applied during the known periods of synthesis and translocation of the floral stimulus indicated that stress (relative turgidity of the leaf falling below 78%) inhibited both processes. Any pre-existing floral stimulus was not dissipated during a 32-hour period of stress, however.

The effect of water stress on the water status of the apex was explored under various stress conditions by floating the apices in a series of concentrations of polyethylene glycol solutions and evaluating the osmotic potential of the solution in which no change in apex length occurred. The apex was resistant to stress relative to the leaves, in that the water potential of the apex was unaffected until the relative turgidity of the leaves had fallen below 65%.

The possible involvement of translocation and assimilation in the response of the apex to water stress was indirectly assessed by the imposition of a water stress on plants growing in different combinations of light intensities. It was noted that primordium production, when limited by water stress, was stimulated by an increase in light

intensity, suggesting that at least some of the effects of water stress on the apex are indirectly mediated through effects on assimilation.

CHAPTER 1. INTRODUCTION

1.1. Morphology and histogenesis of the shoot apex in grasses.

1.1.1. Developmental morphology.

The developmental morphology of the shoot apex in Gramineae has been extensively investigated and the morphological changes described for barley (Bonnett 1935), wheat (Bonnett 1936, Barnard 1955), Lolium (Evans and Grover 1940, Cooper 1950, 1951, 1956, Barnard 1957) and many other members of the Gramineae (Sharman 1945, 1947, 1960a and 1960b, Bonnett 1936, Barnard 1957).

The sequence of events is as follows. At first the apical dome is hemispherical with a few simple ridge leaf primordia. As the apical meristem grows leaf primordia are formed acropetally and the apical dome elongates. A leaf primordium is initiated by a small protuberance on one side of the apex which is transformed by growth at the flanks of the primordium into a crescent and then into a collar almost surrounding the apex.

The commencement of the change of the apex to floral development is marked by the appearance of small bulges in the axils of presumptive leaf primordia, the bases of which at this stage completely encircle the axis. As the apex continues to elongate axillary primordia are formed in rapid succession in the axils of leaf initials. This results in

a double ridge appearance. These axillary primordia develop into spikelets in barley whereas in other species they may give rise to branch primorida. Differentiation of spikelet structure begins in primordia central on the spike and then successively in others basipetally and acropetally.

In barley the dome of the apical meristem does not at any stage differentiate to form a floral structure; the primary branches form spikelets and are terminated by ovaries (Nicholls and May 1963). The spikelets arise in the plane of axis and bear one flower. Differentiation of the spikelet after the establishment of the lateral spikelet initials (sterile in two-row barley) proceeds with the appearance of glume initials followed by lemma, pale and stamen initials. The pistil is formed finally from an area of the meristem located between the anthers, differentiating considerably later than those structures. In awned varieties, awn development on the lemma is distinguishable shortly after stamen initiation (Bonnett 1935).

The spike of Triticum and Lolium, on the other hand, is a determinate inflorescence, and the secondary branches are terminated by ovaries and bear fertile tertiary branches (Barnard 1955, Evans and Grover 1940). The sequence of events is very similar to barley. Stamen and pistil initials arise from the flower primordia which are formed in the axils of the lemmas. The spikelets are multiflowered and the

floral branch is indeterminate. In Lolium the spikelets arise at right angles to the plane of the axis whereas in Triticum these originate in the same plane (Barnard 1957).

1.1.2. Histogenesis.

The histological organisation of the shoot apex in Gramineae can be adequately described by the tunica and corpus zonation on the basis of cell pattern (Sharman 1945, 1947). The surface layer of cells of the tunica surrounds an inner core of cells, the corpus. The cells of both the outer layer, dermatogen, and the inner layer, hypodermis, of the tunica divide anticlinally except where a leaf primordium is initiated. The cells of the corpus, on the other hand, divide in all planes. The various organs originating from the apex are derived either from tunica alone or from tunica and corpus together and on this basis are classified as of foliar or cauline origin respectively.

A leaf primordium is initiated by the periclinal division of cells in the tunica and so are the glume, lemma and pale initials. The spikelets, flowers and stamens, on the other hand, are cauline in origin and compare with the tiller buds and main shoot meristems (Sharman 1945, 1947, 1960a, Barnard 1955, 1957).

The spike of a gramineous plant is a branched inflorescence bearing fertile and unfertile flowers. In barley the spike does not terminate in a spikelet. The

rates of development of the different spikelets on barley spike vary and the number that mature is determined by the conditions of growth (Bonnett 1935). In Triticum and Lolium, on the other hand, the spike is a determinate inflorescence and growth conditions affect the number of fertile flowers in a spikelet rather than the number of spikelets (Bonnett 1936).

1.2. Effect of day length on apical morphogenesis.

Day length has been found to exert a profound influence on apical development in grasses (Cooper 1950, Evans 1958, 1960a, Aspinall and Paleg 1963, Aspinall 1966). The major response classes include long day (Evans, 1958, Evans and Allard 1934, Allard and Evans 1941, Koller and Highkin 1960), and short day plants (Best 1959), although several grasses are unaffected by day length (Allard and Garner 1940).

1.2.1. The response types.

The photoperiodic requirement may be an obligate or quantitative response. In obligate long day plants flowers initiate only above a critical photoperiod as in Lolium temulentum (Evans 1958). The quantitative long day plants, on the other hand, are able to flower in short days but do so more rapidly in long days. This class includes most of the barley varieties (Aspinall 1966).

1.2.2. Changes after induction.

Wetmore, Gifford and Green (1959) studied apical morphogenesis in Xanthium, Chenopodium and Papaver, by

subjecting them to appropriate photoperiodic regimes. It was shown that a recognisable increase in meristematic activity in the corpus cells was the first sign of transition. Increased cell division was followed by cell enlargement resulting in elongation of the apex. The meristematic activity extended to the peripheral zone resulting in the formation of a capitulum or head of flowers in Xanthium, an inflorescence axis in Chenopodium and a single flower in Papaver.

Gifford and Tepper (1961) also found increased meristematic activity in the corpus cells of the Chenopodium shoot apex following induction. The nucleoli of the corpus cells were smaller than those of the tunica cells in the vegetative apex but began to enlarge following induction and continued to do so until the nucleoli of all the cells of the apex were approximately equal in size. Meristematic activity led to the first macroscopic change in the morphology of the shoot apex in the appearance of precocious bud primordia. Subsequently a primordial compound inflorescence was formed consisting of a terminal conical apex with numerous subjacent primordial inflorescences.

Histochemical changes following induction and paralleling these morphological changes included an increase in the concentration of RNA followed by total protein content (Gifford and Tepper 1962). These increases were not

localised but were general throughout the apex.

The transformation of the vegetative to the floral apex in the Gramineae involves activation of a number of sites on the apex leading to spikelet differentiation, in contrast to the overall activation outlined for Chenopodium. In wheat, Barnard (1955) has shown that the spikelet primordium is initiated by periclinal divisions at sub-hypodermal depth curving to the outside and anticlinal divisions in the dermatogen. This meristematic activity extends transversely around half the circumference of the axis. The bulge or horizontal ridge over the presumptive leaf primordium marks the first sign of transition from the vegetative to the floral state and is usually designated as 'double ridges'. Knox and Evans (1966) working with Lolium temulentum have demonstrated that the double ridge is initially marked by an increase in the size of the nuclei of the cells of the hypodermis and to a lesser extent of the dermatogen and sub-hypodermal cells.

Histochemical changes following induction in Lolium temulentum were also investigated by these workers. The first sign of induction in the apical meristem was found on the second day after long day exposure in that RNA increased in isolated pockets at a number of sites along the flanks of the apex where spikelet primordia differentiated subsequently. This build up was more prominent a day later. The cells with increased RNA content had large rounded nuclei. In

contrast, the cells of the subtending leaf primordia rapidly lost the RNA stainability that was evident during the vegetative phase of apical growth.

The changes in gross apex morphology during the transition from the vegetative to the floral state have been studied by various workers (Allard and Evans 1941, Cooper 1950, 1951, Barnard 1955, Williams 1960). The first discernible event is an increase in apex length, this being a general phenomenon in the members of the Gramineae (Evans 1960a and 1960b, Aspinall and Paleg 1963). The control of this response by day length has been demonstrated (Aspinall and Paleg 1963).

Subsequent to induction, an acceleration in the rate of primordium production at the apex occurs in barley (Nicholls and May 1963, Aspinall and Paleg 1963), Chenopodium amaranticolor (Thomas 1961), and in a number of short and long day plants (Langer and Bussell 1964, Gifford and Tepper 1961).

Data on the response of leaf differentiation, as contrasted with leaf initiation, to photo-induction are conflicting. Thomas (1961) found that floral induction in Chenopodium amaranticolor led to a dramatic increase in the rate of initiation of new leaves. He concluded that transition from vegetative to reproductive development was accompanied by pronounced mitotic activity in the apical meristem which resulted in an acceleration of all

morphogenetic processes. Langer and Bussell (1964) have similarly reported an increase in the rate of leaf initiation during induction from a number of short and long day plants. They concluded from their evidence that the first manifestation of induction is an elongation of the apex leading to an overall acceleration in morphogenesis.

In contrast to the observations of Thomas, Popham and Chan (1952) found that the first evidence of transition from a vegetative to a reproductive state in Chrysanthemum morifolium was a period of little or no growth following induction. In the same species, Schwabe (1959) found a similar situation where leaf differentiation was greatly depressed following induction. Williams (1960) demonstrated that the transformation of the vegetative to the reproductive apex in wheat was associated with a progressive decrease in the initial values of relative growth rates for successive leaves and a simultaneous explosive growth of corpus derived tissues. In barley, depression in the rate of leaf differentiation during the transition from the vegetative to the reproductive condition was noted by Aspinall and Paleg (1963). It is noteworthy that the measure of leaf differentiation in these experiments was the time taken for each leaf primordium to change from a single ridge to a lamina initial 1 mm long. Nicholls and May (1963) found no change in the rate of leaf differentiation in barley as between 8 and 16 hour photoperiods,

whereas the rate of primordium production was significantly increased. When the transition of a vegetative to a floral apex occurred, the further development of at least two basal simple ridge primordia to form either a leaf or a spikelet was dependent on the prevailing photoperiod. The trend of basipetallous development of spikelets from the point of first initiation at the centre of the spike was intensified in a longer photoperiod.

It is possible to conclude that the presence of a simple ridge primordium during the transition of the apical meristem from a vegetative to a reproductive condition does not necessarily mean that it will eventually form a leaf. The direction of its subsequent development into a leaf or a floret is in all probability dependent on the level of the stimulus. The observations of Thomas (1961) need further elaboration in terms of leaf initiation and differentiation in that the present evidence does not distinguish between initiation and development of the leaf.

A depression in the rate of leaf differentiation can possibly be explained on the basis of an increased demand for assimilates and other factors that are diverted towards floral development at the expense of the leaf (Aspinall and Paleg 1963). The phenomenon needs further experimentation however, with separation of the two growth processes in order to understand the situation more clearly.

The inductive demands of different plants vary, ranging from one long day (Evans 1958) to several weeks (Hamner and Naylor 1939). Once the minimum photoperiodic requirement is fulfilled, the plant initiates flower primordia irrespective of whether the plant is maintained in the promotive day length or returned to non-inductive conditions. Continuance of a photo-inductive environment accelerates the rates of inflorescence development. Evans (1960a), with a number of grass species in different day lengths, concluded that the effects of day length on induction and floral development are related. He presumed that, in induction, a stimulus to organs of cauline origin is involved whereas further floral development is controlled by the production of cell elongating substances. Aspinall and Paleg (1963) found similarity in the response of primordium production and floret differentiation in barley, suggesting that the same endogenous mechanism controlled the two phenomena.

1.2.3. Photoperiodic induction and perception.

The formulation of the principle that light perception leading to photo-induction is located in the leaves whereas the induced response, differentiation of flower primordia, takes place at the apex is the basis of the concept of a flowering hormone (Garner and Allard 1920). An optimal flowering response can be obtained in photoperiodically sensitive plants by exposing the leaves alone to inductive

conditions; the rest of the plant remaining in a non-inductive regime (Withrow and Withrow 1943, Hamner and Naylor 1939, Hamner and Bonner 1938). In Lolium temulentum 6 cm^2 of leaf lamina exposed to an inductive day length is sufficient to lead to initiation of an inflorescence (Evans 1960b). A single leaf, if at the right sensitive stage (Borthwick and Parker 1940), is hence sufficient to produce an inductive response.

1.2.3.1. Floral stimulus.

Evidence for the production of a flowering hormone in the leaves and for its translocation to the apex has been obtained by the separation of the sites of perception and of response with grafting experiments in both short day (Hamner and Bonner 1938, Zeevart 1958) and long day plants (Lang 1965). Grafting an induced plant to a receptor in a non-inductive regime resulted in floral initiation in both donor and receptor. Further evidence for the transmission of an inductive stimulus ('florigen') is provided by studies of the effects of removing the induced leaf at intervals following photoperiodic induction. Judging from the effects of leaf removal on subsequent apical development, florigen translocation appears to commence some time following the provision of an inductive environment. In Xanthium (Salisbury 1955) translocation lasts for 12 hours under optimal conditions. Evans (1960c) studied the translocation of

florigen in Lolium temulentum in a similar manner. All leaves excepting the sixth fully expanded leaf were removed from the plants before exposure to a long day. The remaining leaf was removed at intervals during and following the long day treatment (extension of an 8 hour period in day light with 16 hour of incandescent light of either 2 or 15 foot-candles intensity) and subsequent apex development was recorded. Most of the translocation appeared to take place in the 8 hrs of high intensity light following the long day treatment; it was slowed down, but not stopped, if the high light intensity was replaced with low light intensity. It was concluded that the high light intensity was not required to complete the long day processes. Experiments with Xanthium pennsylvanicum by Carr (1957) and Searle (1961) suggested a passive transport of floral stimulus with photosynthates from the leaf. There is, however, a great discrepancy in the velocity of movement of the photosynthates and the floral stimulus. The velocity of movement of the floral stimulus in Xanthium (Zeevart 1962) and in Pharbitis (Imamura and Takimato 1955, 1956) were estimated as less than 0.4 cm/hour. These values were based on the minimum threshold amount of floral stimulus required to produce flowering.

The measurement as such is of the threshold concentration that is required for flower formation and not the genuine translocation rates which would undoubtedly be higher. The problem of threshold was overcome by Evans and

Wardlaw (1964) in Lolium temulentum by establishing the minimum area of leaf tissue needed to obtain a flowering response and precisely determining the time in which the floral stimulus moved the distance of the leaf blade into the sheath by defoliating the plant at intervals after the critical light period. The rate of translocation of the floral stimulus thus worked out was 2 cm/hour.

The disparity in this improved estimate of the translocation rate of the floral stimulus and the known velocity of assimilate in some members of the Gramineae, such as 80-100 cm per hour in wheat (Wardlaw 1965) and 84 cm per hour in sugar-cane (Hartt, Kartschak, Forbes and Burr 1963), and the ability of a small leaf to export floral stimulus and no, or very little, assimilate prompted Evans and Wardlaw (1966) to explore the translocation pattern further. They made a determination of the velocity of the movement of the floral stimulus, at the same time as a measurement of the velocity of movement of ^{14}C labelled assimilates in Lolium temulentum plants of the same age and under the same environmental conditions. The translocation rates were 1-2.4 cm/hr for the floral stimulus and 77-105 cm/hr for ^{14}C labelled assimilates. On the basis of the evidence it was concluded that, although both the floral stimulus and assimilate may move in the phloem, they

probably do so independently and by different mechanisms. This slow rate of movement of the floral stimulus compares with that of auxins and of gibberellins, and is comparable with the rates of cytoplasmic streaming.

1.2.3.2. Inhibitory effect of non-induced leaf.

It has been claimed that the essential element in the photoperiodic response of long day plants is the inhibitory effect of long dark periods. Flowering is obtained if this inhibition is absent, as in the long light period. For instance Snyder (1948) with Plantago lanceolata and Takimato (1955) with Silene armeria found that a combination of short light periods with short dark periods or long light periods with short or long dark periods were favourable for flower formation, while combinations of short light periods with long dark periods were not.

Evans (1960c), working with Lolium temulentum, found that exposure of a single leaf to a long day was capable of initiating inflorescence development both in the presence and absence of leaves simultaneously kept in a short day. It was concluded that a positive floral stimulus to flowering was formed in the leaves during exposure to a long day. Pronounced enhancement of the flowering response under relatively high temperatures during the light period also supported the conclusion that the effect of the light period was not through suppression of a dark inhibitor.

In the same plant, Evans (1960c) demonstrated that short day leaves reduce the flowering response. The effect was dependent on the ratio of the numbers of long and of short day leaves on the plant and the time that these were retained. The inhibitory effect of a short day leaf was present even if it was not inserted between the long day leaf and the apical meristem suggesting that the effect was not on translocation or distribution of the floral stimulus. Evans and Wardlaw (1964) have confirmed this view by the use of labelled assimilates. Promotive processes in the leaves of Lolium temulentum can proceed in the absence of oxygen whereas inhibitory processes cannot do so (Evans 1962b) suggesting an oxidative step in the generation of the inhibitory effect.

1.2.3.3. Stability of the floral stimulus.

There is no direct evidence in respect of the stability of florigen. It has been shown that the effects of induction below a threshold level can be summated when these are separated by periods of non-inductive conditions; the possibility of fractional induction has thus been demonstrated for both short and long day plants (Hamner 1940, Carr 1955). The possibility of fractional induction indicates a high degree of stability of the floral stimulus (Lang 1965). Further indirect evidence on the stability of florigen can be drawn from experiments designed to demonstrate the effects of anti-metabolites on induction at the apex.

The flowering response in Pharbitis (Zeevart 1962) was inhibited by the application of anti-metabolites of DNA to the apex and was restored by precursors of DNA. The anti-metabolite, 5 fluorodesoxyuridine, was rapidly metabolised by the tissue and its depressing effect on cell division at the apex lasted for 40 hours only. As floral induction was nevertheless suppressed, this suggests that florigen cannot be maintained in the apex at a level sufficient to produce floral initiation for as long as 48 hours. It is difficult to define the stability of the floral stimulus in Lolium temulentum with this accuracy as the experiments of Evans (1964b) provide no indication of the period within which the anti-metabolites inhibited cell division at the apex. The apparent contradiction between the implications of experiments using fractional induction and of those involving anti-metabolites remains to be resolved.

1.3. Plant Water Stress.

1.3.1. Water Balance.

The stage at which plant growth departs from its maximum possible rate due to water stress cannot be defined in terms of the soil water status alone. The rates of physiological processes which control plant growth are closely related to the internal water balance of the plant system which is controlled by both the relative rate of water loss and of water absorption.

Transpiration, that is, loss of water from plants, involves movement of water in both the liquid and vapour state. The change from the liquid to the gaseous phase requires energy and thus the process is energy dependent. The sun's radiant flux at the plant surface supplies this energy. The site of evaporation is within the leaf, either at or within the walls of the mesophyll cells surrounding the intercellular spaces. There are thus two major portions of the transpiration stream from the soil to the external atmosphere (Milthorpe 1961). The first of these, from the soil spaces to the mesophyll cells, is concerned with the transport of water in the liquid state, whereas the second, from the mesophyll cell surface to the external atmosphere is concerned with the diffusion of water in the gaseous phase. The overall flux of water through the system is controlled by the water potential gradient from the soil to the external atmosphere and the resistances to water movement in the pathway. The potential gradient, except under extreme conditions either of moisture stress or of atmospheric humidity, is large so that effective regulation of the transpiration stream rests in the resistances to water movement. Resistances to movement of water in the liquid stage can only effectively limit water flux by increasing the energy required for evaporation at the cell surface. It has been suggested that this may take place due to the retreat of the effective water surface

for evaporation within the cellulose matrix of the cell wall hence increasing surface tension and imbibitional forces limiting evaporation (Preston 1954). This effect has been shown to be of relatively minor importance, however, (Milthorpe 1959) and the effective resistances to water flux in the transpiration stream must be sought in the vapour phase.

The diffusion of water in the vapour phase from the mesophyll cell to the external atmosphere follows two pathways. The first and major pathway is through the stomata. Some diffusion also takes place through the leaf cuticle but generally the amount is much less. The resistances that are encountered in the movement of water through these diffusion paths can be expressed on the basis of the following equation (Milthorpe 1960)

$$L_s = \frac{1}{S_c + S_D / \left[1 + S_D(1/S_1 + 1/S_w)\right]}$$

where L_s is the resistance between the external atmosphere and the evaporating surface, S_c is the conductance of the cuticle, S_D is the conductance of stomata, S_1 is the conductance of sub-stomatal cavities and S_w is the conductance of cell walls (conductance = $\frac{1}{\text{resistance}}$).

The resistances involved at the level of sub-stomatal cavities and cell walls are very small and can be

neglected without great loss of precision (Milthorpe 1960). The resistance of cuticle is high in relation to stomata (Slatyer and Bierhuizen 1964) and in wheat it has been estimated to be 70 times that of the minimal stomatal resistance (Milthorpe 1960). A further large but variable resistance to water loss from the plant occurs during diffusion through the boundary layer of still air at the leaf surface. The dimensions of this resistance depend upon such factors as leaf size and shape, surface properties and wind speed. In normal conditions, transpiration can be expressed in the form of the following equation on the basis of Frick's law (Penman and Schofield 1951)

$$T = {}^{\Delta c}/_{r_a + r_l}$$

where Δc is the difference in water vapour potential at the evaporating sites and in the free air, r_l is the resistance of the stomatal pores and r_a is the external resistance of the boundary layer of air. In conditions of water stress where the stomata are closed, or nearly so, a term for the water flux through the cuticle becomes of greater importance in determining the overall flux. Loss of water from the plant leaf by transpiration results in lowering of the leaf water content and as a consequence the potential energy of water in the leaf is reduced. Water moves from the soil into the plant when the potential energy of the water in the

leaf is lower than that in the soil. Several terminologies have been used to define the status of water in the plant and soil system but the term 'water potential' which gives a more comprehensive description of both the plant water and the soil water energy status on a thermodynamic basis has been widely accepted (Slatyer and Taylor 1960). Water in soils and plant systems encounters forces due to the presence of the solid phase, dissolved salts, external gas pressure and the gravitational field. These effects are expressed in terms of the potential energy of water (Gardner 1965). Water potential, which is the chemical potential of the water in the plant or soil, has been defined as the difference between the partial specific Gibbs free energy of water in the system under consideration and of free pure water at the same temperature (Slatyer and Taylor 1960). As a result of the dehydration of the plant leaves by transpiration, the water potential is lowered. This establishes a water potential gradient which is transmitted through the intact water columns of the plant system across the root cortex to the soil water. Lowering of water potential at the root surface below that in the soil results in the passive movement of water along a potential gradient from the root (Slatyer 1960, Gardner 1965, Vaadia, Raney and Hagan 1961). Movement of water in the plant due to active absorption

requiring the expenditure of respiratory energy is, however, evident in plants where very slow or negligible transpiration occurs and little soil water stress exists (Slatyer 1960). In pepper and sunflower plants, water movement from the roots to the leaves when the leaf water potential was higher than that of the solution bathing the roots was considered (Barrs 1966) to be due to root pressure moving water against the potential gradient. However, in conditions where a temperature gradient existed, water movement in a direction opposite to the gradient of water potential has been previously reported (Taylor and Cavazza 1954). In water culture experiments, where a temperature gradient is difficult to avoid, an inverted gradient of water transport may be due to the difference in temperature between the leaves and the solution bathing the roots. Quantitatively, passive absorption is of far greater significance and most water is absorbed by the plant in this manner (Slatyer 1960).

The internal water balance of the plant depends on the relative rates of water absorption and of water loss. The two processes are influenced by a complex of atmospheric, soil and plant factors which modify the rates of uptake and loss. The factors controlling the two processes differ, such that a change in any one of the factors upsets the balance. Water deficits caused by excessive transpiration are usually less severe as the plants regain turgor during the night.

On the other hand, a lag of absorption behind transpiration due to increasing depletion of water in the soil becomes increasingly severe with time. In dry soils, this lag of absorption is caused by the low potential of water at the root surfaces, occasioned by inadequate movement of water to the root surface and the reduced potential of soil water held in soil matrices (Richards and Wadleigh 1952, Philip 1957). In soil which is not uniformly dry, the rate of root growth into moist zones controls this lag (Philip 1958, Petinov 1961). When the soil is adequately supplied with water, the major resistance to uptake is within the root (Kramer 1938). Thus there is an increase in water absorption following death or removal of roots. Crafts, Currier and Stocking (1949) have experimentally shown that longitudinal movement of water in plants can take place through non-living elements of the xylem. This suggests that there is only a low resistance to the movement of water in the xylem vessels. In certain conditions, where the xylem elements are less than 20μ in diameter, movement of water may face a resistance at this level as has been reported for some grass species (Wind 1956). The lag of absorption behind transpiration results in a water deficit in the plant. This lag is determined by the difference between resistance to water flux into the plant (soil matric potential, osmotic potential, flow through soil, etc.) and resistance to water flux out of the plant (stomatal resistance, boundary layer, cuticular resistance, etc.).

1.3.1.1. Measurement of water stress in the plant.

Internal water balance has long been recognised as an important aspect of the physiological status of the plant. Water content as a percentage of fresh and dry weight has been used to provide an index of internal water deficit (Miller 1938). The water content of plant tissue varies with species, organ, tissue and age (Stocking 1956). It also varies diurnally (Wilson, Boggess and Kramer 1953) and seasonally (Ackley 1954, Clark and Gibbs 1957). Hence, a measurement of water content expressed as a function of fresh or dry weight is not a satisfactory indicator of the water status of a plant or plant tissues (Kramer 1959).

It has been suggested (Weatherley 1950, 1951) that turgid water content provides a more satisfactory basis for reference. The term 'relative turgidity' was introduced and defined as the ratio, expressed as a percentage, of the amount of water in a known quantity of tissue when sampled to the amount of water in the same quantity of tissue when fully turgid:

$$\text{Relative turgidity} = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

Since the introduction of the method it has been widely used (Slatyer 1955, 1957, 1961a, Rutter and Sands 1958, Clausen and Kozlowski 1965) and has proved a quantitative and valuable index of internal plant water deficit, except in conditions of slight water deficit where it has been found

insensitive (Slatyer and Barrs 1965). It has been shown that an approximate relationship exists between the relative turgidity and the water potential of the tissue (Weatherley and Slatyer 1957, Slatyer 1960) which enhances the significance of the measurement.

The osmotic pressure of sap expressed from plant organs has been used as an indicator of the water status of plants (Walter 1955) but due to limitations in the significance of this (Kramer 1959), attention has shifted to 'diffusion pressure deficit'. The diffusion pressure deficit (Meyer 1945) is a measure of the pressure with which water tends to move into cells and tissues. Fully turgid tissue has a zero diffusion pressure deficit and the diffusion pressure deficit increases as the water deficit increases. The measurement technique involves balancing the diffusion pressure deficit of the tissue with the osmotic potential of the solution using a graded series of concentrations. The solution in which the tissue neither gainsnor loses water from or to the solution is considered to have an osmotic potential equivalent to the diffusion pressure deficit of the tissue. Although floating the tissue in the solution and determining the change in length (Usprung 1923) or weight (Meyer and Wallace 1941) of the tissue has the advantage of great simplicity, it introduces errors due to infiltration of the solution into the intercellular spaces, and in the case of plasmolysis,

into intracellular spaces of the tissue. To overcome this, a vapour pressure method for measuring diffusion pressure deficit has been used (Slatyer 1958). This method utilizes the same general principle as the solution method except that the diffusion pressure deficit of the tissue is balanced against the water potential of vapour over a solution rather than the solution itself. The use of psychrometers for direct measurement of tissue water potential by vapour means has been employed with the advantage of speed and precision (Brix 1962, Ehlig 1962). The psychrometer is used to measure the wet bulb depression of air over a sample enclosed in a thermally constant chamber. A constant reading indicates vapour pressure equilibrium has been established between the air and the sample. The corresponding water potential relates to that of the sample.

1.3.1.2. Plant water status and response.

Although the stage at which a reduction in growth due to water stress takes place cannot be defined in terms of soil water stress alone, evidence is available from numerous studies that plant growth is retarded at a soil water potential of less than 1 bar and is reduced progressively with decreasing potential (Richards and Wadleigh 1952). It appears that transpiration does not decrease below the potential rate until lower potentials are reached than those at which growth is reduced (Slatyer 1957). Most of the

evidence suggests that a progressive decline in transpiration takes place with increasing stress (Slatyer 1955, 1956).

The soil water stress at which transpiration is reduced varies greatly, being a function of the amount of root growth and potential transpiration. For instance, Slatyer (1956) estimated that the transpiration rates of ground nuts, cotton and sorghum grown in the same environment, departed from the potential rates with soil water stress of about 12, 20 and 70 millimeters respectively. The amount by which the transpiration rate is reduced below the potential rate is a function of stomatal closure and the size of the cuticular conductance of water vapour (Milthorpe 1960). In wheat, it has been shown that complete closure of stomata reduced the transpiration to about 15% of the potential (Milthorpe 1960). The resistances encountered in the transpiration pathway also restrict CO_2 flux in the reverse direction. Since CO_2 must also diffuse from the mesophyll cell walls to photosynthetic sites in the chloroplast, an additional resistance in the liquid phase is involved in this process, the magnitude of which is several times as high as the other two resistances (Gaastra 1959, 1962). Slatyer and Bierhuizen (1964) using phenyl mercuric acetate as a transpiration suppressant obtained a reduction in transpiration rate due to the closure of stomata without any apparent effects on photosynthetic rates.

1.3.2. Plant responses to water stress.

1.3.2.1. Growth.

Soil water becomes progressively less available to plants as the soil water stress increases (Richards and Wadleigh 1952, Stanhill 1957). Evidence to the contrary, that is, that soil water is equally available to plants over the range from field capacity to permanent wilting (Veihmeyer and Hendrickson 1950, Veihmeyer 1956), was based on field experiments where a decline in water availability was complicated by uneven root distribution. For instance, it has been shown by Majmudar and Hudson (1957) and Salter (1957) that water uptake, growth and transpiration continued at the maximum rate so long as roots could grow into regions of moister soil. A soil water stress of 1.5 atm has been found to reduce the yield and quality of cigarette tobacco (Van Bavel 1953) and dry weights and stem elongation in Scots pine seedlings (Sands and Rutter 1959). Similarly, a reduction in vegetative and fruit growth in apple has been shown with a soil water stress of less than 1 atm (Goode 1956). Retardation in differentiation of leaf and spikelet primordia has been shown to occur with a soil water stress of less than 0.8 atm (Nicholls and May 1963). In several other investigations (Jarvis and Jarvis 1963a, Slatyer 1960), where the water balance of the plant was the measure of stress, reduction in growth when the relative turgidity of leaf fell below

90 per cent has been demonstrated. In general, water stress leads to a reduction in growth and this departure is apparent as the water becomes less available to the plant and is progressively more acute with increasing stress.

It has generally been found that, with a mild water stress of short duration, root growth is initially favoured over shoot growth (Wangermann 1961, Jarvis and Jarvis 1963c). An increase in the root/shoot ratio with decreasing soil water contents has been reported in wheat (Harris 1914). Wangermann (1961) commenting on the stimulation of root growth by a slight water stress attributed it to the optimum conditions for root growth arising from an interaction between aeration and the availability of water. However, Jarvis and Jarvis (1963c) obtained similar stimulation in the root growth of Lupinus albus when water stress was applied in water culture experiments by the addition of polyethylene glycol. Relative stimulation of root growth of corn, tomato and wheat has also been found by Eaton (1942) with increasing concentrations of chloride and sulphate in culture solution. The initial stimulation of root growth which is followed by a reduction in growth with progressive water stress is difficult to explain on the basis of evidence available at the present time.

Water stress leads to a general reduction in growth but its influence on different plant organs may be different. Williams and Shapter (1955) studied the effects

of moderate intermittent water stress at different stages of development in barley and rye. They found that the effects varied with the stage of growth at the time of stress and the past history of the plant in relation to water regime. The responses to the treatment also varied with the species. For instance stem height was reduced in barley but not in rye. They found that plant parts growing most actively were the ones most affected. Aspinall, Nicholls and May (1964) with barley, have also reported that the most rapidly growing tissues at the time of water stress are most affected. In the tomato plant the growth of the leaf laminae was consistently retarded, whereas that of the stem was promoted under water stress (Gates 1955a). On recovery, however, the situation was reversed in that the growth of leaves was promoted and that of the stem retarded. It has also been shown by Catsky (1962) that, under water stress, the younger leaves were at a better water status than the old leaves resulting in death of old leaves and survival of younger ones. Milthorpe (1950) found that this differential in drought-resistance was related to the proportion of meristematic to elongating cells with the former being completely resistant. It appears then, that high water potential is associated with high growth activity and such growth suffers a greater check when water becomes limiting. However, the greater resistance of meristematic cells to water stress enables the actively

growing tissues to survive under desiccation and to resume growth soon after recovery.

A relationship between fluctuating soil water potential and growth is difficult to assess. This is at least partially due to an elevation of the relative growth rate during recovery from stress as compared to that of plants on adequate water supply throughout (Gates 1955a and 1955b). Thus, plants can in some circumstances make good the loss sustained and consequently the overall performance of the plant depends on the intensity and duration of water stress and the stage of development at which the plant experiences the stress (May and Milthorpe 1962).

