WATER STRESS AND GROWTH AND DEVELOPMENT IN RADISH

by

Daryl C. Joyce B.App.Sc.(Hort.Tech.)Hons.

Department of Plant Physiology
Waite Agricultural Research Institute
University of Adelaide
South Australia

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SUMMARY

The effect of water deficit on the growth and yield of biennial 'root' crops has been studied in some detail. However, little attention has been paid to explaining the underlying mechanisms of observed yield reductions in such crops. With this in mind, Radish was chosen as an example of this group of plants and its response to a range of types, intensities and durations of water stress investigated. The development of the fleshy axis was studied in detail.

Water stress induced with polyethylene-glycol solutions (-5, -10 and -15 bars) resulted in considerable disruption to plant growth. These effects however, were largely of a transient nature. As intensity, and to a lesser extent duration (24, 48 and 72 hr), of stress were increased the reduction of plant growth was increasingly marked until eventually shoot and also fleshy axis death occurred. Tissue death around leaf margins, apparently as a consequence of steep water potential gradients, was a feature of PEG-induced stress. Monitoring of fleshy axis diameter changes throughout stress episodes revealed that water loss was continuous, probably as a consequence of poor stomatal regulation. Repeated episodes of PEG-induced water stress caused highly significant yield reductions.

Water stress imposed through soil water depletion (i.e. water regimes) caused more persistent reductions in Radish plant yield. Again these effects were more marked as stress intensity was increased, and the yield reduction was proportionally greater in fleshy axis than shoot tissue. Both cell division and cell expansion were significantly reduced by stress in both the shoot (reduced leaf area) and the fleshy axis (reduced volume). Although cell division was less obviously decreased by stress than was cell expansion, its reduced rate during stress appeared

to be the primary limitation to plant recovery following stress relief. Cell expansion was more markedly responsive to both imposition and alleviation of stress. The general pattern of fleshy axis cellular differentiation and development was not disrupted even by severe stress, however.

In older plants, the rate of leaf senescence was accelerated by stress, whilst in younger plants only the rate of leaf appearance was reduced. In plants of both ages individual leaf area was decreased. It was determined that older leaves had less ability, possibly because of larger cell sizes and a poorer ability to osmoregulate, than younger leaves to maintain a favourable water balance.

Rapid reduction in leaf water status (PEG treatment) initiated massive proline accumulation. Young, slowly-droughted Radish plants did not display the same considerable reduction in plant water status as did PEG-treated plants and, with time, were able to recommence very slow growth under such conditions. Soluble sugar concentrations in the leaves, cotyledons, hypocotyls and roots of these plants rose, and cell wall thickening in the hypocotyl (through lignin deposition) was observed.

It was concluded that Radish can accomodate single short, episodes of water stress, but that repeated episodes or very severe stress results in a significant yield reduction. The Radish does not have any stage of growth during fleshy axis development which is particularly sensitive to water stress. Long-term depletion of substrate moisture results in reduced plant yield through reduced rates of cell division and inhibited cell expansion. Restoration of a favourable water supply results in a persistent stress effect due to the reduction in cell numbers. When stressed early, before the plant has commenced substantial cell expansion, the Radish can adapt to survive in severely water limited environments.

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.

(Daryl C. Joyce)

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ABBREVIATIONS AND SYMBOLS USED IN THIS THESIS

ABA	abscisic acid
$AgNO_3$	silver nitrate
BA	benzyl adenine
^{14}C	radioactive carbon
^{o}C	degrees centigrade
cm	centimetre(s)
cm^2	square centimetre(s)
CO_2	carbon dioxide
cpm	counts per minute
dpm	disintergrations per minute
DNA	deoxyribonucleic acid
FAA	formalin-aceto-alcohol
g	gram(s)
GA_3	gibberellic acid
hr(s)	hour(s)
kg	kilogram
KOH	potassium hydroxide
l	litre
µ	micron(s)
µci	microcurie(s)
µein	microeinstein(s)
µg	microgram
µl	microlitre(s)
MCW	methanol : chloroform : water
mg	milligram(s)
min	minute(s)
MW	molecular weight
MP	melting point
ml	millilitre(s)

ABBREVIATIONS contd.

mm	millimetre(s)
N	normal
NaCl	sodium chloride
Na_2CO_3	sodium carbonate
$NaHCO_3$	sodium bicarbonate
NaOH	sodium hydroxide
nm	nanometre(s)
PEG	polyethylene glycol
RNA	ribonucleic acid
RWC	relative water content
sec	second(s)
W	watt(s)
w/v	weight/volume (concentration)
Ψ	water potential
Ψ_m	matric potential
Ψ_p	turgor potential
Ψ_π	osmotic (solute) potential
*	statistically significant at 95% probability level
%	percent

CHAPTER 1.

INTRODUCTION

1.1 Plant Water Stress Response

1.1.1 Tissue Water Relations

The most widely accepted criterion for plant water status in water stress studies is the tissue water energy status or water potential. Water potential (Ψ) is in its simplest form a function of three variables (Gardner and Ehlig, 1965);

$$\Psi = \Psi_{\pi} + \Psi_{p} + \Psi_{m}$$

where Ψ is total water potential, Ψ_{π} is the osmotic or solute potential, Ψ_{p} the turgor or pressure potential and, Ψ_{m} the component due to adsorption forces i.e. the matric potential. Also a widely used criterion is relative water content which estimates the water held in a tissue relative to its potential capacity at full turgor (Barrs, 1968). The relationship between water potential and relative water content (RWC) is slightly curvilinear such that as RWC falls below around 60% the rate of decline in Ψ increases (Warren Wilson, 1967).

Growth and expansion of the plant is related particularly to turgor potential, no expansion occurring unless turgor potential is substantially positive, and the ability to maintain positive turgor has been used as a criterion of the relative drought resistance of different species. For instance, Eggplant is able to maintain a more favourable water balance during stress than some other vegetables (Behboudian, 1977a). Possible responses to water stress allowing the maintenance of a relatively better water status include the retention of a higher RWC for a given fall in Ψ, more effective control of transpiration, better osmoregulation through solute accumulation and, rapid recovery of water status on stress alleviation. Intraspecific differences may not be as great as interspecific differences in this respect (Fischer and Sanchez, 1979). The lower growth rates

incurred at reduced turgor may be compensated for in part in some crops eg. Sorghum (Stout *et al.*, 1978) through extended growth periods, however in other crops no such compensation is apparent (eg. Beans, Elston *et al.*, 1976).

Plant water status fluctuates markedly even in well watered plants during the course of the diurnal period. In the field minimum daily water potentials occur at around 1400 hours (Ehrler *et al.*, 1978). Water stress thus involves the maintenance of reduced water potentials throughout much or all of the diurnal period; however, only slight reductions in water status (which may involve the maintenance of positive turgor) can result in reduced reproductive and vegetative growth.

In short-term stress episodes reduced stomatal conductance offers the main resistance to water loss. The rapid stomatal response appears to be directly effected by reduced turgor and precedes the action of secondary physiological stomatal regulators such as abscisic acid (Davies and Lakso, 1978). Not all species of plants however are able to effectively limit water loss through stomatal control, which is dependant upon a high cuticular resistance to water flow. Aspen, for instance, has very low drought tolerance because the cuticle has very little resistance to water passage (Jarvis and Jarvis, 1963). In plants such as the Eggplant (Behboudian, 1977b) and Bean (Solarova *et al.*, 1977) stomata close and restrict water loss at a leaf water potential of around -10 bars.

Several other resistances to water flow exist within the plant. The common phenomenon of midday wilt, which occurs even on moist soils when the atmospheric demand for moisture is highest, serves to illustrate the existence of at least one such resistance which causes reduced leaf water potential. Studies on Sunflower have suggested that leaf resistance to water flow operates as a variable independently of stomatal control and that it limits water loss more than do the stem resistances to flow (Black, 1979). Root resistance to water flow is the largest within the plant and

is probably the primary factor regulating leaf water status under conditions conducive to midday wilt. Radial passage of water from the root epidermis to the xylem elements forms the rate limiting step (Newman, 1976). However, in situations where soil water is not abundant a considerable resistance (reduced hydraulic conductivity) is generated in the zone immediately surrounding the roots, and thus an external resistance can be created in excess of that which could be predicted from the general soil water potential. It is important therefore to consider the plant in respect to its central position in the soil-plant-air continuum.

1.1.2 Growth Effects of Water Stress

In this study the prime concern is the vegetative growth of fleshy axis tissues derived from hypocotyl and upper radicle tissues. Work on some vegetable species has shown that germination (Ross and Hegarty, 1979) and hypocotyl elongation (Ronnike, 1957) are more sensitive to water stress than radicle growth, but that the drought resistance of the hypocotyl may improve in the course of time (Obloj and Kacperskapalacz, 1978). During development subsequent to the hypocotyl stage, stress during rapid fleshy axis swelling may have greater effects on yield compared to stress at earlier and later stages when growth rates are lower eg. Turnips (Stanhill, 1958) and Sugarbeet (Singh *et al.*, 1978). In the culture of field crops, it may be beneficial to attempt to strike an irrigation balance to achieve maximum yield with acceptable quality as for instance, copious water supply has been shown to produce maximum yield of Turnips but to reduce cooking quality (Stanhill, 1958). On the other hand, irrigation of a previously non-irrigated Sugar Beet crop increased sugar yields per hectare (Draycott and Messem, 1977), however, discriminatory use of episodes of water deficit in the later periods of Sugar Beet development can be employed to further improve sugar yields (Singh *et al.*, 1978). Water management for the Sugar Beet crop based on the regular assessment of xylem water potential has been suggested

(Stegman and Bauer, 1977) with irrigation controlled so that the average potential does not fall below -12 bars. Similar programs may be desirable for other crops where the fleshy axis is harvested.

In less applied studies it has been shown that there is a linear relationship between leaf water potential and yield of Radish, Red Beet and Onion plants subjected to a range of salt and humidity stresses (Hoffman and Rawlins, 1971). Differences in mean water potential between full and almost no yield were only 6 bars in Radish, 13 bars for Beet and 4 bars for Onion. These responses are probably controlled by changes in plant turgor, a suggestion which is strengthened by the observation that the application of an external pressure of 8.5 bars to counteract completely the internal turgor resulted in cessation of expansion of the fleshy axis of Radish (Kibreab and Danielson, 1977). In this study, with no excessive external salt supply, the osmotic potential of tissue extract was linearly related to the pressure applied. This indicates a role for osmoregulation, the lowering of internal osmotic potential by the active accumulation of organic solutes and ions, in the growth of the fleshy axis.

Leaf growth proceeds in a manner similar to fleshy axis growth through processes of cell division and cell expansion, and thus many principles established for leaf growth during stress may apply directly to fleshy axis development. However, each organ has its own particular functions and thus similarities can be expected to be limited to the physical rather than the physiological implications of lower water status. Leaf growth is dynamically highly responsive to reduced water potential (Barlow and Boersma, 1972; Bunce, 1978; and Boyer, 1970). For example, the elongation of Maize leaves is reduced at -0.2 bar soil water potential (-7 bar leaf water potential) and the process ceases at -2.5 bar soil water potential (Acevado *et al.*, 1971). In Soybean and Cotton (Bunce, 1978) however, leaf expansion continued at night even when the net assimilation rate was reduced by stress and, in a similar fashion renewed leaf expansion

occurred towards the end of a 4 day dessiccation period in Corn, Sunflower and Soybean despite earlier severe inhibition (Boyer, 1970). It is suggested that reduced atmospheric water demand in the first case and the accumulation of osmotic solutes resulting in improved turgor in the second account for these apparent anomalies. Leaf number may also be reduced by stress, but in Field Beans (Karamanos, 1978) this was only a minor factor in the reduction of total plant leaf area. An increased rate of leaf senescence was deemed more important, but still only a minor factor relative to the reduction in leaf area. In stressed Soybean, the rate of leaf expansion corresponded closely to leaf water potential, and relative growth rate was negatively correlated with stomatal conductance, leaf water potential and the rate of leaf area expansion (Sivakumar and Shaw, 1978). When subjected to wilting, the relative growth and net assimilation rates of Tomato plants were also reduced and normal translocation was modified, as was evident from higher stem weight ratios (i.e. stem dry weight : total plant dry weight) and lower lamina weight ratios (Gates, 1955).

The dependence of vegetative growth on plant water status was also apparent in a study of *Pinus resinosa* and *Betula papyrifera* (Braekke *et al.*, 1978). Contrary, however, to the situation with stems and the other vegetative organs discussed above, stress may result in enhanced root growth in some crops eg. Cotton (Malik *et al.*, 1979). In young Cotton an increase in the root : shoot ratio of droughted plants was due to an absolute increase in root weight with no change in shoot weight. This growth phenomenon is of importance with respect to the ability of a plant to exploit a greater soil volume and take up more water.

Although the prime aim of this study is to examine vegetative growth during stress with emphasis on fleshy axis tissues, effects of stress on reproductive growth warrant brief mention. Many investigations on the effects of water stress have demonstrated that stress at critical periods of fruit or seed formation lead to the greatest yield reductions

in such crops. This response has been found in the following crops : Wheat (Bole and Dubetz, 1978; Chinoy, 1962), Rapeseed (Richards and Thurling, 1978), Bush Beans (Dubetz and Mahalle, 1969), Soybean (Doss *et al.*, 1974; Yazdi-Samadi and Saddati, 1978), Peas (Salter, 1962; Miller *et al.*, 1977) and, Corn (Denmead and Shaw, 1960; Robins and Domingo, 1953) to mention a few.

Very relevant to plant growth in stress environments is the ability of plants to recover from water stress episodes. Rapid recovery of a high water status confers a growth advantage leading to increased plant production (Nulsen and Thurtell, 1978). Recovery times vary with the severity of stress; for instance, Maize plants at -11 bar leaf water potential regain lost turgor within 40 to 50 minutes, but more severely stressed plants require longer periods (up to 300 minutes). The recovery of leaf water potential is interrupted in severely stressed Maize plants by a plateau phase lasting around 30-45 minutes and occurring at -8 to -9 bars. This phase is attributable to undetermined root processes as this study was carried out in the dark to avoid stomatal interaction effects. The stomata of Maize and Sorghum plants reopen normally after wilted plants are rewatered and 2 days later their leaf diffusive resistance is close to control levels (Sanchez-Draz and Kramer, 1971). In the recovery period following stress, leaf expansion may be accelerated in comparison with control plants. Previously stressed *Panicum* plants have a leaf expansion rate greater than that of plants not subjected to stress for up to 33 hours after stress alleviation (Ludlow and Ng, 1977). This rate of expansion is related to the intensity and duration of previous stress and, in the light, displays a marked transient burst soon after rewatering. However, despite the increased rate of expansion, total recovery in leaf length is not attained. It is suggested that the increased leaf expansion rate is due to the enlargement of cells from a pool of unexpanded cells generated through continued cell division activity during stress. A similar increase in leaf

expansion following stress relief has also been reported for Tomato (Gates, 1955) and was accompanied by a rising lamina weight ratio and a decreasing stem weight ratio. Again recovery was not total.

The reduction in growth during stress may be due to biochemical responses to stress as well as to the purely physical effects, and the recovery of normal metabolism following stress relief is also of importance. For instance, in dark grown Wheat, chlorophyll formation on stress alleviation in the light shows a prolonged lag phase and yields a decreased chlorophyll content. This effect became more marked with increased duration of the previous stress (Bengston *et al.*, 1978). Accumulated ABA and proline fell rapidly to return to control levels within 10 hours of stress alleviation. Similarly, the activities of L-Phenylalanine ammonia- lyase and nitrate reductase, which were markedly reduced in water stressed Maize seedlings, both showed partial to complete recovery within 24 hours after rehydration of stressed plants (Bardzik *et al.*, 1971).

1.1.3 Physiology of Plants during Water Stress

The following discussion aims to broadly outline physiological responses to water stress, first considering those responses that affect tissue water status (stomatal aperture and osmotic adjustment), then the altered hormonal balance of stressed plants, and finally examining the changes in carbon and nitrogen metabolism.

An intricate "timing" of plant physiological responses to water stress exists (figure 1.1) in relation to the rate of onset of stress, the duration of stress, the severity of stress and the varied responses of individual species to stress. It is important to consider these points during any attempt to compare and relate findings from different studies.

Figure 1.1

Diagrammatic generalization of the relative sensitivity of some plant processes to water stress (after Hsiao, 1973).

PROCESS AFFECTED	SENSITIVITY TO STRESS	REMARKS
	very sensitive → relatively insensitive	
	REDUCTION IN TISSUE WATER POTENTIAL REQUIRED TO AFFECT THE PROCESS (- bars)	
	0 — 10 — 20	
Cell Growth		fast growing tissue
Wall Synthesis		fast growing tissue
Protein Synthesis		fast growing tissue
Protochlorophyll Formation		etiolated tissue
Nitrate Reductase Level		
ABA Accumulation		
Cytokinin Level		
Stomatal Opening		Depends on species
CO_2 Assimilation		Depends on species
Respiration		
Proline Accumulation		
Sugar Accumulation		

1.1.3.1 Stomatal Behaviour in Response to Stress

Stomatal control is a turgor-mediated phenomenon with regulation of aperture being subject to a complex system of controls (Zelitch, 1969). During water stress, stomatal aperture is the major factor determining the plant water exchange and carbon dioxide flux, and regulation through internal CO_2 concentration and endogenous ABA level has been proposed (Raschke, 1976). However, in short term stress such as occurs when the atmospheric environment exerts a strong demand for moisture, aperture control via direct humidity sensing by guard cells may be involved (Edwards and Meidner, 1978). Evidence of the importance of subsidiary cells in regulating guard cell activity, independently of the bulk leaf turgor, supports this notion (Maier-Maercker, 1979; Beadle et al., 1978). It can thus be concluded that the importance of the various control mechanisms can vary, and indeed they may interact (Schulze and Kuppers, 1979), according to the water supply and demand conditions. Whatever the sensing and control mechanism, the loss of potassium ions from guard cells to surrounding cells appears to be the primary effector of reduced guard cell turgor (Ehret and Boyer, 1979).