1.3.2.2. Photosynthesis and Respiration.

A reduction in photosynthesis with increasing water deficit has been reported by various workers (Ashton 1956, Dastur 1925, Verduin and Loomis 1944). That this may be due to closure of stomata even before the soil water stress reaches the permanent wilting percentage has been shown by Schneider and Childers (1941). On the other hand, Baker and Musgrave (1964) found a reduction in photosynthetic rate in corn when the soil water stress was 1 atm and the transpiration rate had not departed from the potential rate. An actual increase in photosynthesis in apple leaves when soil water content fell below field capacity has been reported (Negisi and Satoo 1954) but this was followed by a marked

reduction before wilting. Similarly, an increase was reported by Schneider and Childers (1941) at slightly less than maximum leaf turgor, which they attributed to wider opening of stomata. Verduin and Loomis (1944) found that the porosity of the corn leaf to gases was greatly reduced by wilting, but the lack of a high correlation between measured porosity and photosynthesis indicated that factors in addition to stomatal closure were responsible for the reduction in photosynthesis. Transpiration is more dependent on the opening of stomata than photosynthesis since an additional resistance of much higher magnitude is involved at the mesophyll cells in which diffusion occurs in the liquid phase during photosynthetic processes (Gaastra 1959).

A close relationship between the net assimilation rate and the water content of the leaf has been demonstrated (Dastur 1925, Dastur and Dessai 1933). With a decrease in water content of the leaf there was a corresponding decrease in the rate of assimilation. The influence of water stress on the translocation of photosynthates in sunflower plants was studied by Wiebe and Wihrheim (1962) by exposing single leaves of the plants to a $C^{14}O_2$ labelled atmosphere and determining the translocation by measuring the amount of C^{14} in various portions of the plant. They found that translocation of photosynthates was reduced in water stressed plants. Similar reductions in woody species have also been reported

(Roberts 1964). In experiments (Plaut and Reinhold 1965) where effects of water stress on the passage of (^{14}C) sucrose was investigated by applying a drop of the labelled solution to the primary leaf of bean plants and following the course of movement in various plant parts, it was found that an initial stimulation of transport occurred in water stressed plants within a period of 30-45 minutes but the rates subsequently declined and fell far below controls. The stimulation of transport soon after the application of a drop of the solution to the water stressed plants might be a result of a favourable change in the water status of the tissue due to the drop of solution applied.

The evidence for a marked effect of water stress on translocation suggests that some at least of the effects of water stress on photosynthesis may be indirect. There is an increasing body of evidence that photosynthetic rates may be markedly influenced by the size of the 'sinks' utilizing assimilates and the rate of translocation to the sinks (Thorne ~~Trome~~ and Evans 1964, Humphries and Thorne ~~Trome~~ 1964, Nosberger and Humphries 1965). It is conceivable, then, that water stress may influence photosynthesis through an inhibition of the transport of the products of photosynthesis.

The influence of respiration on water stress has been studied by a number of workers. A fall in water content of Phalaris tuberosa and Lolium multiflorum from 90 to

80 per cent was shown to be associated with increases in repiration (Petrie and Wood 1938). The respiration rates, however, declined with increasing loss of water. Similarly, increases in respiration rates with slight water stress followed by a decline due to increasing stress were found in apple leaves (Schneider and Childers 1941). Negisi and Satoo (1954) working with apple leaves and Moss, Musgrave and Lemon (1961) with corn showed increased respiration due to mild stress. In Brassica roots that were allowed to desiccate gradually in dark, maximum respiration rates were recorded when the water contents of the tissue ranged between 90 and 80 per cent (Bouillenne-Walraud and Bouillenne-Walraud 1926). Kaul (1965) has reported a 20 per cent increase in carbon-di-oxide output with slight water stress followed by a decrease of 50 per cent with increasing water stress in wheat. It appears, then, that respiration rates increase with slight water stress and decline with further stress. The initial increase in respiration may be associated with decreased resistance to gaseous diffusion by the increased opening of stomata. A direct stimulation of hydrolytic and oxidative activity due to stress has been suggested as a possible explanation for the increase in respiration (Iljin 1957). In severe water stress, however, the decline in respiration has been found to be associated with decreases in respiratory metabolites (Kaul 1965) indicating that factors other than

stomatal closure are involved in the decreasing CO_2 evolution.

1.3.2.3. Plant Composition.

The nature and course of various biochemical reactions are often changed by water stress, resulting in changes in overall chemical composition. An accelerated conversion of starch to sugars during water stress has been reported by various workers (Eaton and Ergle 1948, Wadleigh and Ayers 1945, Woodhams and Kozlowski 1954). Petrie and Wood (1938) found that there was an increase in the net rate of starch hydrolysis with decreased water content and an increase in the level of sucrose. This rapid reduction in starch was not compensated by a corresponding increase in sugars. On the other hand, the rate of respiration was increased.

There is a decreased net formation of protein from amino acids with a reduction in leaf water content in Phalaris tuberosa L. and Lolium multiflorum (Petrie and Wood 1938a, 1938b). Marked reduction in protein contents with water stress has also been reported in corn (West 1962) and cabbage (Goff and Levitt 1965). In cabbage this progressive decrease was attributed to the reduction in the SH contents of soluble and non-soluble protein fractions and presumably water stress reduced SH-dependent enzyme activity. Wood and Barrien (1939a, 1939b) also found that the amount of protein sulphur decreased with a decrease in water content.

Under these conditions the amount of the sulphur-containing amino acid cystine did not increase as the other amino acids increased, but the amount of inorganic sulphate did. It may be possible that the rate of oxidation of cystine increases as the water content decreases and this could be one cause of the net decrease of protein synthesis (Gates 1964). The predominance of hydrolytic reactions under water stress may indicate a failure of energy producing mechanisms (May and Milthorpe 1962).

The RNA content of the leaf decreases under water deficit although there is no change in concentration on a dry weight basis (Gates and Bonner 1959). The decrease in ribonucleic acid per leaf was intimately related to the slower growth rate of the leaf. The rate of P^{32} incorporated into nucleic acid under water stress did not differ from that in unstressed plants indicating that the net reduction was due to accelerated destruction rather than to decreased synthesis. Kessler (1961) also found a decline in RNA constituents with increasing water stress in tomato plants. Incorporation of uracil C^{14} into RNA in water stressed plants was similar to that of control plants and the decline in RNA was possibly due to increased hydrolysis. However, a lower incorporation of thymine C^{14} in the water stressed plants suggested impairment of DNA synthesis. In contrast to these reports, some evidence for a reduction in RNA synthesis under conditions

of water stress has been obtained (Shah and Loomis 1965). Uracil ^{14}C turnover in water stressed sugar beet plants is reduced suggesting inhibition of RNA synthesis under water stress. Similarly, Zholkevich and Koretskaya (1959), who followed changes in nucleic acid components of pumpkin roots under water stress, found that the content of RNA increased linearly in controls whereas it changed little in water stressed plants. The authors suggested that synthesis was affected although the net response may have been due to increased RNA destruction.

It is apparent that almost all metabolic reactions are influenced by water stress, but, with the evidence at present available, it is difficult to assign a key role to any mechanism.

1.4. Effects of Water Stress on Apical Morphogenesis.

The effects of water stress on apical morphogenesis, particularly reproductive development, have not been explored extensively. It has been recognised, however, that certain stages of plant development are more susceptible to the effects of water stress than others (Williams and Shapter 1955, Aspinall, Nicholls and May 1964), although responses vary with the duration and intensity of stress.

1.4.1. Early stages of development.

Water stress in the early stages of growth was found to reduce spikelet differentiation in maize (Petinov

and Berko 1965). In wheat, water stress during spikelet formation reduced the number of spikelets (Domanskii 1959, Konovalov 1959) and in oats, stress during spikelet differentiation and the flowering stage resulted in a delay in spikelet development and a reduction in the number of fertilised flowers due to pollen sterility (Novikov 1952, 1954). In all the above experiments, however, the level of stress that the plants were suffering was not well defined. In a more precise experiment Nicholls and May (1963) found that gradually increasing soil water stress in barley resulted in an appreciable retardation in the differentiation of both leaf and spikelet primordia at a soil water potential of 0.8 atmosphere and that primordium formation ceased at a soil water potential between 2 and 2.5 atmospheres. Two abnormalities due to water stress were also found to occur on the apex either separately or together. One was a failure in the formation of a primordium on the apex resulting in the absence of a spikelet in that position and the other was a rotation in the plane of symmetry of the whole apex. The effect of water stress was more pronounced in reducing primordium formation than in affecting spikelet differentiation.

1.4.2. Later stages of development.

In later stages of plant development, water stress has been found to reduce the yield in various ways. In barley, water stress imposed between stamen initiation and

anthesis markedly reduced stem internode elongation and the number of grains on the ear (Aspinall et al 1964). The reduction in the number of grains was considerable when stress was imposed shortly after the formation of spikelet primordia and diminished rapidly the later the stress was imposed, particularly after anthesis. In wheat, water stress during the stem elongation phase was found to reduce the number of ears per plant (Asana, Mani, Pillay and Gahlot 1955). A reduction in the number of ears was reported when the water stress was applied at ear emergence in wheat (Asana, Saini and Ray 1958, Chinoy 1962a), and at panicle emergence in oats (Paauw 1949). Adequate soil water during the 'tasselling' stage of corn was found to be essential (Robins and Demingo 1953), water stress during this period causing an irreversible reduction in yield. In the same plant, the silking stage has also been reported to be particularly susceptible to water stress (Denmead and Shaw 1960). Water stress at the pollen formation stage in barley resulted in a reduction in pollen viability, as a consequence of which the number of fertilized flowers per spike was reduced (Zavadskaya and Skazkin 1960). At the pollen meiosis stage in wheat, water stress was found to cause male sterility in the lower florets of each spikelet without affecting female fertility (Bingham 1966). Although megasporogenesis was found to be more susceptible to water stress than microsporogenesis, prolonged stress caused the

female gametophyte to become infertile also (Skazkin and Lukomskaya 1962, Matkalyak 1965). Water stress between anthesis and ripening was found to reduce grain size considerably (Aspinall et al 1964) and the effect was greater the earlier the stress was imposed after anthesis. Similarly, in oats, water stress between emergence and ripening was reported to reduce grain size (Paauw 1949, 1952). Water stress reduces the yield at all stages of plant ontogeny but apparently internode elongation and anthesis are the two stages when damage is greatest. The components of growth that are most effective in contributing to yield are in actively growing stages on both these occasions.

1.4.3. Mechanism of action.

1.4.3.1. Indirect effects.

From this survey, it is evident that the effects of water stress are most severe on plant organs growing most rapidly at the time the stress is imposed. The mechanism underlying these responses is, however, not well understood. An indirect effect of reduced water potential in restricting the translocation of photosynthates and of hormones to the actively metabolising tissue has been suggested as a possibility (Nicholls 1962). Similarly, adverse effects of water stress on grain development after ear emergence have been attributed to a reduction in the assimilate supply to the grain (Denmead and Shaw 1960). Evidence from experiments

where the velocity and pattern of assimilate transport were investigated show that during the early stages of grain development, the velocity of movement of photosynthates towards the ear was twice as high as that of downward transport (Wardlaw 1965) and was markedly decreased by the removal of grains. Reduced translocation of carbohydrates and mineral nutrients during water stress has been demonstrated in tomato (Gates 1955a, 1955b, 1957). Of more direct interest to the study of grasses, however, is the observation by Petinov and Berko (1965) that retardation of spikelet differentiation in maize due to water stress was accompanied by inhibition of both translocation of amino acids to the apex and synthesis of protein in the leaves.

1.4.3.2. Direct effects.

In contrast to these indirect influences of water stress on the growth of meristamatic regions a direct effect may arise from the greater water requirement of actively metabolising cells. It has been suggested that this greater susceptibility arises because of a greater requirement for water during cell elongation (Maximov 1929). May and Milthorpe (1962), on the other hand, considered that the effects are more likely to be associated with cell division processes, which are involved in the differentiation of spikelets and flowering. Some recent information on this conflict has been provided by Gardner and Nieman (1964).

In radish leaves, where DNA content is linearly related to cell number, water stress was imposed by submerging leaves in mannitol solutions in a graded series of concentrations ranging from 1 to 16 bars. DNA content was reduced to 60% by a stress of 1 to 2 bars but there was little subsequent decrease as the osmotic potential was increased to 16 bars. This suggested that cell division was affected at low water potential but was not further decreased at higher potentials. They also obtained a measure of cell size from the ratio of leaf water content to DNA and found that, with increasing osmotic potential, cell enlargement decreased more gradually than cell division up to 8 bars but thereafter cell enlargement was completely prevented. These data may resolve the conflict between the views of Maximov (1929) and of May and Milthorpe (1962).

It has been shown that meristematic tissues are more resistant to desiccation than are elongating cells in wheat (Milthorpe 1950). Similarly, Iljin (1953) demonstrated that cells with larger vacuoles were less resistant to desiccation than cells with small vacuoles. In experiments where the response of barley grain growth was investigated (Aspinall 1965), it was found that the rate of grain growth in earlier stages of development was reduced only by severe water stress and this effect was not associated with changes in water potential of the grains. This indicated a greater

resistance of the grain than of other plant organs to water stress. Similarly, a greater resistance of the spike to water stress has been shown in wheat (Chinoy 1962a, 1962b) and in barley (Zavadskaya and Skazkin 1960). The need for a high hydration level for maximum meristematic activity of the spike and its greater resistance to increasing soil water potential might explain the persistance of root and shoot primordia over long periods of drought in some perennial grasses (Lucanus, Mitchell, Pritchard and Calder 1960).

Little is known about the mechanism of the effects of water stress on floral development in the Gramineae. Hence, it seemed desirable to investigate the influence of water stress on the processes involved in the reproductive development of the apex.

CHAPTER 2. MATERIALS AND METHODS

The general cultural practices and the design of the experiments are described here but further details pertaining to particular experiments are given in the Results section along with the appropriate experiment.

2.1. Materials.

2.1.1. Plant Material.

Barley (*Hordeum vulgare* L.) and *Lolium temulentum* were studied as examples of quantitative and qualitative long day plants respectively. The barley variety, Prior A, a mildew resistant strain of the variety Prior, was grown in all experiments. In addition, the variety CI 3576, which is reputedly more drought resistant, was compared with Prior A in one experiment. Seeds of both these varieties originated from Dr. K.W. Finlay of the Agronomy Department. *Lolium temulentum* seed was obtained from a single plant selection supplied by Dr. L.T. Evans of the Plant Industry Division, C.S.I.R.O., Canberra.

2.1.2. Controlled Environment Cabinet.

The cabinets employed had space (4' x 4') for one hundred pots (10 cm x 10 cm) arranged in 10 rows on the plant bed. The light source was a bank of 32 80 watt 'cool white' fluorescent tubes (Philips TLF 80/33) and four standard clear incandescent lamps (60 W). Two incandescent strip lamps

(75 W) were used for prolonging photoperiods. The light intensity in the main light period was 2000 foot-candle of fluorescent light plus 80 f.c. of incandescent light, the basic photoperiod was extended with incandescent light alone with an intensity of 80 f.c. Light energy in the main photoperiod was varied by changing the vertical distance of the plants from the light source. Light intensities were measured with an Eel broad spectrum photocell. Temperature was maintained at 17°C ± 1°C with a maximum gradient of 1°C between the air inlet and outlet at the sides of the cabinet.

2.2. Methods.

2.2.1. Short-period Water Stress Experiment.

In this experiment the influence of pre-sowing hardening, as defined by Genkel (see May, Milthorpe and Milthorpe 1962), on the response of barley to short term water stress was investigated with two varieties, Prior A and CI 3576.

The hardening treatment was as follows. Samples were drawn from the bulk grain to determine the original moisture content. The remaining grain was weighed and placed in a flask with distilled water equivalent to 30% of the air dry weight of the grain; this was then rotated horizontally on a mechanical mixer for one hour. All the water was taken up by the grains during this period, and the grains were then thinly spread in shallow dishes covered with moist

blotting paper and left in a germination cabinet for 48 hours at 20°C in dark. The moist grains were then dried down to the original air dry moisture content, using an electric fan at 25°C in the dark for 72 hours. This cycle of wetting and drying was repeated once. In the second cycle the moist grains were left in the germination cabinet for 24 hours instead of 48 hours. The hardened and control seeds were planted in 6" plastic pots in a glasshouse. Treatments were randomised between five blocks on the glasshouse bench. Each pot contained a layer of coarse gravel at the bottom, 1.6 kg dry weight organic loam and a surface mulch of fine gravel. Ten seeds were planted in each pot and thinned to five after recording emergence. Water stress was imposed by withholding water for various periods.

2.2.2. Continuous Water Stress Experiments.

Seeds of barley (c.v. Prior A) were pre-germinated for 24 hours at 20°C before planting in 10 cm plastic pots containing a loam and sand mixture (Nicholls and May 1963). The soil mixture was obtained by mixing Urrbrae loam with Plympton sand in the ratio of four parts of loam to one part of sand (volume by volume) followed by steam sterilization. For each batch of one hundred pots, 80 kg dry weight of soil was mixed with a mineral nutrient supplement and then moistened gradually with distilled water to approximately 14 per cent soil water content. The final moistening was

done when the soil had been placed in the pots and its water content determined. The moisture characteristics of the mixture have been determined previously in the Plant Physiology laboratory, and a 14 per cent moisture content corresponds to a water potential of 0.1 atmosphere where the soil mixture is easily workable.

The mineral nutrient supplement added to each batch of soil was as follows: KNO_3 31.6 g, $Ca(NO_3)_2.4H_2O$ 106.5 g, $MgSO_4.7H_2O$ 33.0 g, $NaH_2PO_4.2H_2O$ 63.0 g, $FeCl_3$ 2.4 g and $NaNO_3$ 36.4 g.

The pots were covered with aluminium foil to minimize losses from evaporation. This was pierced in two places, once for the plant and once for watering. A half inch layer of sand was used as surface mulch in experiments where larger numbers of plants were grown in each pot.

Treatments were randomized within blocks in the cabinet. The position of each block was changed daily in a cyclic pattern to reduce the variance due to positional effects. Water was applied daily to bring the weight of each pot to a pre-determined level. A correction for plant fresh weight was computed from harvested material. Water stress was imposed by withholding water for various periods.

2.2.3. Water Stress and Photoperiodic Experiments.

Lolium temulentum seeds were pre-germinated for 48 hours at 20°C in dark and transplanted to 10 cm x 10 cm plastic

containers. The rooting medium consisted of a vermuculite and sand mixture in the ratio of 1:3 (volume by volume) (Aspinall and Paleg 1963). Five seeds were sown in each pot and the plants were watered daily with 200 ml of a nutrient solution containing the following nutrients: $NaNO_3$ 91 mg, $Ca(NO_3)_2.4H_2O$ 25.6 mg, KNO_3 79 mg, $NaH_2PO_4.2H_2O$ 157 mg, $MgSO_4.7H_2O$ 92.5 mg, $FeCl_3$ 6 mg, H_3BO_3 0.7 mg, $MnSO_4.4H_2O$ 0.35 mg, $ZnSO_4.7H_2O$ 0.05 mg, $CuSO_4.5H_2O$ 0.02 mg and MoO_3 0.025 mg.

The plants were grown in an 8 hour photoperiod for approximately 5 weeks when the blade of the sixth leaf was fully expanded. The plants were then exposed to one inductive cycle by extension of the 8 hours of high intensity light with 16 hours of incandescent illumination of 80 foot-candle intensity at plant height. The plants were then returned to the 8 hour photoperiod regime for 3 further weeks before harvest. In the first experiment, the whole shoot was exposed to the long photoperiod, whereas in latter experiments only the blade of the sixth leaf of the main stem was retained, the other leaves being removed during the light period immediately preceding the long day. Evans (1960b and 1960c) has shown that the sixth leaf alone is sufficient to obtain a full photoperiodic response in this strain of Lolium temulentum. The blade of the sixth leaf was removed at different times either during the long day exposure or

the following period of high intensity light.

These treatments were combined with periods of water stress. Water stress was imposed by the application of polyethylene glycol (molecular weight 4000) solutions to the rooting medium at the rate of 200 ml. per pot. Solutions were prepared to give the desired osmotic potential and the water stress was relieved by thoroughly washing out the solution with distilled water. Seven cycles of completely filling the pot with distilled water and allowing it to drain was found sufficient to completely wash out the polyethylene glycol solution.

Polyethylene glycol has been widely used as an agent to increase the osmotic potential of the rooting medium (Jarvis and Jarvis 1963c, Janes 1961, 1964 and Barrs 1966). In certain instances injurious effects in terms of chlorosis and browning of plants after four days of polyethylene glycol application have been reported (Leshem 1966). In these cases, however, the possible presence of impurities in the polyethylene glycol was not evaluated. Purified polyethylene glycol has been used as an osmotic agent with no discernible interference with normal metabolic processes (Largerwerff, Ogata and Eagle 1961), but in one experiment (Janes 1964) with a low molecular weight (400) polyethylene glycol, there was some entry of the osmotic agent (500 p.p.m.) into the plant in five days.

To avoid possible complications due to impurities, the polyethylene glycol 4000 was tested prior to its use. Plants were subjected to water stress by application of polyethylene glycol solution to the rooting medium for a period of 72 hours when it was removed by washing. The plants recovered from stress and grew normally with no visible injurious effects following relief of the water stress.

The possibility that polyethylene glycol 4000 could enter into the plant system, despite the high molecular weight of the polyethylene glycol and the short duration of the stress, was checked by determinations of leaf water content (fresh weight - dry weight) during application of polyethylene glycol to the roots and after its removal. It has been shown (Slatyer 1961a) that solutes entering the plant system during osmotic stress result in a recovery of plant water content due to the consequent increase in the internal osmotic potential. It was found that such a recovery did not occur with polyethylene glycol application. Further details on this method are presented in Appendix I.

The experiments were randomized in five blocks, each pot containing five plants giving 25 plants for each determination. Each block was rotated daily in a cyclic pattern to minimize the variance due to positional effects.

2.3. Measurements.

2.3.1. Dry Weight.

Measurements of dry weight were made for the whole shoot, roots and plant parts. Fresh plant material was placed in 2 x 1 cm specimen tubes and dried in a forced-draught oven at 90°C for 48 hours.

2.3.2. Apical Measurements.

The main shoots of the plants were dissected at each harvest under a binocular microscope and the lateral appendages at each node were recorded. The lateral appendages were grouped into the various developmental stages as fully-expanded, expanding, differentiating (not visible before dissection) leaves, simple ridges and spikelets. The apex passes through certain well-defined stages of development during its ontogeny (Bonnett 1935, Cooper 1951) and these were used to assess the development of the apex. The whole apex was classified on the stage of development of the most advanced primordium.

The scale used by Aspinall and Paleg (1963) was employed in describing the development of barley apex and was as follows:

Stage of Development	No.
Apex not elongating	1
Apex elongating	2
Double ridge on apex	3
Upper ridge elongating	4
Lateral spikelets visible as simple mounds	5
Glume initials visible	6
Lemma initials visible	7
Stamen initials visible	8
Awn initials visible	9
Awns longer than spikelets	10
Anthesis	11
Inflorescence fully emerged	12

The scale used by Vince (1965) was employed in describing the development of the Lolium temulentum apex and was as follows:

Stage of Development	No.
Apex not elongating	1
Apex elongating	2
Double ridge on apex	3
Upper ridge enlarging	4
Glume ridges visible	5
Lemma ridges visible	6
Flower primordia swelling	7

2.3.3. Apex Length.

Apex length was measured with an ocular micrometer under a binocular microscope. The length from the top of the apex to the base of the lowest simple ridge or spikelet primordium was recorded.

2.3.4. Relative Turgidity Measurements.

The water status of the plant was assessed from leaf relative turgidity (R.T.) measurements. The technique used by Barrs and Weatherley (1962) for measuring relative turgidity of Ricinus communis was checked and standardized for the barley leaf. Details are given in Appendix II. The method finally adopted was as follows.

Samples of 10 discs, 8 mm in diameter, were taken from the middle portion of the fully-expanded intact leaves with a sharp wad punch. A rubber bung was used to support the leaves during punching. The discs were then ejected into small gas tight weighing bottles and the fresh weight of the sample recorded. In experiments where less than 10 disc samples were taken, weighing was recorded to 1/100 th of a milligram and surgical gloves were used for handling the weighing bottles. After fresh weight determinations, the leaf discs were floated on distilled water in a 4 cm closed petri dish and left for 4 hours at 20°C in the dark. The discs were then removed with small forceps and placed on a four-sheet layer of No. 4 Whatman filter paper covered with another four-sheet layer and surface dried by applying a 500 g weight to the top layer for 20 seconds. The sample was then returned to the weighing bottle and the turgid weight recorded. Finally, the samples were placed in a forced-draught oven at 90°C for 48 hours after which dry weights were measured and

the Relative Turgidity expressed as follows (Weatherley 1950):

$$R.T. = \frac{\text{Fresh weight - dry weight}}{\text{Turgid weight - dry weight}} \times 100$$

2.3.5. Water Potential of the Apex.

The water status of the apex was assessed by a modification of the solution equilibration method. The technique was standardized and the details are given in Appendix III. The method employed was as follows.

Five concentrations of polyethylene glycol giving a graded series of osmotic potentials from $^{-}4.9$ to $^{-}6.9$ bars were employed. Apices were dissected under a binocular microscope and cut with a sharp needle at the base of the oldest simple ridge primordium. After measuring the initial apex length, apices were floated on the polyethylene glycol solutions in a closed 4 cm petri dish and left at a constant temperature of 20°C in the dark for 4 hours. Each treatment had three replicates for each solution. The water potential of the tissue was determined by plotting the relative change in apex length and estimating the osmotic potential for zero apex length change.

CHAPTER 3. RESULTS

3.1. Short Period of Water Stress and Growth.

3.1.1. Methods.

The influence of two relatively short periods of water stress of differing severity on the growth and development of two varieties of barley, Prior A (V_1) and CI 3576 (V_2), was investigated. The opportunity was also taken to assess the effects of a pre-sowing hardening treatment (Genkel, see May and Milthorpe 1962) in this system. The general cultural practices and the design of the experiments have been described earlier (see chapter 2.2.1) but further details pertaining to the experiment alone are described here. Ten seeds were sown in each pot and thinned to five, 8 days from planting. Seedling emergence was recorded from the second day after sowing and the final percentage emergence calculated from the total number of seedlings on the eighth day. Harvests were taken at 15, 23, 27, 35 and 48 days from planting. Water stress was imposed by withholding water from the fifteenth day and the timing of the two subsequent harvests was determined by the visible response of the plants to water stress. Harvest 2 was taken when the plants first showed signs of wilting and harvest 3 when the plants failed to recover from wilting overnight. The mild water stress treatment (W_1) ended at harvest 2 when normal watering was resumed and the

severe water stress treatment (W_2) at harvest 3, the non-stressed series (W_0) were watered adequately throughout. The water status of the plants was assessed from leaf relative turgidity measurements taken at each harvest.

The plants at each harvest were dissected to reveal the apex of the main shoot and the total number of primordia, stage of development of the most advanced primordium and the shoot dry weight were recorded.

The total primordium number was assessed as the total number of lateral appendages on the main shoot axis, and the stage of development on the basis of the scale described earlier (sub-chapter 2.3.2). The shoot dry weights were obtained by drying the plant material in a forced-draught oven at 90°C for 48 hours.

3.1.2. Results.

3.1.2.1. Leaf water status.

Water status of the plants was assessed by the relative turgidity measurements on leaves. These measurements were taken for all treatments at each harvest and are given in Table 3.1.1.

Relative turgidity was not affected by the hardening treatment. Water stress reduced the relative turgidity and reduction in both the varieties was significantly apparent in harvest 3. With relief of stress relative turgidity returned to the level of controls.

TABLE 3.1.1

RELATIVE TURGIDITY (MEANS)

Treatments	Harvests				
	1	2	3	4	5
V_1 W_o H	95.6	96.7	85.8	88.1	92.8
V_1 W_1 H	-	96.5	95.1	89.3	95.2
V_1 W_2 H	-	-	74.8	89.3	92.8
V_1 W_o U	97.0	95.6	87.0	89.3	92.7
V_1 W_1 U	-	93.3	96.2	88.5	87.4
V_1 W_2 U	-	-	73.2	90.7	87.5
V_2 W_o H	95.5	93.3	91.7	82.4	83.6
V_2 W_1 H	-	91.0	95.3	86.7	84.7
V_2 W_2 H	-	-	75.8	87.5	82.6
V_2 W_o U	95.4	95.2	87.5	88.0	86.0
V_2 W_1 U	-	88.2	92.4	89.7	86.0
V_2 W_2 U	-	-	70.2	89.0	84.3

Harvest 3 LSD (P = .05) = 4.4

3.1.2.2. Seedling emergence.

Seedling emergence data for each treatment was computed from the means of 56 pots, each containing 10 seeds. The final emergence percentage (Table 3.1.2) was reduced in both varieties by the hardening treatment although the effect on Prior was far more pronounced than on CI 3576. In CI 3576 there was an initial stimulation to emergence due to the hardening treatment, in that 17% of the hardened seedlings

had emerged 2 days after sowing whereas none of the untreated seedlings had emerged at that time.

TABLE 3.1.2

FINAL SEEDLING EMERGENCE

Treatment	Percentage Seedling Emergence
Prior hardened	88.57
Prior unhardened	99.00
CI 3576 hardened	95.57
CI 3576 unhardened	98.71

LSD (P = .001) = 5.31
(P = .05) = 3.09

3.1.2.3. Rate of primordium production.

Hardening had little effect on the number of primordia in either variety (Table 3.1.3) and produced a barely significant increase in primordium number on the third harvest in plants in the mild water stress. These effects were not of a permanent nature and disappeared in the later harvests.

Primordium production in either variety was reduced by water stress. This effect was significantly apparent by Harvest 3.

At Harvest 3, primordium number was least in treatment W_2 but by Harvest 5 the difference between treatments W_1 and W_2 had disappeared, although both were

TABLE 3.1.3

NUMBER OF PRIMORDIA ON MAIN SHOOT

	Harvest 1	Harvest 2	Harvest 3		Harvest 4		Harvest 5	
	Treat. Means	Treat. Means	Treat. Means	Water Means	Treat. Means	Water Means	Treat. Means	Water Means
V_1 W_o H	11.2	15.2	19.8	W_o 20.7	21.8	W_o 22.6	25.0	W_o 26.5
V_1 W_1 H		15.0	19.0	W_1 19.3	21.2	W_1 21.3	24.6	W_1 25.4
V_1 W_2 H		-	17.6	W_2 17.6	20.4	W_2 21.0	24.2	W_2 25.2
V_1 W_o U	11.4	14.6	19.4		21.8		25.0	
V_1 W_1 U		14.2	18.4		21.2		23.4	
V_1 W_2 U		-	17.2		21.0		23.8	
V_2 W_o H	10.4	14.2	21.2		24.0		28.0	
V_2 W_1 H		14.4	21.4		20.8		26.8	
V_2 W_2 H		-	17.6		20.6		25.4	
V_2 W_o U	10.6	14.6	22.4		22.8		28.0	
V_2 W_1 U		14.4	18.6		22.2		27.0	
V_2 W_2 U		-	18.0		22.2		27.4	

Significant Effects:

Harvest 1. Variety*

Harvest 2. Variety x Hardening*

Harvest 3. Variety***
Hardening x Water**
Variety x Water*
Water - LSD Water means only (P = 0.05) = 0.6

Harvest 4 Variety*
Water - LSD Water means only (P = 0.05) = 1.0

Harvest 5. Variety***
Water - LSD Water means only (P = 0.05) = 0.8

* Significant at 5% level
** Significant at 1% level
*** Significant at 0.1% level

significantly less than the control (W_o) series. In this experiment, then, primordium number was lastingly affected by water stress although the two levels of stress did not have a differential effect.

The rates of primordium production in the two varieties varied with time. Initially, the number of primordia was significantly higher in Prior A but with a later increased rate of primordium production in CI 3576 this situation was reversed.

At Harvest 2, there were more primordia on hardened than on unhardened plants in the variety Prior. As with the other effects of hardening, however, the difference was not large and disappeared with time.

3.1.2.4. Apical Development.

The rate of spikelet differentiation was significantly reduced in both varieties by water stress (Table 3.1.4) throughout the experiment. Hardening had little influence apart from a barely significant interaction between water and hardening in Harvest 4.

The rate of spikelet development was significantly higher in CI 3576 initially and remained so until the final harvest. Apart from a slight difference in initial reaction (Harvest 2) both varieties responded similarly to water stress.

TABLE 3.1.4

STAGE OF APICAL DEVELOPMENT

	Harvest 1	Harvest 2	Harvest 3		Harvest 4		Harvest 5	
	Treat. Means	Treat. Means	Treat. Means	Water Means	Treat. Means	Water Means	Treat. Means	Water Means
V_1 W_o H	2.0	2.2	3.2	W_o 3.5	4.8	W_o 5.0	7.4	W_o 7.5
V_1 W_1 H		2.0	3.0	W_1 3.3	4.8	W_1 5.0	7.2	W_1 7.5
V_1 W_2 H		-	3.0	W_2 3.1	4.2	W_2 4.6	7.0	W_2 7.2
V_1 W_o U	2.0	2.0	3.0		5.0		7.0	
V_1 W_1 U		2.0	3.0		5.0		7.2	
V_1 W_2 U		-	3.0		4.8		7.0	
V_2 W_o H	1.8	3.0	4.0		4.6		8.0	
V_2 W_1 H		3.2	3.8		6.4		7.8	
V_2 W_2 H		-	3.2		4.6		7.4	
V_2 W_o U	1.6	3.0	4.0		5.6		7.6	
V_2 W_1 U		3.2	3.6		5.2		7.8	
V_2 W_2 U		-	3.4		5.0		7.4	

Significant Effects:

Harvest 2. Variety***
Variety x Water*

Harvest 3. Variety***
Variety x Water*
Water - LSD Water means only (P = 0.05) = 0.2

Harvest 4. Variety**
Water x Hardening*
Water x Variety x Hardening*
Water - LSD Water means only (P = 0.05) = 0.4

Harvest 5. Variety***
Water - LSD Water means only (P = 0.05) = 0.3

* Significant at 5% level
** Significant at 1% level
*** Significant at 0.1% level

3.1.2.5. Shoot dry weight.

Shoot dry weight was unaffected by the hardening treatment in either variety (Table 3.1.5), was higher in CI 3576 than in Prior in the later harvests (4 and 5) and was reduced by the severe water stress (W_2) alone. This reduction in dry weight due to water stress disappeared once the plants were re-watered. The lack of effect of the mild stress (W_1) on dry weight contrasts with the inhibition of apical development already discussed.