The importance of stomatal regulation of plant water balance and the limiting of water loss during stress has been recognised for many decades (Maximov, 1929; Fasehun, 1979). The degree to which stomata control water loss during stress may vary, however. In Snap Beans, stomatal resistance increased sharply at fairly high leaf water potentials (-8 to -11 bars) and the response approximated an effective on-off mechanism (Kanemasu and Tanner, 1969). However, in some herbage species (White Clover, Perennial Ryegrass, Phalaris and Tall Fescue) stomatal conductance fell only slowly with developing stress and did not reach a minimum value until much of the herbage was dead (Johns, 1978). Even at this stage, water loss per unit foliage area was reduced only 20 to 30%, and the greater survival ability of Tall Fescue was largely conferred by the leaf-rolling

trait. A similar degree of variation may exist in the value of stomata in conferring intraspecific differences in drought tolerance. An investigation into the relative drought resistance of two *Pinus radiata* clones indicated that the greater survival capacity of one was due to its ability to limit transpiration to around half that of the other (Bennett and Rook, 1978).

Similarily, stomatal frequency (or density) may also have a role in respect to relative drought resistances. However, when field grown Barley cultivars were screened for the relationship between flag leaf stomatal frequency and either yield or morphological traits, no significant correlations were obtained (Ledent and Jouret, 1978), which tends to contradict this concept.

1.1.3.2 Osmotic Adjustment During Water Stress

Osmotic adjustment or turgor regulation occurs with the accumulation of soluble compounds which decrease the osmotic potential, and hence the water potential, of cells whilst maintaining turgor. Such maintenance of unchanged turgor potential between irrigated and non-irrigated treatments has been found in Apple (Davies and Lakso, 1978). Similarly Sorghum has been shown to adjust to a water deficit through osmotic adjustment and leaf senescence more effectively than through stomatal control (Jones and Turner, 1978). Sorghum appears to osmoregulate more efficiently than Corn (which relies more heavily on stomatal control); and thus, through reducing the amount of tissue water loss per unit drop in water potential, attains greater drought resistance (Sanchez-Draz and Kramer, 1971).

The solutes which act in osmoregulation vary, but in water stress where ionic adjustment is limited (in comparison to salt stress) they are of organic nature. For instance, water stressed Beans accumulated higher concentrations of soluble solids whilst salt stressed Beans adjusted

ionically (Janes, 1966). During severe stress, Wheat accumulated carbohydrates and amino acids in the apex and in both enclosed elongating and expanding leaves (Munns *et al.*, 1979). In this study K^+ was also a major component of general osmotic adjustment, and additionally a role for malate accumulation in leaves was suggested.

1.1.3.3 The Role of Growth Regulators during Water Stress

Major growth regulators studied in relation to water stress are abscisic acid (ABA), cytokinins, auxin, gibberellins and ethylene. For instance, during stress root cytokinin concentration decreases and leaf ABA level increases (Vaadia, 1976). These changes result in increased root permeability and decreased stomatal conductance and represent an example of the interaction of growth regulators effecting plant adaptation to water stress. The application of growth regulators, such as these, has been used to mimic the response to water stress and to gather evidence supporting their proposed roles (Mizrahi and Richmond, 1972).

ABA applied exogenously can be used as an effective anti-transpirant provided plants are only mildly stressed (Talha and Larsen, 1976). The effects may be transient, however, and the effectiveness of repeated applications may decline with time (Goode *et al.*, 1978). A large increase in the level of endogenous ABA in a stressed plant is a widespread phenomenon causing reduced stomatal aperture (Blake and Ferrell, 1977); short chain fatty acids (eg. undecanoic acid) may also accumulate and a synergistic action with increased ABA has been proposed (Willmer *et al.*, 1978).

ABA synthesis can occur in the chloroplasts of plant cells and the compound can readily migrate to other plant parts (Loveys, 1977). The accumulation of ABA is due to increased synthesis (Milborrow, 1978) and increased levels are probably maintained because the rate of synthesis and metabolism are both elevated and approximately equal (Harrison and Walton,

1975). Plants, having experienced stress episodes, appear to retain some persistent hormonal adjustment which confers greater stomatal sensitivity to subsequent stress periods and elevated ABA levels (Davies, 1978).

Accumulated ABA has effects other than on the stomatal response. It is a potent stem growth inhibitor (During and Scienza, 1975) and, in interaction with other hormones, can effect changes in general growth patterns of plants subjected to stress; for instance accumulated ABA interacts with apical dominance (auxin) factors in the Corn tassel and causes growth promotion of lower axillary inflorescences (Damptey *et al.*, 1978). It has also been suggested that proline accumulation during stress may be caused by ABA accumulation and partially regulated by the level of accumulated ABA (Rajagopal and Andersen, 1978). Other suggested consequences of ABA accumulation include reduced leaf growth, enhanced senescence and the production of epidermal hairs (Jones, 1978).

Reduced cytokinin levels are associated with water stress and have been correlated with decreased protein synthesis (Ben-Zioni *et al.*, 1967). Reduced levels may also interact with increased ABA in synergistic effects on stomatal closure (Bengston *et al.*, 1978); additionally they may regulate the rate of increase in transpiration in the wake of stress alleviation.

Auxin levels also fall during stress. Reduced levels may be involved in decreasing cell extensibility (Cleland, 1967) and in reducing the hydraulic conductivity of tissues (Boyer and Wu, 1978).

Little change in gibberellin levels occurs during stress. In an investigation of leaf abscision, for example, GA_3 was found to play only a minor role (Davenport *et al.*, 1979). However it has been suggested that the tissues of plants previously subjected to stress may be more responsive to GA_3 during recovery (Rai *et al.*, 1978).

During water stress, ethylene levels and emmission tend to rise in some tissues (Sivakumaran and Hall, 1978), and have been associated with

reduced growth rates and enhanced senescence (Wright, 1979). Exogenous ethylene has been shown to decrease the extension period and extensibility of Barley roots (Hall *et al.*, 1977), and the ability of some species to continuously generate increased levels of ethylene during stress is correlated with the leaf shedding reaction (Aharoni, 1978).

1.1.3.4 Carbon Distribution and Metabolism during Water Stress

This section considers the effects of water stress on carbon fixation, its distribution throughout the plant and, its expenditure in respiration processes. The reduction of photosynthesis during water stress is a widely reported phenomenon (Kriedemann and Smart, 1971; Rawson, 1979; Planchon, 1979; Parsons *et al.*, 1979). Reduction in total plant photosynthesis occur as a result of both reduced photosynthetic activity per unit leaf area and of reduced production of new leaf area (Boyer, 1976). Photosynthetic activity declines as a result of reduced stomatal aperture, increased mesophyll resistance (Pospisilova *et al.*, 1978) and, inhibited chloroplast activity.

At the metabolic level, stress reduced ribulose 1,5-diphosphate activity (O'Toole *et al.*, 1977), inhibited carbon flux from serine to sucrose and inhibited carbon metabolism via oxaloacetate induce a shift from sugar to amino acid metabolism and increased photorespiration activity (Lawlor, 1976; Lawlor and Fock, 1977, 1978). The level of reduction in photosynthesis following identical stress treatments has been used to rank the relative stress resistance of Rice cultivars (Jones, 1979). This study also detected photosynthetic adaptation resulting from a previous stress.

The rate of translocation is less sensitive to water stress than is photosynthesis. Photosynthetic rates of Bean, for instance, decline as a result of rapid stomatal closure, whereas translocation rate in the first few hours after the onset of stress appears relatively insensitive (Hoddinott *et al.*, 1979). In Strawberry subjected to drought, the pattern

of carbon distribution was unaffected but the amount translocated was considerably reduced (Udovenko and Goncharova, 1977). Stress in Bean plants caused a brief initial stimulation of translocation followed by a virtual cessation of acropetal assimilate distribution (Plaut and Reinhold, 1965). On stress alleviation, acropetal translocation resumed rapidly. Both here and in a study on Wheat and Bean plants (Couch and Todd, 1972) stress resulted in a retention of carbon in ethanol soluble fractions. In contrast, studies on Tomato and Bean (Woodhams and Kozlowski, 1954) revealed that leaf, stem and root concentrations of starch and both reducing and non-reducing sugars fell during stress, and that on stress alleviation starch concentrations alone recovered. However, bearing in mind the process of osmoregulation, it would appear that increased sugar concentration during stress is the more general plant response.

Respiration is less sensitive to stress than is photosynthesis (Lawlor and Fock, 1978), and, it has been suggested that the decline in respiration rate on stress is associated with the decreased growth and not with maintenance (Penning de Vries *et al.*, 1979).

1.1.3.5 Effects of Stress on Nitrogen Metabolism and Nitrogen Containing Compounds

The uptake of nitrate and, to a lesser extent, ammonium is reduced by water stress through reduced root permeability and reduced water flow (Frota and Tucker, 1978a). In relation to dry matter production, however, plant nitrogen content increases in stressed plants.

The enzymes of nitrate assimilation are reduced in activity during drought. Reduced nitrate reductase synthesis has been reported in the wake of reduced polysome levels, with this sequence being reversed on stress alleviation (Morilla *et al.*, 1973). Nitrite reductase activity also declines with stress but the response is less sensitive than that of

nitrate reductase. This difference in the effect of stress on the two enzymes has been proposed as a mechanism which prevents toxic nitrite accumulation (Heuer *et al.*, 1979). It was further suggested that the reduction in nitrate reductase activity is a result of direct inhibition and of reduced synthesis, whilst the reduction in nitrite reductase activity is due to direct inhibition by stress alone.

Fundamentally related to growth is the production of proteins for various functions. Accumulation of amino acids, nitrate and nitrite in stressed tissues has been reported as a consequence of decreased protein synthesis (Frota and Tucker, 1978b). Protein synthetic rates are reduced more in drought sensitive than drought tolerant Maize cultivars (Botha and Botha, 1979). Increased rates of protein degradation are not suspected as a major cause of reduced protein levels (Maranville and Paulsen, 1972), and the reduced synthetic rates may be due to dissociation of polysomes to monosomes (Hsiao, 1970). Conversion of polysomes to monosomes is a reversible process on stress alleviation. This phenomenon appears to be directly proportional to growth reductions in stressed plants (Rhodes and Matsuda, 1976). However, directly relating to protein synthesis, RNA levels in stressed tissues have been found to fall due to increased destruction (Gates and Bonner, 1959) and decreased synthesis (Sheoran and Sihag, 1978). Thus an interaction of processes may serve to reduce protein levels, for example in drought hardy Black Locust seedlings polysome levels fall, RNAse activity increases and only reduced rates of protein synthesis are maintained during stress (Brandle *et al.*, 1977).

The most spectacular change in chemical composition of many plants experiencing water stress is the accumulation of high tissue concentrations of the amino acid proline (Palfi *et al.*, 1974). Another organic molecule, the quarternary ammonium compound glycinebetaine, may also accumulate. In stressed Barley the accumulation of glycinebetaine is proportionally less than that of proline (Wyn Jones and Storey, 1978), however on stress

alleviation proline levels fall more rapidly than those of glycinebetaine (Ahmad and Wyn Jones, 1979). A general increase in amino acid levels during stress has been reported and attributed to proteolysis and also to increased net synthesis in some (eg. proline) instances (Thompson *et al.*, 1966). Moreover, it has been demonstrated that proline accumulation is independent of protein synthesis (Boggess and Stewart, 1980). The biochemical mechanisms leading to proline accumulation such as inhibited oxidation, enhanced production, and protein breakdown, may differ in importance between species (Boggess *et al.*, 1976). The proline accumulated during stress may serve to protect cell constituents during periods of water stress (Jager and Meyer, 1977; Schobert and Tschesche, 1978) and the relative magnitude of proline accumulation has been correlated with drought hardiness in a group of Barley cultivars (Singh *et al.*, 1972). This view has been questioned however, with the suggestion that proline accumulation is merely a non toxic product of the interruption of normal metabolism (Hanson *et al.*, 1979; Tal *et al.*, 1979; Ferreira *et al.*, 1979).

1.1.4 The Effect of Water Stress on Cell Growth and Morphological Development

Cell division and cell expansion in leaves, stems, roots and cotyledons are reduced by water stress (Ordin, 1966). Cell expansion requires the exertion of positive internal pressures against the cell wall and also the presence of auxin which has a role in cell wall loosening. Critical values of water potential exist in relation to auxin-induced cell wall loosening in Avena coleoptiles. Elastic stretching is reduced to a low level, and is auxin insensitive, at low water potential. Irreversible plastic stretching, although less sensitive, is similarly reduced at a water potential below about -3 bars in the bathing solution (Cleland, 1959). At higher water potential, plastic stretching is proportional to the water potential of the bathing solution.

The growth rate (expansion) of a single cell can be thus expressed in terms of two major variables (Green, 1968), viz;

Rate = Cell Extensibility x Turgor Pressure

Extensibility, in *Nitella* for example, is not constant but falls to zero as turgor is reduced to 80% of full turgor. In this study it was shown that the critical turgor value could be reduced, however, by previous stress episodes.

The elasticity of cell walls may decrease (i.e. increasing elastic moduli) as cell turgor increases to high values and also as cell volume increases during normal cell growth (Steudle *et al.*, 1977). Thus a direct control link between turgor and cell extensibility may serve to mediate cell growth both during stress and during favourable growth periods. The following formula illustrates the major parameters involved in cell growth and can be used to determine the half time ($T_{\frac{1}{2}}$) of swelling or shrinkage of individual plant cells;

$$T_{\frac{1}{2}} = \frac{0.693}{Lp} \cdot \frac{V/A}{(\varepsilon+\pi)}$$

where V is cell volume, A is cell area, Lp is hydraulic conductivity, ε the internal osmotic pressure and, π the elastic modulus. The flow of water into or out of the cell is determined by the magnitude and direction of the water potential gradient between the cell and its environment. The water potential of the cell is determined primarily by the balance between turgor pressure and osmotic potential. Through breaking down, and defining and measuring, the parameters of cell growth discussed above a greater understanding of the physical effects of water stress on plant growth is being attained.

Turgor pressure is not only important for cell expansion, but also is an important factor in relation to cell division. In fact, comparatively, cell division may have a greater turgor pressure requirement if maximum rates are to be attained (Kirkham *et al.*, 1972). However, cell

division may proceed at a reduced rate at a turgor pressure below that which halts cell expansion (Gardner and Nieman, 1964). In support of this suggestion, it was found that *Vicia faba* root elongation rate was reduced 6-fold by a stress which only prolonged the mitotic cycle 1.86-fold (Murin, 1979). The S-phase of cell division (including DNA synthesis) was the period most sensitive to stress.

The relative sensitivities of cell expansion and of cell division may vary between species and possibly between organs on the same plant. Studies with Sugar Beet leaves have shown that a small reduction in turgor has far more effect on cell division than on cell expansion (Terry *et al.*, 1971) but the two processes appear equally sensitive in Soybean hypocotyls although in the absence of any osmotic adjustment cell expansion may be slightly more sensitive (Meyer and Boyer, 1972). Effects of stress on other plant processes may influence the more direct effects on division and expansion. Although cell division is reduced by stress during the early phase of endosperm development in the Wheat grain, the relative effect during the subsequent expansion period is difficult to gauge, as the severely reduced assimilate supply causes shrivelled grains (Brocklehurst *et al.*, 1978).

Morphological consequences of stress on cell division and expansion include reduced leaf thickness due to smaller mesophyll and palisade cells, and thus reduced intercellular airspace and substomatal cavity volume (Manning *et al.*, 1977). The number and cross-sectional area of leaf xylem elements may also be reduced. Smaller leaf cells resulting from stress have thicker walls which may confer greater plant tolerance to low water potential (Cutler *et al.*, 1977). Advantages of smaller cells include reduced susceptibility to mechanical damage on shrinkage, lower osmotic potential and a lower turgor requirement for growth. The increased internal to external leaf area ratio resulting from reduced leaf area may confer an added advantage through improved water use efficiency (Smith and Nobel, 1978).

Stress can also alter the morphology of reproductive development which may result in lower yields. The formation of new primordia in the developing Barley apex is an example of an extremely sensitive yield-related process (Husain and Aspinall, 1970). Primordium formation is considerably more sensitive than the growth and morphogenesis of lateral spikelets.

Cambial activity may also be reduced by stress and may be regulated through a requirement for daughter cells to expand to a critical size before they in turn can divide. This was apparently the case in excised and stressed *Fraxinus* stems in which the critical size is around 6 μ diameter (Doley and Leyton, 1968).

Altered root anatomy under water deficit conditions has also been recorded. Rice grown in dryland condition when compared with the same variety grown in paddy has a reduced root diameter, increased stele diameter, reduced numbers and sizes of cortical cells, and a reduced number of metaxylem elements (El-Aishy, 1979). These alterations however should be considered in relation to the fact that no drought treatment was applied. Also regarding roots, the importance of the maintenance or acceleration of root growth during stress may exceed that of shoot adaptations in conferring drought tolerance. This situation was found in an experiment comparing two *Eucalyptus* species, which demonstrated that a deep and ramifying root system conferred the greatest degree of drought tolerance (Pereira and Kozlowski, 1976).

Relevent to stress effects on tissue morphology is the point that stress may also be involved, both directly and indirectly, in irreversible physiological disorders. An example of a direct effect is pithiness in Celery. This disorder may be triggered by ABA accumulation and results in tissue breakdown within the petiole (Aloni and Pressman, 1979). Indirect involvement is instanced by the synergistic effect water stress can have in the development of blossom end rot in Tomatoes. Tissue breakdown at the distal end of the fruit is believed to be due to limited

calcium supply, with calcium supply being a process easily inhibited by restricted water supply (Geraldson, 1957).

1.1.5 The Effect of Water Stress on Cell Wall Metabolism and on the Structure and Function of Cells and their Organelles

Cell wall metabolism is reduced during water stress. In *Avena* coleoptiles the hot-acid soluble and cellulose fractions are first affected and with increasing severity, the other non-cellulosic polysaccharide fractions are then decreased (Ordin, 1960). However, the synthesis of all cell wall fractions appears to be uniformly reduced in stressed Sunflower and Almond leaves (Plaut and Ordin, 1961). The wall metabolism of tracheid elements and other cambial derivatives is also reduced when excised Pine stem tissue is stressed (Whitmore and Zahner, 1967).

At the ultrastructural level, water stress disrupts symplastic transport when plasmolysis causes either breakage of plasmodesmatal connections or leaves only protoplasmic connections through cytoplasmic strands (Hechtian strands). Callose production around plasmodesmata occurring during plasmolysis is removed during recovery, with subsequent restoration of electrical coupling (Drake *et al.*, 1978).