TABLE 3.1.5

MEAN SHOOT DRY WEIGHT PER PLANT ($\log_e$ mg)

Treatments	Harvests 1	2	3	4	5
V_1 W_o H	3.9	4.4	4.6	4.7	5.0
V_1 W_1 H		4.4	4.6	4.7	5.0
V_1 W_2 H		-	4.3	4.7	5.1
V_1 W_o U	4.0	4.4	4.6	4.7	5.1
V_1 W_1 U		4.3	4.6	4.7	5.0
V_1 W_2 U		-	4.1	4.6	4.9
V_2 W_o H	4.0	4.4	4.6	4.8	5.2
V_2 W_1 H		4.3	4.6	4.8	5.0
V_2 W_2 H		-	4.3	4.8	5.0
V_2 W_o U	3.9	4.4	4.6	4.7	5.3
V_2 W_1 U		4.3	4.6	4.8	5.2
V_2 W_2 U		-	4.2	4.7	5.2

Harvests 1 and 2.	Not significant
Harvest 3.	LSD (P = 0.05) = 0.1
Harvests 4 and 5.	Variety Significant at 0.1% level

3.2. Continuous Water Stress and Growth.

3.2.1. Experiment 1.

3.2.1.1. Methods.

The influence of continuous water stress on the growth and apical development of barley (c.v. Prior A) was investigated under a photoperiod of 16 hours of high intensity light (2080 f.c.) in controlled environment conditions. The methods used and the design of the experiment have been described earlier (sub-chapter 2.2.2).

One seed was sown in each pot and harvests were taken at six-day intervals during the duration of the 48-day experiment. Water stress was imposed by withholding water from planting onwards. The treatments were W_o - not stressed, W_1 - stress continued from planting to day 42, and RW - stressed from planting until day 30, thereafter watered as W_o.

The water status of the plants was assessed by leaf relative turgidity measurements. The data on apical development were recorded from day 6 onwards at regular intervals of 6 days whereas growth measurements were only made from day 12 onwards. The following data were taken:

1. Top growth dry weight
2. Individual leaf dry weight
3. Root dry weight
4. Number of expanded and unexpanded leaves
5. Total number of primordia
6. Stage of development of the most advanced primordium on the apex.

The total number of primordia was recorded as the total number of lateral appendages on the main shoot axis and the stage of development on the basis of the scale described earlier (sub-chapter 2.3.2). The dry weights were obtained by the usual technique. The roots were washed as free as possible of adhering soil particles before placing them in a forced-draught oven for drying.

3.2.1.2. Results.

3.2.1.2.1. Leaf water status.

Leaf water status was assessed from relative turgidity measurements taken from day 12 onwards (Fig. 1). Leaf relative turgidity was already reduced by water stress on day 12 and fell continuously with further stress. Initially the rate of fall was slow but accelerated later. On day 24 the relative turgidity was 84% but in a further 18 days it had fallen to 35.4%. With relief of stress, recovery was rapid and within 12 days watered plants were comparable with the not stressed control plants.

3.2.1.2.2. Growth measurements.

Total plant dry weight: Water stress reduced total plant dry weight ($\log_e$), as compared to controls, the difference becoming statistically significant on day 24 and increasing with time (Fig. 2). The production of dry matter ceased entirely from day 30 when the relative turgidity was 73.8% (Plate 1). With the relief of stress at this

PLATE 1

Effect of water stress on growth of barley (c.v. Prior A) 30 days after planting - Continuous water stress experiment 3.2.1.

Left: Not water stressed

Right: Water stressed - water withheld after planting


96
WS

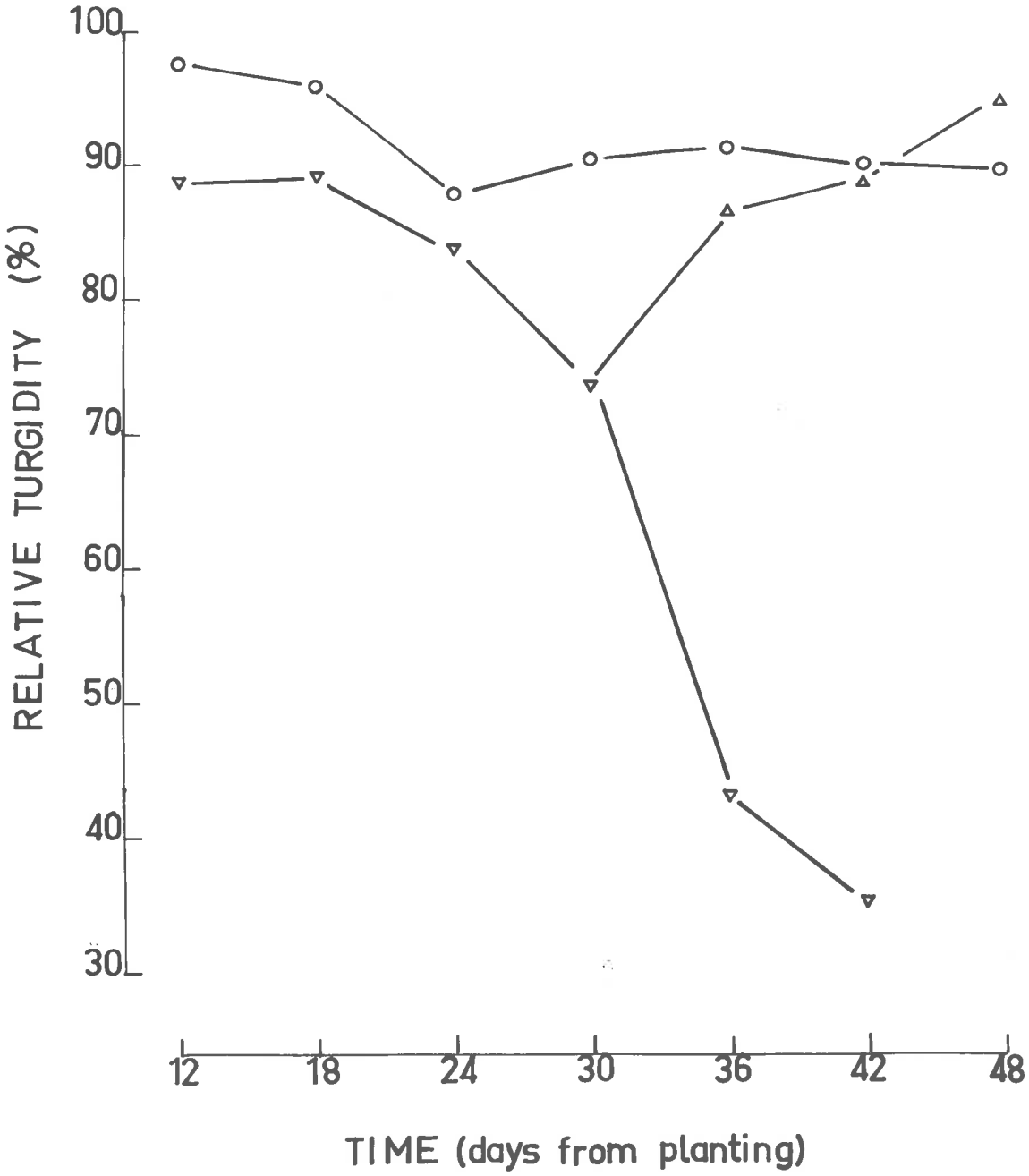
FIGURE 1

Effect of water stress on the relative turgidity of the barley leaf - Continuous water stress experiment 3.2.1.

○ Not water stressed

▽ Water stressed - water withheld after planting

△ Water stressed until day 30, thereafter watering resumed as with non-stressed plants


100
90
80
70
60
50
40
30
RELATIVE TURGIDITY (%)
12
18
24
30
36
42
48
TIME (days from planting)

stage, there was little effect on dry matter production in the first 6 days but a rapid increase later (Plate 2). The total dry weight production of the re-watered plants was, however, significantly lower than that of the control plants and remained so until the final harvest.

Relative growth rates were calculated for each time interval (Table 3.2.1) and illustrate the points noted above. In particular, it may be seen that the relative growth rate of the stressed plants was little affected in the first period (RT 88.6 - 88.9) but fell to very low levels subsequently (RT < 88.9). Growth after re-watering was slight initially (RT 73.8 —— 86.3) but then, for a short period (RT 88.5) was more rapid than that of control plants of the same age. This phase did not last long, however, and the relative growth rates of these plants fell to that of the controls.

TABLE 3.2.1

RELATIVE GROWTH RATES OF TOTAL PLANT DRY WEIGHT (mg/mg./day)

Time Interval (Days)	Treatments		
	Control	Water-stressed	Re-watered
12 to 18	0.159	0.147	
18 to 24	0.150	-0.007	
24 to 30	0.127	0.029	
30 to 36	0.136	0.005	0.003
36 to 42	0.050	-0.004	0.152
42 to 48	0.074	-	0.063

PLATE 2

Effect of water stress on growth of barley (c.v. Prior A) 42 days after planting - Continuous water stress experiment 3.2.1.

Left: Water stressed - water withheld after planting

Middle: Re-watered - water stressed until 30 days from planting, thereafter watering resumed as with non-stressed plants

Right: Not water stressed


74
WS

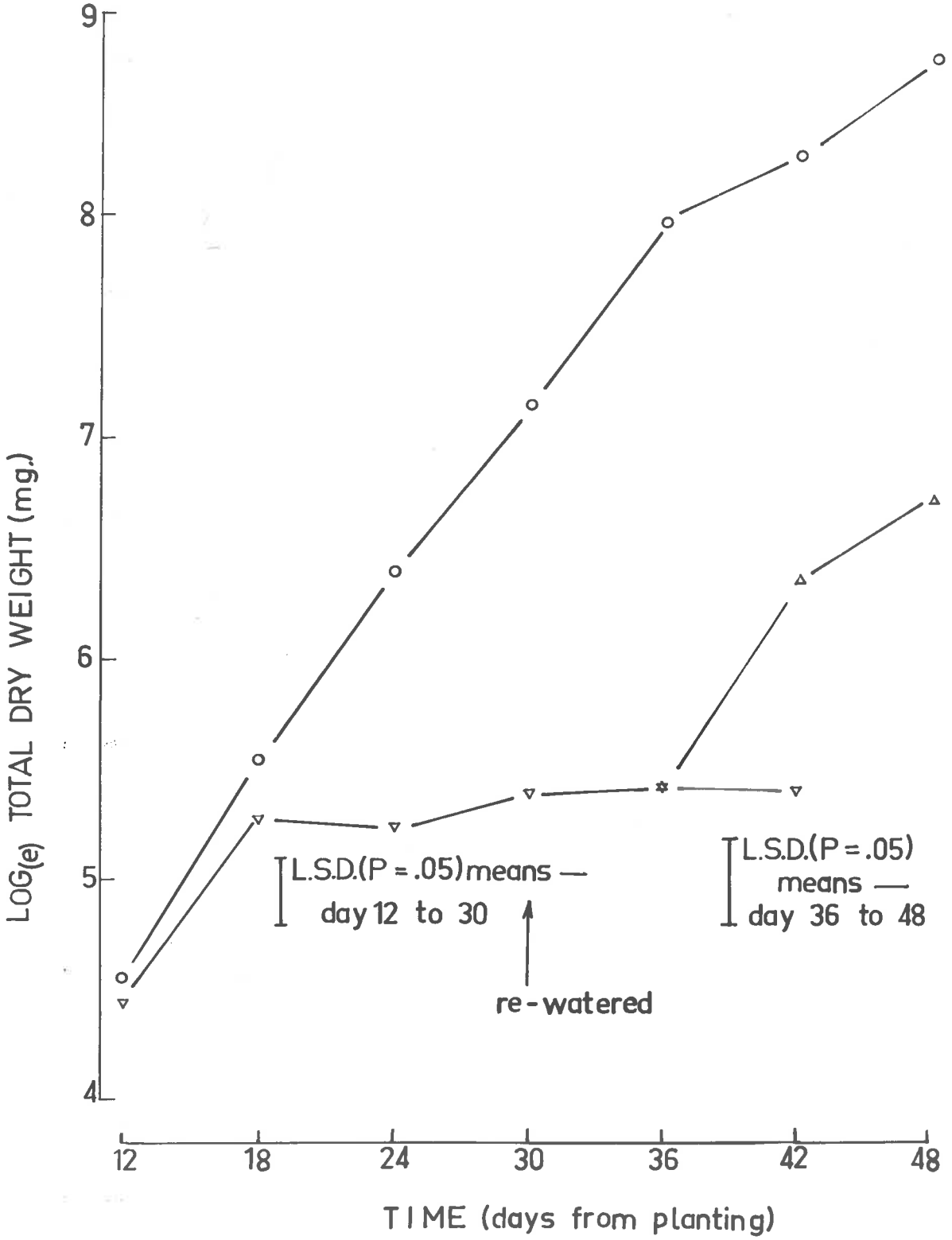
FIGURE 2

Effect of water stress on the production of plant dry weight in barley c.v. Prior A ($\log_e$ mg) - Continuous water stress experiment 3.2.1.

○ Not water stressed

▽ Water stressed - water withheld after planting

△ Water stressed until day 30, thereafter watering resumed as with non-stressed plants


9
8
7
6
5
4
LOG(e) TOTAL DRY WEIGHT (mg.)
L.S.D.(P = .05) means — day 12 to 30
L.S.D.(P = .05) means — day 36 to 48
re-watered
12
18
24
30
36
42
48
TIME (days from planting)

Shoot and root dry weight: Shoot and root dry weight ($\log_e$), the two components of total plant dry weight, were affected differently by water stress (Fig. 3a and 3b). Water stress reduced shoot dry weight from day 24 (RT 84%) and this effect became larger with time. Although production of shoot dry weight was considerably reduced it did not cease until the final harvest (RT 35.4%). On the other hand, root dry weight increase was not inhibited before day 24, but thereafter production of roots ceased entirely with a final loss in dry weight. With relief of stress there was little effect on the dry weight production in the first 6 days in either of the components but a rapid increase was apparent 12 days from re-watering. The dry weight production of both roots and shoots of the re-watered plants remained significantly lower than that of the control plants.

Leaf dry weight: Leaf dry weight ($\log_e$) in regularly watered plants showed a linear increase in the initial phase of plant development (Fig. 4). Later on, the rate of increase fell and between days 36 and 42 there was no apparent increase. Thereafter there was a reduction in leaf dry weight. A significant reduction in leaf growth due to water stress was apparent on day 12. After day 30 (when the relative turgidity had fallen to 73.8%) there was no further increase in leaf dry weight in the stressed plants.

FIGURE 3a. Effect of water stress on the production of shoot dry weight in barley c.v. Prior A ($\log_e$ mg.)

FIGURE 3b. Effect of water stress on the production of root dry weight in barley c.v. Prior A ($\log_e$ mg.)

Continuous water stress experiment 3.2.1.

○ Not water stressed

▽ Water stressed - water withheld after planting

△ Water stressed until day 30, thereafter watering resumed as with non-stressed plants

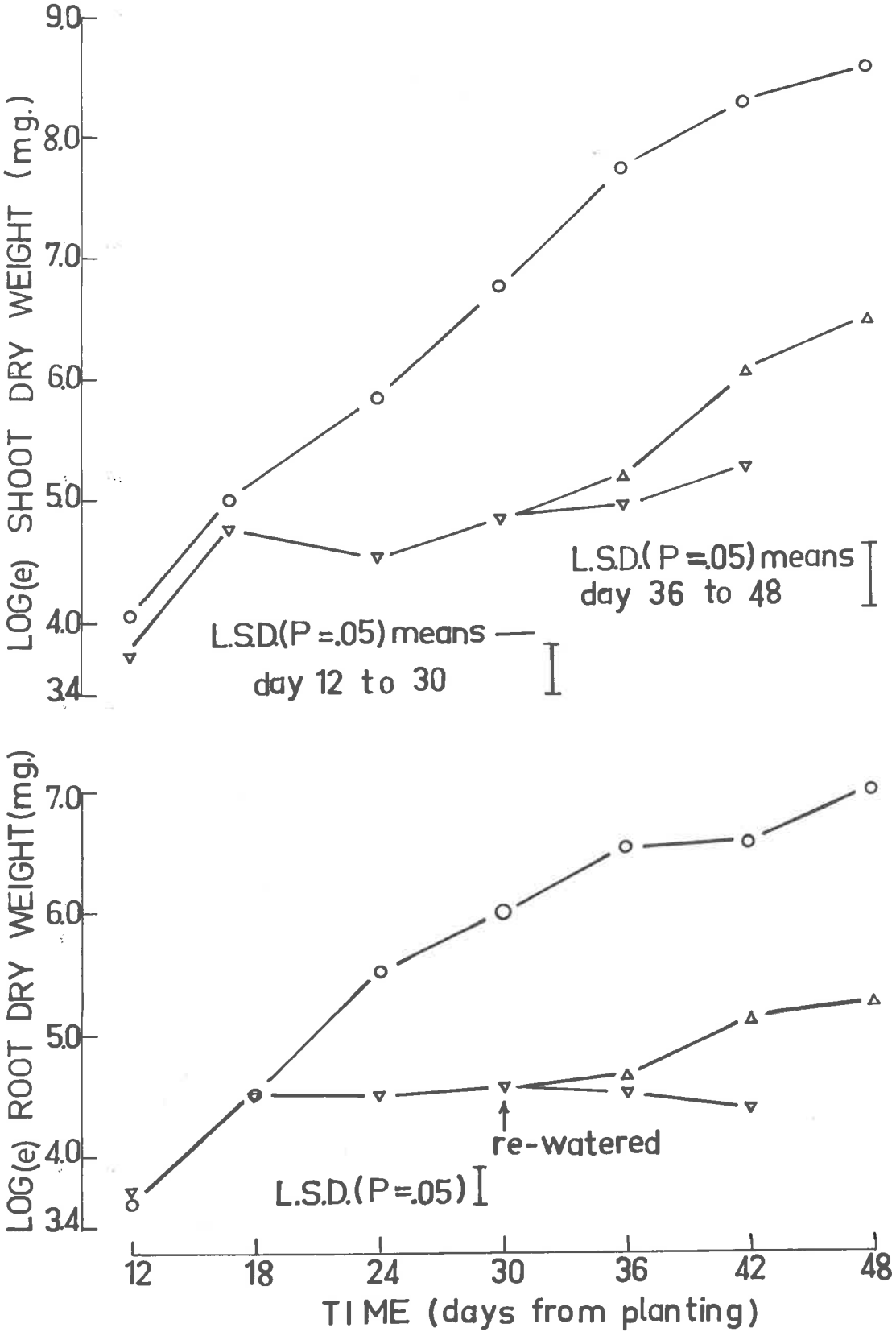

LOG(e) SHOOT DRY WEIGHT (mg.)
9.0
8.0
7.0
6.0
5.0
4.0
3.4
L.S.D.(P=.05) means
day 12 to 30
L.S.D.(P=.05) means
day 36 to 48
LOG(e) ROOT DRY WEIGHT(mg.)
7.0
6.0
5.0
4.0
3.4
re-watered
L.S.D.(P=.05)
12
18
24
30
36
42
48
TIME (days from planting)

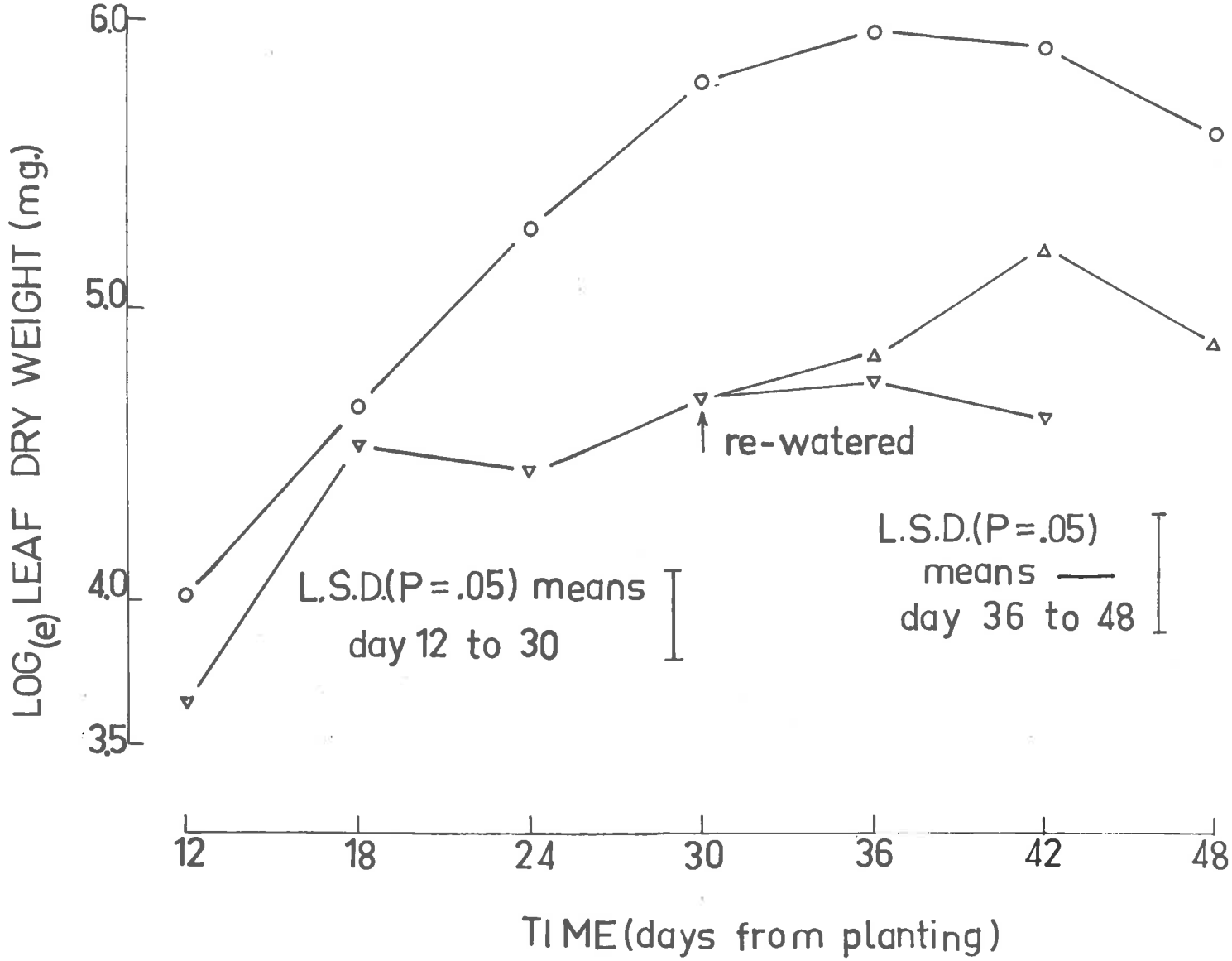
FIGURE 4

Effect of water stress on the production of leaf dry weight ($\log_e$ mg) - Continuous water stress experiment 3.2.1.

○ Not water stressed

▽ Water stressed - watering withheld after planting

△ Water stressed until day 30, thereafter watering resumed as with non-stressed plants


6.0
5.0
4.0
3.5
LOG(e) LEAF DRY WEIGHT (mg.)
re-watered
L.S.D.(P=.05) means
day 12 to 30
L.S.D.(P=.05)
means
day 36 to 48
12
18
24
30
36
42
48
TIME (days from planting)

With re-watering, leaf dry weight increased but total leaf dry weight of re-watered plants remained below that of the controls throughout.

The maximum dry weight attained by each leaf on the stem was also measured (Fig. 5). It may be seen that leaf 1 reached a greater dry weight under water stress than in the control treatment but that the weight of all the subsequent leaves was less than that of the control plants. The difference was greater the higher the leaf was inserted on the stem. With relief of stress, the first two leaves attained a higher dry weight than the control but the other leaves remained considerably reduced in weight.

Leaf development: Both expanded (leaves with the ligule exposed) and unexpanded (including all unexpanded leaves greater than a simple ridge primordium on the apex) leaves were counted in each treatment at each harvest (Table 3.2.2).

It is evident that there was little increase in the total number of leaves from Harvest 1 onwards, indicating that the majority of the leaf primordia had been initiated at the beginning of the period under discussion. Interest centres, therefore, on the effect of water stress on the expansion of these leaf primordia to form leaves. There was a very pronounced reduction in the rate of leaf expansion with water stress although a few leaves continued

FIGURE 5

Maximum leaf dry weight at each position on the main stem -
Continuous water stress experiment 3.2.1.

○ Not water stressed

▽ Water stressed - water withheld after planting

△ Water stressed until day 30, thereafter watering resumed as with non-stressed plants

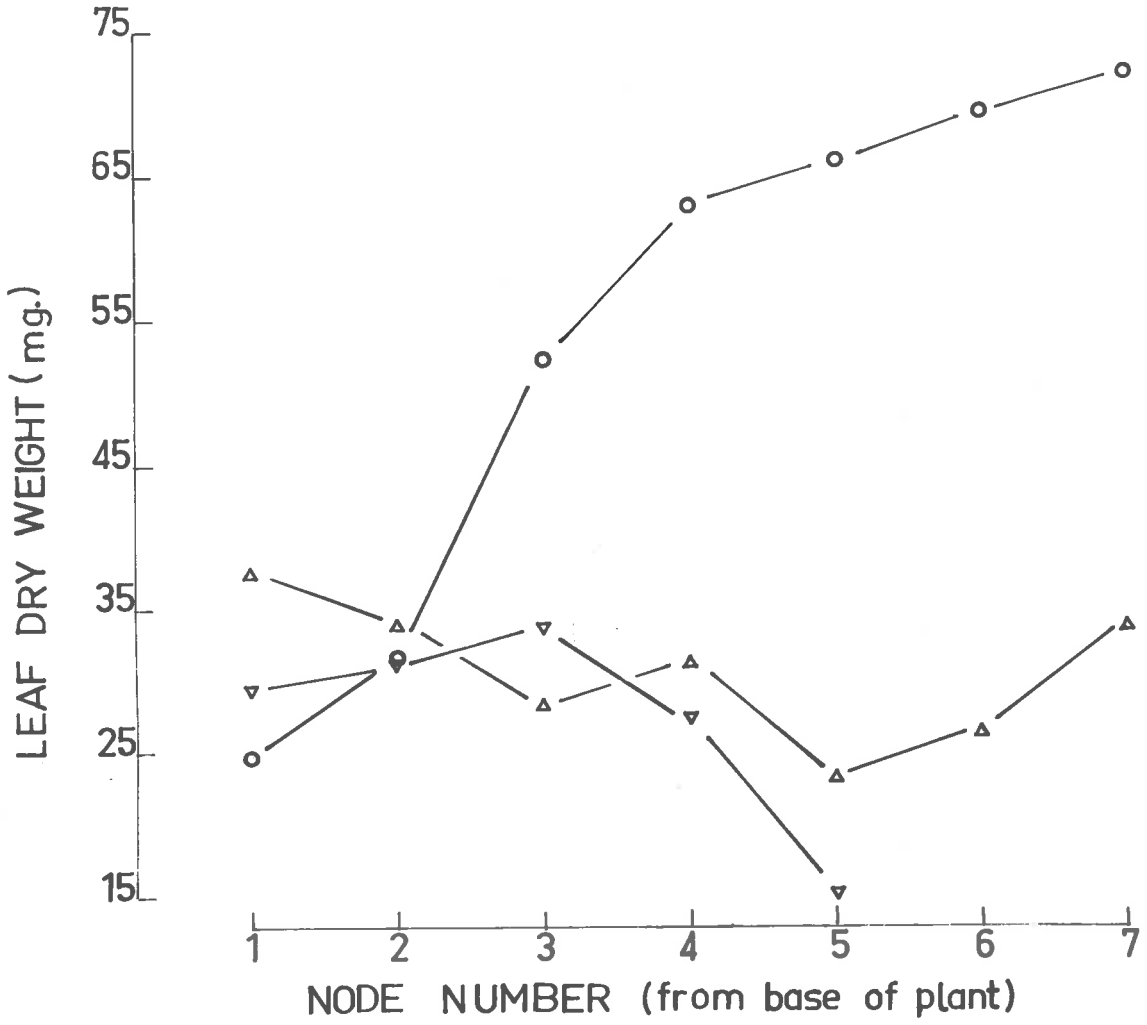

75
65
55
45
35
25
15
LEAF DRY WEIGHT (mg.)
1
2
3
4
5
6
7
NODE NUMBER (from base of plant)

TABLE 3.2.2

NUMBER OF EXPANDED LEAVES

	Harvests							
	1	2	3	4	5	6	7	8
Control	0	0.8	2.8	5.0	7.2	7.4	7.4	7.8
Water-stressed	0	0.4	2.0	2.6	2.6	3.0	3.6	-
Re-watered	-					6.0	7.2	7.0

LSD (P =0.05) Harvest 1-7 (control and water-stressed) = 0.3
Harvest 6-8 (control, water-stressed and re-watered) = 0.2

NUMBER OF UNEXPANDED LEAVES

	Harvests							
	1	2	3	4	5	6	7	8
Control	7.4	8.6	6.0	4.2	1.2	0	0	0
Water-stressed	8.0	9.0	6.4	6.0	6.2	6.0	5.8	-
Re-watered						2.4	0.6	0.4

LSD (P =0.05) Harvest 1-7 (control and water-stressed) = 0.2
Harvest 6-8 (control and re-watered) = 0.6
Harvest 6 and 7 (control, water-stressed and re-watered) = 0.4

to unfold even at the more severe stress levels. Re-watering ~~to~~ previously stressed plants led to an immediate and dramatic expansion of pre-existing unexpanded leaves.

3.2.1.2.3. Apical Measurements.

Primordium production: Initially primordium production was significantly stimulated in the water stress treatment as compared to the control (Fig. 6, day 6); this may not be a strict water-stress response but rather due to increased aeration in the rooting medium. The rate of primordium formation fell considerably with continuing stress, so that the total number of primordia produced in the water stressed plants was significantly lower than that of the control plants. This reduction became more pronounced with increasing stress and from day 18 the formation of primordia completely ceased (RT 88.9). Water stress was relieved on day 30 when the relative turgidity had fallen to 73.8%. Primordium formation resumed with the relief of water stress, the increase over water stress plants being apparent within 6 days. The total number of primordia produced in the re-watered plants remained significantly lower than the controls, however. It was apparent that primordium production on the main apex had ceased in the control and re-watered plants by the end of the experiment. The effects of water stress on this parameter were, therefore, of a permanent nature.

Apical development: The rate of spikelet differentiation was reduced due to water stress by day 12, when the relative turgidity of the stressed plants had fallen to 88.6% as against 96.5% for the controls (Fig. 7).

FIGURE 6

Relationship of primordium number with time during water stress - Continuous water stress experiment 3.2.1.