During severe stress, which causes vacuolar and inter-cellular space shrinkage, lipid droplets and vesicles proximal to dictysomes appear and rupture of the plasmalemma and/or the tonoplast occurs in Sunflower mesophyll cells (Fellows and Boyer, 1978). Chloroplasts however, retain their integrity at a low water potential which results in the loss of many other cellular organelles. Below certain water potentials tissues lose their ability to recover, but this capacity is apparently tissue-specific with Sunflower phloem tissue for example having greater dessication tolerance than mesophyll tissue. Similarly in Sorghum, and to a lesser extent in Maize, mesophyll cells are more prone to damage (assessed by tonoplast rupture) than bundle sheath cells (Giles *et al.*, 1974). In

Mitchell grass, moreover, multiple vesiculation occurs in mesophyll cells during stress, but not in bundle sheath cells (Mittelheuser, 1977).

Chloroplasts of Sorghum have swollen outer membranes during stress, and, in the case of bundle sheath chloroplasts, have reduced starch contents (Kenneth *et al.*, 1976). Both the loss of starch and the vesiculation of the tonoplast observed during stress are reversible on stress alleviation. Despite the maintenance of Sunflower chloroplast integrity during severe stress, photosystem II activity is considerably reduced. This reduction has been attributed to changes in conformation of the lamellar membranes (Fellows and Boyer, 1976).

Root tissues suffer ultrastructural damage, similar to that described for leaf tissues, during stress. The meristematic cells of Maize roots exposed to stress display rearranged chromatin, reversion of polysomes to monosomes, and damaged and rearranged membranes of mitochondrial cristae, the endoplasmic reticulum and plastids. All these effects are reversible provided not more than 60% of the cellular water is lost (Nir *et al.*, 1970). Root tissue dehydrated to a lethal state, however, has total structural disruption with only large chromatin masses being recognisable (Nir *et al.*, 1969).

Alteration in the activity of certain enzymes may be related to this ultrastructural damage caused by stress. Increased hydrolysis and membrane damage has been linked to increased activity of acid phosphatase and acid and alkaline lipases within chloroplasts of stressed Cotton (Da Silva *et al.*, 1974). Similarly acid lipase activity rose in stressed mitochondria. Coupled with damage and increased degradative enzyme activity may be impairment of cellular oxidation processes. In air dried Rice leaves decreased activities of peroxidase and catalase could result in toxic levels of compounds such as hydrogen peroxide (Mishra *et al.*, 1978).

1.2 The Radish Plant

1.2.1 General Introduction and Discussion

The Radish (*Raphanus sativus* L.) is thought to have originated in an area to the west of the Mediterranean (Banga, 1976). Numerous cultivars within four generally acknowledged varieties exist. The round red cultivars belong to the variety *radicula*, the fleshy axis of which is generally eaten raw as a salad vegetable noted for its pungent flavour. The pungent principle is believed to be 4-methylthio-3-butanyl isothiocyanate (Friis and Kjoer, 1966). The fleshy axis has little nutritional value, being around 93% water with low fibre and protein, and only trace quantities of vitamins (Purseglove, 1968). It is however, an important vegetable crop in many Asian countries, and is grown commercially throughout the world.

1.2.2 The Radish Fleshy Axis

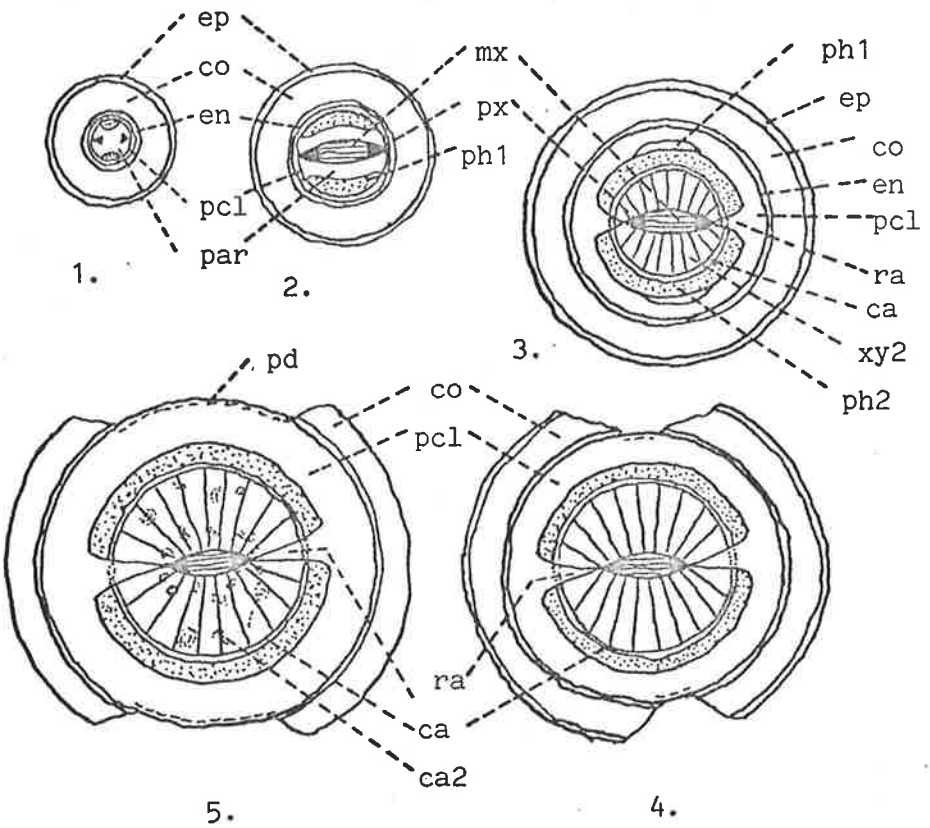
The edible fleshy axis is derived from the hypocotyl and upper radicle tissues. Soon after emergence tissues within the hypocotyl core begin to divide and expand, resulting in rupture of the cortex at opposite poles and subsequent rapid development until 40-50 days after sowing (Hayward, 1938; Weaver and Bruner, 1927; Paliwal and Kavatheker, 1971). The anatomical composition (figure 1.2) of the mature fleshy axis consists of large parenchyma cells arranged in irregular rays and surrounded by a relatively thin band of pericycle tissue. A cambial ring runs between the pericycle and inner parenchymatous region. The parenchymatous region contains the xylem elements, a large proportion of the phloem tissue, and, areas of secondary cambium.

It has been proposed that the early growth of the hypocotyl occurs entirely by cell expansion (Billington, 1970). However, the subsequent growth and the transition from hypocotyl to fleshy axis is associated with both active cell division and expansion. Division occurs primarily in

Figure 1.2

Diagrammatic representation of the progressive development of the Radish fleshy axis, where: ep, epidermis; co, cortex; en, endodermis; pcl, pericycle; par, parenchyma; mx, metaxylem; px, protoxylem; ph1, primary phloem; ra, ray; ca, cambium; xy2, secondary xylem; ph2, secondary phloem; and, ca2, secondary cambium.

1. cross section of hypocotyl soon after germination, through to 5., cross section of young fleshy axis showing all tissue types of the fully mature axis.
(After Hayward, 1938.)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the cambium, although as mentioned above, secondary sites of cell division become obvious in the parenchymatous region as roots approach maturity.

Cambial activity is closely correlated with cytokinin levels. Over a 40 days growth period the concentration of zeatin, and zeatin derivatives, rises in a sigmoid fashion similar to that displayed by fleshy axis diameter, and, as the increase in diameter slows the concentration of an unidentified cytokinin increased (Radin and Loomis, 1971). It was suggested from the evidence obtained, that zeatin and its derivatives regulate cambial activity in Radish and are synthesised in the shoot, whilst the unidentified cytokinin may be synthesised in the roots and transported to the shoot.

Translocation of photosynthetic assimilates to the developing fleshy axis has been shown to be determined by the storage capacity of that organ (Starck and Ubysz, 1976; Starck and Stradowska, 1977). The fleshy axis however, does not store sucrose before maturation because high invertase activity, particularly acid invertase, exists (Ricardo and Sovia, 1974). Prior to maturation reducing sugars are present in far higher concentrations than sucrose, but at maturation invertase activity falls and sucrose accumulates in the fleshy axis.

1.2.3 Growth of the Radish Plant and its Response to Environmental Variables

Poor correlations between shoot and fleshy axis growth parameters have been consistently reported in Radish studies (Muthukrishnan and Arumugam, 1977; Thamburaj, 1973), and phenotypic selection towards decreasing this aspect of variability has found little success (Prasad and Prasad, 1978). However, plant selection procedures do offer ample scope for the overall improvement of varieties. Effects of seed size and vigour add to this genetic variability with Radish crops. Seeds less than 2.5 mm diameter yield significantly less than seeds greater than 2.5 mm diameter (Kononokov and Kravchuk, 1974). Low-vigour seed also

reduces the yield of fleshy axes through causing an earlier cessation of rapid growth (Chen *et al.*, 1972).

Radish cultivars in general, are highly responsive to light and other environmental factors. Radish is a long-day flowering plant, but earlier maturing cultivars respond to a long photoperiod less than do late maturing cultivars (Obraztsov, 1971). Fleshy axis growth of the European cultivar 'Comet' under long-day conditions can be accelerated by high light intensity despite bolting and flowering (Yoo, 1977). Under identical conditions, two Chinese cultivars exhibited decreased fleshy axis development and required vernalization before responding normally to light intensity and photoperiod. Far-red light has been shown to inhibit Radish seed germination and, in one cultivar tested, blue light had a similar effect (Swarnker and Kumar, 1977). Red and White light, and also dark, have no inhibitory effects. In a study on the enlargement growth of detached Radish cotyledons, red light caused increased reducing sugar concentrations (probably through increased amylase activity), and, zeatin applied after light stimulation enhanced the effects (Huff and Ross, 1975). It was postulated that increased sugars, through increased osmotic potential, caused greater cell expansion in response to light triggered phytochrome activation of amylase. Another effect of light is to inhibit the potential elongation of Radish hypocotyls which can occur if seedlings are grown in the dark. In Sakurajima seedlings, applied regulators (1AA, GA_3 and BA) have little or no effect against the inhibitory effect of light on elongation, and, from a study of endogenous growth inhibitors, it was concluded that inhibitors control elongation either through their depressed destruction, promoted biosynthesis or both (Hagesgawa and Miyamoto, 1978).

By varying irradiance and carbon dioxide concentrations, increased growth rates may be obtained in Radish. Higher levels of assimilates in the plant, due to increased shoot growth and increased

net photosynthesis, result in more favourable dry matter distribution to fleshy axis tissues (Combe, 1979).

Temperature, like light, has both qualitative and quantitative effects on Radish growth and metabolism. Flowering in Japanese Radish is hastened by vernalization whilst time to flowering in unvernalized plants fluctuates in an annual rhythm according to the period of seed storage (Yoo and Uemoto, 1976). The gibberellin content of Radish plants doubles in response to seed or seedling vernalization (Suge, 1970). In the longer term temperature has important effects on growth. For instance, in a root temperature experiment (shoots at ambient temperature) germination did not occur at 5°C, was optimal and followed by normal shoot and fleshy axis growth at 20°C, and occurred at 25 and 30°C, but subsequent fleshy axis malformation and abnormal shoot growth was obvious (Wendt, 1977). Similarly, bed grown Radish with bottom heat (14-16°C) significantly outyield unheated controls (7-13°C) (Goldsberry and Halbett, 1977). A constant ambient temperature between 20 and 30°C appears optimal for rapid growth and maximum yield (Nieuwhof, 1976 and 1978). Radish growth at above optimal temperatures results in increased axis elongation, increased shoot dry weight and decreased axis girth (Suzuki and Takano, 1978; Suzuki, 1978).

The interaction of temperature and relative humidity has significant effects on fleshy axis production. High humidity (100%) and low temperature (20°C) causes elongated 'sausage-shaped' fleshy axes, whilst both high humidity and high temperature (30°C) result in normal globe-shaped axes. Low humidity (50%) at either temperature also results in normal fleshy axes (Pareek and Heydecker, 1970).

In view of the theme of this thesis, some discussion of Radish response to stress situations is warranted. Although the observations mentioned above consider high temperatures, such temperatures of up to around 30°C are not uncommon under normal growth conditions. Both high (39°C) and low (4°C) stress temperatures were used to compare Radish

response with control plants at 20°C. Both stress treatments caused massive increases in the proline concentration of 'White Icicle' leaves and the high temperature treatment caused reduced plant height and lower leaf chlorophyll concentrations (Chu *et al.*, 1974). The cold treatment inhibited the increase in plant fresh, but not dry, weight. In the heat stress treatment proline only accumulated when the relative humidity was low, viz. 50% as against 93%. Salinity stress and soil water deficit also reduce growth and chlorophyll concentration, and result in increased proline levels (Chu, 1974). Salt (NaCl) and alkali (Na_2CO_3) stress applied to germinating and young Radish seedlings causes stunted seedlings, decreased rootlet formation, reduced respiration, and a delay in the translocation of carbohydrates and fat derivatives from cotyledons to the embryonic axis (Raman and Rama Das, 1978). Seedlings were more sensitive to alkaline than saline conditions. A less commonly encountered stress, atmospheric pollution, has also been shown to significantly reduce Radish growth. Low concentrations of ozone and sulphur dioxide, which together have additive effects, results in reduced shoot and fleshy axis fresh weights and reduced fleshy axis diameter and length (Tingey *et al.*, 1971).

CHAPTER 2.

MATERIALS AND METHODS

2.1 Plant Material

In the course of experimentation two round red Radish cultivars were used, 'Fireball' (supplied by Arthur Yates and Co. Pty. Ltd.) and 'Mars' (supplied by Hendersons Seed Co. Pty. Ltd.). Use of 'Fireball' was discontinued because of pregermination noted in the seed supplied. Both cultivars are common commercial lines. Samples of seed (100) from each cultivar yielded 98% germination in water soaked filter paper lined petri dishes.

On the basis of an unreplicated preliminary investigation, seeds were graded by mesh sieves and only those in the range of 2.5 to 3.5 mm diameter were used in the experiments. Table 2.1.1 presents the data (means for five plants) on which this decision was based. All harvest growth parameters seem to be reduced with smaller seed size. Kononokov and Kravchuk (1974) established that seed size greater than 2.5 mm resulted in greater yields of fleshy axes than seed size less than 2.5 mm. Small seeds demonstrate a slower rate of fleshy axis diameter development compared with large seeds (figure 2.1.1) and by a predetermined harvest data show reduced diameters (ca. 2 mm).

2.2 Growth Environments

Plants were grown in a glasshouse and in a controlled-environment cabinet.

The glasshouse was whitewashed during the hot summer months and cooling was provided by an evaporative cooler and supplementary air conditioning unit. Winter heating by steam pipes was supplemented with a sealed oil element unit. Temperature was thus maintained between 15-20^{o}C.

Table 2.1.1

The effect of parent seed size on growth parameters of Radishes harvested 50 days after sowing.

Category	Seed Size Range	Seedling Height at 8 days	Leaf Area	Fresh Weight		Fleshy Axis Volume	Dry Weight		Moisture Content	
				Shoot	Fleshy Axis		Shoot	Fleshy Axis	Shoot	Fleshy Axis
	(mm)	(mm)	(cm^2)	(g)	(g)	(cc)	(g)	(g)	(%FW)	(%FW)
Small	2.0-2.5	22.7	288	12.04	15.68	16.4	0.62	0.80	95	95
Medium	2.5-3.5	25.3	355	15.24	21.83	22.0	0.87	1.12	94	95
Large	3.5-4.5	33.7	352	15.90	23.72	22.6	1.01	1.50	94	94

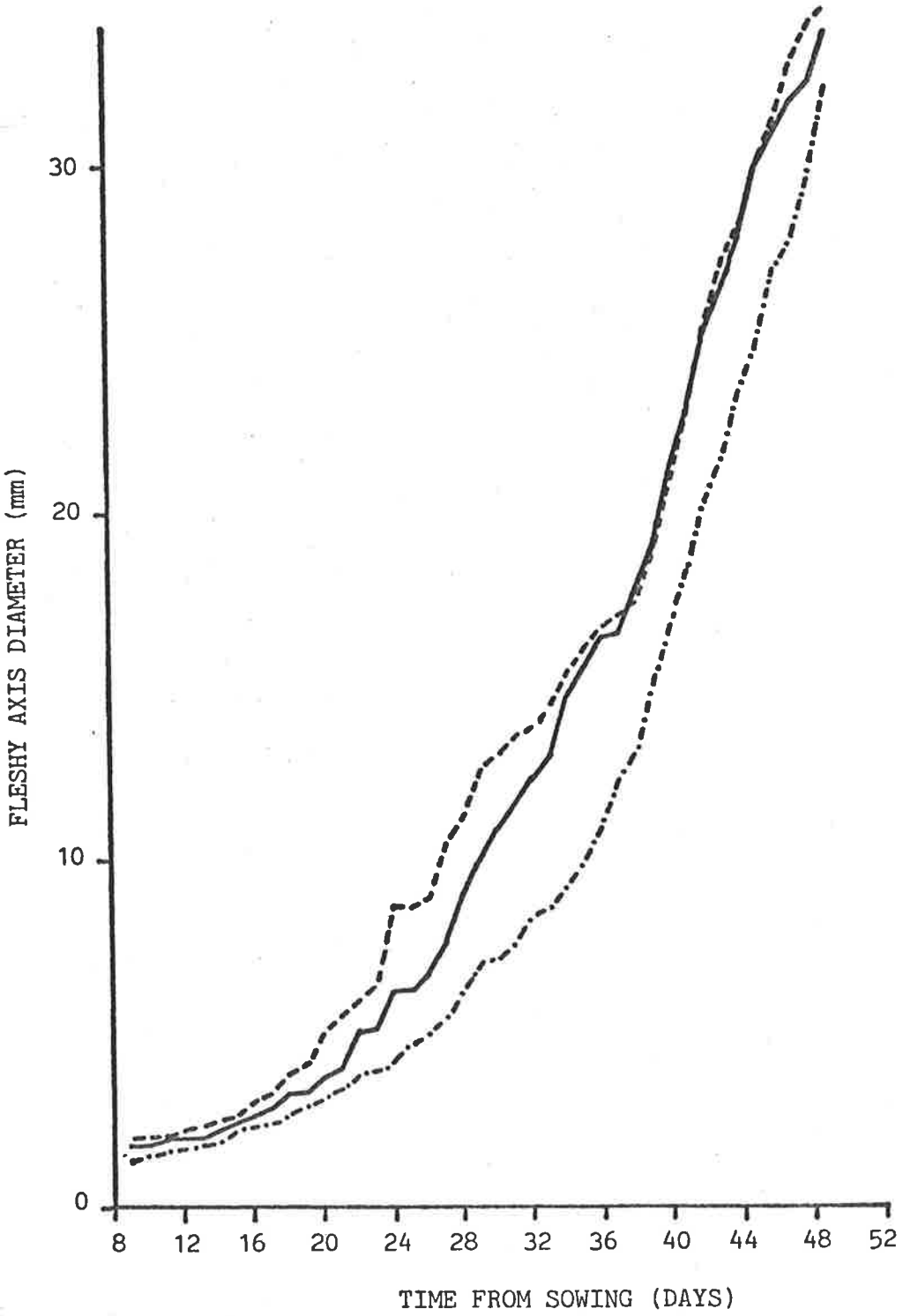
Figure 2.1.1

Parent seed size effect on maximum fleshy axis diameter growth of Radish.