○ Not water stressed

▽ Water stressed - water withheld after planting

△ Water stressed until day 30, thereafter watering resumed as with non-stressed plants

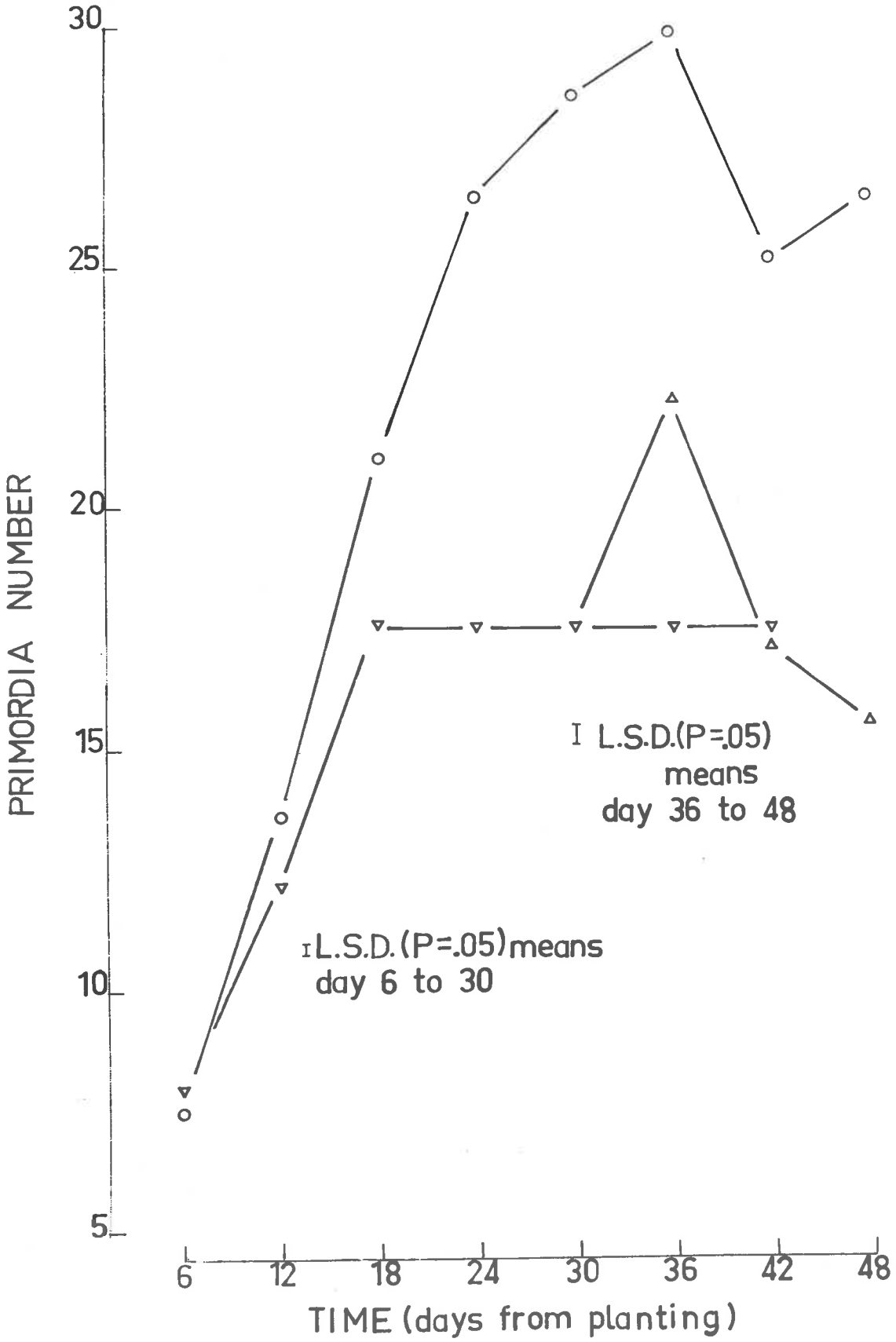

30
25
20
15
10
5
PRIMORDIA NUMBER
I L.S.D.(P=.05)
means
day 36 to 48
I L.S.D.(P=.05)means
day 6 to 30
6
12
18
24
30
36
42
48
TIME (days from planting)

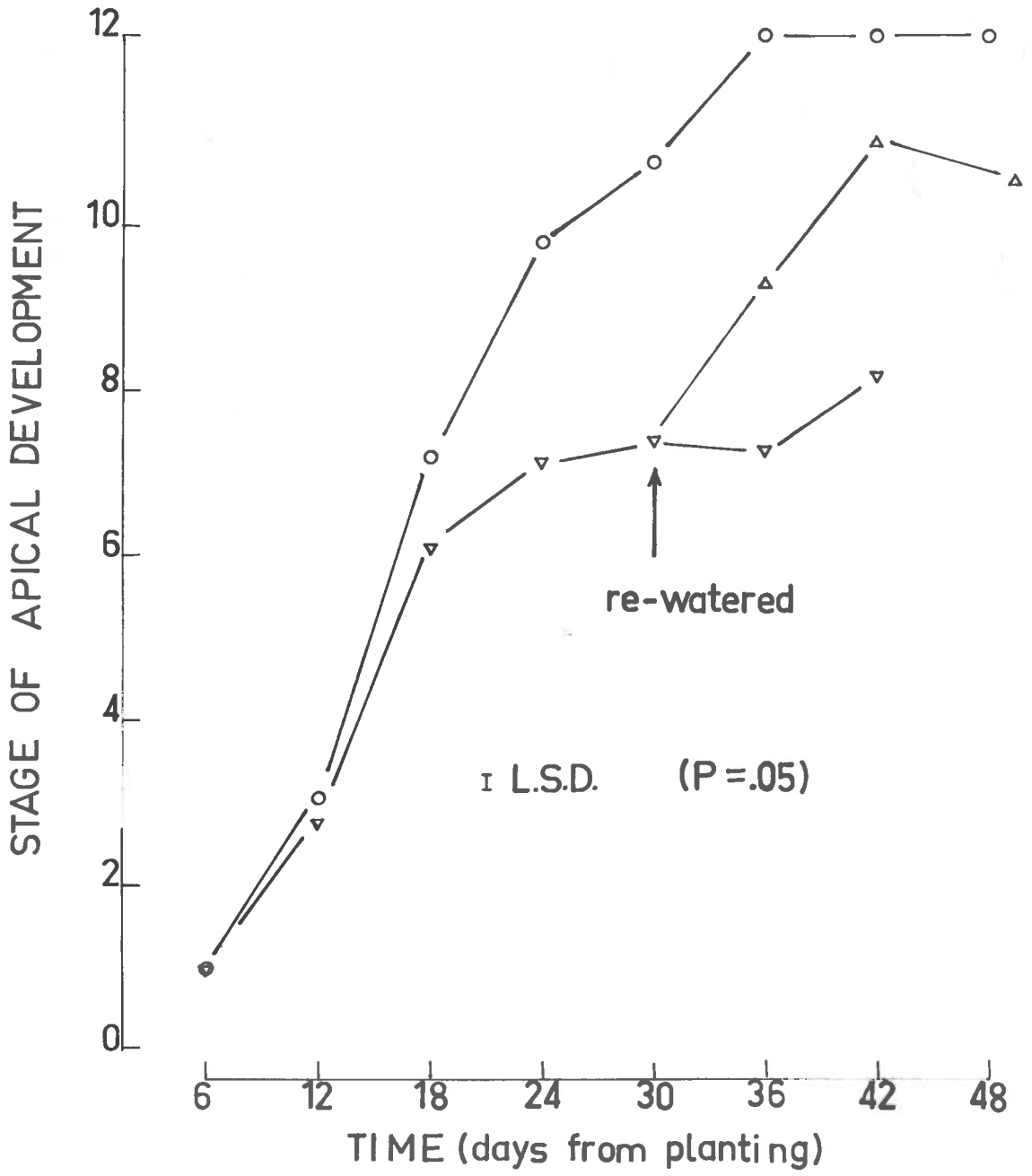
FIGURE 7

Relationship of apical development with time during water stress - Continuous water stress experiment 3.2.1.

○ Not water stressed

▽ Water stressed - water withheld after planting

△ Water stressed until day 30, thereafter watering resumed as with non-stressed plants


STAGE OF APICAL DEVELOPMENT
12
10
8
6
4
2
0
re-watered
I L.S.D. (P=.05)
6
12
18
24
30
36
42
48
TIME (days from planting)

Although water stress significantly retarded apical development, spikelet differentiation, at a reduced rate, continued until the final harvest when the relative turgidity had fallen to 35%. When water stress was relieved the rate of spikelet development increased within the first 6 days from re-watering.

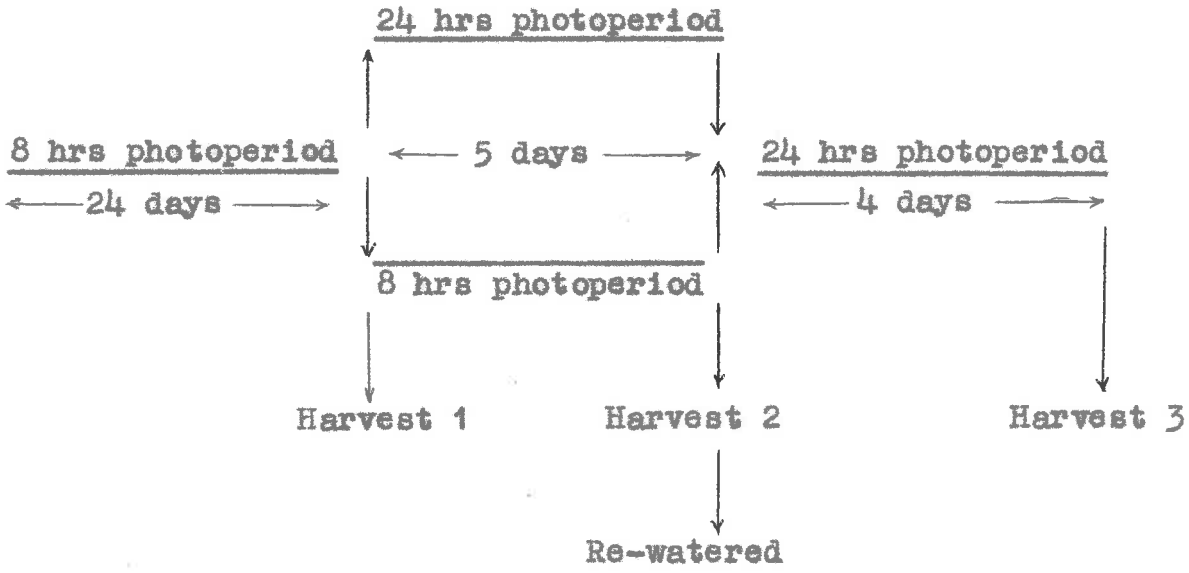
3.2.2. Experiment 2.

It was clear from the foregoing experiment that continuous progressive water stress retarded the development of the barley apex both in terms of primordium production and differentiation of the spikelets. Primordium formation ceased when the leaf relative turgidity fell below 90% but the differentiation of spikelets continued, although at a reduced rate, until the relative turgidity had fallen to 35%. It was possible that this differential response of the two parameters of apical growth to water stress may have arisen from interference with the photoperiodic control of floral initiation. This was investigated by different combinations of water stress and photoperiod in two further experiments.

3.2.2.1. Methods.

The duration of the experiment was 33 days. Two seeds were sown in each pot containing soil mixture (sub-chapter 2.2.2). Water stress was imposed by withholding water soon after planting (W_1), 11 days after planting (W_2), 16 days after planting (W_3), or 24 days after planting (W_4). In the control treatment (W_o) optimum soil water content

was maintained throughout by daily watering. The plants were grown for 24 days in an 8-hour photoperiod of high intensity light (2080 foot candles) whereafter Harvest 1 was taken by harvesting one plant from each pot. The remaining plants were then divided into two groups and exposed to short (L_1) and long (L_2) photoperiods for a further 5 days when Harvest 2 was taken. The short photoperiod consisted of 8 hours light from fluorescent and incandescent sources (2080 f.c.) whereas in the long photoperiod the 8 hours basic photoperiod was extended to 24 hours with incandescent light (80 f.c.). Water stress was relieved following Harvest 2 and the plants were grown for a further 4 days under the long photoperiod, when Harvest 3 was taken. Each treatment was replicated five times and randomized within blocks. A diagramatic representation of the experiment is given below.



3.2.2.2. Results.

3.2.2.2.1. Leaf water status.

Relative turgidity measurements on the leaves were taken from all treatments at each harvest (Table 3.2.3). It is apparent that extension of light period did not affect the water status of the plants.

TABLE 3.2.3

RELATIVE TURGIDITY
(means of 5 samples)

Treatments	Harvests 1	2	3
W_o L_1	95.2	94.0	89.6
W_o L_2		92.0	89.6
W_1 L_1	53.7	50.6	87.0
W_1 L_2		50.4	87.8
W_2 L_1	44.7	46.9	84.0
W_2 L_2		47.0	84.3
W_3 L_1	60.8	54.5	86.5
W_3 L_2		54.0	85.8
W_4 L_1	74.9	58.0	56.2
W_4 L_2		60.3	85.2

3.2.2.2.2. Primordium production.

It may be seen that water stress reduced the rate of primordium production (Table 3.2.4). With increasing stress, the reduction became more pronounced and primordium formation ceased in all but treatment W_4.

TABLE 3.2.4

NUMBER OF PRIMORDIA
(means of 5 samples)

Treatments	Harvests 1	2	3
W_o L_1	17.8	21.0	22.8
W_o L_2		22.4	25.8
W_1 L_1	10.2	10.2	10.6
W_1 L_2		10.4	15.4
W_2 L_1	12.6	12.6	13.0
W_2 L_2		12.6	13.6
W_3 L_1	14.2	14.2	16.0
W_3 L_2		14.4	16.8
W_4 L_1	15.0	16.2	17.4
W_4 L_2		16.8	18.2

Harvest 1. LSD ($P = 0.05$) = 0.9
Harvest 2. LSD ($P = 0.05$) = 1.0
Harvest 3. LSD ($P = 0.05$) = 0.7

In this treatment, the duration of water stress was considerably less than in the others and primordium production continued at a reduced rate. An increase in the photoperiod did not significantly increase the number of primordia in any of the plants during water stress even in treatment W_4. The control plants, however, showed a response. On re-watering primordium formation was resumed and the increased photoperiod during the period of stress resulted in a significant stimulation of the rate of primordium formation.

3.2.2.2.3. Apical development.

Apical development was retarded by the two more severe water stress treatments initially (Table 3.2.5).

TABLE 3.2.5

APICAL DEVELOPMENT
(means of 5 samples)

Treatments	Harvests 1	2	3
W_o L_1	3.0	3.0	7.0
W_o L_2		6.0	8.0
W_1 L_1	1.0	1.0	1.6
W_1 L_2		1.4	3.0
W_2 L_1	2.8	3.0	3.0
W_2 L_2		3.0	4.0
W_3 L_1	3.0	3.0	3.0
W_3 L_2		4.0	4.0
W_4 L_1	3.0	3.0	5.0
W_4 L_2		4.0	6.0

Harvest 1. LSD (P =0.05) = 0.2
Harvest 2. LSD (P =0.05) = 0.9
Harvest 3. LSD (P =0.05) = 0.7

The leaf relative turgidity in these two treatments had fallen below 54% at this time. In the two other stress treatments where relative turgidity had fallen to 60.8% (W_3) and 74.9% (W_4) as against 95.2% in control (W_o) no effect of stress on apical development was apparent. Water stress reduced the subsequent response of the plants to photoperiod.

There was no appreciable increase in development stage between Harvests 1 and 2 in the short photoperiod in any treatment, but a rapid change in the control plants in long photoperiod. In treatments W_1 and W_2 there was no such response to photoperiod and in treatments W_3 and W_4 only a barely significant response. Following relief of the water stress and placing all plants in a long photoperiod, all the treatments showed a significant effect of the previous exposure to long photoperiods. Thus, plants under water stress can apparently be sensitive to photoperiod although the response is not immediately expressed in increased apical development.

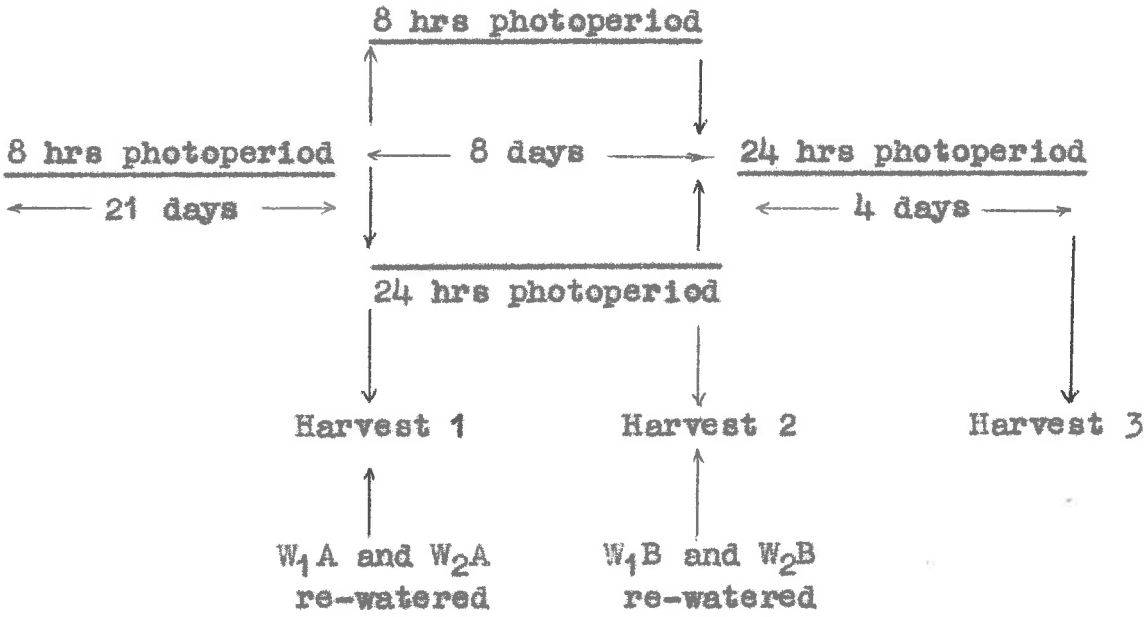
From the above experiment it was evident that water stress reduced the growth of the apex both in terms of primordium production and spikelet development. However, the level of water stress that resulted in a cessation of primordium production did not necessarily stop spikelet development which continued at a reduced rate in some treatments. Since various water stress treatments were imposed by withholding water at different periods prior to the photoperiodic treatments, it is possible that the response to photoperiod may have been influenced by the duration of stress prior to photoperiodic treatment and its effect on the receptive status of the plant rather than by the level of water stress alone. This situation was further investigated under different combinations of the duration of water stress and photoperiod.

3.2.3. Experiment 3.

3.2.3.1. Methods.

The duration of this experiment was 33 days and as in the two foregoing experiments it was conducted in the controlled environment cabinet. Two seeds were sown in each pot containing the usual soil mixture. There were three water regimes. Water stress was imposed by withholding water directly after planting (W_1), and 16 days after planting (W_2). In control pots (W_0) an optimum soil water content was maintained throughout. The plants were grown in an 8-hour photoperiod of 2080 foot candle intensity light for 21 days when Harvest 1 was taken by harvesting one plant from each pot. The two water stress treatments (W_1 and W_2) were divided into two equal batches. In one batch stress was relieved (W_1A and W_2A) before exposing them to two photoperiodic regimes whereas in the other stress was continued (W_1B and W_2B). The plants were then grown for a further period of 8 days in two photoperiodic regimes of short and long photoperiod. The short photoperiod (L_1) consisted of an 8-hour basic photoperiod whereas in the long light period (L_2) the 8-hour photoperiod was extended to 24 hours by low intensity illumination (80 f.c.). Thereafter Harvest 2 was taken and the plants were grown for a further period of 4 days in a long photoperiod after relieving stress in the continued water stress treatments (W_1B and W_2B) before Harvest 3 was taken.

A diagramatic representation of the experiment is given below.



3.2.3.2. Results.

3.2.3.2.1. Leaf Water Status.

Relative turgidity measurements were taken for all the treatments at each harvest and are given in Table 3.2.6. The leaf relative turgidity was considerably reduced in the W_1 treatment but the effect was slight in W_2 in Harvest 1. With release of stress the stressed plants recovered and in Harvest 2 the relative turgidity of re-watered plants was not apparently different to that of regularly watered plants. Again, extension of the light period did not change the relative turgidity of the plants

as compared to that of corresponding water stress treatments in the short photoperiod.

TABLE 3.2.6

RELATIVE TURGIDITY
(means of 5 samples)

Treatments	Harvests 1	2	3
W_o L_1	92.9	88.9	90.5
W_o L_2		92.6	90.6
W_1A L_1	77.2	90.1	91.0
W_1A L_2		89.5	92.6
W_1B L_1		71.0	89.9
W_1B L_2		70.3	89.6
W_2A L_1	89.7	98.3	91.6
W_2A L_2		92.8	90.7
W_2B L_1		76.5	91.5
W_2B L_2		76.6	91.0

3.2.3.2.2. Primordium production.

Immediately prior to the exposure to the long photoperiod, only the severe water stress (W_1) had affected primordium production in comparison to the control treatment (W_o) (Table 3.2.7). At this time the relative turgidity in this treatment was 76%, as compared to 92% in the control and 89% in W_2. Following a further 8 days of stress in the short photoperiod, primordium production ceased in the severely stressed plants (W_1 B L_1) and was

TABLE 3.2.7

NUMBER OF PRIMORDIA
(means of 5 samples)

Treatments	Harvests 1	2	3
W_o L_1	18.2	28.4	37.8
W_o L_2		34.4	39.4
W_1A L_1	12.6	18.0	27.4
W_1A L_2		23.2	29.4
W_1B L_1		12.6	14.2
W_1B L_2		12.8	15.6
W_2A L_1	18.6	27.6	35.0
W_2A L_2		34.8	43.2
W_2B L_1		23.0	28.4
W_2B L_2		26.0	32.6

Harvest 1. LSD ($P = 0.001$) = 4.0
Harvest 2. LSD ($P = 0.05$) = 2.3
Harvest 3. LSD ($P = 0.05$) = 3.2

reduced in the moderately stressed plants (W_2 B L_1). During this period, an increase in the photoperiod had no effect on primordium production in the severely stressed plants (W_1 B L_2) but stimulated initiation in the less severely stressed plants (W_2 B L_2). Re-watering the plants prior to exposure to the long photoperiod resulted in approximately the same photoperiodic stimulation to primordium production as in the control series (W_o - stimulation due to long photoperiod - 6.0, W_1A - 5.2, W_2A - 7.2). After all the plants

had been re-watered and placed in the long photoperiod for a further 4 days, it was apparent that any response to photoperiod had been suppressed by the severe water stress (W_1B) whereas all the other treatments demonstrated a response. It would appear, therefore, that although prior water stress does not render a plant incapable of responding to photoperiodic stimulation, a severe stress during exposure to long days can completely prevent any response.

3.2.3.2.3. Apical Development.

The overall responses of spikelet development in this experiment (Table 3.2.8) were very similar to

TABLE 3.2.8

STAGE OF APICAL DEVELOPMENT
(means of 5 samples)

Treatments	Harvests 1	2	3
W_o L_1	3.0	4.0	6.0
W_o L_2		6.6	7.8
W_1A L_1	2.0	3.0	4.6
W_1A L_2		5.2	7.0
W_1B L_1		2.2	2.6
W_1B L_2		2.6	3.0
W_2A L_1	3.0	3.4	6.0
W_2A L_2		6.6	8.0
W_2B L_1		3.0	5.0
W_2B L_2		5.0	6.9

Harvest 1. LSD ($P = 0.05$) = 0
Harvest 2. LSD ($P = 0.05$) = 2.2
Harvest 3. LSD ($P = 0.05$) = 2.2

those of primordium production already discussed. Thus, severe stress (W_1) delayed development initially and prevented any response to photoperiod either immediately following exposure to the long photoperiod or following re-watering. The moderate stress (W_2) permitted a reduced response to photoperiod and re-watering prior to exposure to the long photoperiod restored the response more or less completely.

3.2.3.2.4. Shoot dry weight.

It was apparent in the previous experiments that dry matter production was significantly reduced by water stress. The possibility that the extended light period reduced this adverse effect of water stress rather than acting through a photoperiodic mechanism was investigated by measuring shoot dry weight (Table 3.2.9).

Water stress significantly reduced shoot dry weight but extension of the light period had no effect. The effect of long photoperiod on apex growth was evidently mediated through a photoperiodic effect alone rather than through an influence on overall plant growth.

TABLE 3.2.9

MEAN SHOOT DRY WEIGHT PER PLANT ($\log_e$ mg.)

Treatments	Harvests 1	2	3
W_o L_1	4.6	5.6	6.1
W_o L_2	-	5.6	6.0
W_1A L_1	3.7	4.2	4.9
W_1A L_2		4.2	4.7
W_2A L_1	4.6	5.4	5.9
W_2A L_2		5.5	6.1
W_1B L_1		3.7	4.0
W_1B L_2		3.7	3.9
W_2B L_1		4.9	5.3
W_2B L_2		4.9	5.3

Harvest 1. LSD ($P = 0.05$) = 0.2
Harvests 2 and 3. LSD ($P = 0.05$) = 0.3

3.3. Water Stress and Photoperiodic Induction

The effects of water stress on apical growth and morphogenesis as reported in the preceding section (3.2) strongly suggested an interference with the photoperiodic response. Lolium temulentum, a qualitative long day plant requiring one long day for induction (Evans 1958) was chosen to further investigate this possibility. Plants were subjected to various periods of water stress before, during and following an inductive treatment (Evans 1960c).

The general cultural practices and the design of the experiments have been described earlier (sub-chapter 2.2.3)

but further details pertaining to each experiment are described along with the respective experiment.

3.3.1. Preliminary experiment on the effect of water stress.

3.3.1.1. Methods.

Plants were grown in an 8-hour photoperiod of 2080 f.c. light intensity until the sixth leaf stage. These were then exposed to one inductive cycle consisting of a 16-hour extension of the basic photoperiod with incandescent light of 80 f.c. intensity. The plants were then returned to the 8-hour photoperiod environment and grown for a further three weeks when the apices were dissected and both the stage of morphological development and the apex length recorded. Water stress was imposed by the application of five (W_1) and 10 (W_2) atmosphere osmotic potential polyethylene glycol solutions to the rooting medium 24 hours before the inductive cycle (D_1), during induction (D_2) and at the expiry of the inductive cycle (D_3). Water stress was of 24 hours duration in each case. In addition one set of plants was not stressed (W_0) and was grown with the rest under the same conditions.

3.3.1.2. Results.

3.3.1.2.1. Leaf water status.

Leaf water status was assessed from relative turgidity measurements at 1, 4, 8 and 24 hours after the imposition of stress (Table 3.3.1).

TABLE 3.3.1

RELATIVE TURGIDITY

Treatments	Time of sampling after imposition of the stress			
	1 hour	4 hours	8 hours	24 hours
Control	93.8	94.6	92.8	94.6
5 atmosphere stress	93.8	93.6	92.6	89.8
10 atmosphere stress	93.2	92.0	91.0	89.6

LSD (P = 0.05) = 1.6

Relative turgidity was reduced by water stress but it was only after 24 hours of stress that the relative turgidity fell below 90%.

3.3.1.2.2. Apical development.

There was no significant effect of water stress on spikelet development (Table 3.3.2).

TABLE 3.3.2

Treatments	Stage of development (means of 25 samples)
W_o	3.4
W_1 D_1	3.4
W_2 D_1	3.7
W_1 D_2	3.8
W_2 D_2	3.8
W_1 D_3	3.7
W_2 D_3	3.4
	Not significant

It is apparent that water stress which reduced the relative turgidity of the plants to 89.6% before, during or after an inductive cycle did not affect development of the spikelets.

3.3.1.2.3. Apex length.

In contrast to apical development, apex length was significantly reduced by water stress (Table 3.3.3).

TABLE 3.3.3

Treatments	Apex length (mm) (means of 25 samples)
W_o	1.48
W_1 D_1	1.21
W_2 D_1	1.18
W_1 D_2	1.23
W_2 D_2	1.16
W_1 D_3	1.29
W_2 D_3	1.15

LSD (P = 0.05) = 0.08

This reduction was more pronounced with severe stress (W_2) but the effects of the two stress treatments were only significantly different when stress was applied after the inductive cycle (D_3). It is of interest that all the water stress treatments, including those imposed before the inductive cycle, reduced apex length. This suggests that this effect, noted in the barley experiments in the form of a reduction in primordium production, may be unrelated to floral induction.

3.3.2. Effect of water stress on generation of the floral stimulus.

Osmotic stress of 5 or 10 atmospheres which reduced leaf relative turgidity to a minimum of 89.6% had no effect on photoperiodic induction. This is a relatively mild water stress, however, and it was decided to further explore the effects of stress on photoperiodic induction by exposing the plants to osmotic pressures of 18 and 24 atmospheres in the rooting medium.

3.3.2.1. Methods.

Lolium temulentum plants were subjected to 18 (W_1) and 24 (W_2) atmosphere osmotic potentials polyethylene glycol solutions during an inductive cycle in an 8-hour photoperiod of 2080 f.c. light intensity and compared with plants which were not stressed (W_o). All plants were grown until the sixth leaf expanded fully and were then reduced to the sixth leaf before exposure to an inductive cycle. The inductive cycle consisted of an extension of the basic photoperiod with 16 hours of incandescent light of 80 f.c. intensity. Water stress was imposed two hours before the extension of the photoperiod and released two hours before (T_1) and at the end (T_2) of the extended light period. The stage of morphological development of each apex together with its length was recorded three weeks after the inductive treatment.

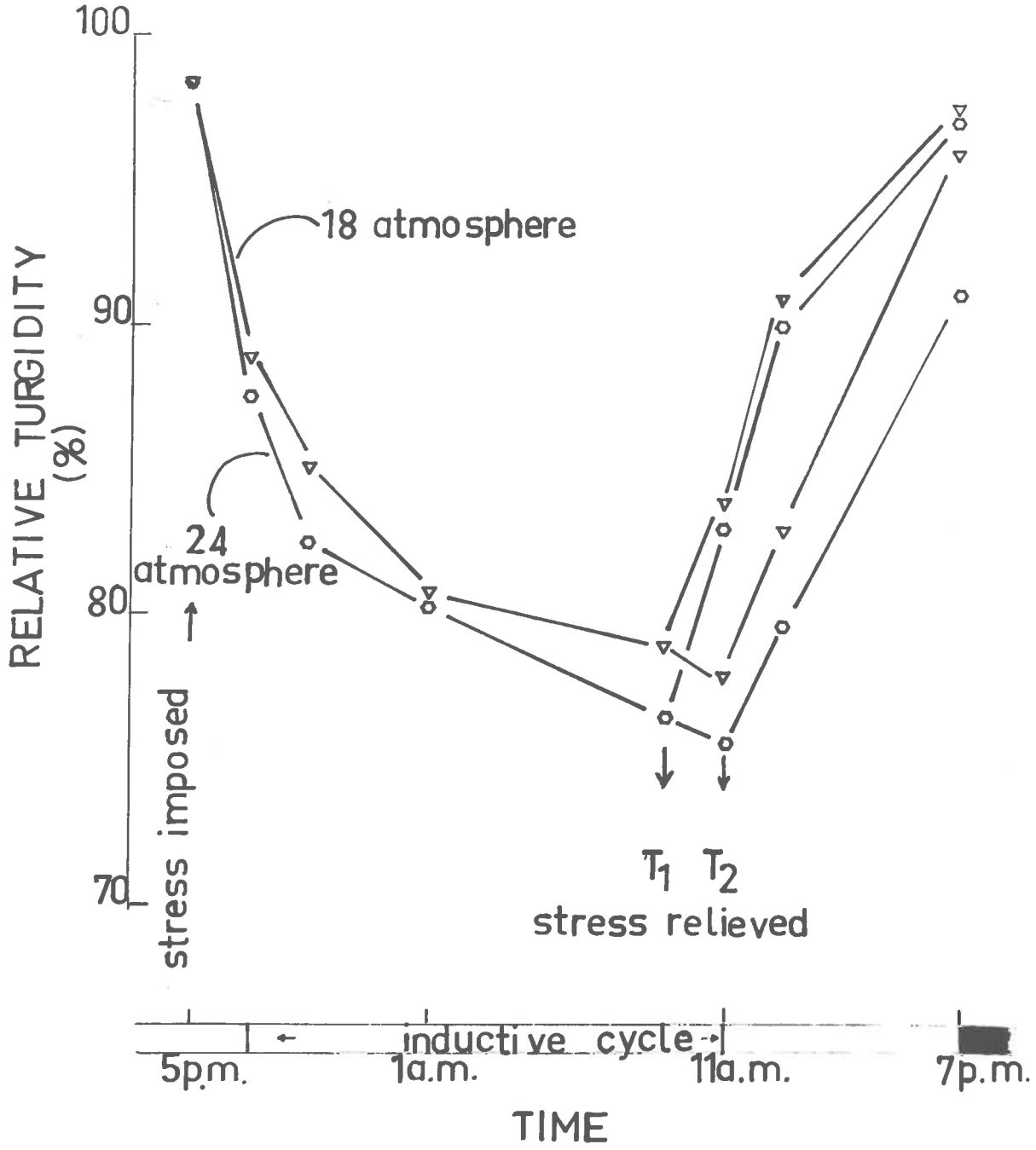
3.3.2.2. Results.

3.3.2.2.1. Leaf water status.

Leaf relative turgidity was reduced from 98 to 89 and 87% by 18 and 24 atmospheres osmotic potential, respectively, two hours after imposition of the stress (Fig. 8). A maximum reduction to 78 and 75% was produced by the two stress treatments in 18 hours. On release of the stress, recovery of leaf turgidity was rapid and after four hours values returned to 90% excepting in W_2T_2 treatments where it took six hours to reach that level.

FIGURE 8

Relative turgidity of *Lolium temulentum* leaf retained during exposure to long photoperiod with water stress - Water stress and photoperiodic induction experiment 3.3.2.


100
90
80
70
RELATIVE TURGIDITY (%)
18 atmosphere
24 atmosphere
stress imposed
T1 T2
stress relieved
inductive cycle
5p.m.
1a.m.
11a.m.
7p.m.
TIME

3.3.2.2.2. Apical development.

Water stress significantly reduced spikelet development (Table 3.3.4), the two water stress treatments being equally effective in reducing development. The majority of the stressed plants had not initiated double ridges (score 3) by the time that the harvest was taken, suggesting that water stress had prevented floral induction.

TABLE 3.3.4

STAGE OF APICAL DEVELOPMENT

Treatments	Stage of development (means of 25 samples)
W_o	4.8
W_1 T_1	2.3
W_2 T_1	2.4
W_1 T_2	2.3
W_2 T_2	2.0

LSD (P =0.05) = 0.6

The time of relief of stress did not have a significant effect on the response of the plants. This indicates that synthesis of a floral stimulus during the final two hours of the extended light period was not the critical factor affected by water stress.

3.3.2.2.3. Apex length.

Apex length was significantly reduced by water stress (Table 3.3.5). This reduction was similar in the case of mild stress (W_1) treatment regardless of the

TABLE 3.3.5

Treatments	Apex length (mm) (means of 25 samples)
W_o	1.58
W_1 T_1	1.26
W_2 T_1	1.27
W_1 T_2	1.29
W_2 T_2	1.18

LSD (P = 0.05) = 0.08

time when stress was relieved. On the other hand, in the severe water stress (W_2) treatment, there was a significant effect of the time of relief of stress. A further reduction in apex length was produced in W_2T_2 treatment as compared to W_2T_1. This may have been related to the slower recovery of leaf relative turgidity in this treatment as compared with all others (Fig. 8).

3.3.3. Effect of water stress on the translocation of floral stimulus.

3.3.3.1. Methods.

The effect of water stress during the known period of translocation of floral stimulus (Evans 1960c) was examined in the following experiment. The plants were grown in an 8-hour photoperiod until the sixth leaf was expanded. All the plants were then reduced to the sixth leaf before exposure to an inductive cycle of 16 hours of low intensity incandescent light. These plants were then grown for a

further three weeks in the 8-hour basic photoperiod after which each apex was dissected. Water stress was imposed by the application of polyethylene glycol solutions of 18 (W_1) and 24 (W_2) atmospheres osmotic potential to the rooting medium 2 hours before the expiry of the inductive cycle and was relieved 2 (RL_1), 6 (RL_2) and 10 (RL_3) hours later during the light period following the inductive cycle. The long day leaf that had generated the floral stimulus was removed simultaneously with the relief of stress. Translocation of the floral stimulus in relation to various periods of stress was thus estimated along with the non-stressed plants (W_o) where the induced leaf was removed in the same sequence. A diagramatic representation of the experiment is given below.

Inductive Cycle	Light		Dark
9 a.m.	11 a.m.	3 p.m.	7 p.m.
↑ Water stress (imposed)	↑ Water stress relieved and leaf defoliated simultaneously (RL_1)	↑ Water stress relieved and leaf defoliated simultaneously (RL_2)	↑ Water stress relieved and leaf defoliated simultaneously (RL_3)

3.3.3.2. Results.

3.3.3.2.1. Leaf water status.

Water stress reduced the relative turgidity to 87.5 (W_2) and 88% (W_1) after two hours of imposition

of the stress (Fig. 9). Again, with relief of stress, recovery was rapid and the values returned to above 90% after four hours. For measurement of relative turgidity, leaves were retained on plants after the relief of stress.

3.3.3.2.2. Apical development.

Water stress significantly reduced spikelet development (Table 3.3.6). Reduction in the stage of development was of about the same order with both stress treatments. Time of leaf removal had a profound influence on the flowering response. Apical development was significantly increased the later the leaf was removed in the control treatment (W_0). On the other hand, time of leaf defoliation had no effect in either of the stress treatments. It may also be seen that, in the two stress treatments (W_1 and W_2), the stage of development did not reach score 3 corresponding to the double ridge stage.

TABLE 3.3.6

STAGE OF APICAL DEVELOPMENT
(means of 25 samples)

Stress	Time of Leaf Removal		
	2 hours	6 hours	10 hours
Control	2.2	2.6	3.1
18 atmospheres	2.0	2.1	2.1
24 atmospheres	2.0	2.0	2.0

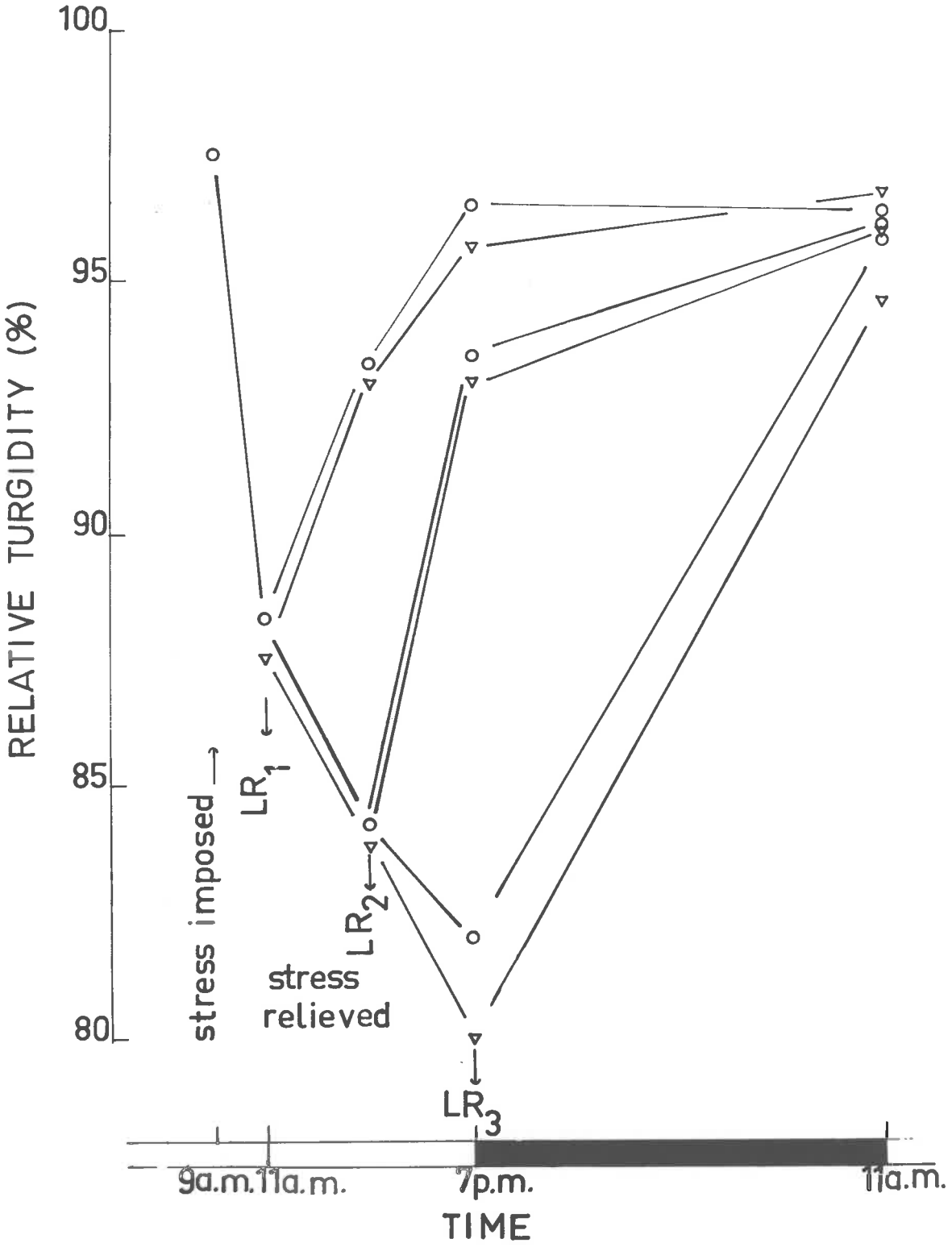
LSD (P =0.05) = 0.1

FIGURE 9

Relative turgidity of *Lolium temulentum* leaf with water stress during the translocation of floral stimulus - Water stress and photoperiodic induction experiment 3.3.3.

○ Water stress imposed by 18 atmospheres polyethylene glycol solution

▽ Water stress imposed by 24 atmospheres polyethylene glycol solution


100
95
90
85
80
RELATIVE TURGIDITY (%)
stress imposed
LR1
LR2
stress relieved
LR3
9a.m.
11a.m.
7p.m.
11a.m.
TIME

3.3.3.2.3. Apex length.

Apex length was significantly reduced by both of the water stress treatments (Table 3.3.7).

TABLE 3.3.7

APEX LENGTH (mm.)
(means of 25 samples)

Stress	Time of Leaf Removal		
	2 hours	6 hours	10 hours
Control	1.18	1.29	1.39
18 atmospheres	1.14	1.14	1.17
24 atmospheres	1.12	1.15	1.19

LSD (P =0.05) =0.06

Water stress, both the mild (W_1) and severe (W_2) levels, reduced apex length and prevented any increase in length with longer retention of the induced leaf, a response apparent in the control (W_0) series. The reduction due to water stress (in comparison with the control series) was statistically significant in all but the plants in the mild stress series where the induced leaf was removed after two hours.

It is clear from the results of the foregoing experiment that retention of the induced leaf in the control treatment significantly increased the development of the apex both in terms of morphological development and apex length. This indicates that no morphogenetic stimulus was

being translocated from the leaf to the apex during the period of water stress.

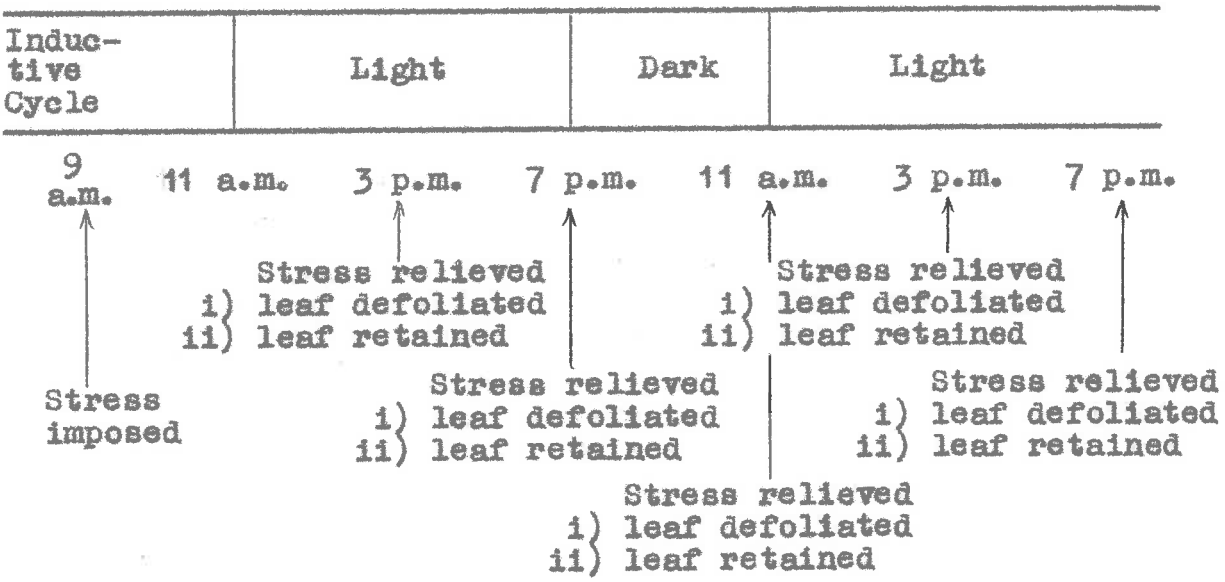
3.3.4. Effect of water stress on the stability of the floral stimulus.

The previous experiments with *Lolium temulentum* can be interpreted as indicating that water stress can inhibit both the process of floral induction in the leaf and the translocation of the floral stimulus to the apex. A further possible effect of water stress would be on the stability of the floral stimulus. Thus, water stress subsequent to floral induction, which prevents movement of the floral stimulus out of the leaf, might lead to destruction of the stimulus in the induced leaf. This possibility was examined in the final experiment with *Lolium temulentum*.

3.3.4.1. Methods.

Plants were subjected to combinations of water stress and defoliation of the induced leaf during the translocation period. Water stress was imposed by the application of a polyethylene glycol solution of 24 atmospheres osmotic potential to the rooting medium 2 hours before the expiry of the extended light period and relieved after 6, 10, 26, 30 or 34 hours. The plants were divided into three main groups of which two were stressed and one was not stressed. In one of the stressed groups the induced leaf was removed simultaneously with the relief of stress. In the

alternative group the leaf was retained and, thus, provided a source of stimulus which, if not dissipated during stress, would be translocated when the plant was re-watered. Leaves were removed in the same sequence in the third group of plants which were not stressed. One further set of plants was not subjected to stress and the induced leaf was not removed. The treatments are summarized in the diagram below.



3.3.4.2. Results.

3.3.4.2.1. Leaf water status.

Water stress reduced the relative turgidity to 80.5% after 6 hours of imposition of the stress (Table 3.3.8). A maximum reduction to 62.5% was produced by the stress treatment in 34 hours.

TABLE 3.3.8

RELATIVE TURGIDITY

	Time after imposition of the stress				
	6 hrs	10 hrs	26 hrs	30 hrs	34 hrs
24 atmospheres	80.5	79.5	73.9	70.6	62.5

3.3.4.2.2. Apical development.

As in the previous experiment, where the induced leaf was defoliated simultaneously with relief of the water stress, there was no floral induction, indicating that stress prevented translocation of the floral stimulus out of the leaf (Fig. 10). On the other hand, retention of the induced leaf after relief of the stress resulted in floral differentiation and increased development of the apex. Thus, the inductive stimulus retained in the leaf during water stress was not completely destroyed during this period. Even where the leaf was retained, however, stress reduced the subsequent floral development of the apex, particularly if the stress was not relieved within four hours following induction. As this effect did not increase after the first four hours of stress, it is possible that it is indicative of an inhibition of the formation of floral stimulus rather than of a stimulation of its breakdown. In contrast, in plants not subjected to water stress, the longer the leaf was retained the greater the apical development, indicating the course of translocation

FIGURE 10

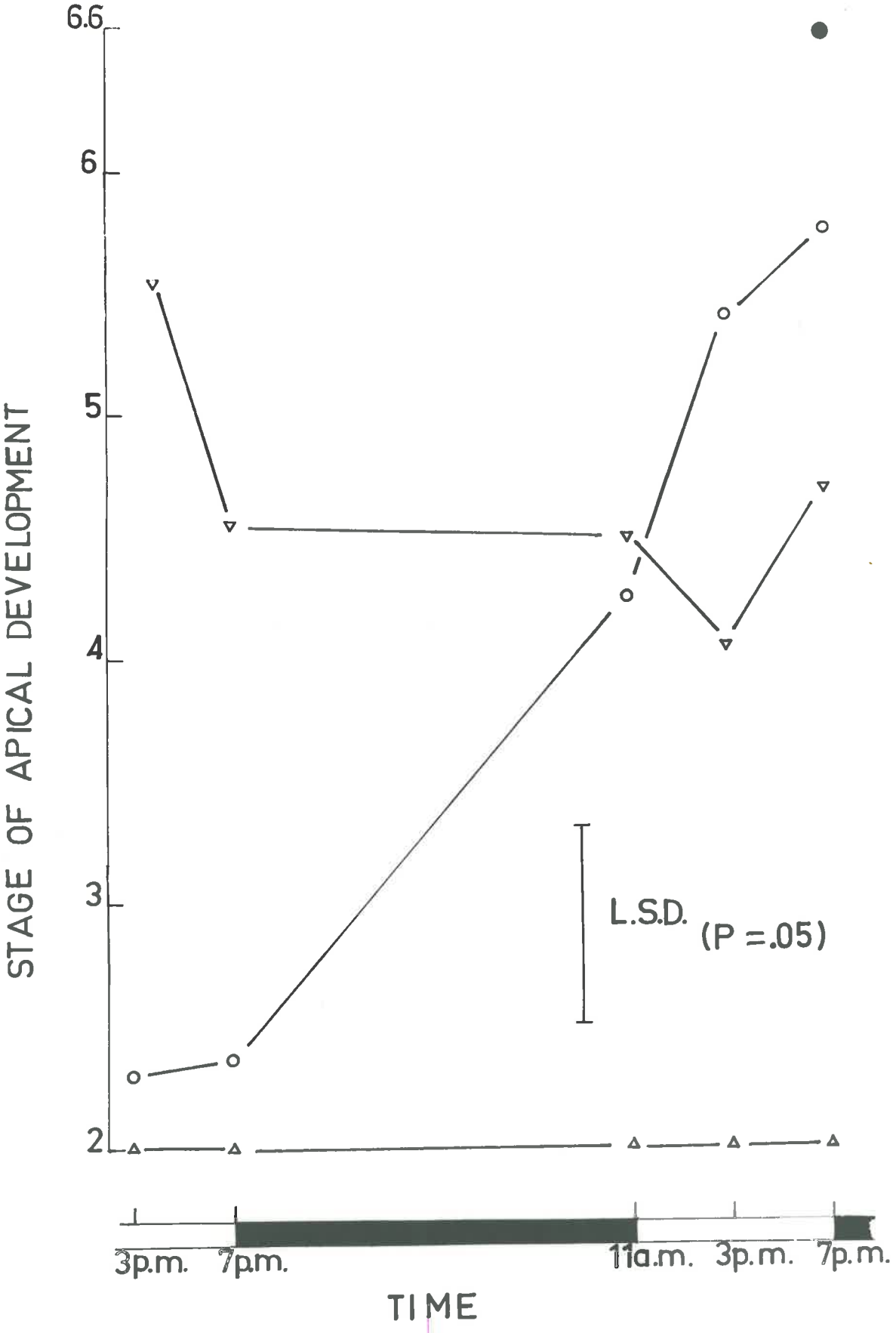
The effect of water stress and defoliation during translocation of the floral stimulus on floral development - Water stress and photoperiodic induction experiment 3.3.4.

▽ Water stress imposed on the roots by the application of a polyethylene glycol solution of 24 atmospheres osmotic potential, plants not defoliated

△ Water stressed, plants defoliated after stress relieved

○ Not water stressed, plants defoliated at same times

● Not water stressed, plants not defoliated as in previous treatment


6.6
6
5
4
3
2
STAGE OF APICAL DEVELOPMENT
L.S.D. (P =.05)
3p.m.
7p.m.
11a.m.
3p.m.
7p.m.
TIME

of the floral stimulus from the leaf. Retention of the leaf beyond 32 hours had a barely significant stimulative effect.

3.3.4.2.3. Apex length.

The data for the apex length measurements (Table 3.3.9) parallel completely the development of the apex. Thus, water stress followed by defoliation inhibited elongation. Stress without defoliation allowed apical elongation, particularly when the stress was relieved early. Apical elongation of non-stressed plants was greater the later they were defoliated. These support the conclusions from the development data.

TABLE 3.3.9

APEX LENGTH (mm)
(means of 25 samples)

Treatments	Hours following induction				
	4	8	24	28	32
Stressed not defoliated	1.62	1.48	1.44	1.38	1.46
Stressed defoliated	1.04	1.03	1.09	1.07	1.06
Not stressed defoliated	1.10	1.27	1.33	1.39	1.56
Not stressed and not defoliated	1.83	1.83	1.83	1.83	1.83

LSD (P = 0.05) = 0.14

3.4. Effect of water stress on the water status of the apex.

From the results of the foregoing experiments it was concluded that water stress imposed during the generation and translocation of the floral stimulus inhibited both

processes. A further interpretation is possible, however, in that water stress may directly lower the capacity of the apex to respond to the floral stimulus. Such an effect must result from a change in the water status of the apical tissues, and this was measured directly.

3.4.1. Effect of short term water stress.

3.4.1.1. Methods.

The experiment was conducted in a controlled environment cabinet with an 8-hour photoperiod (2080 f.c.) at 17°C. Barley plants (c.v. Prior A) were grown in plastic pots containing a vermiculite and sand mixture saturated with nutrient solution and water daily. Water stress was imposed three weeks after planting by the application of a polyethylene glycol solution of 18 atmospheres osmotic potential to the rooting medium. Plants were harvested 8 and 24 hours after imposition of the stress when the water potential of the apex and the relative turgidity of the leaves were measured. The water potential of the apex was measured by the solution equilibration method which has been described in detail earlier (sub-chapter 2.3.5).

3.4.1.2. Results.

3.4.1.2.1. Leaf water status.

Relative turgidity of leaves was considerably reduced due to stress (Table 3.4.1). After 8 hours

of stress, it fell to 78.2% as compared to 98.2% in the non-stressed plants at the same time. With increasing duration of stress, relative turgidity fell further and after 24 hours of application of the stress it was reduced to 75%.

TABLE 3.4.1

RELATIVE TURGIDITY

Treatments	Hours after imposition of the stress	
	8	24
Water stressed	78.2	75.0
Not stressed	98.4	97.3

3.4.1.2.2. Water potential of the apex.

The change in apex length in the various solutions was plotted relative to the initial length (Fig. 11). The water potentials of the apex calculated from the data show a maximum increase of -0.03 and -0.07 bars due to 8 and 24 hours of water stress respectively (Table 3.4.2). Thus, a short term water stress that considerably reduced the water status of the leaves (RT 75%) had an insignificant effect on the water status of the apex.

TABLE 3.4.2

WATER POTENTIAL OF THE APEX (BARS)

Treatments	Hours after imposition of the stress	
	8	24
Water stressed	-5.26	-5.21
Not stressed	-5.23	-5.14

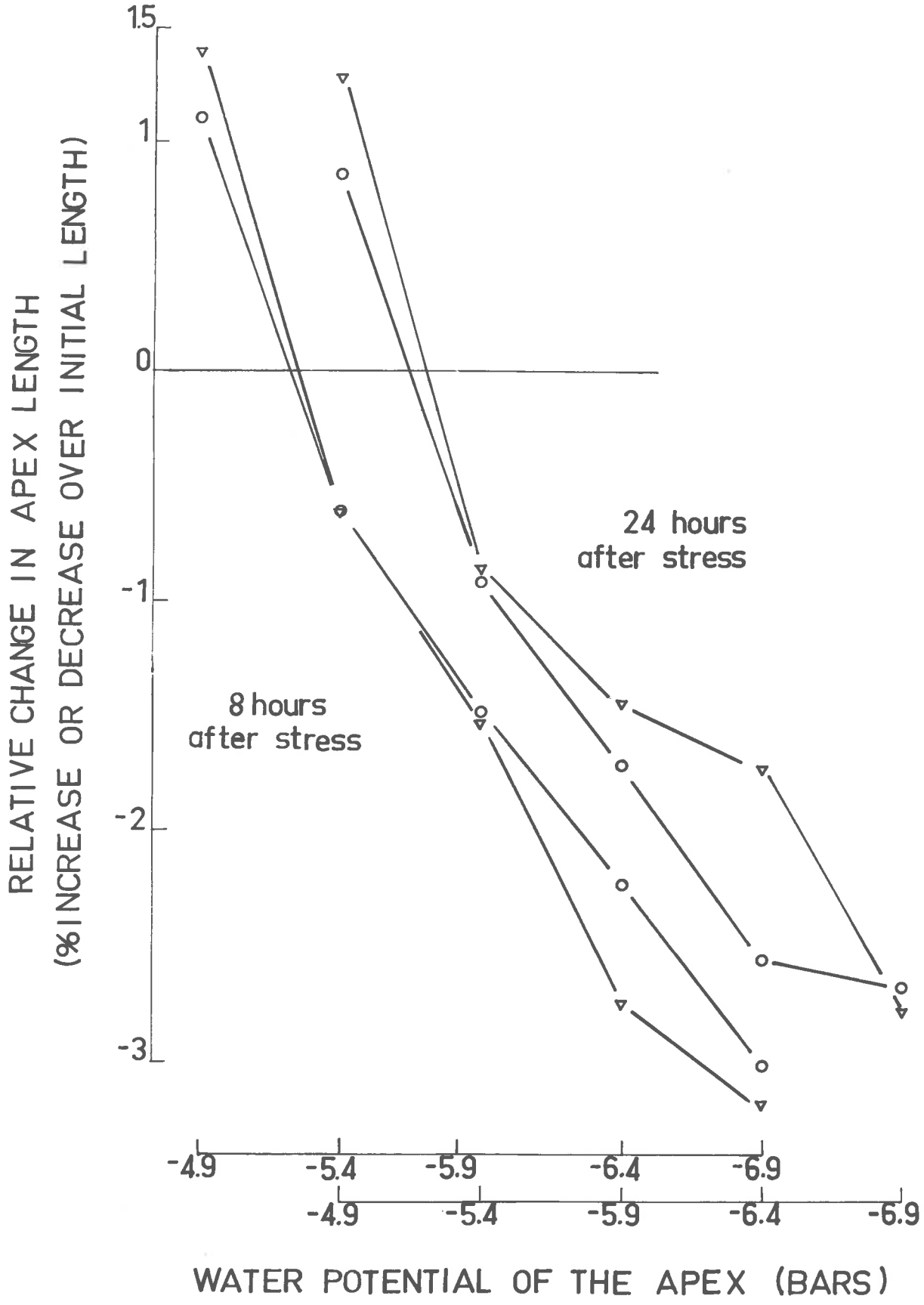
FIGURE 11

Relative change in length of excised apices after 4 hours in solutions of different osmotic concentrations. Plants subjected to water stress by the application of a polyethylene glycol solution of 18 atmospheres for 8 and 24 hours before apical excision -

Effect of water stress on the water status of the apex experiment 3.4.1

○ Not water stressed

▽ Water stressed


RELATIVE CHANGE IN APEX LENGTH
(% INCREASE OR DECREASE OVER INITIAL LENGTH)
1.5
1
0
-1
-2
-3
24 hours
after stress
8 hours
after stress
-4.9
-5.4
-5.9
-6.4
-6.9
-4.9
-5.4
-5.9
-6.4
-6.9
WATER POTENTIAL OF THE APEX (BARS)

3.4.2. Continuous water stress.

3.4.2.1. Methods.

The experiment was conducted in a controlled environment cabinet with an 8-hour photoperiod at 17°C. Barley plants (c.v. Prior A) were grown in pots containing a soil mixture prepared in the manner described earlier (sub-chapter 2.2.2). Water stress was imposed by withholding water after planting and Harvests 1 and 2 were taken 20 and 25 days from planting. Water stress was relieved after Harvest 2 by resuming watering. Plants were grown for a further 4 days after re-watering when Harvest 3 was taken. At each harvest the water potential of the apex was measured along with the relative turgidity of the leaves.

3.4.2.2. Results.

3.4.2.2.1. Leaf water status.

Water stress reduced the relative turgidity of the leaves to 80.7% on Harvest 1 (Table 3.4.3).

TABLE 3.4.3

RELATIVE TURGIDITY

Treatments	Harvest 1	Harvest 2	Harvest 3
Water stressed	80.7	62.4	-
Not stressed	99.3	99.3	97.6
Re-watered			95.1

With increasing water stress the relative turgidity fell further to as low as 62.4% as compared

to 99.3% in the non-stressed treatment. With the relief of stress, recovery was rapid and the relative turgidity returned to the level of non-stressed plants (Harvest 3).

3.4.2.2.2. Water status of the apex.

Fig. 12 presents the curves of the relative change in apex length as a percentage against the five graded series of concentration of polyethylene glycol solutions for Harvests 1, 2 and 3. A progressive water stress until Harvest 1 when the relative turgidity of the leaves had fallen to 80.7% had little effect on the relationship as compared to the non-stressed treatment. With increasing stress, however, the curve departed from that of the non-stressed treatment on Harvest 2 (RT 62.4%). Water stress produced an increase of -0.03 (Harvest 1) and -0.49 (Harvest 2) bars in the water potential of the apex. With the relief of stress, the water potential of the apex returned to the level of non-stressed treatment (Table 3.4.4).

TABLE 3.4.4

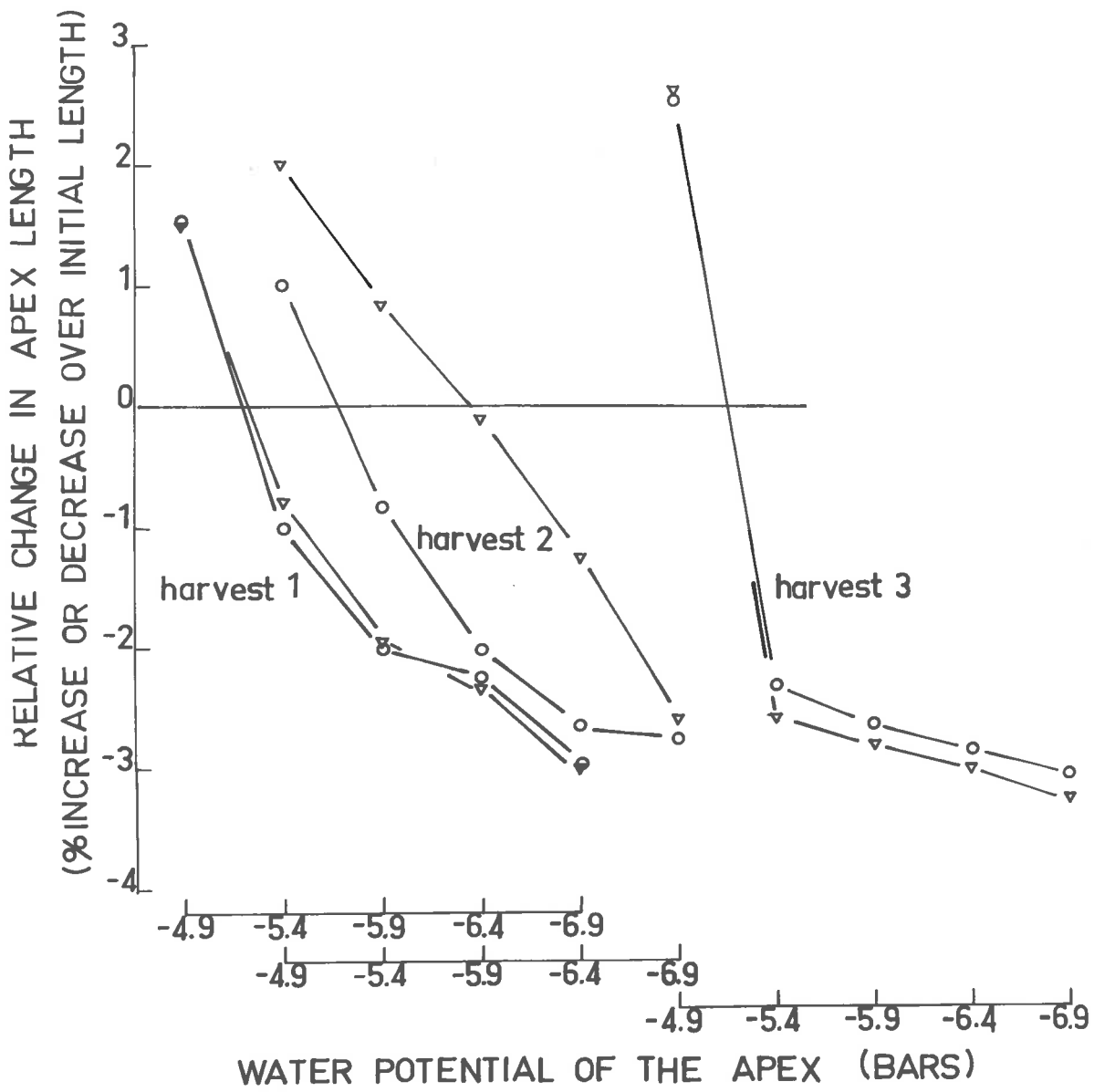
WATER POTENTIAL OF THE APEX (BARS)

Treatments	Harvest 1	Harvest 2	Harvest 3
Non-stressed	-5.21	-5.19	-5.17
Water stressed	-5.24	-5.68	
Re-watered			-5.17

FIGURE 12

Relative change in length of excised apices after 4 hours in solutions of different osmotic concentrations. Apices excised from plants grown with adequate water throughout (○) or subjected to water stress (▽) for 25 days and then re-watered -

Effect of water stress on the water status of the apex experiment 3.4.2.


RELATIVE CHANGE IN APEX LENGTH
(%INCREASE OR DECREASE OVER INITIAL LENGTH)
3
2
1
0
-1
-2
-3
-4
harvest 1
harvest 2
harvest 3
-4.9
-5.4
-5.9
-6.4
-6.9
WATER POTENTIAL OF THE APEX (BARS)

It was clear from the above experiments that the apex was resistant to water stress relative to the leaves in that water potential of the apex was unaffected until the relative turgidity of the leaves had fallen below 75% and possibly as low as 65%.

3.5. Indirect effects of water stress on apical development due to inhibition of translocation or assimilation

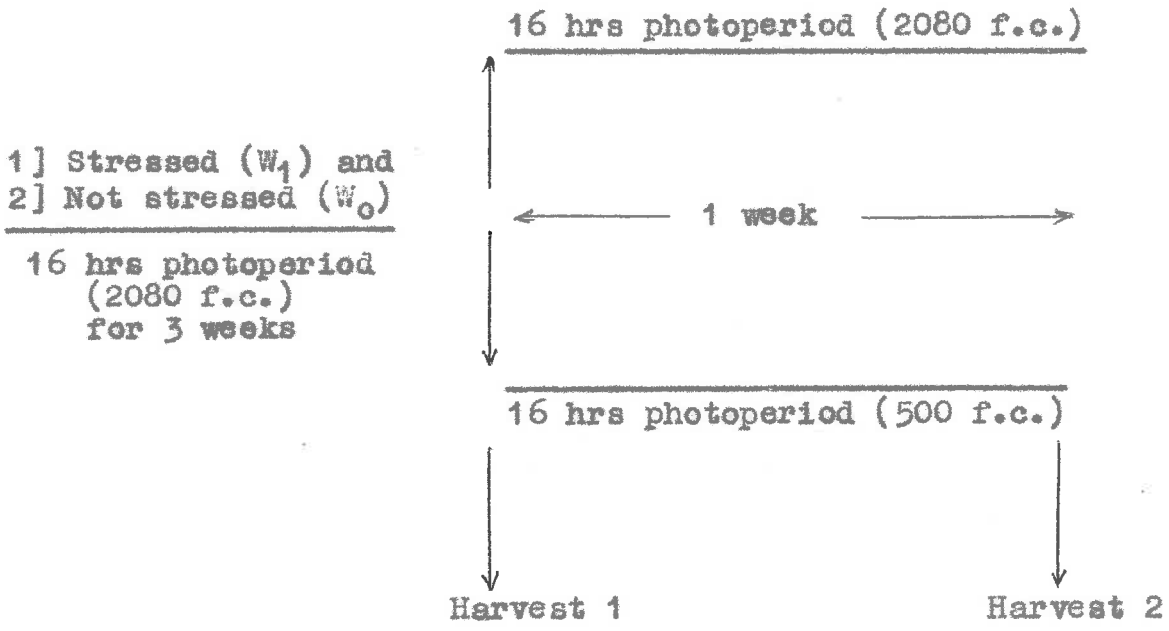
It was apparent from the previous experiments that the effects of water stress on primordium formation at the apex were unlikely to be due to a direct influence of water stress on the water status of the apex. Primordium formation was affected when leaf R.T. fell below 90% (sub-chapter 3.2.1.2.3), but apex water status not until leaf R.T. reached at least 75%. It was conceivable then, that these effects were mediated indirectly through the effects of water stress on assimilation and translocation of photosynthates. This possibility was investigated in the following experiments by the imposition of water stress on plants growing in different combinations of light intensities. The experimental design and general methods have been described earlier (sub-chapter 2.2.2) but further details pertaining to the treatments are described here along with the respective experiment.

3.5.1. Experiment 1.

3.5.1.1. Methods.

Barley plants (c.v. Prior A) were grown in

plastic pots (10 cm x 10 cm) containing soil mixture prepared in the manner described earlier (sub-chapter 2.2.2). The experiment was of 4 weeks' duration and was conducted in a controlled environment cabinet with a 16-hour photoperiod (2080 f.c.). Water stress was imposed by withholding water after planting (W_1) whereas in the control series (W_o) adequate soil water content was maintained throughout. Harvest 1 was taken 3 weeks after planting. Thereafter, plants in the two treatments were sub-divided into two groups for high (L_1) and low (L_2) intensity light measurements. High light intensity consisted of 2080 f.c. and low light intensity of 500 f.c. All the plants were grown in two light regimes for a further week when Harvest 2 was taken. A diagramatic representation of the experiment is as follows:



3.5.1.2. Results.

3.5.1.2.1. Leaf water status.

Water stress reduced the relative turgidity to 82.5% as against 91.6% in the control series (Table 3.5.1). With increasing stress the relative turgidity fell further to 80.5% (L_1) and 81.1% (L_2) in the two light regimes.