-.-.-. Small

——— Medium

------ Large


FLESHY AXIS DIAMETER (mm)
0
10
20
30
8
12
16
20
24
28
32
36
40
44
48
52
TIME FROM SOWING (DAYS)

Photoperiod control, when required, was achieved with incandescent globes (60 W Phillips 'Pearl') supported one metre above canopy level.

The growth cabinet had a height-adjustable plant platform of 1.25 square metres. The light source was a bank of 28 fluorescent tubes (65 W Phillips 'White') and 8 incandescent globes (60 W Phillips 'Pearl'). Photoperiod was 12 hours in most experiments. A light flux density of about 400 μein/cm^2/sec was maintained at canopy level, and temperature was 20°C ± 1°C constant in most experiments.

2.3 Growth Systems

Several systems for growing, and methods for stressing, plants were used. There was marked interplant variability and a preliminary experiment was conducted to examine the validity of using the mean of a small number (5) of plants to compare treatments. The Bartlett Test does not assume a normal distribution and was used to test the homogeneity of variance of growth parameters measured on plants grown in three pots in the glasshouse. Despite large natural variation between individual plants within pots (figure 2.3.1) no significant differences between means were found for any parameter. Thus although estimates of the coefficients of variance indicated that sample variance was large for each parameter (table 2.3.1), each pot has been shown to represent a true population sample. In other words, each pot had a similar parameter mean with like variance about that mean in the absence of any treatment likely to have an adverse or promotive effect on plant performance. With this information available, Spearmans Rank Correlation Coefficient tests were carried out with the pooled data (15 plants). There was a surprisingly poor correlation between shoot and fleshy axis growth (table 2.3.2). For example, a correlation coefficient of only 0.39 between shoot and fleshy axis fresh weight. On the other hand fleshy axis parameters showed high correlations with each other. Prasad and Prasad (1978) and Muthukrishan and Arumugam

Figure 2.3.1

Natural variation in Radish shoot and fleshy axis organs between 15 plants cultured as three replications.

Table 2.3.1

Coefficients of variation for a range of growth parameters collected from 15 plants constituting three replications.

Growth Parameter	Coefficient of Variation			
	Pot 1	Pot 2	Pot 3	Pooled Data
Shoot Fresh Weight	0.436	0.349	0.582	0.435
Shoot Dry Weight	0.411	0.379	0.600	0.438
Fleshy Axis Fresh Weight	0.565	0.662	0.823	0.635
Fleshy Axis Dry Weight	0.551	0.529	0.771	0.584
Leaf Area	0.314	0.281	0.573	0.403
Leaf Number	0.197	0.206	0.267	0.244
Fleshy Axis Maximum Diameter	0.405	0.356	0.562	0.412
Fleshy Axis Volume	0.582	0.671	0.829	0.645

Table 2.3.2

Correlations between various growth parameters based on data from 15 individual Radish plants.

Parameter	Shoot Fresh Weight	Shoot Dry Weight	Fleshy Axis Fresh Weight	Fleshy Axis Dry Weight	Leaf Area	Leaf Number	Fleshy Axis Maximum Diameter	Fleshy Axis Volume
	1.	2.	3.	4.	5.	6.	7.	8.
8.	0.384	0.300	0.998	0.970	0.457	0.016	0.904	1.000
7.	0.339	0.324	0.907	0.879	0.371	-0.006	1.000	
6.	0.619	0.582	0.008	0.101	0.576	1.000		
5.	0.975	0.930	0.421	0.539	1.000			
4.	0.493	0.405	0.964	1.000				
3.	0.386	0.297	1.000					
2.	0.942	1.000						
1.	1.000							

(1977) have also reported poor correlations between shoot and fleshy axis parameters. As a consequence of this preliminary experiment, further experiments were conducted using small numbers of plants in pots, in which detailed measurements of shoot and fleshy axis growth were taken separately.

Although not proven, an apparent decline in seedling vigour occurred as the seed aged and direct sowing of dry seed was abandoned during the period of experimentation. Seeds were thereafter pregerminated and only vigorous seedlings were transplanted. In later experiments surface sterilization of seed was practised (1 part White King^{R} [Cl 4% w/v] to 1 part distilled water and shaken for 5 minutes, followed by 2 hours washing under flowing tap water).

Seeds were germinated on distilled water soaked filter paper in petri dishes. Germination proceeded normally whether the petri dishes were wrapped with Alfoil or placed unwrapped into the growth cabinet. The seeds were allowed to grow on for 48 hours (pregerminated seed) up to one week (small seedlings) before being transplanted. Seedlings kept in petri dishes for longer than 48 hours were moistened with half-strength Hoagland's nutrient solution.

Dark coloured plastic pots (100 to 210 mm diameter) were used. The plants were grown in a variety of rooting media: fine river sand alone, coarse river sand alone, a mixture of isolite and coarse sand (1:3), coarse river sand and clay loam mixture (1:1) and vermiculite alone. A layer of gravel and, for sand, fine nylon mesh was used to facilitate drainage.

Mineral nutrients were supplied by watering with various dilutions of Hoagland's solution (Hoagland and Arnon, 1938) in most experiments. In one experiment Osmocote^{R} (3 month), a slow release fertilizer formulation, was incorporated into the soil to a depth of 10 cm (3 g/7 kg soil).

2.4 Imposition of Stress

Water stress was imposed either by allowing depletion of the water in the rooting medium or by substituting for the water in the medium with a solution of known osmotic potential. Polyethylene glycol (PEG 4000 M.W.) was used as the osmoticum and was dissolved in half-strength Hoagland's solution (osmotic potential -0.7 bars). PEG solutions in the range of -5 to -15 bars (Slavik, 1974) were used to impose water stress. Several studies have reported toxic and undesirable effects of PEG in addition to those caused by the decrease in osmotic potential (Lawlor, 1970; Lesham, 1966; Mexal *et al.*, 1975). No visible toxic effects were noted in the present study. In addition a comparison of the light transmission over the spectral range of 300 to 700 nm for commercial PEG (Union Carbide) with that of analytical grade PEG (BDH) and with PEG purified by the method of Albertsson (1971) revealed no differences in absorbance. This indicated that the PEG used was free of impurities.

The volume of PEG solution applied to impose stress was generally equal to twice that volume of water required to wet air dried rooting medium to field capacity. At the end of a period of stress the PEG solution was removed by flooding the rooting medium with either distilled water or nutrient solution at double the volume of PEG originally applied.

In the alternative technique, the rate of depletion of water in the rooting medium was recorded by weighing pots daily. The weights obtained daily were used to calculate the timing and amount of water required to maintain regimes where either a) the rooting medium was cycled between field capacity and a predetermined lower water content, or b) was cycled between two levels of water content below field capacity.

Salt and cold stress were also imposed in one experiment. Salt stress was imposed and alleviated as was that imposed with PEG, but using a 17 g/l NaCl solution (-10 bars osmotic potential). Cold stress was imposed by transferring plants to $5^{o}C \pm 0.5^{o}C$ with 400 $\mu ein/cm^{2}/sec$ irradiance for 12 hours per day.

2.5 Measurement of Plant Water Status

Plant water status was monitored, as a general rule, through measurements of leaf relative water content. Estimates of water potential and some estimates of osmotic potential were also carried out.

2.5.1 Relative Water Content

Relative water content was determined with either whole leaves or with leaf discs (1 cm diameter). Whole leaves were used when the oldest 'fully expanded' leaf on the plant was the first formed true leaf. Leaf discs (3 to 5) were punched from the blade tissue of larger leaves with a cork borer. The last fully expanded leaf was used in all estimates. The following formula was used to calculate relative water content (Barrs, 1968):

$$(\%)\ \text{R.W.C.} = \frac{\text{f.w.} - \text{d.w.}}{\text{t.w.} - \text{d.w.}} \times 100$$

Fresh weight (f.w.) was measured immediately after excision of leaf material, leaves being placed in a sealed plastic bag during the short interval between excision and weighing. Turgid weight (t.w.) was obtained after floating the material for 4 hr on distilled water under diffuse light. The leaf was then blotted dry with soft absorbant tissue and weighed. Dry weight (d.w.) was obtained following oven drying (80°C) for 48 hours.

2.5.2 Water Potential

Leaf water potential was measured by two techniques, thermocouple psychrometry and with the pressure bomb (Barrs, 1968). For psychrometry a leaf (or part thereof) was placed immediately after excision into the psychrometer cup so as to line the whole surface. The cup was then sealed with the thermocouple plug and placed into a water bath (25°C ± 0.001°C). Tissue was allowed to equilibrate with the chamber atmosphere for a minimum of 2 hrs. The Spanner type psychrometer used had 24 channels

(thermocouples). Deflections recorded on a moving chart recorder were quantified by comparison with a standard curve prepared with salt solutions of known water potential (Barrs, 1968).

Pressure bomb estimations of leaf water potential were obtained on a commercial instrument (Soilmoisture Equipment Corp.). The leaf was placed through the chamber head and the seal around the petiole tightened until no light was visible along the petiole channel. Approximately 5 mm of petiole protruded above the seal. After securing the chamber head to the chamber the pressure was increased slowly until xylem fluid appeared at the vascular bundles. A CO_2/air mix was used to pressurize the chamber. Determination of fluid appearance at the vascular bundles was facilitated with a travelling microscope. Water potential was read directly off the pressure gauge which was calibrated in bars.

2.5.3 Osmotic Potential

Leaf osmotic potential was measured with a micro-osmometer (Barrs, 1968). Plant material was sampled into a plastic tube which was immersed in liquid nitrogen and then sealed before storage at -20°C. The stored material was thawed (30 min) and sap was expressed through a fine wire mesh with a micropress. A 10 to 50 µl sample was placed into the Knauer^R micro-osmometer to determine osmotic potential by freezing point depression. The data obtained were compared with a standard curve prepared with NaCl solutions of known osmotic potential (Slavik, 1974).

2.6 Macroscopic Growth Parameters

The growth of the shoot and fleshy axis was assessed from measurements of leaf area and number, shoot and fleshy axis fresh and dry weights, fleshy axis maximum diameter, fleshy axis volume. Other data (eg. water content) were calculated from this primary data. An unreplicated preliminary experiment yielded the fresh and dry weight data

(means for five plants) presented in table 2.6.1. A stage of rapid shoot growth precedes a similar stage in fleshy axis development. Sigmoid growth patterns for both organs are clearly discernible (figure 2.6.1). As good correlations existed between fleshy axis volume, fleshy axis fresh weight and fleshy axis maximum diameter (table 2.3.2) the convenient and nondestructive measurement of diameter was used to follow fleshy axis development, and stress effects, over time.

2.6.1 Fresh Weight

Tissue fresh weight was determined immediately after harvest using a top-pan balance accurate to 0.01 g. Shoot fresh weight included all above ground parts to the clear demarcation 'collar' at its junction with the fleshy axis. Fleshy axis tissue included all tissue below the shoot junction to the transition between swollen and normal tap root tissue. Lack of anthocyanin and a considerable reduction in diameter marked this transition.

2.6.2 Dry Weight

Dry weight was determined after placing tissue into paper bags and drying it at 80^{o}C for 72 hours in a forced-draught oven.

2.6.3 Leaf and Cotyledon Area

Leaf and cotyledon areas were measured with an electronic planimeter and included the petiole to its junction with the stem. The Paton Electronic Planimeter produced a digital readout of the area of tissue presented on a transparent conveyor and passed between a line of photocells and a light source at a constant speed.

Table 2.6.1

Changes in fresh and dry weights, and moisture content, of the shoot and fleshy axis of developing Radish plants.

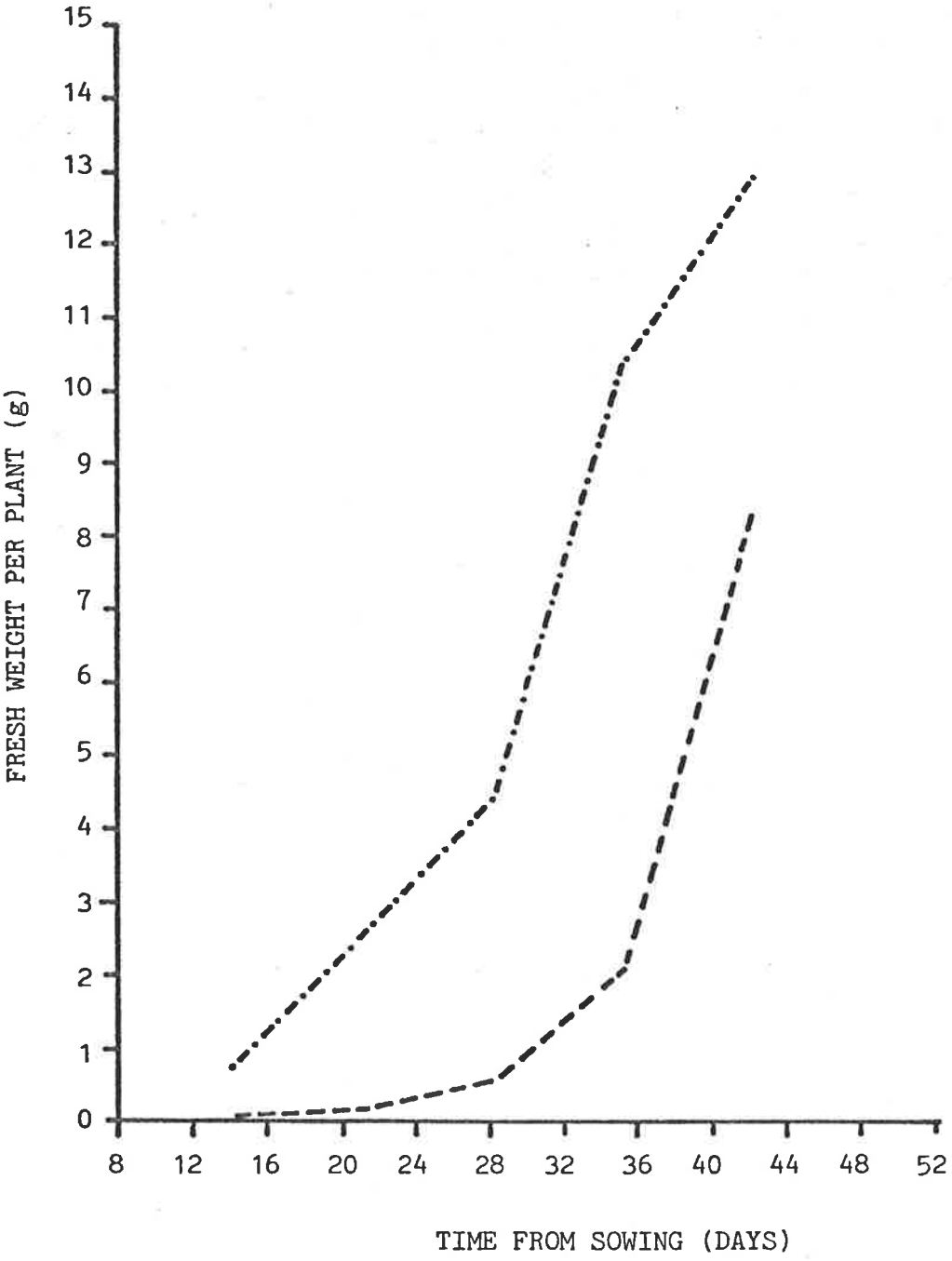
Days from Sowing	Fresh Weight (g)		Dry Weight (g)		Moisture Content (% F.W.)	
	Shoot	Fleshy Axis	Shoot	Fleshy Axis	Shoot	Fleshy Axis
14	0.70	0.09	0.05	0.01	93	89
21	2.58	0.17	0.17	0.01	93	94
28	4.40	0.55	0.27	0.07	94	87
35	10.30	2.07	0.47	0.11	95	95
42	12.39	8.31	0.78	0.47	94	94

Figure 2.6.1

Relative fresh weight development of Radish shoot and fleshy axis organs.

-.-.-.-.Shoot

------- Fleshy Axis


FRESH WEIGHT PER PLANT (g)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
8
12
16
20
24
28
32
36
40
44
48
52
TIME FROM SOWING (DAYS)

2.6.4 Leaf and Cotyledon Number

Cotyledons and leaves were counted provided they retained their structural integrity. Only dead and clearly disintegrating tissue was excluded. A young leaf was counted if the two halves of its blade had unfolded to be on or about the same axis.

2.6.5 Maximum Fleshy Axis Diameter

This measurement was made using vernier scale callipers. The 'jaws' of the instrument were adjusted to touch either side of the fleshy axis at its visually assessed widest point. Diameter was measured to an accuracy of 0.1 mm.

2.6.6 Fleshy Axis Length

Fleshy axis length (mm) was measured from the shoot/fleshy axis transition zone to the fleshy axis/root transition zone with vernier callipers.

2.6.7 Fleshy Axis Volume

Volume was determined by lowering the organ on the end of a long fine needle into a measuring cylinder containing distilled water. The meniscus level before and after the organ was fully submerged was read and the difference between the two measurements gave fleshy axis volume. The density of the fleshy axis was calculated from the fresh weight and volume.