TABLE 3.5.1

RELATIVE TURGIDITY

Treatments	Harvest 1	Harvest 2
W_o L_1	91.6	88.7
W_o L_2	-	90.1
W_1 L_1	82.5	80.5
W_1 L_2	-	81.1

3.5.1.2.2. Primordium production.

Water stress significantly reduced primordium production on Harvest 1 (RT 82.5%) (Table 3.5.2).

TABLE 3.5.2

NUMBER OF PRIMORDIA
(means of 10 samples)

Treatments	Harvest 1	Harvest 2
W_o L_1	31.7	33.4
W_o L_2	-	33.8
W_1 L_1	25.0	27.6
W_1 L_2	-	25.2

Harvest 1. LSD ($P = 0.05$) = 5.9
Harvest 2. LSD ($P = 0.05$) = 3.9

With increasing stress, the reduction became larger on Harvest 2. Light energy had no effect in either of the treatments.

3.5.1.2.3. Apical development.

Water stress significantly reduced the development of spikelets (Table 3.5.3) particularly in the second harvest. Light intensity had no effect on the control or water stressed plants.

TABLE 3.5.3

STAGE OF DEVELOPMENT
(means of 10 samples)

Treatments	Harvest 1	Harvest 2
W_o L_1	7.9	11.0
W_o L_2	-	10.6
W_1 L_1	6.6	8.4
W_1 L_2	-	8.6

Harvest 1. N.S.
Harvest 2. LSD ($P = 0.05$) = 1.4

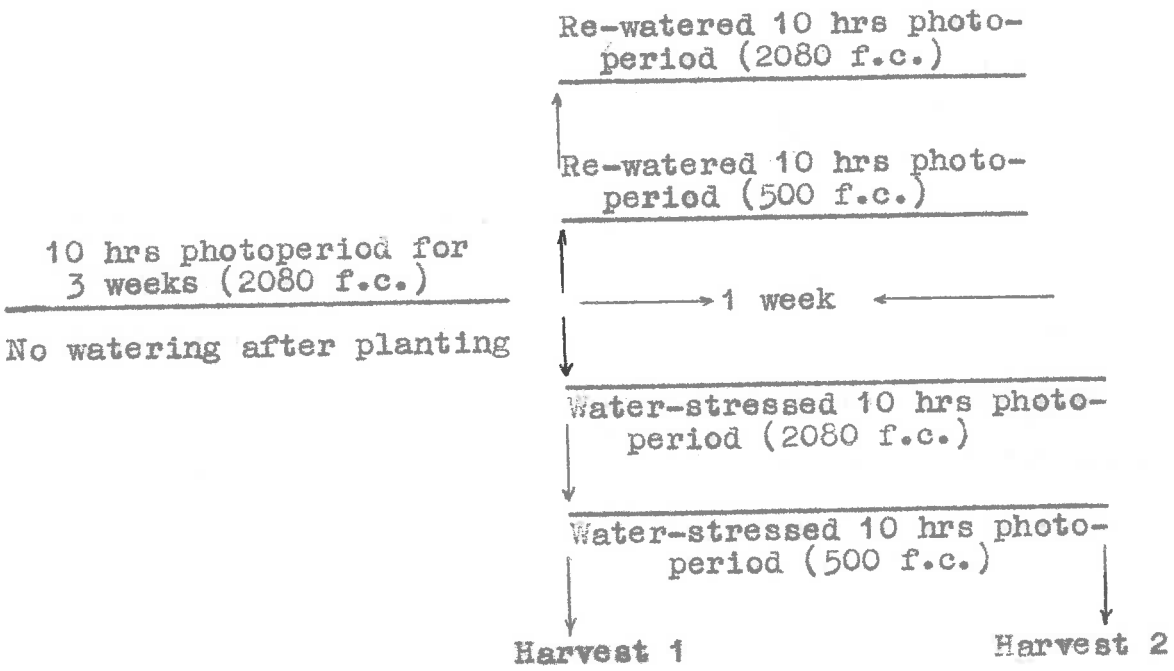
3.5.2. Experiment 2.

In the previous experiment, exposure to different light intensities had no effect on apical development in either the control or water-stressed series. This was attributed to the long photoperiod which reduced the response to light intensity (Aspinall and Paleg 1963) and resulted in the plants being at a relative unresponsive stage of development at the commencement of the treatment. Accordingly,

the experiment was repeated in a shorter photoperiod.

3.5.2.1. Methods.

Barley plants were grown in a controlled environment cabinet with a 10-hour photoperiod (2080 f.c.) for 3 weeks. Watering was stopped for all the plants immediately after sowing and an initial harvest (Harvest 1) was taken 3 weeks from planting. The plants were then divided into two groups. In one group water stress was relieved (RW) whereas in the other it was continued (WS). These two water treatments were sub-divided into four batches and exposed to high (L_1 2080 f.c.) and low (L_2 500 f.c.) light intensity regimes and grown for a further 1 week when Harvest 2 was taken. A diagrammatic representation of the experiment is as follows:



3.5.2.2. Results.

3.5.2.2.1. Leaf water status.

The relative turgidity at Harvest 1 was 92.3% (Table 3.5.4). Since the relative turgidity at the second harvest, where stress had been relieved, was of about the same order, it appears that stoppage of the water supply did not materially affect the water status of the plants before Harvest 1. With increased duration the effects of water stress became apparent at Harvest 2 when the relative turgidity fell to 88%.

TABLE 3.5.4

RELATIVE TURGIDITY

Treatments	Harvest 1	Harvest 2
WS L_1	92.3	88.3
WS L_2		87.8
RW L_1		94.3
RW L_2		92.7

3.5.2.2.2. Primordium production.

Water stress significantly reduced primordium production (Table 3.5.5). An increase in light intensity significantly stimulated production in both the water treatments, and, although primordium production was considerably reduced by stress, it did not cease, even at the low light intensity.

TABLE 3.5.5

NUMBER OF PRIMORDIA
(means of 10 samples)

Treatments	Harvest 1	Harvest 2
WS L_1	15.1	21.4
WS L_2		17.6
RW L_1		25.6
RW L_2		22.7

LSD (P =0.05) = 2.0

3.5.2.2.3. Apical development.

Apical development was significantly reduced by water stress (Table 3.5.6). A significant stimulation due to an increase in light intensity was only apparent in the re-watered plants. Light energy had no effect in the water stress treatment.

TABLE 3.5.6

STAGE OF DEVELOPMENT
(means of 10 samples)

Treatments	Harvest 1	Harvest 2
WS L_1	2.6	3.2
WS L_2		3.0
RW L_1		3.5
RW L_2		3.1

LSD (P =0.05) = 0.4

This experiment demonstrates that a stimulation of primordium production by an increase in light intensity is possible even if primordium production is limited by stress and leaf relative turgidity has fallen to 88%.

CHAPTER 4. DISCUSSION

4.1. Growth of the plant.

The best objective measure of plant growth is the total dry weight. Using this criterion, plant growth was affected as soon as leaf relative turgidity fell below 90% (day 12 Fig. 2). A similar reduction in plant dry weight increment where relative turgidity fell below 90% has been noted in Lupinus albus (Jarvis and Jarvis 1963a). In experiments where only soil water potential was measured, water stress before the permanent wilting point, presumably before leaf relative turgidities had reached very low values, reduced elongation in sunflower (Furr and Reeve 1945) and produced a general reduction in the dry matter production of tomato plants (Gates 1955a). It has been argued, however, that water is equally available to plants in a drying soil over the range from field capacity to permanent wilting when water suddenly becomes unavailable (Veihmeyer and Hendrickson 1950). This would presumably imply that leaf relative turgidities would remain high and then drop rapidly and growth parameters would follow the same pattern. This argument was based on field experiments where uneven root distribution complicated the interpretation of the data. Evidence from numerous others, better controlled studies, suggested to Richards and Wadleigh (1952) that water became progressively

less available in drying soil with the necessary presumption that leaf relative turgidity would also progressively fall as the soil dried out. Results from the present study demonstrating a progressive decline in leaf relative turgidity (Fig. 1) and a departure of growth from the control level (Fig. 2) as water became less available to the plants support this latter suggestion.

Although total plant dry weight was reduced by water stress, the different plant organs were differently affected. A slight initial stimulation of root growth was noted in the stress treatment (day 12 Fig. 3b). It is difficult to interpret this stimulation in terms of water stress as relative turgidity measurements on day 6 are not available. A reduction in root growth in this treatment was not apparent until day 18, however, whereas shoot dry weight increase was reduced from day 12. It appears reasonable to assume, therefore, that there was a differential effect of water stress on these two parameters of plant growth in this initial phase. Stimulation of root growth with slight water stress and an increase in root/shoot ratio with decreasing soil water content have been previously noted (Harris 1914, Eaton 1942, Jarvis and Jarvis 1963c). It is possible that optimum conditions for root growth may arise as a result of an interaction between aeration and water availability (Wangermann

1961). Alternatively, during slight water stress, the internal conditions of the root tips may be more favourable than those of the growing regions of the shoot, thus shifting the balance of the growth processes in favour of roots (Oppenheimer 1960).

The increase in shoot dry weight was considerably reduced by water stress but production of dry matter continued until the leaf relative turgidity had fallen below 43% (day 36 Fig. 3a). Apart from leaf 1 which attained a greater dry weight under water stress than in the control plants, all the leaves were reduced in weight, the reductions being greater the higher the leaf was inserted on the stem (Fig. 5). Since the majority of the leaf primordia were initiated before water stress became effective (Table 3.2.2), it must have been the expansion of leaf primordia that was drastically reduced. In Lupinus albus where water stress reduced plant growth it was shown that a primary contribution to this reduction was the failure of the leaves to expand (Jarvis and Jarvis 1963a). Gates (1955a) also found that a water stress which reduced the growth of tomato plants had a more severe effect on the dry weights of the leaf laminae than on the stem. In the same plant, leaves of different ages were found to be differently sensitive to water stress with the younger leaves suffering greatest inhibition (Gates 1955b).

With relief of the water stress there was a dramatic expansion of the leaf primordia (Table 3.2.2) but the reduction in leaf growth produced during the stress remained apparent. This indicates that a change had occurred during stress which limited further growth. It has been shown by Alekseev (see Gates 1964) that the growth processes of an individual leaf have their own critical period when the leaf is particularly sensitive to water stress. Alekseev suggested that this period is not the stage of elongation but an earlier stage during embryonic growth. In the present study, where reduction in leaf growth remained apparent after relief of the stress, it was noted that the influence of water stress on leaf dry weight was relatively more severe the higher the leaf was inserted on the stem (Fig. 5). This indicates that the earlier development of the leaf was the critical stage affected by water stress. During water stress a preferential transport of water to younger leaves rather than to older ones has been shown in fodder cabbage and rape plants (Catsky 1962). The superior water status of the young leaves led to their survival whereas the older leaves died. The survival of the embryonic tissues during dessication was found to be related to the proportion of meristematic (resistant) to elongating (susceptible) cells (Milthorpe 1950). It seems, then, that high growth activity is associated with a higher hydration level and

suffers a greater inhibition when water becomes limiting. Thus a water stress at a critical stage of meristematic activity may arrest growth but due to the relatively greater resistance of actively dividing cells these will survive during stress.

Relief of water stress resulted in a transient elevation of plant growth rate above that of plants on adequate water supply throughout (Table 3.2.1). Any reduction in growth during the period of stress was made good on the relief of stress in the short duration water stress treatments (Table 3.1.5) but this elevation of growth rate in the recovery phase was not sufficient to overcome the reduction produced by a more prolonged stress (Fig. 2). A similar elevation in the growth rates of tomato plants during recovery from a brief period of water stress has been noted by Gates (1955a, 1955b); in this case the increase in growth rate during recovery was sufficient to overcome the effects of the period of stress. The growth effects of intermittent water stress appears to depend on the intensity and duration of the period or periods of stress. The differences in growth responses of individual plant organs to water stress may well be related to their stages of development.

It has been shown that translocation of mineral nutrients and photosynthates is markedly reduced during water stress. Gates (1957) has reported inhibition of translocation of dry

matter, nitrogen and phosphorus from the older to younger tomato leaves during water stress. Similarly inhibition of translocation of photosynthates by water stress has been demonstrated in other plants. (Wiebe and Wihrheim 1962, Roberts 1964). Photosynthesis is closely related to the water content of the leaves (Dastur 1925, Dastur and Dessai 1933), but maximum photosynthesis is not always associated with the maximal value of leaf water content but often with a slightly lower level (Schneider and Childers 1941, Negisi and Satoo 1954). Recent evidence suggests that photosynthetic rates are influenced by the size of the 'sinks' utilizing assimilates and the rate of translocation to the sinks (Thorne and Evans 1964, Noseburger and Humphries 1965). It would appear that some of the effects of water stress on assimilation rates could arise through an inhibition of transport. It is conceivable then that some of the effects of water stress may be indirectly mediated through an inhibition of translocation of minerals and products of the photosynthesis. The increase in dry weight of leaf 1 during water stress noted in the present study may be due to the accumulation of photosynthates due to such a block to transport.

Protoplasmic structure has often been referred to as of importance in the response of plants to water stress (Stocker 1960). It has been claimed that changes in the

colloido-chemical state of the protoplast during water loss intensify hydrolytic breakdown of carbohydrate and protein in the plant cell (Iljin 1957, Kursanov 1956, Genkel 1961). Structural changes, as measured by viscosity and permeability, have been claimed to occur within the cell during changes in turgidity (Stocker 1960). Genkel (see May, Milthorpe and Milthorpe 1962) claimed that a pre-sowing treatment of grain could induce protoplasmic qualities favouring drought resistance. Thus subjecting the grain to various cycles of wetting and drying has been claimed to result in a greater hydration of colloid, higher viscosity and elasticity of the protoplasm and a greater amount of bound water. These changes are held by Genkel (see May, Milthorpe and Milthorpe 1962) to be responsible for a lower water deficit in the hardened plant during stress.

The results of the present study do not support these claims by Genkel. A pre-sowing hardening treatment had no effect on the relative turgidity of the plants during a short period of water stress (Table 3.1.1). Thus the contentions of Genkel that the water deficit of the plant is lowered by the hardening treatment do not find support from the results. A change in protoplasmic structure due to hardening favouring resistance to stress was also not observed, whether it was measured by the effects of stress on plant development (3.1.3, 3.1.4) or on dry matter production (3.1.5).

4.2. Growth of the apex.

The growth of the apex was reduced by water stress both in terms of primordium production and morphological development (3.1, 3.2). These two parameters of apical growth were affected differently, however. Primordium production ceased when leaf relative turgidity fell below 90% (day 18 Fig. 6). On the other hand, morphological development of the apex continued, albeit at a reduced rate, even with severe stress when the leaf relative turgidity had fallen to 43% (day 42 Fig. 7). A reduction in primordium formation in the barley apex at a soil water stress of 0.8 atmosphere has been noted by Nicholls and May (1963). Such a mild water stress would presumably not produce low leaf relative turgidities. These authors also demonstrated that primordium production was relatively more sensitive to water stress than spikelet differentiation. Morton and Watson (1948), on the other hand, claimed that the rate of formation of leaf initials in sugar-beet was relatively insensitive to water supply. Their conclusions were inferred from count of expanding leaves, however, not from direct apical observations.

Primordium production which ceased during water stress was rapidly resumed when the stress was relieved (Fig. 6). This indicates that no permanent changes were induced in the apex by the stress and that the inhibition of growth was due to contemporary factors. The apex is capable of organised

growth when supplied with nutritional and hormonal factors either from the plant or from an artificial medium (Ball 1946, Wetmore and Morel 1949, Wetmore 1954, 1956). It is therefore apparent that the profound effects of water stress on apical activity could be mediated through either an effect on the supply of essential factors from the rest of the plant or a direct effect on the cells of the apex.

In dealing with the second of these alternative mechanisms, it is evident that any direct effect of water stress on the metabolism of apical cells must be mediated through a change in the water potential of those cells. Results of the determination of water potential of the whole apex (3.4) during water stress demonstrated that the barley apex is relatively more resistant to stress than the leaves. The water status of the apex was not affected even when the relative turgidity of the leaves had reached to 75%. Since a reduction in primordium production was affected as soon as leaf relative turgidity fell below 90% it can be concluded that it is unlikely that observed effects on the growth of the apex were produced by a direct effect of water stress on the apical cells. It is, of course, conceivable that measurement of the gross water status of the whole apex concealed changes in the water status of cells at the presumptive sites of primordia but this seems highly unlikely.

The more likely alternative, then, is that water stress influenced apical growth through an effect on the essential factors supplied to the apex by the rest of the plant. It is possible to divide such factors into two broad categories - controlling agents such as hormones and gross nutritional factors such as carbohydrates. Evidence for the first of these factors come from the acceleration of primordium production often associated with photoperiodic induction (e.g. Aspinall and Paleg 1963). In barley, water stress during photoperiodic induction prevented any immediate acceleration of primordium formation (Tables 3.2.4, 3.2.7). After stress was relieved, however, there was a promotion of primordium formation by the photoperiodic stimulation given during the period of stress. One possible explanation is that transport of a substance, which promotes primordium formation directly, was inhibited by stress. In contrast, however, a water stress imposed prior to the inductive treatment in Lolium temulentum (3.3.1) inhibited elongation of the apex. This suggests that the effect of water stress on primordium production may not be directly related to the photoperiodic stimulus alone. Rather, water stress may limit the supply of two 'substances' to the apex. Firstly it may limit the supply of the photoperiodic stimulus which perhaps only indirectly affects primordium formation through the processes

consequent upon floral induction. Secondly, and more importantly in this context, it may inhibit the translocation of some other substance necessary for the normal primordium production by the apex, irrespective of the development of the apex.

The substance or substances involved in the normal production of primordia and translocated to the apex may be hormonal or nutritional in nature. Some indirect evidence for considering a nutritional control of primordium production at the shoot apex has been advanced by Milthorpe (1959a). Reduced translocation of carbohydrates and mineral nutrients during water stress has been demonstrated by Gates (1957) and adverse effects of stress on grain development have been attributed to this effect (Denmead and Shaw 1960). Similarly a water stress that reduced spikelet differentiation in maize was found to be accompanied by inhibition of translocation of amino acids to the apex (Petinov and Berko 1965). In the present study primordium production at the apex was accelerated by an increase in light intensity even when the overall rate of apical growth was limited by water stress (3.5). This could be interpreted to suggest that only carbohydrate or some other product of photosynthesis was here limiting apical growth. Increasing the rate of photosynthesis increased the supply to the apex although the translocation was partially blocked by water stress. Such evidence is

far from direct, however, as many other parameters could be affected by an increase in the light intensity.

A further complication to hypotheses which envisage water stress blocking the transport of some essential substance to the apex is the possible importance of the distribution of that substance between the several parts of the apex. Monopolisation of food reserves by the developing florets during water stress has been reported by a number of workers reviewed by Kozlowski (1965). Competition in the more mature barley spike even in the absence of water stress has been demonstrated where the removal of grains resulted in an increase in the weight of the remaining grains (Buttrose and May 1959). In a more pertinent study, where the growth of barley grain was investigated, it was suggested that assimilate from the leaves was shared equally among the majority of the grains in plants not subjected to water stress, but was limited in distribution by water stress in such a way that the lower grains received a larger proportion (Aspinall 1965). A similar situation may occur in the earlier stages of development. Aspinall and Paleg (1963) noted that the rate of leaf differentiation was depressed during the transition of the barley apex from the vegetative to the reproductive state. This depression in the rate of leaf differentiation was considered to be due to an increased demand for assimilates and other factors by developing floral primordia at the expense of the

developing leaves. Since leaf expansion (Table 3.2.2) and primordium production were both severely curtailed by water stress whereas spikelet development was less affected, it is possible that essential factors, either nutritional or hormonal, were increasingly channelled to the developing spikelets when limited by water stress.

The possible reasons for such a preferential distribution of materials to developing spikelets during stress require examination. Evidence is accumulating that minerals and carbohydrates are translocated preferentially to regions of high auxin content (Booth, Moorley, Davies, Jones and Wareing 1962, Hill 1962). Developing florets (Mayer 1956, Stitchel 1959) and seeds (Leopold 1955) appear to be regions of high auxin production and it is reasonable to presume that they may act as preferential sinks for translocated material. These speculations require conformation, however, as dome of the apex and the developing leaves, which appear to be most inhibited during stress, are also regions usually associated with a high auxin content.

The growth of the apex, with or without stress, is necessarily related to its metabolism but as yet comparatively little is known about either its general physiology or biochemistry. In some of the analytical studies (Steward, Wetmore, Thompson and Nitsch 1954, Steward, Wetmore and

Pollard 1955) where efforts have been made to locate and identify the chemical reactions in the apical meristem, the apex, because of its small size, has usually been treated as a whole. Thus substances that are of special importance in maintaining the characteristic organisation of the apex, as in the inception of a primordium, are as yet unknown. The sustained growth of the apex, however, depends entirely on supplies of substances from below. As these move upwards at different stages of differentiation, changes are likely to occur in the gradient of their movement, as no two cross sectional levels in the meristem and no two concentric layers of the tissue are likely to be metabolically identical. The superimposition of water stress on this system may exert profound effects, such as those noted, through relatively minor effects on rates of movement and gradients of essential metabolites.

4.3. Water status of the plant and the apex.

A meaningful approach to the study of water stress and plant growth can only be achieved by defining the stress in terms of plant water status. The water potential of the plant cells alone controls the physiological processes which in turn regulate growth. Kramer (1963) has cautioned against the interpretation of the effects of water stress on the basis of a given degree of soil water stress. Such measurements

need not necessarily be accompanied by an equivalent degree of plant water stress.

A convenient, although arbitrary, index of the water status of the plant tissue is the 'relative turgidity' (Weatherly 1950). This is defined as the ratio of the water actually present in the tissue to that held by the same tissue when fully turgid. This index was used in the present study to estimate the leaf water status. It has been argued by Gaff and Carr (1961) that the water in the plant cell protoplast and in the cell wall are not necessarily in potential equilibrium. They envisage the plant cell wall acting as a buffer protecting the protoplast from rapid changes in water potential. If this view is correct, then measurements of relative turgidity and vapour equilibration measurements of water potential would tend to over-estimate the stress suffered by the plant protoplast, as such measurements integrate the water potential of the whole tissue including the cell walls. Gaff and Carr based their views on data from Eucalyptus leaves where the cell wall is relatively thick and may be envisaged to act in such a way. In a thin-leaved species such as barley, however, it is difficult to accept that water in the protoplast and the cell wall, which are in intimate contact, could get far out of potential equilibrium for a significant time period. In such plants, until it is

unequivocably demonstrated to the contrary, it would seem preferable to accept a measurement of tissue water potential as a good measure of protoplasmic water potential. A close relationship between leaf relative turgidity and leaf water potential has been frequently demonstrated (Weatherley and Slatyer 1957, Slatyer 1960a, Carr and Gaff 1961). This being so, it is expected that the measurements of leaf relative turgidity in the present study are indicative of the water potential of the tissue, which in turn regulated plant growth during water stress. Simultaneous readings of leaf water potential were not made and it is therefore impossible to calibrate the corresponding relative turgidity measurements in terms of water potential.

A water stress which reduced leaf relative turgidity to 75% had no effect on the water potential of the apex (3.4). Apparently there was a difference in the water status of the apex and of the leaf during stress. It has been shown by Catsky (1962) that, during water stress, young leaves of cabbage and rape plants were at a superior water stress in comparison with the old leaves. Attention has been drawn by Kramer (1959a) to the ability of apical tissues to obtain water at the expense of the older tissue. Zavadskaya and Skazkin (1960) found that a water stress in barley plants which resulted in the death of leaves did not kill the apex.

Similarly, in wheat, Chinoy (1962b) has shown that leaves were the first to be damaged during water stress, followed by the stem and last of all by the spike. Stem tips of tomato plants (Wilson 1948) and sunflower (MacDougal 1920) continue to elongate when the remainder of the stem was shrinking during water stress. Anderson and Kerr (1943) reported that the growth rate of developing cotton bolls was uniform during both day and night. Other plant organs showed a reduced growth during the day, attributed to a temporary water stress. Kerr and Anderson (1944) considered that the high imbibitional forces of the developing cotton bolls enable them to retain an adequate supply of water at the expense of more mature cells in other parts of the plant. Somewhat in contrary to this data is the finding that water stress which reduced grain growth in barley was not associated with any change in the water potential of the grains (Aspinall 1965).

Some of this evidence suggests a difference in the water status of the apex compared with the rest of the plant. It is possible that apical tissues may be able to obtain water at the expense of older tissues. The results of the present study demonstrate, however, that when leaf relative turgidity fell to 75% during stress, the water potential of the apex did not change from approximately -5 atmosphere. The relationship between water potential and relative turgidity

estimated by Slatyer (1960a) for tomato leaves gives the corresponding value to 75% relative turgidity as -15 atmosphere water potential. If an analogous relationship exists in barley, it would seem highly unlikely that water could be transported from the leaf (-15 atm) to the apex (-5 atm). This relationship requires conformation, as it would vary with the plant species, but it does not seem feasible that the water potential of the apex would be higher (negatively) than that of the leaves. It seems more likely, then, that the resistant apical tissues may possess some barrier to the loss of water to the rest of the plant.

The remarkable tolerance, or perhaps avoidance, of water stress by the apical tissues may be of significant value in the maintenance of perennial species in arid conditions. It has been noted that perennial grasses of semi-arid pastures (Walter 1939), and in long periods of drought in arid lands (Lucanus et al 1960), survive death from water stress and resume growth after a shower of rain because of the survival of the apical meristems.

4.4. Development of the apex.

Water stress has profound effects on apical growth, whether it be on a vegetative apex or a mature flowering apex. Thus, stress in the earlier stages of development retards spikelet development in oats (Novikov 1952, 1954), maize (Petinov and Berko 1965) and barley (Nicholls and May 1963).

In the later stages of development it reduces the number of ears in wheat (Asana et al 1958, Chinoy 1962a) and stem elongation and grain formation in barley (Aspinall et al 1964). Where such effects have been observed with greater precision, it has been found that water stress during spikelet development reduces pollen viability in barley (Zavadskaya and Skazkin 1960) and male fertility in wheat (Bingham 1966). Such varied observations have given rise to the hypothesis that water stress has its greatest influence on the most active process occurring at the time of stress (Aspinall et al 1964). This concept helps to explain the many ultimate manifestations of periods of stress during ontogeny, but does not provide any insight into the areas of metabolism which may be most influenced by water stress.

In the light of the present data, it does not even appear that this hypothesis completely describes the situation. In an early floral apex during water stress it has been found that primordium production at the terminal apex ceased at a lower water stress than did floret differentiation. Although the observations of floret differentiation were necessarily subjective there seems no doubt that the growth of floral organs continued even until leaf relative turgidity had been reduced to 43%. The reason for this remarkable difference in the susceptibility of the various regions of the apex to inhibition by water stress have already been discussed at

length. It suffices here to note that floral development was not greatly affected by a water stress reducing leaf relative turgidity to 90% but was limited when relative turgidity fell to 75% or lower.

It is possible that the effects of water stress on apical development - such parameters as spikelet differentiation and grain production - may be due to an influence on some control process affecting all these aspects of development. The photoperiodic stimulation of development is possibly such a control process. Photoperiod profoundly influences apical development in many grasses, including barley and Lolium temulentum and this effect is not confined simply to an acceleration of floral initiation (Aspinall 1966). Guitard (1960) has demonstrated an effect of photoperiod on all stages of apical development in barley, excluding the growth occurring after the fertilization of the ovules. An effect of water stress on the process of photoperiodic stimulation may well produce at least some of the effects of floral development which have been noted in the literature and here.

In this connection it may be significant that photoperiodic induction in Lolium temulentum was not inhibited by water-stress which only reduced the relative turgidity of the leaves to 89% (Table 3.3.2) but was completely inhibited when leaf relative turgidity fell to 78% or below (Fig. 8). These limits are very similar to those for an effect of water stress

on spikelet development in barley.

Conclusive evidence for an effect of water stress on the photoperiodic process has been obtained with Lolium temulentum. In summary, water stress during the period of induction and synthesis of the floral stimulus in the leaf completely prevented floral initiation. Similarly, water stress during the period of translocation of the stimulus to the apex prevented floral initiation. This was due to an inhibition of transport of the stimulus and not to an acceleration of its destruction, as releasing the stress without simultaneous defoliation resulted in very little inhibition of floral initiation (Fig. 10).

It is more difficult to obtain similar evidence for an effect of water stress on the process of photoperiodic induction in barley as the plant requires more than one inductive cycle to produce a flowering response. The data obtained in the present study do, however, support the conclusions drawn with Lolium temulentum. At relatively low levels of water stress (R.T. 89%), an increase in the length of photoperiod stimulated apical development as in the non-stressed plants. This demonstrates that, in barley as well as in Lolium, the photoperiodic system is not sensitive to water stress until this reaches a moderately severe level. Where water stress was more severe, any immediate response to an increase in the length of the photoperiod was inhibited.

Relief of the water stress, however, resulted in a stimulation from the lengthened photoperiod being displayed in increased apical development. This resembles the inhibition of transport of the floral stimulus from the leaf demonstrated in Lolium temulentum, but has a further facet in that it suggests that in barley, at least, some synthesis of the floral substance can continue during a water stress sufficient to block its transport from the leaf. Such an effect may account for reports of accelerated developmental changes occurring following relief of water stress in the field.

In discussing the data so far it has been assumed that water stress affects the photoperiodic response through an effect on the leaf. Alternatively, it could be argued that water stress affects the sensitivity of the apex to the floral stimulus and hence produces the recorded responses. The reasons for rejecting this hypothesis need elaboration. Direct measurements of the water status of the apex reveal that this did not alter until the leaf relative turgidity fell below 75% at least. As this is within the range in which the photoperiodic response is affected this evidence does not conclusively support either hypothesis. The effectiveness of water stress during the inductive period, however, before any stimulus could have reached the apex (Evans 1960c) can only be explained by supposing that the water stress inhibits leaf processes or that it so affects the apex as to render it insensitive to the floral stimulus even after stress is

relieved. This second suggestion is not supported by the rapid growth of the apex following stress relief. Similar arguments hold for the effects of stress during translocation of the floral stimulus from the leaf.

It would appear, then, that water stress inhibits at least two processes occurring in the leaf during photoperiodic stimulation. Effect of water stress on the translocation of metabolites have been recorded (Gates 1957) but evidence is accumulating (Evans and Wardlaw 1966) that the floral stimulus is translocated from the leaf by a different pathway and at a different rate than are the products of photosynthesis. It is possible that this apparently slower route involves transfer from cell to cell rather than through sieve tubes and it is not difficult to conceive of water stress interfering with this process.

The point at which water stress interferes with the synthesis of the floral stimulus is impossible to pin-point. Evidence has been produced, at one time or another, for effects of water stress on many areas of metabolism (see May and Milthorpe 1962) and it is impossible to state that impairment of any particular metabolic pathway is a characteristic of the effects of water stress. Also, our knowledge of the chemical aspects of photo-induction has not reached the stage where even a single biochemical reaction or intermediate can be identified (Searle 1965). Evidence from

inhibitor studies with Lolium temulentum (Evans 1964b) and other plants (Bonner, Heftmann and Zeevart 1963) suggests that steroid biosynthesis in the leaves is involved in photo-induction. This may be the point of action of water stress. On the other hand, RNA metabolism, which is known to be affected by water stress (Gates and Bonner 1959, Shah and Loomis 1965), is probably not involved in the photo-inductive event in the leaf but is of importance in the apex when the floral stimulus arrives from the leaves.

4.5. Water stress and cereal yield in the field.

It has been frequently observed that a period of water stress under field conditions leads to a reduction in cereal yield due to a decrease in the number of grains per ear (Asana et al 1958, Robins and Domingo 1953). Aspinall et al (1964) associated this component of yield reduction by water stress with stress before anthesis. The data from the present study suggest that primordium production is sensitive to water stress even earlier in plant development, but that spikelet development and the flowering process itself are resistant to transient periods of mild stress. In the southern Australian environment, cereals are sown in late autumn or early winter after the commencement of the winter rainfall season. Water stress during the early phases of vegetative growth is not as frequent as water stress after flowering but can occur in seasons in which the early winter rains are delayed. In such a season, primordium production

at the apex is likely to be curtailed but, unless water stress is unusually severe, spikelet development should proceed if the plants have initiated flowers. The breaking of a period of drought should be accompanied by rapid growth, both in terms of primordium production and spikelet development. The eventual size of the ears will be determined by the balance between primordium production and of spikelet development (Paleg and Aspinall 1966). Periods of water stress in the early stages of development may well lead to reduced ear size as spikelet differentiation is less affected by stress than is primordium production. On the other hand, if short periods of drought are accompanied by clear skies there may be less effect on ear size, as the present evidence suggests that the effects of mild water stress on primordium production can be at least partially overcome by an increase in light energy. Flowering date is not likely to be affected by short periods of drought during the early phases of growth as the flowering process is resistant to stress and shows rapid compensatory recovery after the relief of stress.

These considerations lead to the conclusion that short periods of water stress in the early phases of growth are unlikely to have any major effects on cereal yield. This corresponds with the experience in other studies (Aspinall et al 1964).

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BIBLIOGRAPHY

Ackley, W.B. 1954. Seasonal and diurnal changes in the water contents and water deficits of Bartlett pear leaves. Plant Physiol. 29 : 445-48.

Allard, H.A. and W.W. Garner. 1940. Further observations on the response of various species of plants to length of day. U.S.D.A. Tech. Bull. 727 : 1.

Allard, H.A. and M.W. Evans. 1941. Growth and flowering of some tame and wild grasses in response to different photoperiods. J. Agri. Res. 62 : 193.