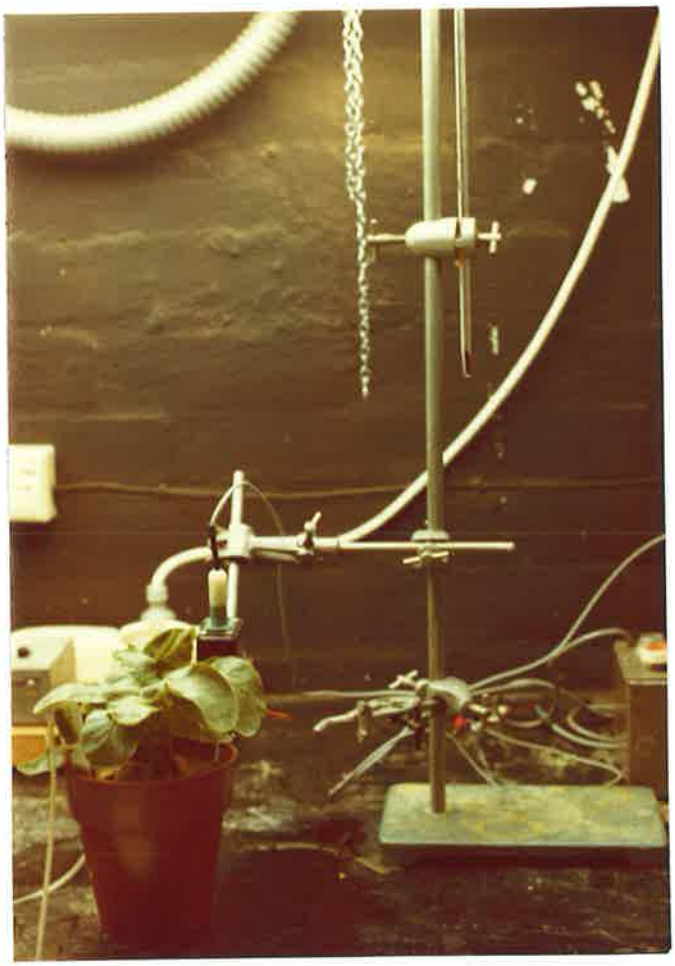
2.6.8 Continuous Monitoring of Fleshy Axis Diameter

Changes in fleshy axis diameter during stress were continuously monitored with a heart/smooth muscle transducer (Harvard, Model 386) connected by means of a light spring tension against a lever resting on the organ (figure 2.6.2). The rotary capacitance-bridge electronic transducer was connected through a variable bridge balance unit to a moving chart recorder. The apparatus was calibrated by gently moving the lever

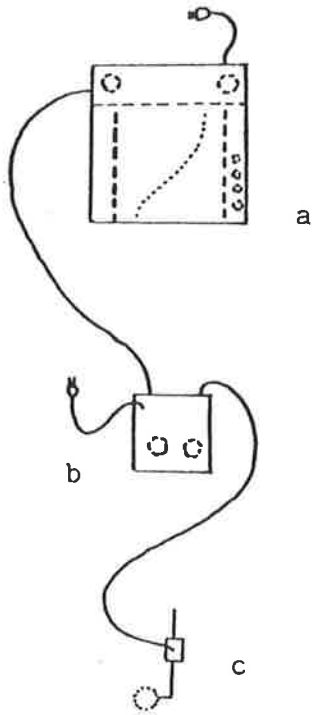
Figure 2.6.2

Coupling system between the Radish fleshy axis and the rotary displacement transducer used for the continuous monitoring of axis diameter.

1. Photograph of transducer coupled to Radish fleshy axis.
2. Schematic diagram of transduction and monitoring system (a, chart recorder; b, bridge balance; c, transducer).
3. Plan diagram of transduction unit (d, spring tension adjustment; e, transducer support arm; f, light tension spring; g, transducer lever [steel rod]; h, rotary displacement transducer casing; i, plastic contact pad; j, Radish fleshy axis; k, lead to bridge balance).

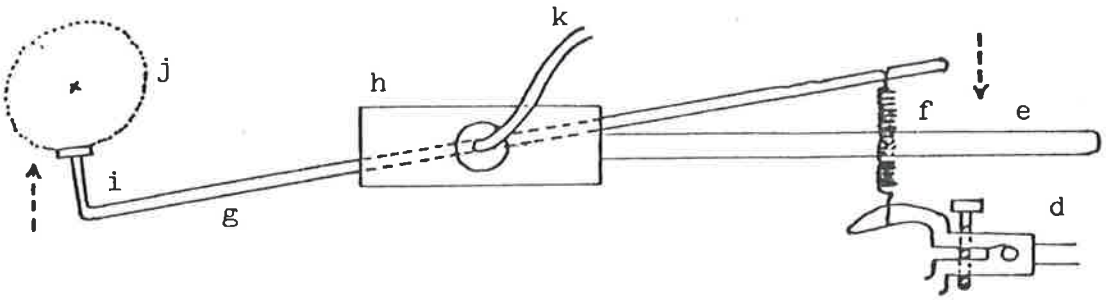


1.



2.

3.



with a known distance adjustment on vernier scales touching the lever, and recording the deflection on the chart recorder. An accuracy of 0.1 mm was detectable.

2.7 Microscopic Growth Observations

Cell numbers and sizes, etc. were recorded in shoot and fleshy axis tissues.

2.7.1 Shoot Tissue

A 1.1 cm disc was excised from either cotyledon or leaf tissue avoiding main veins. The disc was incubated for 20 hr at 20°C in 1.0 ml of 5% Chromic acid (Brown and Rickless, 1949), shaken for 1 min on a vortex mixer, allowed to incubate for a further 4 hr and reshaken. Immediately after the second shaking an aliquot was placed on a Haemocytometer and the number of cells in four grids counted. The count was repeated on another aliquot for each leaf disc. Each grid held 0.1 mm^3 of solution and the number of cells per leaf disc was calculated from these data. Provided the leaf or cotyledon area was known the cell number per leaf could be estimated. However as leaf size and leaf number were highly variable detailed estimations of leaf cell numbers were not made.

2.7.2 Fleshy Axis Tissue

Fleshy axis tissue was sectioned and stained immediately in 1% safranin and then passed through an ethanol series (30, 50, 70 and 90%). Fifteen minutes was allowed for each of the alcohol steps, and prior to the 70% step tissue was stained with 0.5% light green in 70% ethanol. Tissue was finally placed in 100% ethanol for 30 minutes, 50 xylol : 50 ethanol for 15 minutes and then xylol for 2 minutes before being mounted in Canada balsam and covered with a coverslip.

The paraffin wax technique (modified from Johansen, 1940) was used initially to prepare transverse sections for estimation of cell size and cell numbers. A 2-5 mm thick disc of fleshy axis tissue was excised with a new razor blade and infiltrated under vacuum with F.A.A. for 18 hours. After fixing, the tissue was washed in two changes of 50% ethyl alcohol and passed through an ethanol series (5, 10, 15, 20, 30, 50, 70, 85, 95 and 100% ethanol). Two hours were allowed for each step in this series. Tissue was then cleared in a chloroform/ethanol series (1:2, 1:1, 2:1 and pure chloroform) again allowing 2 hours per step. Paraffin infiltration was initiated by holding tissue at 30°C and adding paraffin wax until the chloroform was saturated (ca 48 hours). Tissue was then transferred into pure molten wax (M.P. 56°C) for 12 hours, the wax being renewed at 4 hr intervals. Embedding was carried out by transferring the tissue into an Alfoil cast, into which molten wax was poured. The cast was cooled rapidly in flowing water. After trimming, the cast was attached, with molten wax, to an object holder, and sections (10 μ) cut with the razor blade attachment on a hand operated rotary microtome. Clean slides were labelled and smeared with Mayers albumen. Sections were removed from water filled petri dishes in which they had been floating, onto a thin layer of water covering the slide. The slide was dried on a warm hot plate. The staining series follows: wash in xylol for 5 minutes, in ethanol/xylol (1:1) for 5 minutes, in absolute ethanol:anesthetic ether (1:1) for 10 minutes, after the tissue became opaque it was washed in 70% ethanol for 5 minutes then quickly in commercial alcohol, followed by two changes of absolute alcohol (2 minutes each). The sections were stained for 2 minutes with light green, cleared with clove oil, and passed through 2 x 2 minute changes of xylene. The sections were mounted in Canada balsam, covered with a coverslip and allowed to dry for three days.

This technique was time consuming and a simpler and quicker technique was devised. Tissue was collected and fixed overnight at 4°C in 4% buffered formaldehyde (O'Brien *et al.*, 1977). It was

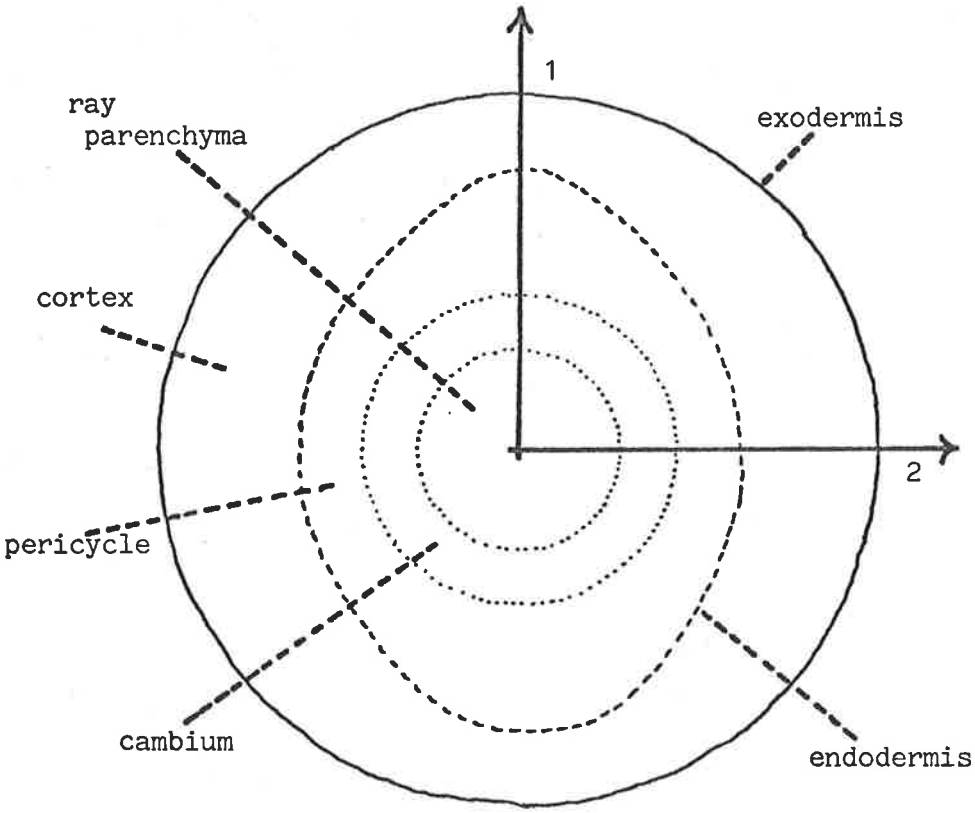
then washed with distilled water and dehydrated in 100% methyl cellosolve (2-methoxyethanol) for 24 hrs at 4°C (Considine, pers. comm.). JB4 was used to infiltrate and embed the tissue (JB4 pamphlet, Polysciences). Embedding was carried out between two small plastic weighing trays as oxygen must be excluded to allow polymerization. Infiltration was at 4°C and embedding at room temperature. The casts were trimmed and fixed to wooden specimen holder blocks with Araldite^{R} cement. The sections (4 µ) were cut with glass knives (Bennett *et al.*, 1976) and floated on warm water. Sections were then transferred to slides and allowed to dry overnight whereupon they adhered tightly to the slides. The sections were stained with toluidine blue (0.05% in benzoate buffer) for five minutes, washed in water, allowed to dry and mounted in Permamount.

The sections prepared by either technique were used to assess cell expansion and division. The transverse section was transected by two radiating lines (figure 2.7.1) at 90°C to each other. These passed through the widest and narrowest points of the pericycle. Cell widths and cell numbers for cortex, pericycle, cambium, parenchymatous ray tissue and pooled tissue types were measured along these transects. An optical micrometer was used. An optical micrometer grid permitted the estimation of the number of cells, and consequently the average cell area, for each tissue type. Estimates of cell area were collected as the mean for five or ten randomly selected areas within tissue types.

Cell wall thickness was estimated by photographing the relevant sections and measuring the wall thicknesses of 10 randomly selected parenchyma cells for each section. A dissecting microscope with eyepiece micrometer was used to measure thicknesses from the developed negatives and these values were then averaged and corrected for magnification.

Figure 2.7.1

Diagrammatic representation of tissue classification and data collection transects used on the Radish fleshy axis.


1
2
ray
parenchyma
exodermis
cortex
pericycle
cambium
endodermis

2.8 Chemical Procedures

Labelled CO_2 was supplied to stressed and unstressed plants. Counts per minute were determined in sugar fractions and in crude alcohol soluble and alcohol insoluble extracts of leaf, cotyledon, hypocotyl and root tissues. Additionally, sugar and proline concentrations were measured.

2.8.1 $^{14}CO_2$ Treatment

Five hundred µci of ^{14}C was released into a 100 l rectangular perspex chamber by adding 5 ml of lactic acid (88%) to 250 µl of $NaH^{14}CO_3$. Plants were exposed to this atmosphere for one hour. Air was circulated within the chamber by means of a small electric fan. The reaction was stopped 15 minutes prior to removing the plants by flooding the reaction vial with 1 N KOH.

2.8.2 Counts in Alcohol Soluble and Insoluble Extracts

Twenty milligrams of coarsely ground freeze-dried plant material was extracted by boiling for 15 minutes in 80% ethanol. The supernatant was removed and the process repeated twice. The supernatants were stored at $4^{o}C$. Residues were also held at $4^{o}C$ overnight, in 80% ethanol, between extractions.

The combined supernatants were evaporated to dryness under vacuum and taken up in 10 ml of double distilled water. The residue was dried in a $40^{o}C$ oven and stored at $4^{o}C$ in a vacuum dessicator.

A 0.1 ml aliquot of the alcohol soluble extract was mixed with 10 ml of ACS^{R} cocktail (xylene-surfactant base) and counted for 10 minutes in a liquid scintillation counter. Counts were adjusted for machine efficiency, counting duration and dilution to yield c.p.m. in the soluble extract from one gram dry weight of tissue.

The insoluble residue was ground in 2 ml of distilled water in a glass Duall homogeniser, and made up to 5 ml volume. An aliquot of 1 ml was removed and made up to 5 ml with distilled water. This was mixed with 15 ml of ACS cocktail and the resultant gel counted for one minute. Counts were adjusted in a similar fashion to those for soluble extract to yield c.p.m.

2.8.3 Total Sugars

Concentrations of total sugars in the alcohol extract were determined by the anthrone colorimetric test (Yemm and Willis, 1954). A 0.1 ml aliquot was made up to 1 ml with distilled water. This was carefully layered onto 5 ml of anthrone reagent in boiling tubes. The tube was vigorously shaken, capped with a hot (50°C) marble, and placed in a boiling water bath for 6 minutes. The tube was then cooled by plunging into an ice bath, and optical density at 620 nm was recorded 30 minutes later. Concentration of total soluble sugars was quantified by relation to a sucrose standard curve (5 to 60 µg).

2.8.4 Separation of Sucrose, Glucose and Fructose

From the alcohol soluble extract bulk (10 ml) 500 µl was removed and spotted onto a Whatman 3 mm chromatography paper. The paper was run using ethyl acetate/pyridine/distilled water (10:4:3) in a descending chromatography tank. Sugar markers (5 µl of 1 mg/ml sucrose, 0.2 mg/ml glucose and 0.2 mg/ml fructose) and a sample extract marker were run on strips at both sides of the paper.

After 20 hours the papers were removed and allowed to dry for one hour. The marker strips were cut off and developed by dipping through first a $AgNO_3$ in acetone solution followed by a NaOH in chloroform solution (Anet and Reynolds, 1954). Papers were allowed to dry between and after dips. The relative positions of the sugars were determined and the corresponding sugar cut out for elution. Four unknowns were run on one paper.

The sugars were eluted by allowing 2 ml of distilled water to run by capillary action up between two glass slides (held together by an elastic band) and down through the paper piece held by one corner at the end of the slides (Dent, 1947). As this paper was bent 90° to the horizontal the water after collecting the sugar was able to drip into vials placed directly below. Many elutions were performed at one time by arranging the slide pairs around the periphery of a large raised petri dish containing distilled water. Elution of the 2 ml volume required around 3 hrs.

2.8.5 Concentrations of Sucrose, Glucose and Fructose

One ml of the eluted sugar was used to determine sugar concentration by the anthrone test (see 2.8.3). Concentrations were adjusted for dilution to yield the concentration per mg dry weight.

2.8.6 Specific Activity of Sucrose, Glucose and Fructose

One ml of the eluted sugar was added to 10 ml of ACS cocktail and counted for 10 minutes. Counts were adjusted to c.p.m. and divided by the sugar concentration (see 2.8.5) to estimate specific activity.

2.8.7 Scintillation Counting

Twenty ml glass scintillation vials and ACS^{R} cocktail (Amersham) were used for all counting. The Packard model 3320 liquid scintillation spectrometer was set for ^{14}C and gave an efficiency of 87.3%. Counts were adjusted for time of counting and machine efficiency to yield counts per minute (c.p.m.) data. Presented data was adjusted for dilution during extraction to provide c.p.m. on either soluble or insoluble extract from 1 mg dry weight. As quench curves were not constructed absolute counts (d.p.m.) were not calculated. Based on the knowledge of plant organ total dry weights counts per organ were estimated.

2.8.8 Proline Determination

Freeze dried tissue (around 20 mg) was homogenised in a Duall homogeniser using 1.5 g DeCalso resin and 5 ml of MCW (12 methanol:5 chloroform:3 distilled water). The homogenate was centrifuged for 5 minutes at 2000 rpm and the supernatant collected. The pellet was resuspended in 5 ml MCW and recentrifuged. Three ml of chloroform and 5 ml distilled water was then added to the combined supernatants, shaken, and centrifuged. The aqueous layer was removed to boiling tubes containing 5 ml glacial acetic acid and 5 ml freshly prepared ninhydrin reagent (125 mg ninhydrin: 3 ml glacial acetic acid:2 ml 6 M orthophosphoric acid), and placed for 45 minutes in a boiling water bath. Toluene (5 to 20 ml) was added after cooling. This mixture was shaken and optical density of proline read at 515 nm (Singh, Paleg and Aspinall, 1973). Concentration was estimated against a proline standard curve (0 to 100 µg). Anthocyanins, from the hypocotyl tissue, were not taken up into the benzene layer and thus did not interfere with the estimate.

CHAPTER 3

RESULTS

3.1 Imposition of Episodes of Water Stress during Growth

3.1.1 Introduction

Radish is a biennial crop species and demonstrates no morphologically distinct growth stages between germination and maturation in the first growing season. Despite this lack of phasic development, it is possible that different stages in the development of the fleshy axis are of varying sensitivity to water stress, and that a critical period can be distinguished. Identification of such a period would be beneficial in the optimal water management of Radish crops, and in elucidating water stress effects on growth and development.

This possibility was explored in an experiment where polyethylene glycol (PEG) was employed to induce water deficit at ten stages during the growth of Radish plants.

3.1.2 Methods

The experiment was conducted in the glasshouse under prevailing winter conditions. Temperature was modified to vary between 15 and 20^{o}C and daylength was extended to 16 hrs.

Radish seeds, cv. 'Fireball', were pregerminated and seeds of uniform radicle protrusion sown into 160 mm (top diameter) pots at 5 mm depth 2 days later. Five healthy seedlings were established in each pot by day 10, and monitoring of fleshy axis maximum diameter of 3 plants in each pot was commenced. Monitoring was continued at 2 day intervals to day 57 when the plants were harvested. Except during an episode of water stress the pots were watered with Hoagland's solution ($\frac{1}{4}$ strength) every second day and with distilled water on alternate days. The experimental

design was a randomized complete block, with 3 replications each on separate benches in the glasshouse compartment.

The remaining 2 plants in each pot (those not used to monitor diameter) were sampled to provide data on the water status of plants just prior to and at the 48^{th} hr of water stress. Plant water status was assessed from the youngest one or two fully expanded leaves which were sampled (3 x 1 cm discs) for the estimation of relative water content. The remaining tissue was used for psychrometric determination of water potential.

Ten identical episodes of water deficit were imposed at 3 day intervals on different pots of plants, commencing 20 days post sowing and continuing until day 47. Use of a control pot increased the number of pots per replication to 11. Water stress was imposed by flooding the isolite/sand (4:1 v/v) filled pots with 600 ml of PEG (-10 bar). The episode was terminated with 900 ml of distilled water. Each episode of water deficit was of 48 hrs duration. The last episode was terminated on day 49 and all plants were grown on to day 57 when they were harvested.

In addition to the plants in the main experiment, a further pot of plants was grown under the control conditions to provide material to relate the diameter at different times to anatomical development occurring within the fleshy axis. Transverse fleshy axis sections were prepared from single plants harvested on each of days 10, 24, 37 and 48. The results of this study are presented in Appendix I (page 110).

3.1.3 Results

There were no residual effects of any of the episodes of water deficit, as was apparent in the lack of significant differences between treatments in the data at the final harvest (table 3.1.1). The table further indicates that shoot and fleshy axis growth have a similar sensitivity to a short period of water deficit.

Table 3.1.1

Final yield data (day 57) for Radish plants subjected to a single episode of water stress at one of 10 stages during growth

Stress Number	Stress Period	Fleshy Axis Volume	Fleshy Axis Fresh Weight	Fleshy Axis Dry Weight	Fleshy Axis Moisture Content	Shoot Fresh Weight	Shoot Dry Weight	Shoot Moisture Content	Leaf Area
	(days)	(ml)	(g)	(g)	(%)	(g)	(g)	(%)	(cm^2)
1	20-22	4.7	4.5	0.37	91.8	2.7	0.26	90.4	77.3
2	23-25	4.8	4.6	0.33	92.7	2.5	0.24	90.4	70.0
3	26-28	4.2	4.2	0.31	92.7	2.7	0.28	89.8	72.0
4	29-31	2.7	2.5	0.21	91.6	2.3	0.25	89.1	62.1
5	32-34	2.6	2.4	0.21	91.6	2.5	0.25	90.3	68.3
6	35-37	3.7	3.6	0.28	92.4	3.7	0.22	94.0	64.6
7	38-40	2.4	2.3	0.20	91.3	2.9	0.28	90.1	71.4
8	41-43	3.0	2.9	0.25	91.6	2.6	0.27	89.8	73.3
9	44-46	3.4	3.2	0.24	92.2	2.3	0.23	90.0	66.6
10	47-49	3.1	2.8	0.23	92.1	2.4	0.24	89.9	64.8
11	Control	4.9	4.7	0.37	92.2	2.5	0.26	89.8	70.4
Significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Water stress caused general and considerable reductions in both leaf water potential and relative water content (table 3.1.2). The variation in both parameters was large, but overall mean water potential was -4.9 bars for the prestress measurements and -19 bars for those during stress; the equivalent values of relative water content were 94.8% and 63.2%. The relative water content was the more consistent measure of plant water status when individual plant values were compared.

Fleshy axis diameters were recorded every second day but the results (some presented in table 3.1.3) were difficult to interpret. There were no significant differences between any treatments before water stress was imposed as may be expected. After stress episodes commenced, however, some significant differences appeared. Only on day 30 were there differences capable of straight-forward interpretation. On that occasion, the mean diameter of the axes of episode 4 plants (which were at the 24^{th} hour of stress) was significantly less than the mean of all treatments not subjected to stress at or before that time. This was not the case for episodes 1, 2 and 3, despite the fact that for episodes 1 and 3 diameters were recorded on the 48^{th} hour of stress. In accordance with these observations, it was noted (table 3.1.2) that episode 4 plants had a stress relative turgidity lower than all others. For all episodes subsequent to episode 4 there were no consistent trends in significance with respect to diameter of plants currently being stressed. This supports the conclusion from the final harvest data that the episodes of stress had no residual effect. The contraction of the fleshy axis which occurred during the period of stress is shown for early, middle and late stress episodes in figure 3.1.1. During the late episode of stress fleshy axis diameter was reduced by 0.5 mm at the 48^{th} hr of stress, a reduction representing a 5% decrease over the prestress diameter. Despite the lack of significant differences attributable to stress episodes figure 3.1.1 indicates that fleshy axis diameter growth after stress alleviation does not continue at the prestress

Table 3.1.2

Water potentials and relative water contents of leaves of Radish plants measured just prior to and at the 48th hour of an episode of stress imposed at 10 timed stages during growth.

Stress Number	Stress Period (days)	Water Potential (- bars)		Relative Water Content (%)	
		Prestress	During Stress	Prestress	During Stress
1	20-22	8.3	24.6	90.3	69.0
2	23-25	9.1	31.1	91.7	62.9
3	26-28	8.3	20.9	91.3	70.1
4	29-31	3.9	20.2	95.5	50.5
5	32-34	4.2	14.4	94.3	63.2
6	35-37	4.5	12.6	97.5	66.3
7	38-40	1.4	8.8	96.6	57.4
8	41-43	3.1	13.9	98.3	62.5
9	44-46	3.6	13.8	96.6	70.9
10	47-49	2.5	19.1	96.1	59.5

Table 3.1.3

Maximum fleshy axis diameters for Radish plants subjected to an episode of water stress at one of 10 timed stages of growth.

Stress Number	Stress Period (days)	Maximum Fleshy Axis Diameters (mm) on days...					
		10	20	30	40	50	56
1	20-22	1.38	1.83	1.91	3.36	11.55	16.45
2	23-25	1.41	1.82	1.86	3.35	13.11	17.23
3	26-28	1.47	1.86	2.06	3.91	12.21	16.29
4	29-31	1.37	1.77	1.63	2.67	8.88	13.16
5	32-34	1.52	1.87	2.21	2.68	8.85	13.91
6	35-37	1.42	1.77	2.50	4.41	12.17	16.46
7	38-40	1.41	1.80	2.12	2.95	7.70	12.48
8	41-43	1.45	1.79	2.40	5.90	9.42	14.36
9	44-46	1.37	1.83	2.19	5.51	11.44	15.52
10	47-49	1.42	1.75	2.08	4.52	11.02	14.50
11	Control	1.45	1.83	2.16	4.54	13.36	17.02
Significance		N.S.	N.S.	*	*	N.S.	N.S.
L.S.D.		/	/	0.58	1.67	/	/

Figure 3.1.1

Development of fleshy axis diameter in plants stressed for 48 hr at -10 bars (PEG) during early, middle and late stages of growth.

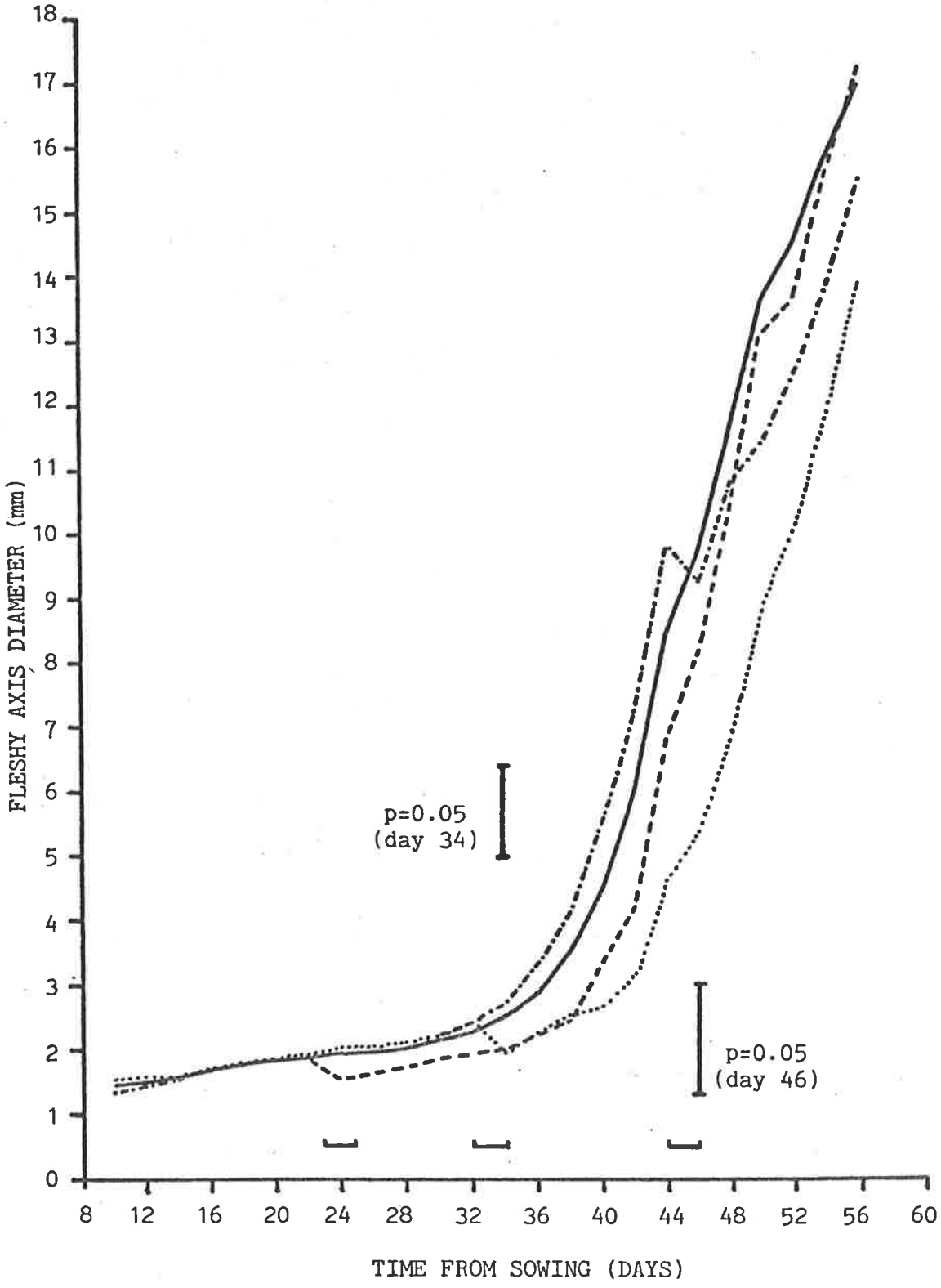
-------- Episode 2 (early)

..... Episode 5 (middle)

-.-.-.-. Episode 9 (late)

_____ Control

⌊⌋ Stress Period


FLESHY AXIS DIAMETER (mm)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
8
12
16
20
24
28
32
36
40
44
48
52
56
60
TIME FROM SOWING (DAYS)
p=0.05
(day 34)
p=0.05
(day 46)

level, nor at the prestress rate, until some time later. Thus a recovery period requirement is in evidence.

A consistent effect of stress in addition to the short-term fleshy axis contraction, was dessication-induced necrosis of the margins of the older leaves.

Discussion

Imposition of a brief period of water deficit resulting in leaf water potential falling to as low as -19 bars (60% RWC) at the end of 48 hrs did not cause a significant residual growth effect. No stage of Radish growth was more sensitive than any other to such an episode of stress.

During the period of stress both shoot and fleshy axis tissues 'wilted', an effect shown by decreased axis diameter. Although the stress imposed was sufficient to slightly reduce photosynthetic area due to marginal leaf necrosis, and, fleshy axis expansion appeared to be slowed, neither effect was significant when compared to the growth of control plants.

These results correspond well with the majority of water stress experiments conducted on root crops, where a simple response to prolonged adverse soil moisture conditions and only indirect relationships with any particular stage of growth are characteristic (Salter and Goode, 1967).

3.2 The Effect of Water, Salt and Cold Stress on Radish Growth

3.2.1 Introduction

The previous experiment demonstrated that a short severe water stress episode imposed at various times had no significant ultimate effects on growth. During the stress episode, however, marked transient responses were recorded. It was possible that short-term extreme stress could result in long-term growth reduction and an experiment was designed to compare the effects of several levels and durations of PEG-induced stress and several durations of salt (NaCl) and cold ($5^{o}C$) stress to see whether or not they had any long-term effects. Following the previous demonstration of a lack of differential sensitivity to stress at different plant ages, the present treatments were applied only once and at the same time.

3.2.2 Methods

The experiment was conducted in the growth cabinet under a 16 hr photoperiod. Pots were watered daily, except during stress episodes, with Hoagland's solution ($\frac{1}{2}$ strength).

Ten Radish seeds, cv. 'Mars' were sown in each pot (100 mm top diameter) in coarse river sand. The number of plants per pot was reduced to 5 on the 10^{th} day after sowing. Plants retained were those of medium size (on the basis of cotyledon area and height), with no apparent abnormality and were evenly spaced within the pot. These plants were further thinned to a final 3, 2 days later. At this time the plants retained were those with a hypocotyl diameter closest to the mean diameter of all plants.

Three levels of PEG (water) stress were imposed (-5, -10 and -15 bars), each for durations of 24, 48 and 72 hours. The salt (NaCl, -10 bars) and cold ($5^{o}C$) stresses were also imposed for similar durations. A control set of plants was provided for each stress duration, and the

experiment was replicated 3 times. The plants were stressed during the rapid phase of growth, commencing 21 days after sowing. Three additional pots in the -5 and -10 bar PEG and -10 bar NaCl treatments were grown to determine plant water status.

In the PEG and NaCl treatments stress was initiated by watering the pots with 200 ml of the appropriate solution. Stress was terminated by watering the pots with 300 ml of nutrient solution.

Maximum fleshy axis diameter was recorded every second day for each plant, commencing on day 12 and continuing until harvest (day 30). Diameter was recorded daily during the episode of stress as well as 4 hr after stress imposition and 4 hr after stress alleviation. Leaf water potential was determined with the Spanner psychrometer on the last fully expanded leaf of each plant in the additional pots just prior to stress alleviation.

The data were subjected to statistical analysis as a factorial design. For each harvest parameter treatment means were then grouped according to whether they fell into the first, second or third group of means between the smallest (most reduced by stress) and largest mean recorded for that character. According to the frequency with which a treatment caused measured attributes to fall into each group, that treatment was assessed as either: (i) lethal, (ii) severe stress, (iii) mild stress or (iv) slight stress.

3.2.3 Results

Leaf water potential fell during stress in the three treatments monitored (table 3.2.1). The reduction was greatest in the -10 bar PEG treatment and least in the -5 bar PEG. Salt stress resulted in the leaf water potential falling to a level intermediate between those of the two water stress treatments.

TABLE 3.2.1

Leaf water potential of plants subjected to stress induced by PEG or NaCl.

Duration of Stress Episode (hr)	Treatment (osmotic potential of solution [Ψs], bars)		
	-5 PEG	-10 NaCl	-10 PEG
	(leaf water potential, bars)		
0	- 4.8	- 5.6	- 4.3
24	- 7.2	-10.1	-12.1
48	- 9.5	-11.3	-21.2
72	-16.1	-17.0	-29.7

In contrast to the previous experiment (section 3.1) all the plant growth attributes measured demonstrated significant persistent effects of stress. At the time of harvest, all the surviving fleshy axes and shoots were found to have the same water content (92.6 and 90.7% respectively) and, it can be concluded that all stressed tissue had returned to a similar degree of hydration.

Using the procedure outlined above, treatments were ranked into categories based on their relative effects on all growth attributes (table 3.2.2). Cold stress had no deleterious effect on growth, nor did the -5 bar water and the -10 bar salt stress treatments imposed for 24 hours only. In contrast, the -15 bar PEG treatments for 48 or 72 hours resulted in plant death. A degree of interaction between the intensity and duration of water stress treatments was noticable.

Neither salt nor cold stress reduced leaf area, in marked contrast to the considerable reductions caused by PEG induced water stress (figure 3.2.1). The severity of the effects of PEG were governed by the concentration of the PEG solution rather than the duration of stress as all durations of -15 bar stress caused leaf area reductions greater than those caused by -10 bars. This reduction in leaf area with stress was due almost entirely to leaf death, as the short period between stress imposition and harvest following stress alleviation (circa 7 days) allowed little time for leaf expansion and initiation provided the plant survived stress. Severe marginal leaf necrosis was common. The effect of stress on shoot fresh weight (figure 3.2.2) was closely related to this reduction in leaf area and shoot dry weight followed fresh weight.

The effects of the various stress treatments on fleshy axis fresh and dry weights (figure 3.2.3) were similar but not identical to the effects on shoot fresh weight already described. For example, -15 bar imposed for 48 hours or even 72 hours did not cause the virtual total loss of tissue which was evident in the shoot. With a less severe

TABLE 3.2.2

Classification of stress treatments as to their severity based on fleshy axis diameters recorded at 2 day intervals after the stress episode and on final harvest growth data.

Classification	Treatments				
	-5 bar PEG	-10 bar PEG	-15 bar PEG	-10 bar NaCl	5^{O}C Cold
	duration (hrs)				
Killing stress	/	/	48 & 72	/	/
Severe stress	72	48 & 72	24	/	/
Mild stress	48	24	/	72	/
Slight stress	/	/	/	48	/
No Effect	24	/	/	24	24, 48 & 72

FIGURE 3.2.1

The effect of three durations (24, 48 and 72 hr) of salt- and cold- or three levels of water-stress on Radish leaf area recorded 9 days after stress commencement.