Anderson, D.B. and T. Kerr. 1943. A note on the growth behaviour of cotton bolls. Plant Physiol. 18 : 261-9.

Asana, R.D., V.S. Mani, K.P. Pillay and K.N.S. Gahlot. 1955. Analysis of drought resistance in crop plants. I. The influence of soil drought on the relation between yield and ear characters in wheat (pot culture). Indian J. Genet. Pl. Breed. 15 : 59-79.

Asana, R.D., A.D. Saini and D. Ray. 1958. Studies in physiological analysis of yield. III. The rate of grain development in wheat in relation to photosynthetic surface and soil moisture. Physiol. Plant. 11 : 655-65.

Ashton, F.M. 1956. Effect of a series of cycles of alternating low and high soil water contents on the rate of apparent photosynthesis in sugar cane. Plant Physiol. 31 : 266-74.

Aspinall, D. and L.G. Paleg. 1963. Effects of day length and light intensity on growth of barley. I. Growth and development of apex with a fluorescent light source. Bot. Gaz. 124 : 429-37.

Aspinall, D., P.B. Nicholls and L.H. May. 1964. The effect of soil moisture on the growth of barley. I. Vegetative development and grain yield. Aust. J. Biol. Sci. 15 : 729-45.

Aspinall, D. 1965. The effects of soil moisture stress on the growth of barley. II. Grain growth. Aust. J. Biol. Sci. 16 : 265-75.

Aspinall, D. 1966. Effects of day length and light intensity on growth of barley. IV. Genetically controlled variation in response to photoperiod. Aust. J. Biol. Sci. 19 : 517-34.

Baker, D.N. and R.B. Musgrave. 1964. The effects of low level moisture stress on the rate of apparent photosynthesis in corn. Crop Sci. 4 (3) : 249-53.

Ball, E. 1946. Development in sterile culture of stem tips and subjacent regions of Tropeaolum majus L. Lupinus albus L. Amer. J. Bot. 33 : 301-18.

Barnard, C. 1955. Histogenesis of the inflorescence and flower of Triticum aestivum L. Aust. J. Bot. 3 : 1-20.

Barnard, C. 1957. Floral histogenesis in the monocotyledons. I. The gramineae. Aust. J. Bot. 5 (1) : 1-20.

Barrs. H.D. and P.E. Weatherley. 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. Aust. J. Biol. Sci. 15 : 413-28.

Barrs. H.D. 1966. Root pressure and leaf water potential. Science 152 (3726) : 1266-68.

Barrs. H.D. 1966a. Re-assessment of the role of root pressure in the movement of water through plants. C.S.I.R.O. Irrigation Res. Lab. Ann. Rep. 1965-66 : 10-11 (Griffith N.S.W.).

Best, R. 1959. Photoperiodism in rice. Field Crop Abst. 12 : 85-93.

Bingham, J. 1966. Varietal responses in wheat to water supply in the field and male sterility caused by a period of drought in a glasshouse experiment. Ann. Appl. Biol. 57 : 365-77.

Bliss, L.C., P.J. Kramer and F.A. Wolf. 1957. Drought resistance in tobacco. Tob. Sci. 1 : 120-23.

Bonnet, O.T. 1935. The development of barley spike. J. Agri. Res. 51 : 451-57.

Bonnet, O.T. 1936. The development of wheat spike. J. Agri. Res. 53 : 445-51.

Bonner, J., E. Heftmann and J.A.D. Zeevart. 1963. Suppression of floral induction by inhibitors of steroid biosynthesis. Plant Physiol. 37 : 43-9.

Booth, A., J. Moorley, C.R. Davies, H. Jones and P.F. Wareing. 1962. Effects of indolyl 3-acetic acid on the movement of nutrients within plants. Nature 194 : 204-5.

Borthwick, H.A. and M.W. Parker. 1940. Floral initiation in Biloxi soya beans as influenced by age and position of leaf receiving photoperiodic treatment. Bot. Gaz. 101 : 806-17.

Bouillenne-Walraud, M. and R. Bouillenne-Walraud. 1926. Contribution à l'étude de la respiration en fenction de l'hydration. Ann. Physiol. Physiochem. Biol. 2 : 426.

Brix, H. 1962. The effect of water stress on the rates of photosynthesis and respiration in tomato plants and labolly pine seedlings. Physiol. Plant. 15 : 10-20.

Buttrose, M.S. and L.H. May. 1959. Physiology of cereal grain. I. The source of carbon for the developing barley kernel. Aust. J. Biol. Sci. 12 : 40-52.

Carr, D.J. 1955. On the nature of photoperiodic induction. III. The summation of the effects of inductive photoperiod cycles. Physiol. Plant. 8 : 512-26.

Carr, D.J. 1957. On the nature of photoperiodic induction. IV. Preliminary experiments on the effect of light following the inductive long dark period in Xanthium pennsylvanicum. Physiol. Plant. 10 : 249-65.

Carr, D.J. and D.F. Gaff. 1959. The role of the cell-wall water in the water relation of leaves. Plant Water Relationships in Arid and Semi-Arid conditions, Proc. Madrid Symp., Unesco, Paris.

Carr, D.J. and D.F. Gaff. 1961. The role of the cell-wall water in the water relations of leaves. Plant Water Relationships in Arid and Semi-Arid conditions, Proc. Madrid Symp., Unesco, Paris.

Catsky, J. 1962. Water saturation deficit in the wilting plant. The response of young leaves and the translocation of water from old into young leaves. Biol. Plant Acad. Sci. Bohemslor 4 : 306-14.

Chinoy, J.J. 1962a. Physiology of drought resistance in wheat. IV. Effects of wilting at different growth and developmental stages on plant characters determining yield of grain in eight varieties of wheat. Phyton 19 : 5-10.

Chinoy, J.J. 1962b. Physiology of drought resistance in wheat. V. Drought coefficients in relation to drought intensity. Phyton 19 : 11-20.

Clark, J. and R.D. Gibbs. 1957. Studies in tree physiology. IV. Further investigations of seasonal changes in moisture content of certain Canadian forest trees. Can. J. Bot. 35 : 219-53.

Clausen, J.J. and T.T. Kozlowski. 1965. Use of relative turgidity technique for measurement of water stress in gymnosperm leaves. Can. J. Bot. 43 : 306-16.

Cooper, J.P. 1950. Day length and head formation in the rye grass. J. Brit. Grass Soc. 5 : 105-12.

Cooper, J.P. 1951. Studies on the growth and development in Lolium. II. Pattern of bud development of the shoot apex and its ecological significance. J. Ecol. 39 : 228-70.

Cooper, J.P. 1956. Developmental analysis of populations in the cereals and herbage grasses. I. Methods and techniques. J. Agri. Sci. 47 : 262.

Crafts, A.S., H.B. Currier and C.R. Stockings. 1949. Water in the physiology of plants. Pub. Chronica Botanica Co. Waltham, Mass. U.S.A.

Dastur, R.H. 1925. The relation between water content and photosynthesis. Ann. Bot. (Lond.) 39 : 769-86.

Dastur, R.H. and B.L. Dessai. 1933. The relation between water content, chlorophyll content and the rate of photosynthesis in some tropical plants of different temperatures. Ann. Bot. (Lond.) 47 : 69-88.

Denmead, O.T. and R.H. Shaw. 1960. The effects of soil moisture stress at different stages of growth on the development and yield of corn. Agron. J. 52 : 272-4.

Domanskii, R. 1959. Investigations concerning the effect of drought on spring barley. Trans. Fiziol. Rastt. 6 : 354-5.

Eaton, F.M. 1942. Toxicity and accumulation of chloride and sulfate salts in plants. J. Agri. Res. 64 : 357-99.

Eaton, F.M. and D.R. Ergle. 1948. Carbohydrate accumulation in the cotton plant at low moisture levels. Plant Physiol. 23 : 169-87.

Ehlig, C.F. 1962. Measurement of energy status of water in plants with a thermocouple psychrometer. Plant Physiol. 37 : 288-90.

Evans, L.T. 1958. Lolium temulentum a long day plant requiring only one inductive cycle. Nature (Lond.) 182 : 197.

Evans, L.T. 1960a. The influence of environmental conditions on inflorescence development in some long day grasses. New Phytol. 59 : 163-74.

Evans, L.T. 1960b. Inflorescence initiation in Lolium temulentum L. I. Effect of plant age and leaf area on sensitivity to photoperiodic induction. Aust. J. Biol. Sci. 13 : 123-31.

Evans, L.T. 1960c. Inflorescence initiation in Lolium temulentum. II. Evidence for inhibitory and promotive photoperiodic processes involving transmissible products. Aust. J. Biol. Sci. 13 : 424-40.

Evans, L.T. 1962a. Day length control of inflorescence initiation in the grass Rottboella exaltata L.f. Aust. J. Biol. Sci. 15 : 291-303.

Evans, L.T. 1962b. Inflorescence initiation in Lolium temulentum L. III. The effect of anaerobic conditions during photoperiodic induction. Aust. J. Biol. Sci. 15 : 281-90.

Evans, L.T. and I.F. Wardlaw. 1964. Inflorescence initiation in Lolium temulentum L. IV. Translocation of the floral stimulus in relation to that of assimilates. Aust. J. Biol. Sci. 17 : 1-9.

Evans, L.T. 1964a. Inflorescence initiation in Lolium temulentum L. V. The role of auxins and gibberellins. Aust. J. Biol. Sci. 17 : 10-23.

Evans, L.T. 1964b. Inflorescence initiation in Lolium temulentum L. VI. Effects of some inhibitors of nucleic acid, protein and steroid biosynthesis. Aust. J. Biol. Sci. 17 : 24-34.

Evans, L.T., I.F. Wardlaw and C.N. Williams. 1964. Environmental control of growth. In "Grasses and Grasslands" Editor C. Barnard. MacMillan and Co., London.

Evans, L.T. 1965. Inflorescence initiation in Lolium temulentum L. VII. The spectral dependence of induction. Aust. J. Biol. Sci. 18 : 745-62.

Evans, L.T. and I.F. Wardlaw. 1966. Independent translocation of ^{14}C-labelled assimilates and of the floral stimulus in Lolium temulentum. Planta 68 : 310-26.

Evans, M.W. and H.A. Allard. 1934. Relation of length of day to growth of Timothy. J. Agri. Res. 48 : 571.

Evans, M.W. and F.O. Grover. 1940. Developmental morphology of the growing point of the shoot and inflorescence in grasses. J. Agri. Res. 61 : 481-520.

Furr, J.R. and J.O. Reeve. 1945. Range of soil moisture percentages through which plant undergo permanent wilting in some soils from semi-arid irrigated areas. J. Agri. Res. 71 : 149-70.

Gaastra, P. 1959. Photosynthesis of crop plants as influenced by light, carbon-di-oxide, temperature and stomatal diffusion resistance. Meded. Landbonwhogesch, Wageningen 59 : 1-68.

Gaastra, P. 1962. Photosynthesis of leaves and field crops. Neth. J. Agri. Sci. 10 : 311-24.

Gaff, D.F. and D.J. Carr. 1961. The quantity of water in the cell wall and its significance. Aust. J. Biol. Sci. 14 : 299-311.

Gardner, W.R. and R.M. Nieman. 1964. Some limit of water availability to plants. Science 143 (3613) : 1460-62.

Gardner, W.R. 1965. Dynamic aspects of soil water availability to plants. Ann. Rev. Plant Physiol. 16 : 323-42.

Garner, W.W. and H.A. Allard. 1920. Effect of relative length of day and night and other factors of environment on the growth and reproduction in plants. J. Agri. Res. 18 : 553-606.

Gates, C.T. 1955a. The response of the young tomato plant to a brief period of water shortage. I. The whole plant and its parts. Aust. J. Biol. Sci. 8 : 196-214.

Gates, C.T. 1955b. The response of the young tomato plant to a brief period of water shortage. II. The individual leaves. Aust. J. Biol. Sci. 8 : 214-30.

Gates, C.T. 1957. The response of the young tomato plant to a brief period of water shortage. III. Drifts in Nitrogen and Phosphorus. Aust. J. Biol. Sci. 10 : 125-46.

Gates, C.T. and J. Bonner. 1959. The response of the young tomato plant to a brief period of water shortage. IV. Effects of water stress on the ribonucleic acid metabolism of tomato leaves. Plant Physiol. 34 : 49-55.

Gates, C.T. 1964. The effect of water stress on plant growth. J. Aust. Inst. Agri. Sci. 30 : 3-22.

Genkel, P.A. 1961. Drought resistance in plants: Methods of recognition and of intensification. Plant Water Relationships in Arid and Semi-Arid conditions, Proc. Madrid Symp., Unesco, Paris.

Gifford, E.M. and H.B. Tepper. 1961. Ontogeny of the inflorescence in Chenopodium album. Amer. J. Bot. 48 : 657-67.

Gifford, E.M. and H.B. Tepper. 1962. Histochemical and autoradiographic studies of floral induction in Chenopodium album. Amer. J. Bot. 49 : 706-14.

Goff, D.F. and J. Levitt. 1965. The sulfahydryl hypothesis in relation to drought tolerance. Plant Physiol. 40 : suppl. xxxv.

Goode, J.E. 1956. Soil moisture relationships in fruit plantations. Ann. Appl. Biol. 44 : 525-30.

Guitard, A.A. 1960. The influence of different day lengths on ear emergence and seed setting in oats. J. Agri. Sci. 57 : 279-85.

Hamner, K.C. and J. Bonner. 1938. Photoperiodism in relation to hormones as factors in floral initiation and development. Bot. Gaz. 100 : 388-431.

Hamner, K.C. and A.W. Naylar. 1939. Photoperiodic responses of dill. Bot. Gaz. 100 : 853-61.

Hamner, K.C. 1940. Interrelation of light and darkness in photoperiodic induction. Bot. Gaz. 101 : 658-87.

Harris, F.S. 1914. Effect of soil moisture, plant food and age on the ratio of tops to roots in plants. J. Amer. Soc. Agron. 6 : 65.

Hartt, C.E., H.P. Kartschak, A.J. Forbes and G.O. Burr. 1963. Translocation of C^{14} in sugarcane. Plant Physiol. 38 : 305-18.

Hill, G.P. 1962. Exudation from aphid stylets during the period from dormancy to bud break in Tilia americana (L). J. Exp. Bot. 13 : 144-52.

Humphries, E.C. and G.N. Thorne. 1964. The effect of root formation on photosynthesis of detached leaves. Ann. Bot. (Lond.) N.S. 28 : 393-400.

Iljin, W.S. 1953. Causes of death of plants as a consequence of loss of water : conservation of life in desiccated tissues. Bull. Torrey Bot. Cl. No. 3 : 166-77.

Iljin, W.A. 1957. Drought resistance in plants and physiological processes. Ann. Rev. Plant Physiol. 8 : 257-74.

Imamura, S. and A. Takimoto. 1955. Transmission rate of photoperiodic stimulus in Pharbitis nil. Bot. Mag. Tokyo 68 : 260-66.

Imamura, S. and A. Takimoto. 1956. Transmission rate of the photoperiodic stimulus across the graft union in Pharbitis nil. Chois Bot. Mag. Tokyo 69 : 23-29.

Janes, B.E. 1961. Use of polyethylene glycols as a solvent to increase the osmotic pressure of nutrient solutions in studies of the physiology of water in plants. Plant Physiol. 36 : suppl. xxiv.

Janes, B.E. 1964. Adjustment of plants to increase in osmotic pressure of root medium. Plant Physiol. 39 : suppl. lvii.

Jarvis, P.G. and M.S. Jarvis. 1963a. The water relations of tree seedlings. I. Growth and water use in relation to soil water potential. II. Transpiration in relation to soil water potential. Physiol. Plant. 16 : 215-53.

Jarvis, P.G. and M.S. Jarvis. 1963b. The water relations of tree seedlings. III. Transpiration in relation to osmotic potential of root medium. Physiol. Plant. 16 : 269-75.

Jarvis, P.G. and M.S. Jarvis. 1963c. Effect of several osmotic substrates on the growth of Lupinus albus seedlings. Physiol. Plant. 16 : 485-500.

Kaul, R. 1965. Effect of water stress on respiration of wheat. Can. J. Bot. 44 : 623-32.

Keesler, B. 1961. Recent advances in botany. 1153-59. (Univ. of Toronto Press, Toronto).

Kerr, T. and D.B. Anderson. 1944. Osmotic quantities in growing cotton bolls. Plant Physiol. 19 : 338-48.

Knox, R.B. and L.T. Evans. 1966. Inflorescence initiation in Lolium temulentum L. VIII. Histochemical changes at the shoot apex during induction. Aust. J. Biol. Sci. 19 : 233-45.

Koller, D. and H.R. Highkin. 1960. Environmental control of reproductive development in Hordeum bulbosum, a perennial pasture grass. Aust. J. Bot. 47 : 843.

Konovalov, J.B. 1959. The effect of a deficiency in soil moisture on grain filling of spring wheat. Fiziol. Rastt. 6 : 183-89.

Kozlowskii, T.T. 1965. Water metabolism in plants. Harper and Row, N.Y., Evanston and London and John Weatherhill, Inc., Tokyo.

Kramer, P.J. 1938. Root resistance as a cause of absorption lag. Amer. J. Bot. 25 : 110-13.

Kramer, P.J. 1959. The role of water in the physiology of plants. Advanc. Agron. 11 : 51-70.

Kramer, P.J. 1959a. Transpiration and the water economy of plants. Plant Physiol.: a treatise, ed. F.C. Steward II : 607.

Kramer, P.J. 1963. Water stress and plant growth. Agron. J. 55 : 31-5.

Kursanov, A.L. 1956. Recent advances in plant physiology in the U.S.S.R. Ann. Rev. Pl. Physiol. 7 : 401-36.

Lang, A. 1965. Physiology of flower initiation. Encycl. Plant Physiol. xv/1 : 1380-1536.

Langer, R.H.M. and W.T. Bussell. 1964. The effect of flower induction on the rate of leaf initiation. Ann. Bot. N.S. 28 : 163-7.

Lagerwerff, J.V., G. Ogata and H.E. Eagle. 1961. Control of osmotic pressure of culture solutions with polyethylene glycol. Science 133 : 1486-7.

Leopold, A.C. 1949. The control of tillering in grasses by auxin. Amer. J. Bot. 36 : 437-40.

Leshem, B. 1966. Toxic effects of carbowaxes (polyethylene glycol) on Pinus halepensis Will. seedlings. Plant and Soil xxiv (2) : 322-24.

Lucanus, R., K.J. Mitchell, G.G. Pritchard and D.M. Calder. 1960. Factors influencing survival of streams of ryegrass during summer. N.Z. J. Agri. Res. 3 : 185-93.

MacDougal, D.T. 1920. Hydration and growth. Carneige Inst. Wash. Pub. 297.

Majmudar, A.M. and J.P. Hudson. 1957. The effects of different water regimes in the growth of plants under glass. II. Experiments with lettuces (Lactuca sativa Linn.) J. Hort. Sci. 32 : 201-13.

Maximov, N.A. 1929. The plant in relation to water. London : Allen and Unwin.

May, L.H. and F.L. Milthorpe. 1962. Drought resistance of crop plants. Field Crop Abs. 15 : No.3. 1-9.

May, L.H., E.J. Milthorpe and F.L. Milthorpe. 1962. Presowing hardening of plants to drought. An appraisal of the contributions by P.A. Genkel. Field Crop Abs. 15 : No.2. 1-6.

Mayer, L. 1956. Wachstum und organbildung an in-vitro kultiverten Segmenten von Pelargonium zonale und Cyclamen persicum. Planta (Berl.) 47 : 401-46.

Meyer, B.S. and A.M. Wallace. 1941. A comparison of two methods of determining the diffusion pressure deficit of potato tuber tissue. Amer. J. Bot. 28 : 838-43.

Meyer, B.S. 1945. A critical evaluation of the terminology of diffusion phenomena. Plant Physiol. 20 : 142-64.

Miller, E.C. 1938. Plant Physiology. N.Y. McGraw Hill Book Co. Inc.

Milthorpe, F.L. 1950. Changes in the drought resistance of wheat seedlings during germination. Ann. Bot. (Lond.) 14 : 79-89.

Milthorpe, F.L. and E.J. Spencer. 1957. Experimental studies of the factors controlling transpiration. III. Interrelations between transpiration rate, stomatal movement and leaf water content. J. Exp. Bot. 8 : 413-37.

Milthorpe, F.L. 1959. Transpiration from crop plants. Field Crop Abst. 12 (1) : 1-9.

Milthorpe, F.L. 1959a. Studies on the expansion of leaf surface. I. The influence of temperature. J. Exp. Bot. 10 : 233-49.

Milthorpe, F.L. 1960. The income and loss of water in arid and semi-arid zones. In Plant-water relationships in arid and semi-arid conditions. Rev. Res., Paris, Unesco.

Milthorpe, F.L. 1961. Plant factors involved in transpiration. Plant water relationships in arid and semi-arid conditions. Proc. Madrid Symp., Unesco, Paris, 107-113.

Morton, A.G. and D.J. Watson. 1948. A physiological study of leaf growth. Ann. Bot. N.S. 12 : 281-310.

Moss, D.N., R.B. Musgrave and E.R. Lemon. 1961. Photosynthesis under field conditions. III. Some effects of light, carbon-di-oxide, temperature and soil moisture on photosynthesis, respiration and transpiration of corn. Crop Sci. 1 : 83-7.

Motkalyuk, O.B. 1965. Effect of insufficient soil moisture during the critical period on formation of the female gametophyte in barley. Bot. Zh. 50 : 874-9.

Namken, L.N. and E.R. Lemon. 1960. Field studies of internal moisture relations of the corn plant. Agron. J. 52 : 643-6.

Negisi, K. and T. Satoo. 1954. The effect of drying of soil on apparent photosynthesis, transpiration, carbohydrate reserves, and growth of seedlings of Akamatu (Pinus densiflora Sieb. et Zucc). J. Jap. For. Soc. 36 : 66-71.

Nicholls, P.B. 1962. On the growth of the barley apex. Ph.D. thesis, Univ. of Adelaide.

Nicholls, P.B. and L.H. May. 1963. Studies on the growth of the barley apex. I. Interrelationships between primordium formation, apex length and spikelet formation. Aust. J. Biol. Sci. 16 : 561-71.

Nosberger, J. and E.C. Humphries. 1965. The influence of removing tubers on dry matter production and net assimilation rate of potato plants. Ann. Bot. (Lond.) 29 : 579-88.

Novikov, V.P. 1952. The effect of a deficiency of water in the soil at different stages of development in oats. C.R. Acad. Sci. U.R.S.S. 82 : 641-3.

Novikov, V.P. 1954. Development of the oat panicle under different conditions of water supply. Bot. U. Zh. 39 : 17-20.

Oppenheimer, H.R. 1960. Adaptation to drought: xerophytism. Plant water relationships in arid and semi-arid conditions. Reviews of Research, Paris, Unesco.

Paauw, F. Vander. 1949. Water relations of oats with special attention to the influence of periods of drought. Plant and Soil 1 : 303-41.

Paauw, F. Vander. 1952. AAnpassing en schade van het gewas haver bij watergebrek. Comm. Hydrol. Res. T.N.O. Proc. Tech. Meet. 1946-50 : 202-5.

Paleg, L.G. and D. Aspinall. 1966. Effect of day length and light intensity on growth of barley.

V. Response by plants in the field to night interruption. Aust. J. Biol. Sci. 19 : 719-31.

Penman, H.L. and R.K. Schofield. 1951. Some physical aspects of assimilation and transpiration. Soc. Exptl. Biol. 5 : 115-29.

Petinov, N.S. 1961. Physiological principles of raising plants under irrigated agriculture. Plant water relationships in arid and semi-arid conditions, Proc. Madrid Symp., Unesco, Paris, 81-92.

Petinov, N.S. and F. Berko. 1965. Free amino acid content in connection with the growth processes of corn growing under different water supply conditions. Fiziol. Rastt. 12 (1) : 56-63.

Petrie, A.H.K. and J.G. Wood. 1938a. Studies on the nitrogen metabolism of plants. I. The relation between the content of proteins, amino acids and water in the leaves. Ann. Bot. (Lond.) N.S. 2 : 33-60.

Petrie, A.H.K. and J.G. Wood. 1938b. Studies on the nitrogen metabolism of plants. III. On the effect of water content on the relationship between proteins and amino acids. Ann. Bot. (Lond.) N.S. 2 : 887-98.

Phillip, J.R. 1958. The osmotic cell, solute diffusibility and the plant water economy. Plant Physiol. 33 : 264-71.

Phillip, J.R. 1957. The physical principles of soil water movement during the irrigation cycle. Proc. III Int. Cong. Irrig. Drainage 8 : 125-54.

Plaut, Z. and L. Reinhold. 1965. The effect of water stress on (^{14}C) sucrose transport in bean plants. Aust. J. Biol. Sci. 18 : 1143-55.

Popham, R. and A.P. Chan. 1952. Origin and development of the receptacle of Chrysanthemum marifolium. Amer. J. Bot. 39 : 329-39.

Preston, R.D. 1954. The transpiration of plants. Proc. Leeds Phil. and Lit. Soc. 6 : 154-67.

Richards, L.A. and C.H. Wadleigh. 1952. Soil water and plant growth. In "Soil Physical Conditions and Plant Growth". (Ed. Byron T. Shaw) (Acad. Press Inc. N.Y.).

Roberts, B.R. 1964. Effect of water stress on the translocation of photosynthetically assimilated carbon-14 in yellow poplar. In "The formation of wood in forest trees". (Ed. M.H. Zimmermann) (Acad. Press Inc. N.Y.).

Robins, J.S. and C.E. Domingo. 1953. Some effects of severe soil moisture deficit at specific growth stage of corn. Agron. J. 45 : 618-21.

Rutter, A.J. and K. Sands. 1958. The relation of leaf

water deficit to soil moisture tension in Pinus sylvestris L. I. The effect of soil moisture on diurnal changes in water balance. New Phytol. 57 : 50-65.

Salisbury, F.B. 1955. Kinetic studies of the physiology of flowering. Doct. dissert. Calif. Inst. of Technol., Pasadena (cited by A. Lang, 1965).

Salter, P.J. 1957. The effects of different water regimes on the growth of plants under glass. III. Further experiments with tomatoes (Lycopersicum esculentum Mill.) J. Hort. Sci. 32 : 214.

Sands, K. and A.J. Rutter. 1959. Studies in the growth of young plants of Pinus sylvestris L. II. The relation of growth to soil moisture tension. Ann. Bot. 23 : 269-84.

Schneider, G.W. and N.F. Childers. 1941. Influence of soil moisture on photosynthesis, respiration and transpiration of apple leaves. Plant Physiol. 16 : 565-83.

Schwabe, W.W. 1959. Some effects of environmental and hormone treatment in reproductive morphogenesis in Chrysanthemum. J. Linn. Soc. Bot. 56 : 254-61.

Searle, N.E. 1961. Persistant and transport of flowering stimulus in xanthium. Plant Physiol. 36 : 656-62.

Searle, N.E. 1965. Physiology of flowering. Ann. Rev. Plant Physiol. 16 : 97-118.

Shah, C.B. and R.S. Loomis. 1965. Ribonucleic acid and protein metabolism in sugar beet during drought. Plant Physiol. 18 : 240-54.

Sharman, B.C. 1945. Leaf and bud initiation in the Gramineae. Bot. Gaz. 106 : 260-89.

Sharman, B.C. 1947. The biology and developmental morphology of the shoot apex in the Gramineae. New Phytol. 46 : 20-34.

Sharman, B.C. 1960a. Development of the inflorescence and spikelets of Anthoxanthum odoratum L. New Phytol. 59 : 60-64.

Sharman, B.C. 1960b. Developmental anatomy of the stamen and carpel primordia in Anthoxanthum odoratum L. Bot. Gaz. 121 : 192-98.

Skazkin, F.D. and Lukomskaya, K.A. 1962. The effect of soil moisture deficiency on the formation of the female gametes of some cereals. Trans. Dokl. Akad. Nauk S.S.S.R. 146 (6) : 1449-51.

Slatyer, R.O. 1955. Studies of the water relations of crop plants grown under natural rainfall in northern Australia. Aust. J. Agri. Res. 6 : 365-77.

Slatyer, R.O. 1956. Evaporation in relation to soil moisture. Neth. J. Agri. Sci. 4 (1) : 73-76.

Slatyer, R.O. 1957. The influence of progressive increases in total soil moisture stress on transpiration growth and internal water relationships of plants. Aust. J. Biol. Sci. 10 (3) : 320-36.

Slatyer, R.O. 1958. The measurement of diffusion pressure deficit in plants by a method of vapour equilibration. Aust. J. Biol. Sci. 11 : 349-65.

Slatyer, R.O. 1960a. Aspects of tissue water relationships of an important arid zone species (Acacia aneura F. Muell.) in comparison with two mesophytes. Bull. Res. Counc. Israel 8D : 159-68.

Slatyer, R.O. 1960b. Absorption of water by plants. Bot. Rev. 26 : 331-92.

Slatyer, R.O. and S.A. Taylor. 1960. Terminology in plant and soil water relations. Nature, Lond. 187 : 922.

Slatyer, R.O. 1961a. Effect of several osmotic substrates on the water relationship of tomato. Aust. J. Biol. Sci. 14 : 519-40.

Slatyer, R.O. 1961b. Internal water balance of Acacia aneura F. Muell. in relation to environmental conditions. Plant water relationships in arid and semi-arid conditions, Proc. Madrid Symp., Unesco, Paris.

Slatyer, R.O. 1962. Internal water relations of higher plants. Ann. Rev. Plant Physiol. 13 : 351-78.

Slatyer, R.O. and J.F. Bierhuizen. 1964. The influence of several transpiration suppressants on transpiration, photosynthesis, and water use efficiency of cotton leaves. Aust. J. Biol. Sci. 17 : 131-46.

Slatyer, R.O. and H.D. Barrs. 1965. Modifications to the relative turgidity technique with notes on its significance as an index of the internal water status of leaves. Methodology of Plant Eco-Physiology. Proc. Montpellier Symp., Unesco, Paris.

Snyder, W.E. 1948. Mechanism of the photoperiodic response of Plantago lanceolata L., a long day plant. Amer. J. Bot. 35 : 520-25.

Stanhill, G. 1957. The effect of differences in soil moisture status on plant growth: a review and analysis of soil moisture regime experiments. Soil Sci. 84 : 205-14.

Steward, F.C., R.H. Wetmore, J.F. Thompson and J.P. Nitsch. 1954. The nitrogen components of certain shoot and apical growing regions. Amer. J. Bot. 41 : 123-34.

Steward, F.C., R.H. Wetmore and J.K. Pallard. 1955. Nitrogenous compounds of the shoot apex of Adiantum pedatum. Amer. J. Bot. 42 : 936-38.

Stichel, E. 1959. Gleichzeitige Induktion von Sprossen und Wurzeln an in vitro kultivertin Gewebestucken von Cyclamen persicum. Planta (Berl.) 53 : 293-317.

Stocker, O. 1960. Physiological and morphological changes in plants due to water deficiency. Plant water relationships in arid and semi-arid conditions. Reviews of research, Unesco, Paris.

Stocking, C.R. 1956. The state of water in cells and tissues. Encycl. Plant Physiol. 3 : 15-21.

Takimoto, A. 1955. Flowering response to various combinations of light and dark periods in Silene armeria. Bot. Mag. (Tokyo) 68 : 308-14.

Taylor, S.A. and L. Cavazza. 1954. The movement of soil moisture in response to temperature gradient. Soil Sci. Soc. Amer. Proc. 18 : 351-58.

Thomas, R.G. 1961. Correlations between growth and flowering in Chenopodium amaranticolor. I. Inhibition of leaf and bud primordia. Ann. Bot. 25 : 138-51.

Thorne, G.N. and A.F. Evans. 1964. Influence of tops and roots on net assimilation rate of sugar beet and

spinach beet and grafts between them. Ann. Bot. N.S. 28 : 499-508.

Usprung, A. 1923. Zur kenntnis der Saugkraft. VII. Eine neue vereinfachte Methode Zur Messung der Sangkraft. Ber. dtsch. bot. Geo. 41 : 338-43.

Vaadia, Y., F.C. Raney and R.H. Hagan. 1961. Plant water deficits and physiological processes. Ann. Rev. Plant Physiol. 12 : 265-92.

Van Bavel, C.H.M. 1953. Chemical composition of tobacco leaves as affected by soil moisture conditions. Agron. J. 45 : 611-14.

Vince, D. 1965. The promoting effect of far red light on flowering in the long day plant Lolium temulentum. Plant Physiol. 18 : 474-82.

Veihmeyer, F.J. and A.H. Hendrickson. 1950. Soil moisture in relation to plant growth. Ann. Rev. Plant Physiol. 1 : 285-304.

Veihmeyer, F.J. 1956. Soil moisture. Encycl. Plant Physiol. 3 : 64-123.

Verduin, J. and W.E. Loomis. 1944. Absorption of carbon-di-oxide by maize. Plant Physiol. 19 : 278-93.

Wadleigh, C.H. and A.D. Ayers. 1945. Growth and bio-chemical composition of bean plants as conditioned by soil moisture tension and salt concentration. Plant Physiol. 20 : 106-32.

Walter, H. 1939. Grassland, Savanna und Busch der ariden Teile Afrikas in ihrer okologischen Bedingtheit. Jb. wiss. Bot. 87 : 750-860.

Walter, H. 1955. The water economy and the hydrature of plants. Ann. Rev. Plant Physiol. 6 : 239-52.

Wangermann, E. 1961. The effect of water supply and humidity on growth and development. In Encycl. Plant Physiol. XVI ed. Ruhland.

Wardlaw, I.F. 1965. The velocity and pattern of assimilate translocation in wheat plants during grain development. Aust. J. Biol. Sci. 18 : 269-81.

Weatherley, P.E. 1950. Studies in the water relations of the cotton plant. I. The field measurements of water deficits in leaves. New Phytol. 49 : 81-97.