———— Controls (1, 2 & 3)

--------- -5 bar PEG

-.-.-.-.- -10 bar PEG

..... -15 bar PEG

-..-..-..- -10 bar NaCl

--.--.--. 5^{o}C Cold

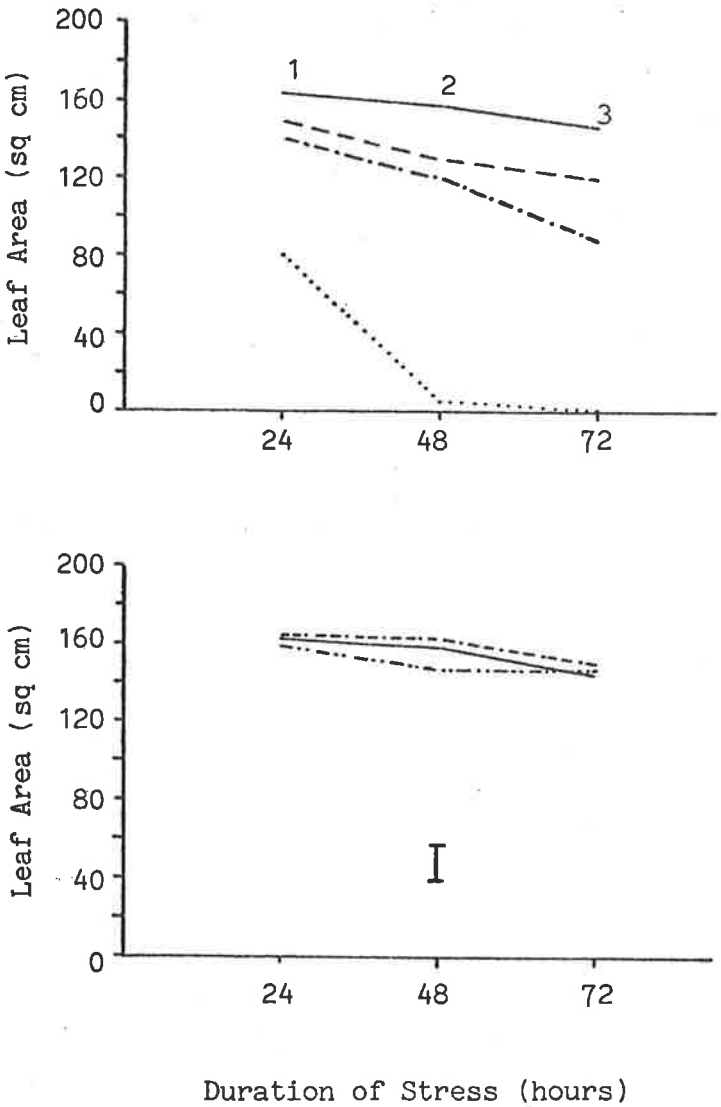

200
160
120
80
40
0
Leaf Area (sq cm)
1
2
3
24
48
72
200
160
120
80
40
0
Leaf Area (sq cm)
I
24
48
72
Duration of Stress (hours)

FIGURE 3.2.2

The effect of three durations (24, 48 and 72 hr) of salt- and cold- or three levels of water-stress on Radish shoot fresh weight recorded 9 days after stress commencement.

———— Controls (1, 2 & 3)

--------- -5 bar PEG

-.-.-.-.- -10 bar PEG

..... -15 bar PEG

-..-..-.. -10 bar NaCl

--.--.--. 5^{o}C Cold

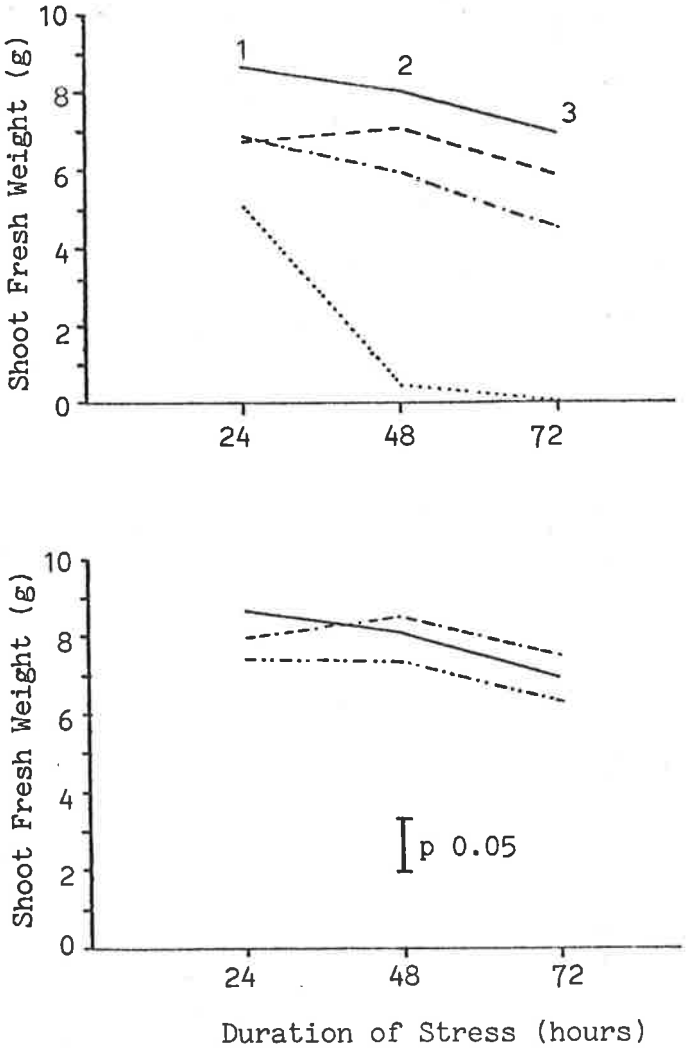

Shoot Fresh Weight (g)
10
8
6
4
2
0
1
2
3
24
48
72
Shoot Fresh Weight (g)
10
8
6
4
2
0
p 0.05
24
48
72
Duration of Stress (hours)

FIGURE 3.2.3

The effect of three durations (24, 48 and 72 hr) of salt- and cold- or three levels of water-stress on Radish fleshy axis fresh weight recorded 9 days after stress commencement.

———— Controls (1, 2 & 3)

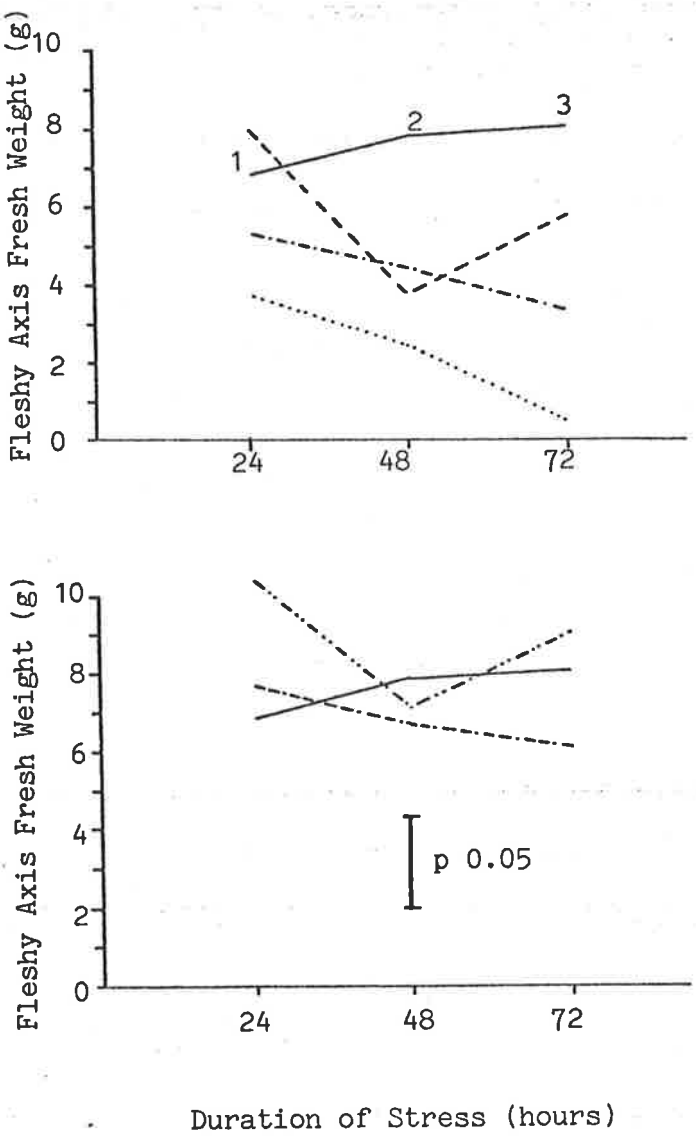
– – – – – – – – -5 bar PEG

-.-.-.-.- -10 bar PEG

. . . . . -15 bar PEG

--.--.--. -10 bar NaCl

-..-..-.. 5^{o}C Cold


Fleshy Axis Fresh Weight (g)
10
8
6
4
2
0
1
2
3
24
48
72
Fleshy Axis Fresh Weight (g)
10
8
6
4
2
0
p 0.05
24
48
72
Duration of Stress (hours)

stress however (-10 bar for 48 hr) the reduction in fleshy axis fresh weight (circa 40%) was greater than the loss in shoot weight (25%). These responses related to the symptoms stress caused in the fleshy axis, with mild stress having no permanent effects, severe stresses (such as -10 bars for 48 hr) causing shrunken, convoluted fleshy axes, and, lethal stresses causing a slow breakdown of the fleshy axis and a rapid breakdown of shoot tissues. Interestingly, despite the apparent death of plants subjected to 48 hours of -15 bar PEG, by harvest some developed axillary shoot buds which were indicative of possible regeneration.

In figure 3.2.4 the effects of stress on the whole plant fresh weight are presented, and, integrate these effects on shoot and fleshy axis tissues. Exposure to -10 bar NaCl for up to 3 days caused no yield reduction. This suggests that the osmotic potential of the NaCl solution was balanced by osmotic adjustment in the plant, possibly through the uptake of Na^+ and Cl^- ions. This suggestion is supported by the observation that plants which wilted upon the imposition of the salt stress had at least partially regained turgor after 24 hours. They subsequently wilted once more if the stress was prolonged, possibly due to the combined effect of the salt and depletion of the water content of the medium. This type of response to salt stress was also reflected in fleshy axis diameter. The fleshy axis lost diameter rapidly during the first 4 hours of stress, but thereafter some recovery towards the prestress diameter was apparent during at least part of the remainder of the various stress episodes (figure 3.2.5).

In comparison with the minor reduction in fleshy axis diameter which resulted from -10 bar salt, -5 bar PEG stress (figure 3.2.6) had a larger effect, and -10 (figure 3.2.7) and -15 bar PEG (figure 3.2.8) produced marked shrinkage. All of these effects were more pronounced as the stress was prolonged. Axis diameter fell rapidly upon stress

FIGURE 3.2.4

The effects of three durations (24, 48 and 72 hr) of salt- and cold- or three levels of water-stress on the combined Radish shoot and fleshy axis fresh weights recorded 9 days after stress commencement.

———— Controls (1, 2 & 3)

– – – – – – – – – -5 bar PEG

-.-.-.-.-.- -10 bar PEG

. . . . . -15 bar PEG

--.--.--. -10 bar NaCl

-..-..-.. 5°C Cold

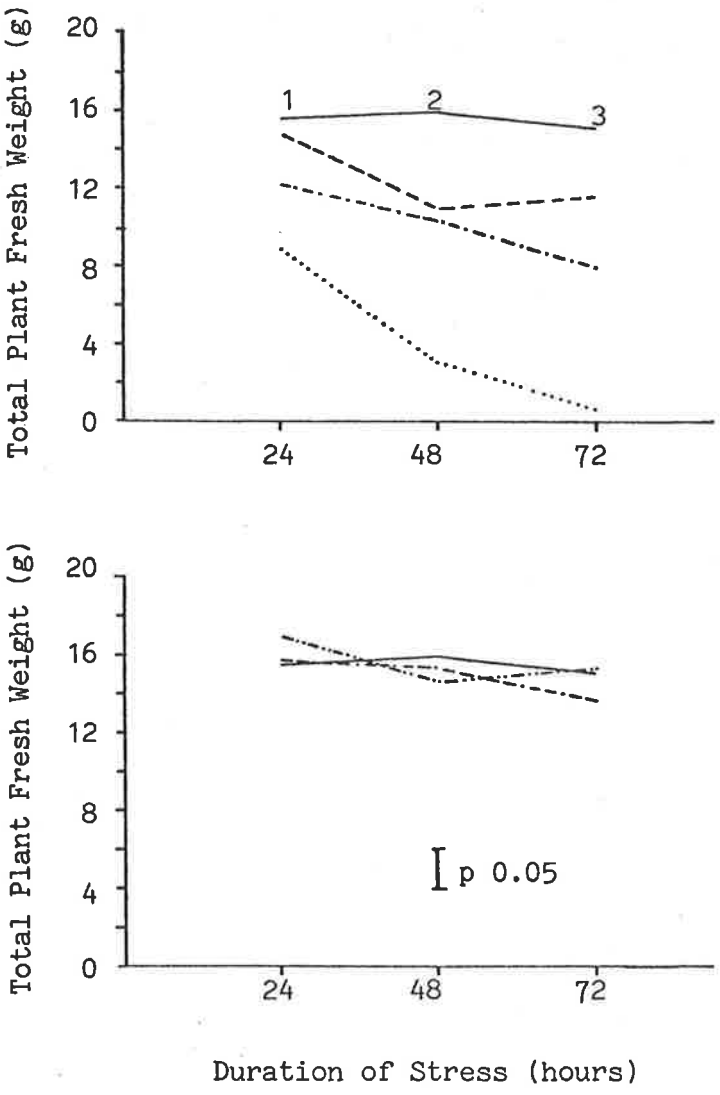

Total Plant Fresh Weight (g)
20
16
12
8
4
0
1
2
3
24
48
72
Total Plant Fresh Weight (g)
20
16
12
8
4
0
p 0.05
24
48
72
Duration of Stress (hours)

FIGURE 3.2.5

The effect of 24, 48 and 72 hours of -10 bar NaCl induced salt stress on Radish fleshy axis diameter growth.

———— 24 hour stress (a)

– – – – – – – – 48 hour stress (b)

· · · · · · · · 72 hour stress (c)

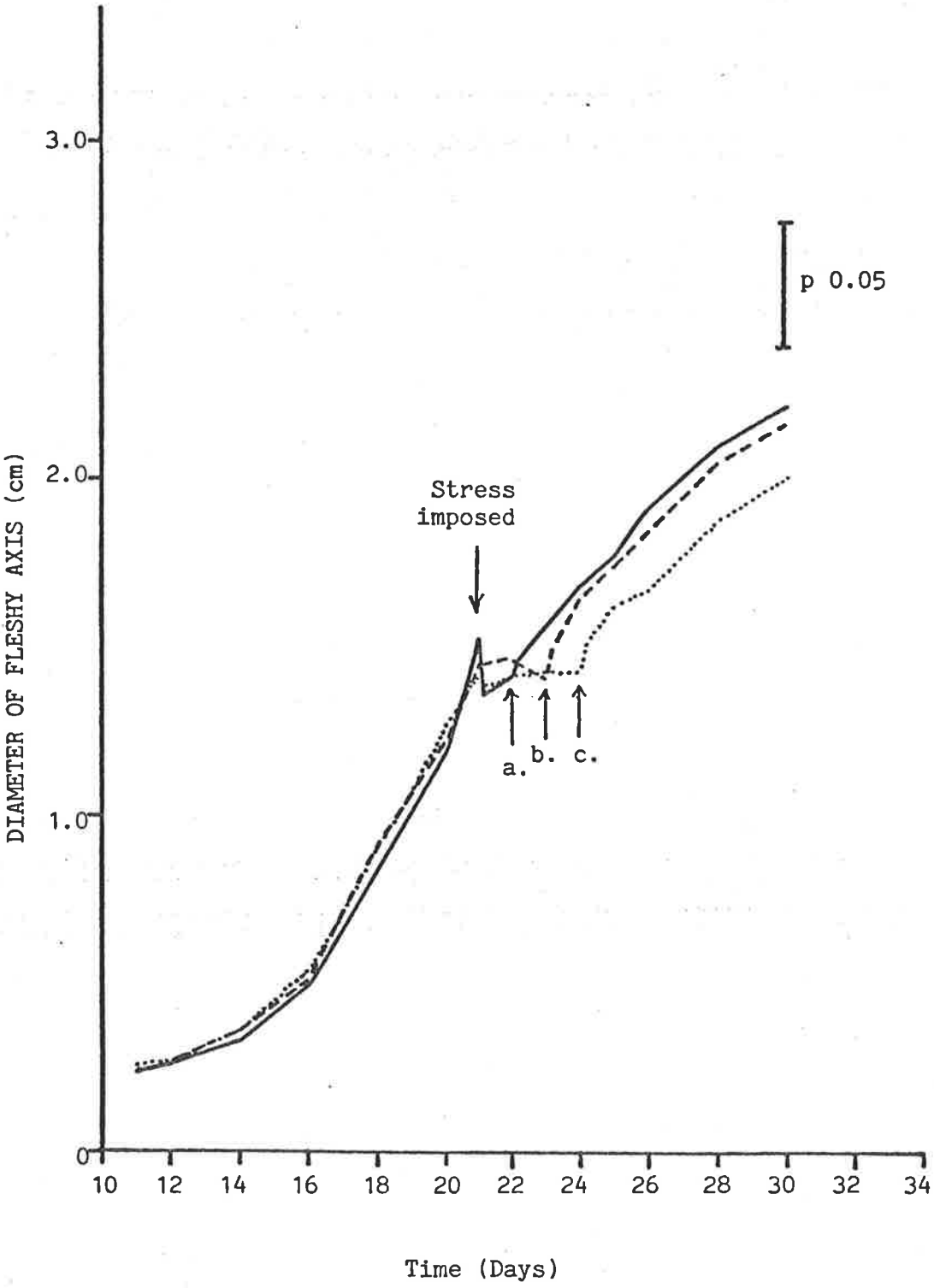

3.0
2.0
1.0
0
DIAMETER OF FLESHY AXIS (cm)
p 0.05
Stress
imposed
a.
b.
c.
10
12
14
16
18
20
22
24
26
28
30
32
34
Time (Days)

FIGURE 3.2.6

The effect of 24, 48 and 72 hours of -5 bar PEG induced water stress on Radish fleshy axis diameter growth.