Weatherley, P.E. 1951. Studies of the water relations of the cotton plant. II. Diurnal and seasonal fluctuations and environmental factors. New Phytol. 50 : 36-51.

Weatherley, P.E. and R.O. Slatyer. 1957. Relationship between relative turgidity and diffusion pressure deficit in leaves. Nature 179 : 1085-6.

West, S.H. 1962. Protein, nucleotide and ribonucleic acid metabolism in corn during germination under water stress. Plant Physiol. 37 : 565-71.

Wetmore, R.A., E.M. Gifford, Jr., and M.C. Green. 1959. Development of vegetative and floral buds. Photoperiodism and related phenomenon in plants and animals. Ed. R.W. Withrow. Amer. Ass. Adv. Sci. Publ. No. 55, Washington D.C. 255-73.

Wetmore, R.H. and G. Morel. 1949. Growth and development of Adiantum pedatum L. on nutrient agar. Amer. J. Bot. 36 : 805-6.

Wetmore, R.H. 1954. The use of "in vitro" cultures in the investigation of growth and differentiation in vascular plants. Brookhaven Symp. Biol. 6 : 22-40.

Wetmore, R.H. 1956. Growth and development in the shoot system of plants. In Cellular mechanisms in differentiation and growth, Princeton, Univ. Press.

Wiebe, H.H. and S.E. Wihrheim. 1962. The influence of internal moisture deficit on translocation. Plant Physiol. 37 (supp.) : i-li.

Williams, R.F. and R.E. Shapter. 1955. A comparative study of growth and nutrition in barley and rye as affected by low water treatment. Aust. J. Biol. Sci. 8 : 435-66.

Williams, R.F. 1960. The physiology of growth in the wheat plant. I. Seedling growth and the pattern of growth at the shoot apex. Aust. J. Biol. Sci. 13 : 401-28.

Wilson, C.C., W.R. Boggess and P.J. Kramer. 1953. Diurnal fluctuations in the moisture content of some herbaceous plants. Amer. J. Bot. 40 : 97-100.

Wilson, E.C. 1948. Diurnal fluctuations of growth in length of tomato stem. Plant Physiol. 23 : 157-8.

Wind, G.P. 1956. Flow of water through plant roots. Neth. J. Agri. Sci. 3 : 259-64.

Withrow, A.P. and R.B. Withrow. 1943. Translocation of floral stimulus in xanthium. Bot. Gaz. 104 : 409-16.

Wormer, T.M. and R. Ochs. 1959. Humidite du sol ouverture des stomates et transpiration du palmier a huile et de l'arachide. Oleagineux 14 : 571-80.

Wood, J.G. and B.S. Barrien. 1939a. Studies on the sulphur metabolism of plants. I. Preliminary investigations on the effects of different external concentrations of sulphate ammonia and cystine on the amount of sulphur containing compounds in leaves. New Phytol. 38 : 125-49.

Wood, J.G. and B.S. Barrien. 1939b. Studies on the sulphur metabolism of plants. III. On changes in amounts of protein sulphur and sulphate sulphur during starvation. New Phytol. 38 : 265-72.

Woodhams, D.H. and T.T. Kozlowski. 1954. Effects of soil moisture stress on carbohydrate development and

growth in plants. Amer. J. Bot. 41 : 316-30.

Zavadskaya, I.G. and F.D. Skazkin. 1960. Microsporogenesis in barley with deficient soil moisture and application of nitrogen at different developmental periods. C.R. Acad. Sci. U.R.S.S. 95 : 190-7.

Zeevart, J.A.D. 1958. Flower formation as studied by grafting. Meded. Landbouwhogesch Wageningen 58 No. 3.

Zeevart, J.A.D. 1962. DNA multiplication as a requirement for expression of floral stimulus in Pharbitis nil. Plant Physiol. 37 : 296-304.

Zholkevich, V.N., T.F. Koretskaya. 1959. Metabolism of pumpkin roots during soil drought. Trans. Fiziol. Rastt. 6 : 690-700.

APPENDIX I. Suitability of polyethylene glycol 4000 as an osmotic agent

In experiments in which Lolium temulentum plants were exposed to water stress during photoperiodic induction it was necessary to impose stress for periods as short as 2 hours. This is impossible using conventional methods and reduced matric potential in a soil medium but is feasible using a liquid medium and reduced osmotic potential. The use of osmotic potential imposes difficulties, however, the major one being the possibility of the entry of the osmotic agent into the plant with the resulting reduction in potential gradient and metabolic effects. Polyethylene glycol of varying molecular weight ranges has been claimed to be the most suitable osmotic agent for such experiments (Jarvis and Jarvis 1963c, Barrs 1966), although some toxic responses to this substance have been reported (Leshem 1966). It was decided to use polyethylene glycol (Carbowax - Union Carbide Australia Ltd.) with an average molecular weight of 4,000 in the present study, but to check the uptake of this substance into the plant during the periods likely to be used experimentally. There is no simple chemical means of estimating polyethylene glycol in the plants but Slatyer (1961a) has suggested an indirect test for the uptake of any osmotic agent by plant roots. If plants exposed to osmotic

stress take up appreciable quantities of the osmotic agent, then the osmotic potential of the plant rises, the plant shows partial recovery and, on relief of the stress, the water content of such plants is temporarily elevated above that of the control, non-stressed plants. This effect was examined in the present system.

Lolium temulentum plants were grown in an 8 hour photoperiod at 17°C ± 1°C temperature until the sixth leaf expanded. Water stress was imposed by the application of a 24-atmosphere osmotic potential polyethylene glycol solution to the rooting medium and was relieved by thoroughly washing out the solution after 2 hours. Plants were allowed to recover for a further 6 hours. Standard leaf discs (8 mm) were punched from the central portion of the sixth fully expanded leaf after 1, 4, 8 and 24 hours of imposition of the stress and 2, 4 and 6 hours after relief of the stress. Leaf discs were punched in the same sequence from plants not subjected to stress. Each sample consisted of ten discs and five plants were used as replicates. After punching, leaf discs were transferred into weighing bottles and the fresh weights were determined. The samples were then placed in a forced-draught oven at 90°C for 48 hours and dry weights were recorded. Water content was obtained as fresh weight - dry weight. Table 1 presents the leaf water content of the stressed leaf tissue relative to that of the control.

TABLE 1

RELATIVE LEAF WATER CONTENT
(% of control)

After imposition of stress				After relief of stress		
1 hour	4 hours	8 hours	24 hours	2 hours	4 hours	6 hours
85	80	78	75	80	96	98

It is apparent from the results that the leaf water content of the stressed leaf tissue relative to that of the not stressed leaf tissue declined progressively for the 24 hour period of stress. After relief of stress, recovery in water content ensued and was almost complete after 4 hours. Since there was no recovery in leaf water content during stress and also no consequent increase above control after relief of stress which is associated with the entry of the solute, it was concluded that within the experimental conditions used in the present study entry of polyethylene glycol into the plant cells did not occur and was not an appreciable source of error.

APPENDIX II. Relative turgidity technique

The term relative turgidity was introduced by Weatherley (1950) and defined as the amount of water in a measured quantity of tissue expressed as a percentage of the amount of water retained by the same tissue when fully turgid.

$$\text{Relative turgidity} = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

The value and repeatability of the relative turgidity method of assessing leaf water deficit in barley and Lolium temulentum in the present study was tested by the methods recommended by Barrs and Weatherley (1962). These authors recognized a number of possible sources of error in the measurement of relative turgidity and each was checked with barley leaves.

1. Sample errors

Fresh weight determinations are relatively free from major errors as long as precautions are taken to prevent evaporation of water from the discs. Sample variations due to inadequate precautions in handling the material have been observed, however (Slatyer and Barrs 1965). The following standard procedure was adopted from the outset to minimize such errors. The leaf to be sampled was first freed from dust particles by a routine wiping of the surface with a tissue. Discs were cut as rapidly as possible with a sharp wad punch supported on a rubber bung and immediately

transferred to a gas tight weighing bottle and weighed. The age of the leaf, its position on the plant and the portion of the leaf sampled could all conceivably influence the relative turgidity measured. The need to standardize these components was assessed in a simple experiment with 4 week-old barley plants. Standard discs (8 mm) were cut from four groups of leaves; leaf 1 (fully expanded with ligule exposed), leaf 2 (expanding leaf, ligule not exposed), leaf 3 (emerged and actively expanding) and leaf 4 (emerging). Discs were taken from three separate regions on each leaf (basal, central and terminal portions). Five discs from each portion were combined as a sample, five plants being used as replicates. The fresh weights, dry weights and water contents (as a percentage of dry weight) were determined and a statistical analysis of the data is presented in Table 1.

TABLE 1

ANALYSIS OF VARIANCE

Source	D.F.	Fresh Weight M.S.	Fresh Weight V.R.	Dry Weight M.S.	Dry Weight V.R.	Water Content M.S.	Water Content V.R.
Leaf number	3	6326.53	21.52 ***	5851.61	27.46 ***	95.06	19.80 ***
Position on leaf	2	57183.12	194.57 ***	137.45	6.44 **	69.32	14.44 ***
Leaf no. x position	6	8412.69	28.62 ***	82.58	3.87 **	12.67	2.63 *
Error	59	293.88	-	21.32	-	4.80	-

* Significant at 5% level
** Significant at 1% level
*** Significant at 0.1% level

It is apparent from the results that significant variation existed between the leaf tissues of different age groups and between different regions of the same leaf. It was concluded that to avoid this source of error, sampling should be restricted to a uniform material and the middle portion of the fully expanded leaf was chosen for further determinations of relative turgidity.

2. Errors due to excess water uptake

A possible error in turgid weight determinations of the discs may arise due to an inadequate surface drying of the tissue after the floating phase is complete. Such a source of error was minimized by adopting a uniform method of surface drying. Leaf discs were placed on a four sheet layer of filter paper, covered with another four sheet layer and surface dried by applying a 500 g weight to the top layer for 20 seconds.

In cotton (Weatherley 1950) and in castor bean (Barrs and Weatherley 1962), the water uptake pattern of leaf tissue is divisable into two distinct phases; an initial rapid uptake during the first few hours of floating followed by a slow persistent uptake as long as the discs remain healthy. In castor bean leaf discs the first phase, lasting 4 hours, represents uptake in response to the water deficit in the tissue and is the period in which full turgidity is attained.

The subsequent slow and steady uptake is due to the growth of the tissue. Errors due to this disc growth have been avoided by limiting the floating period to 4 hours in tobacco (Bliss, Kramer and Wolf 1957) and corn (Namken and Lemon 1960), 2 hours in Eucalyptus globulus Labill (Carr and Gaff 1959) and 1 hour in peanut leaf discs (Wormer and Ochs 1959). The water uptake pattern of barley leaf discs was investigated to assess the duration of the first phase.

The water uptake patterns of discs from fully expanded and expanding barley leaves was followed. Leaf discs of two sizes, 6 or 8 mm diameter, were punched from the central portion of the leaves. Thus there were four treatments replicated five times. Each set of discs was weighed, floated on water and re-weighed at intervals of 2, 4, 6 and 24 hours. The relative changes in weight as a percentage of the original fresh weights are presented in Table 2.

TABLE 2

	Percentage increase in original fresh weight after floating for			
	2 hours	4 hours	6 hours	24 hours
Fully expanded leaf discs (8 mm diameter)	110	116	118	125
Fully expanded leaf discs (6 mm diameter)	108	117	117	122
Expanding leaf discs (8 mm diameter)	117	126	130	135
Expanding leaf discs (6 mm diameter)	137	155	181	181

It is apparent that there was a rapid uptake of water in the first four hours of floating followed by a slow and steady uptake excepting in the smaller leaf discs (6 mm diameter) from expanding leaves where there was no increase after six hours. It may also be seen that water uptake by expanding leaves was greater than that by the expanded leaves, considerably so with smaller discs.

This experiment demonstrated that discs cut from the older leaves exhibited the pattern of water uptake described by Barrs and Weatherley (1962) with full turgidity being reached 4 hours after floating the discs. Discs from younger leaves showed no apparent discontinuity in water uptake due presumably to growth of the discs or the phenomenon of 'plastic flow' described by Milthorpe and Spencer (1957). In future work, therefore, discs were taken only from mature leaves and were floated for 4 hours before the turgid weight was determined.

Excessive water uptake by the discs leading to errors in estimation may also result from injection through the cut edges of the discs. Errors from this source have been reported in Sambucus (Barrs and Weatherley 1962) and Eucalyptus globulus (Carr and Gaff 1959). The importance of this source of error can be assessed from a comparison of the water uptake of discs of different sizes. Since smaller discs have a greater circumference/area ratio it is expected

that edge injection would lead to higher percentage water uptake by small than by large discs. This was not the case with the mature discs in the previous experiment (Table 2) but this factor was investigated further by punching discs of different sizes (6 and 8 mm) from fully expanded leaves of two age groups (leaf 1 and 2 from the base of the plant) floating them for 24 hours and comparing changes in fresh weight. Statistical analysis of the data (Table 3) revealed no evidence of any effect of disc size. It may be assumed, therefore, that this possible source of error is unimportant in barley leaves. It is also of interest that mature leaves of different ages on the same plant gave the same relative turgidity estimates.

TABLE 3

ANALYSIS OF VARIANCE

Source	D.F.	M.S.	V.R.
Blocks	9	113.528	4.81***
Leaf age	1	10.712	0.56 N.S.
Disc size	1	13.340	0.45 N.S.
Leaf age x disc	1	0.931	0.03 N.S.
Error	27	23.593	

*** Significant at 0.1% level
N.S. Not significant

3. Errors due to changes in dry weight during floating

A further source of error is the possible loss of dry weight during the period in which the discs are floating

(Weatherley 1950). This is minimized when the flotation period is short but Barrs and Weatherley (1962) considered it profitable to estimate the compensation point, the light intensity at which no dry weight changes occur, for their discs of Ricinus leaves. This was also attempted with barley leaf discs in the present study. Discs were cut from uniform mature leaves and the duplicate samples were weighed, oven-dried and re-weighed to obtain an estimate of the original water content of the population of discs. Five further sets of discs were weighed and then floated at 0, 10, 50, 100 or 200 foot-candles light intensity at 20°C for 24 hours. These discs were then oven-dried and weighed. The original dry weight of the discs was estimated from the initial fresh weight using the calculated initial water content and the percentage change in dry weight during the 24 hour flotation period was obtained (Table 4).

TABLE 4

Light intensities	Percentage increase over original dry weight due to 24 hours flotation
0	1.01
10	1.09
50	1.06
100	0.69
200	1.21

It is apparent that any change in dry weight due to 24 hours of floating was negligible and there was little difference between the various light intensities to which the leaf discs were exposed. It can be expected that a reduction of the floating period to 4 hours would again considerably reduce the differences in dry weight and this factor can be neglected without loss of precision. Thus a period of 4 hours of flotation in dark was adopted for further relative turgidity measurements.

APPENDIX III. Water potential of the apex

The water potential of the plant tissue can be assessed by immersing the tissue in solutions of a graded series of osmotic potential and measuring the change in weight, volume or length. The solution which does not cause the tissue to change in weight or size is considered to have an osmotic potential equal to the water potential of the tissue. Such determinations are liable to certain errors which may result from inadequate precautions, however, and the vapour equilibration method has been generally preferred (Slatyer 1962). It was not possible to use a vapour equilibration method for the determination of the water potential of the grass apex because of the small size of the object. A solution equilibration method was therefore developed for these determinations.

Due to the configuration of the grass apex, a length measurement is simpler and more reproducible than any estimate of tissue volume or any other dimension. It was possible, however, that the technique would be subject to error if a change in the length of tissue did not reflect an equal change in tissue or vacuolar volume. This would have occurred if the solute penetrated into the tissue and would be more marked it if was metabolised. This possibility was minimized by using the non-electrolyte,

polyethylene glycol (average m.w.-4000) as the osmotic agent. Chemical from the same batch was used to avoid any possible variability. It was not possible to check whether polyethylene glycol entered into the protoplasts and vacuoles of the apical tissue but in view of the results obtained with leaf tissue (Appendix I) this was not likely to be an important source of error. To minimize this, and other possible errors, it was necessary to restrict the time of immersion of the tissue to the shortest possible period giving length changes measurable with a binocular microscope. This was determined as follows. Barley apices (morphological stage of development corresponding to score 5 of the scale, measuring 0.9 mm) were dissected under a binocular microscope and cut with a sharp needle at the base of the oldest simple ridge primordium. After measuring the initial apex length, apices were immersed in polyethylene glycol solutions in a closed 4 cm petri dish and left at a constant temperature of 20°C in the dark. Apex length was recorded after 2, 4 and 8 hours of immersion. The concentrations of the osmotic solutions used were -1.9, -3.9, -5.9, -7.9 and -9.8 bars. Each treatment has three replicates for each solution. Results are presented in Table 1.

TABLE 1

Concentrations of osmotic solutions	Relative change in apex length over initial length (%)		
	after 2 hours	after 4 hours	after 8 hours
-1.9 bars	+ 0.80	+ 3.55	+ 3.00
-3.9 bars	+ 0.10	+ 2.00	+ 2.80
-5.9 bars	- 0.10	- 1.58	- 2.85
-7.9 bars	- 0.15	- 2.50	- 2.65
-9.8 bars	- 0.10	- 3.60	- 3.60

It is apparent that an easily measurable change in apex length had occurred in 4 hours. There was also no evidence of a change with time in the osmotic potential at which no length change occurred; this supports the view that the osmotic agent was not entering the tissue. Immersion time was standardized at 4 hours in all further determinations. The data indicated that water potential of the apices lay between -4 and -8 bars. This factor was further investigated to observe if greater precision could be obtained by restricting the concentration range within these limits. The five polyethylene glycol solutions used were -4.9, -5.4, -5.9, -6.4 and -6.9 bars. Apices were dissected in a similar manner and immersed for 4 hours in the dark at 20^{o}C. The relative change in the apex length compared with the initial length (0.8 mm) is presented in Table 2.

TABLE 2

Concentrations of osmotic solutions	Relative change in apex length over initial length (%)
-4.9 bars	+ 1.00
-5.4 bars	No change
-5.9 bars	- 1.05
-6.4 bars	- 2.50
-6.9 bars	- 3.00

From the above results, it was clear that a concentration range from -4.9 bars to -6.9 bars would accurately estimate the water potential of apices not subject to water stress and this range was used for all the experimental determinations.

APPENDIX IV. Statistical treatment of the data (Analysis of Variance)

The following abbreviations have been used throughout:

D.F.	Degrees of freedom
M.S.	Mean sum of squares
*	Significant at 5% level of probability
**	Significant at 1% level of probability
***	Significant at 0.1% level of probability

SHORT PERIOD OF WATER STRESS AND GROWTH (3.1)

PERCENTAGE OF SEEDS SOWN WHICH EMERGED FINALLY

Source	D.F.	M.S.
Variety	1	157.78**
Hardening	1	644.64***
Variety x Hardening	1	185.79***
Error	52	16.76

NUMBER OF PRIMORDIA ON THE MAIN SHOOT APEX

Source	Harvest 1		Harvest 2		Harvest 3		Harvest 4		Harvest 5	
	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.
Blocks	4	1.07	4	0.16	4	1.56	4	5.37	4	0.19
Water (W)	-	-	1	0.23	2	48.32***	2	13.52**	2	9.52**
Hardening (H)	1	0.20	1	0.63	1	2.82	1	2.40	1	0.15
Variety (V)	1	3.20*	1	1.23	1	25.35***	1	11.27*	1	114.82***
W x H	-	-	1	0.22	2	6.21**	2	3.95	2	2.15
W x V	-	-	1	0.22	2	4.05*	2	2.21	2	0.51
H x V	1	0	1	2.02*	1	0.02	1	0.60	1	6.02
W x H x V			1	0.03	2	5.01*	2	2.45	2	1.81
Error	12	0.67	28	0.44	44	1.10	44	2.43	44	1.55

STAGE OF APICAL DEVELOPMENT ON THE MAIN SHOOT APEX

Source	Harvest 1 D.F.	M.S.	Harvest 2 D.F.	M.S.	Harvest 3 D.F.	M.S.	Harvest 4 D.F.	M.S.	Harvest 5 D.F.	M.S.
Blocks	4	0.07	4	0.03	4	0.18	4	1.20*	4	0.26
Water (W)	-	-	1	0.03	2	0.80**	2	2.45**	2	0.60**
Hardening (H)	1	0.05	1	0.03	1	0.01	1	0.60	1	0.26
Variety (V)	1	0.45	1	11.03***	1	6.01***	1	3.26**	1	4.26***
W x H	-	-	1	0.02	2	0.07	2	1.85*	2	0.27
W x V	-	-	1	0.22*	2	0.47*	2	0.72	2	0.07
H x V	-	-	1	0.02	1	0.03	1	0.27	1	0
W x H x V	-	-	1	0.03	2	0.06	2	1.51*	2	0
Error	13	0.13	28	0.03	59	0.13	59	0.36	59	0.16

SHOOT DRY WEIGHT PER PLANT ($\log_e$ mg)

Source	Harvest 1 D.F.	M.S.	Harvest 2 D.F.	M.S.	Harvest 3 D.F.	M.S.	Harvest 4 D.F.	M.S.	Harvest 5 D.F.	M.S.
Blocks	4	.034	4	.072**	4	.060**	4	.196***	4	.088**
Water (W)	-		1	.019	2	1.176***	2	.021	2	.057
Hardening (H)	1	.003	1	.004	1	.001	1	.020	1	.039
Variety (V)	1	.006	1	.002	1	.016	1	.097**	1	.405***
W x H	-	-	1	.008	2	.031	2	.003	2	.007
W x V	-	-	1	.006	2	.001	2	.022	2	0
H x V	1	.022	1	.002	1	.001	1	0	1	.080
W x H x V	-	-	1	.001	2	.007	2	.003	2	.035
Error	12	.017	28	.013	44	.011	44	.013	44	.018

CONTINUOUS WATER STRESS AND GROWTH

Experiment 1 (3.2.1)

TOTAL DRY WEIGHT PER PLANT ($\log_e$ mg)

(i) Harvests 1 to 6 (water-stressed and non-stressed)

Source.	D.F.	M.S.
Blocks	4	0.009
Harvests	5	7.999***
Treatments	1	30.663***
Harvests x Treatments	5	3.253***
Error	44	0.052

(ii) Harvests 5 to 7 (non-stressed and re-watered)

Source.	D.F.	M.S.
Blocks	4	0.115
Harvests	2	2.161***
Treatments	1	32.210***
Harvests x Treatments	2	0.128
Error	20	0.095

(iii) Harvests 5 to 6 (non-stressed, re-watered and water-stressed)

Source.	D.F.	M.S.
Blocks	4	0.024
Harvests	1	0.820**
Treatments	2	20.073***
Harvests x Treatments	2	0.347
Error	20	0.087

SHOOT DRY WEIGHT ($\log_e$ mg)

(i) Harvests 1 to 6 (water-stressed and non-stressed)

Source.	D.F.	M.S.
Blocks	4	0.016
Harvests	5	9.665***
Treatments	1	39.997***
Harvests x Treatments	5	3.682***
Error	44	0.088

(ii) Harvests 5 to 7 (non-stressed and re-watered)

Source.	D.F.	M.S.
Blocks	4	0.079
Harvests	2	0.687***
Treatments	1	20.891***
Harvests x Treatments	2	0.122
Error	20	0.123

(iii) Harvests 5 to 6 (non-stressed, re-watered and water-stressed)

Source.	D.F.	M.S.
Blocks	4	0.011
Harvests	1	1.068*
Treatments	2	24.325***
Harvests x Treatments	2	0.523
Error	20	0.144

ROOT DRY WEIGHT PER PLANT ($\log_e$ mg)

(i) Harvests 1 to 6 (water-stressed and non-stressed)

Source.	D.F.	M.S.
Blocks	4	0.081
Harvests	5	5.181***
Treatments	1	17.044***
Harvests x Treatments	5	2.173***
Error	44	0.049

(ii) Harvests 5 to 7 (non-stressed and re-watered)

Source.	D.F.	M.S.
Blocks	4	0.079
Harvests	2	0.687***
Treatments	1	20.891***
Harvests x Treatments	2	0.121
Error	20	0.058

(iii) Harvests 5 to 6 (non-stressed, re-watered and water-stressed)

Source.	D.F.	M.S.
Blocks	4	0.111
Harvests	1	0.160
Treatments	2	11.958***
Harvests x Treatments	2	0.202
Error	20	0.070

LEAF DRY WEIGHT PER PLANT ($\log_e$ mg)

(i) Harvests 1 to 6 (water-stressed and non-stressed)

Source.	D.F.	M.S.
Blocks	4	0.021
Harvests	5	3.383***
Treatments	1	9.759***
Harvests x Treatments	5	0.550***
Error	44	0.065

(ii) Harvests 5 to 7 (non-stressed and re-watered)

Source.	D.F.	M.S.
Blocks	4	0.088
Harvests	2	0.219
Treatments	1	5.349***
Harvests x Treatments	2	0.149
Error	20	0.084

(iii) Harvests 5 to 6 (non-stressed, water-stressed and re-watered)

Source.	D.F.	M.S.
Blocks	4	0.001
Harvests	1	0.029
Treatments	2	4.040***
Harvests x Treatments	2	0.173
Error	20	0.095

NUMBER OF EXPANDED LEAVES PER PLANT

(i) Harvests 1 to 7 (water-stressed and non-stressed)

Source.	D.F.	M.S.
Blocks	4	0.039
Harvests	6	8.031***
Treatments	1	6.281***
Harvests x Treatments	6	0.394***
Error	52	0.046

(ii) Harvests 6 to 8 (non-stressed and re-watered)

Source.	D.F.	M.S.
Blocks	4	0.031
Harvests	2	0.059
Treatments	1	0.181**
Harvests x Treatments	2	0.038
Error	20	0.018

(iii) Harvests 6 to 7 (non-stressed, water-stressed and re-watered)

Source.	D.F.	M.S.
Blocks	4	0.032
Harvests	1	0.138*
Treatments	2	2.339***
Harvests x Treatments	2	0.039
Error	20	0.021

NUMBER OF UN-EXPANDED LEAVES PER PLANT

(i) Harvests 1 to 7 (water-stressed and non-stressed)

Source.	D.F.	M.S.
Blocks	4	0.029
Harvests	6	5.069***
Treatments	1	16.924***
Harvests x Treatments	6	2.959***
Error	52	0.035

(ii) Harvests 6 to 8 (non-stressed and re-watered)

Source.	D.F.	M.S.
Blocks	4	0.121
Harvests	2	0.857*
Treatments	1	4.807***
Harvests x Treatments	2	0.857*
Error	20	0.223

(iii) Harvests 6 to 7 (non-stressed, water-stressed and re-watered)

Source.	D.F.	M.S.
Blocks	4	0.182
Harvests	1	0.523
Treatments	2	14.797***
Harvests x Treatments	2	0.449
Error	20	0.137

NUMBER OF PRIMORDIA ON THE MAIN SHOOT

(i) Harvests 1 to 7 (water-stressed and non-stressed)

Source.	D.F.	M.S.
Blocks	4	0.096
Harvests	6	0.469*
Treatments	1	2.594***
Harvests x Treatments	6	0.066
Error	52	0.075

(ii) Harvests 6 to 8 (non-stressed and re-watered)

Source.	D.F.	M.S.
Blocks	4	0.294
Harvests	2	0.304
Treatments	1	3.296***
Harvests x Treatments	2	0.067
Error	20	0.118

(iii) Harvests 6 to 7 (non-stressed, water-stressed and re-watered)

Source.	D.F.	M.S.
Blocks	4	0.096
Harvests	1	0.469*
Treatments	2	2.594***
Harvests x Treatments	2	0.066
Error	20	0.075

APICAL DEVELOPMENT ON THE MAIN SHOOT

(i) Harvests 1 to 7 (water-stressed and non-stressed)

Source.	D.F.	M.S.
Blocks	4	0.027
Harvests	6	6.776***
Treatments	1	2.584***
Harvests x Treatments	6	0.222***
Error	52	1.286

(ii) Harvests 6 to 8 (non-stressed and re-watered)

Source.	D.F.	M.S.
Blocks	4	0.010
Harvests	2	0.048
Treatments	1	0.449***
Harvests x Treatments	2	0.048
Error	20	0.015

(iii) Harvests 6 to 7 (non-stressed, water-stressed and re-watered)

Source.	D.F.	M.S.
Blocks	4	0.024
Harvests	1	0.156*
Treatments	2	1.223*
Harvests x Treatments	2	0.042
Error	20	0.021

Experiment 2 (3.2.2.)

NUMBER OF PRIMORDIA ON THE MAIN SHOOT

Source	Harvest 1		Harvest 2		Harvest 3	
	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.
Blocks	4	0.64	-	-	-	-
Water (W)	4	39.77***	4	189.88***	4	257.53***
Photoperiod (P)	-	-	1	2.42	1	18.00***
W x P	-	-	4	0.92	4	2.55***
Error	16	0.45	40	0.63	40	0.35

APICAL DEVELOPMENT ON THE MAIN SHOOT

Source	Harvest 1		Harvest 2		Harvest 3	
	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.
Blocks	4	0.04	-	-	-	-
Water (W)	4	3.84***	4	14.73***	4	14.58***
Photoperiod (P)	-	-	1	14.58***	1	42.08***
W x P	-	-	4	3.33***	4	0.08*
Error	16	0.04	40	0.55	40	0.30

Experiment 3 (3.2.3.)

NUMBER OF PRIMORDIA ON THE MAIN SHOOT

Source	Harvest 1		Harvest 2		Harvest 3	
	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.
Blocks	4	2.13	-	-	-	-
Water (W)	2	56.27***	4	634.69***	4	967.85***
Photoperiod (P)	-	-	1	192.58***	1	151.38***
W x P	-	-	4	18.80**	4	20.53*
Error	8	1.51	40	3.48	40	6.64

APICAL DEVELOPMENT ON THE MAIN SHOOT

Source	Harvest 1		Harvest 2		Harvest 3	
	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.
Blocks	4	0	-		-	
Water (W)	2	1.67	4	12.83**	4	28.97***
Photoperiod (P)	-	-	1	54.08***	1	35.82***
W x P	-	-	4	2.73	4	1.43**
Error	8	0	40	3.10	40	0.30

SHOOT DRY WEIGHT PER PLANT ($\log_e$ mg)

Source	Harvest 1		Harvest 2		Harvest 3	
	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.
Blocks	4	0.070	4	0.152*	4	0.154
Water (W)	4	1.427***	4	6.642***	4	8.410***
Photoperiod (P)	1	-	1	0.014	1	0.051
W x P	4	-	4	0.012	4	0.060
Error	36	0.025	36	0.040	36	0.063

WATER STRESS AND PHOTOPERIODIC INDUCTION

Preliminary Experiment (3.3.1)

APICAL DEVELOPMENT ON THE MAIN SHOOT

Source.	D.F.	M.S.
Blocks	4	0.587**
Treatments	6	0.208
Error	24	0.098

LENGTH OF APEX ON MAIN SHOOT (mm)

Source.	D.F.	M.S.
Blocks	4	0.002
Treatments	6	0.065***
Error	24	0.003

WATER STRESS AND GENERATION OF FLORAL STIMULUS (3.3.2)

APICAL DEVELOPMENT OF THE MAIN SHOOT

Source.	D.F.	M.S.
Blocks	4	0.480
Treatments	4	6.459***
Error	16	0.209

LENGTH OF APEX ON MAIN SHOOT (mm)

Source.	D.F.	M.S.
Blocks	4	0.0125*
Treatments	4	0.1175***
Error	16	0.0037

WATER STRESS AND TRANSLOCATION OF FLORAL STIMULUS (3.3.3)

APICAL DEVELOPMENT OF THE MAIN SHOOT

Source.	D.F.	M.S.
Blocks	4	0.005
Water (W)	2	1.845***
Time of leaf defoliation (T)	2	0.405***
W x T	4	0.297***
Error	32	0.007

LENGTH OF APEX ON THE MAIN SHOOT (mm)

Source.	D.F.	M.S.
Blocks	4	0.009**
Water (W)	2	0.095***
Time of leaf defoliation (T)	2	0.041***
W x T	4	0.010**
Error	32	0.001

WATER STRESS AND STABILITY OF FLORAL STIMULUS (3.3.4)

APICAL DEVELOPMENT OF THE MAIN SHOOT

Source.	D.F.	M.S.
Blocks	4	0.273
Treatments	15	13.079***
Error	60	0.408

LENGTH OF APEX ON THE MAIN SHOOT (mm)

Source.	D.F.	M.S.
Blocks	4	0.006
Treatments	15	0.307***
Error	60	0.012

WATER STRESS AND LIGHT INTENSITY

Experiment 1 (3.5.1)

NUMBER OF PRIMORDIA ON THE MAIN SHOOT

Source	Harvest 1		Harvest 2	
	D.F.	M.S.	D.F.	M.S.
Blocks	9	10.00	9	12.38
Water (W)	1	224.45*	1	518.40***
Light intensity (L)	-	-	1	19.60
W x L	-	-	1	10.00
Error	9	40.67	27	18.98

APICAL DEVELOPMENT ON THE MAIN SHOOT

Source	Harvest 1		Harvest 2	
	D.F.	M.S.	D.F.	M.S.
Blocks	9	1.80	9	2.45
Water(W)	1	8.45	1	52.90***
Light intensity (L)	-	-	1	0.10
W x L	-	-	1	0.90
Error	9	6.11	27	2.33

Experiment 2 (3.5.2)

NUMBER OF PRIMORDIA ON THE MAIN SHOOT

Source.	Harvest 2	
	D.F.	M.S.
Blocks	9	6.72
Water (W)	1	216.23***
Light intensity (L)	1	112.23***
W x L	1	2.02
Error	27	3.58

Experiment 2 (3.5.2) (cont.)

APICAL DEVELOPMENT ON THE MAIN SHOOT

Source.	Harvest 2 D.F.	M.S.
Blocks	9	0.21
Water (W)	1	0.40
Light intensity (L)	1	0.90*
W x L	1	0.10
Error	27	0.18