_____ 24 hour stress (1)

- - - - - - - - 48 hour stress (2)

. . . . . 72 hour stress (3)

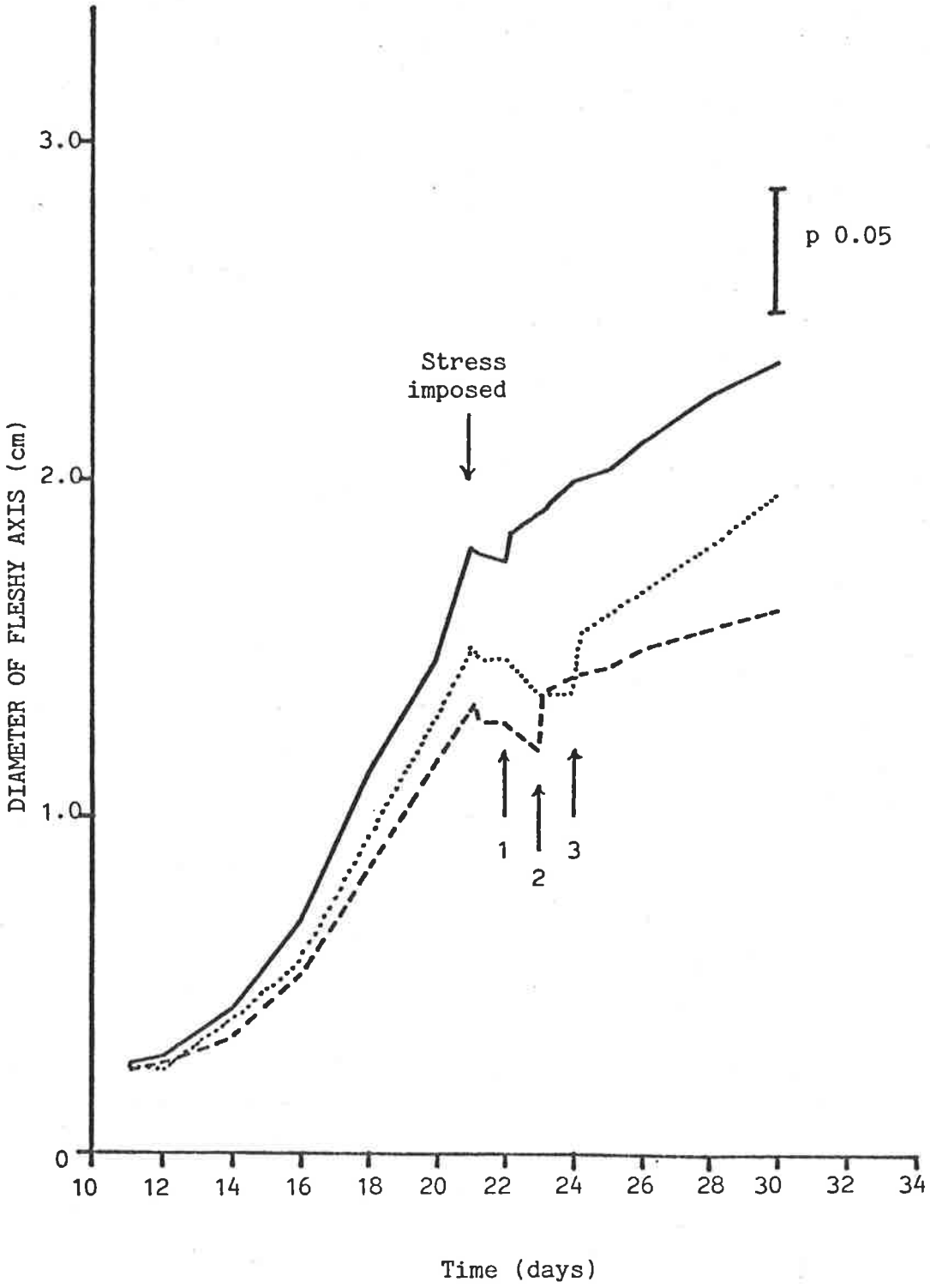

3.0
2.0
1.0
0
DIAMETER OF FLESHY AXIS (cm)
p 0.05
Stress
imposed
1
2
3
10
12
14
16
18
20
22
24
26
28
30
32
34
Time (days)

FIGURE 3.2.7

The effect of 24, 48 and 72 hours of -10 bar PEG induced water stress on Radish fleshy axis diameter growth.

———— 24 hour stress (a)

– – – – – – – – 48 hour stress (b)

..... 72 hour stress (c)

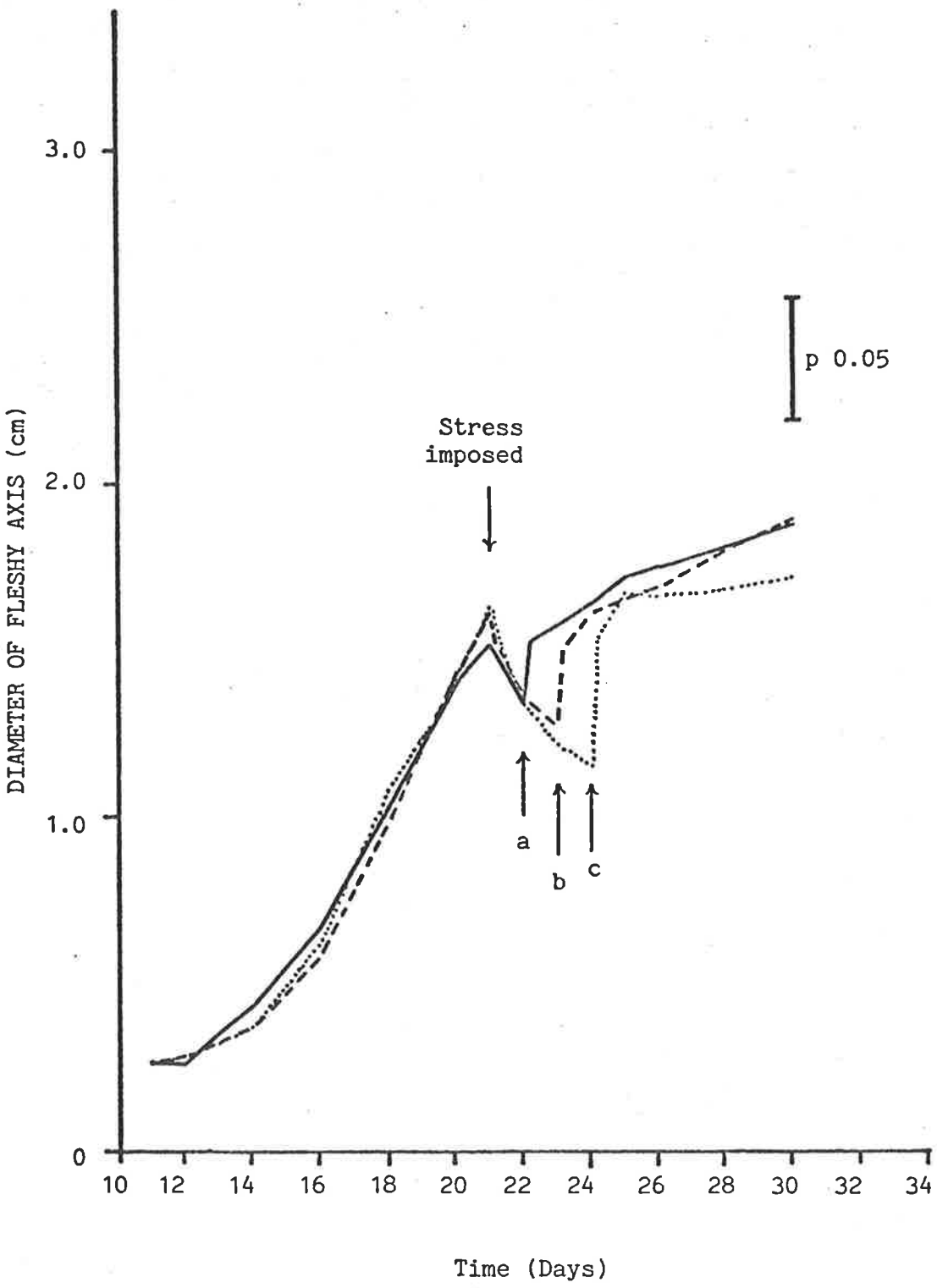

DIAMETER OF FLESHY AXIS (cm)
3.0
2.0
1.0
0
p 0.05
Stress
imposed
a
b
c
10
12
14
16
18
20
22
24
26
28
30
32
34
Time (Days)

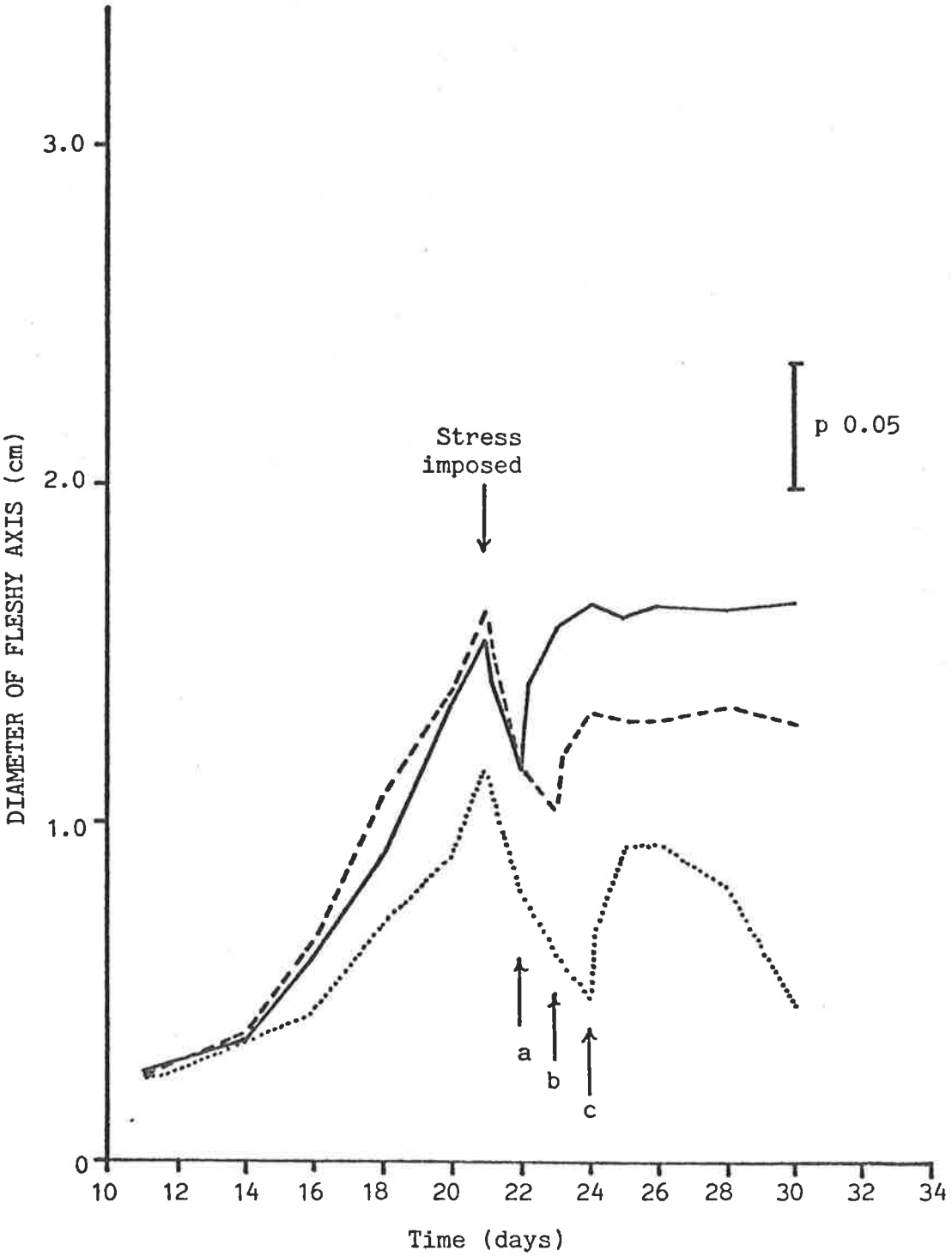
FIGURE 3.2.8

The effect of 24, 48 and 72 hours of -15 bar PEG induced water stress on Radish fleshy axis diameter growth.

———— 24 hour stress (a)

-------- 48 hour stress (b)

..... 72 hour stress (c)


DIAMETER OF FLESHY AXIS (cm)
3.0
2.0
1.0
0
Stress
imposed
p 0.05
a
b
c
10
12
14
16
18
20
22
24
26
28
30
32
34
Time (days)

imposition but then continued to fall during the period of stress. This continuous reduction would have been due to both slow equilibration with the external solution and continued depletion of the water in the rooting medium, and consequent concentration of the PEG solution. There was a decreasing capacity for the plants to expand the fleshy axis upon stress alleviation as the severity of the stress increased. However, in all cases, there was a phase of rapid axis re-expansion during the 4 hours immediately subsequent to rewatering. Where stress had been both severe and prolonged, however, this early expansion upon rewatering was followed by contraction and ultimate death.

In contrast to these major effects of water stress, plants subjected to cold stress were little affected. The plants did not wilt, but comparison between the control (figure 3.2.9) and cold stress (figure 3.2.10) axis diameter curves reveals that cold stress had considerable effects on fleshy axis growth. The axis virtually ceased expansion during exposure to low temperature and, although recommencing soon after the temperature increased, did not rapidly increase to compensate, in the short term for lost girth.

The 16 hour photoperiod to which plants were exposed in this experiment was not a good choice. Bolting occurred frequently and probably contributed to the variability of the plant responses. Radish is a long day plant (Leopold and Kriedemann, 1975) and most plants had elongated internodes by day 30 (provided they survived stress) although the extent of elongation was variable.

3.2.4 Discussion

All levels and durations of water stress caused significant reductions in the final yield of Radish. The significant yield reduction caused by -10 bars PEG for 48 hours, in comparison to an insignificant effect in the previous experiment (section 3.1) was

FIGURE 3.2.9

Fleshy axis diameter growth of Radish control treatments grown to accompany water, cold and salt stress treatments.

———— 1. (24 hour stress control)

-------- 2. (48 hour stress control)

..... 3. (72 hour stress control)

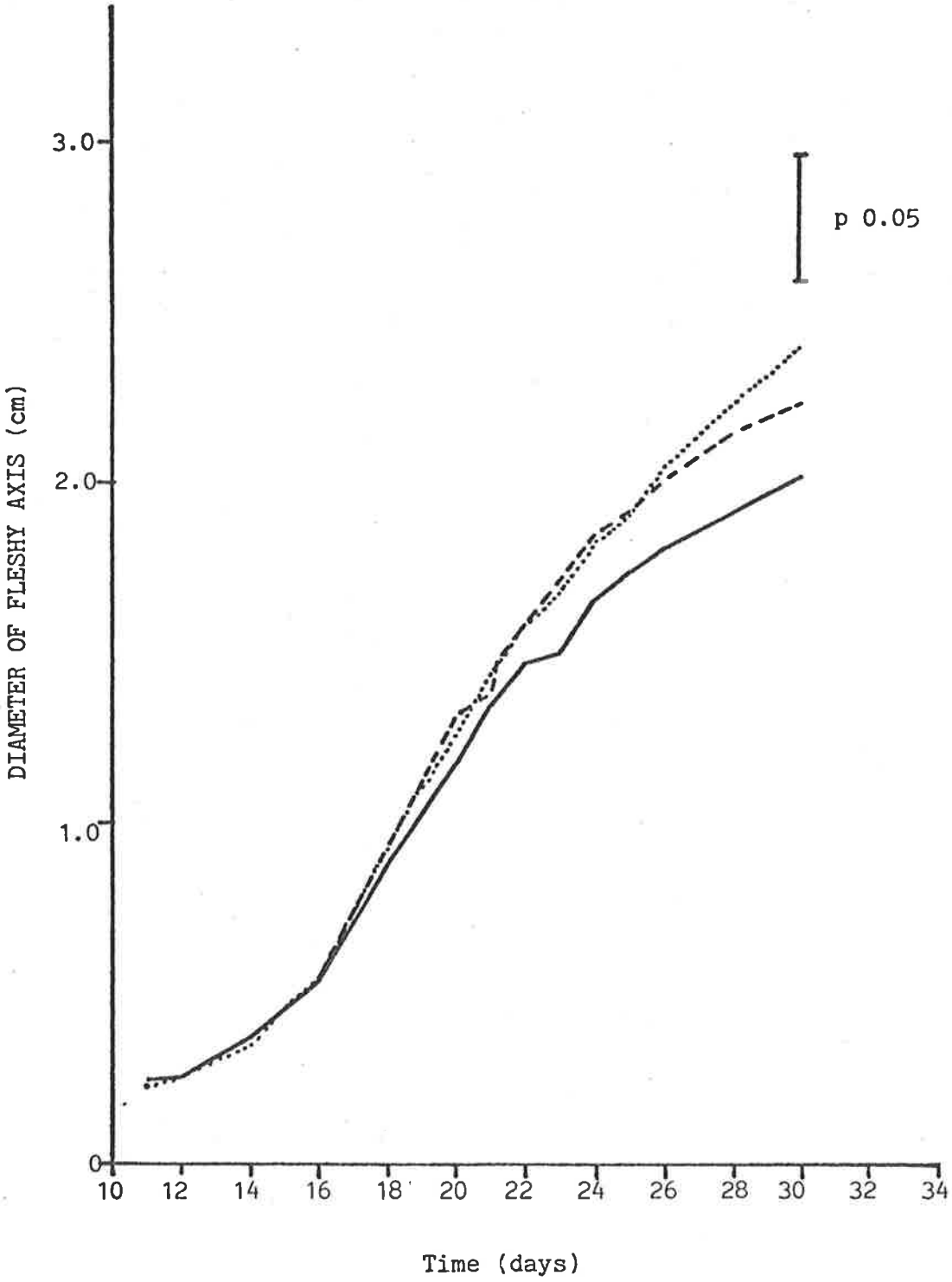

3.0
2.0
1.0
0
DIAMETER OF FLESHY AXIS (cm)
p 0.05
10
12
14
16
18
20
22
24
26
28
30
32
34
Time (days)

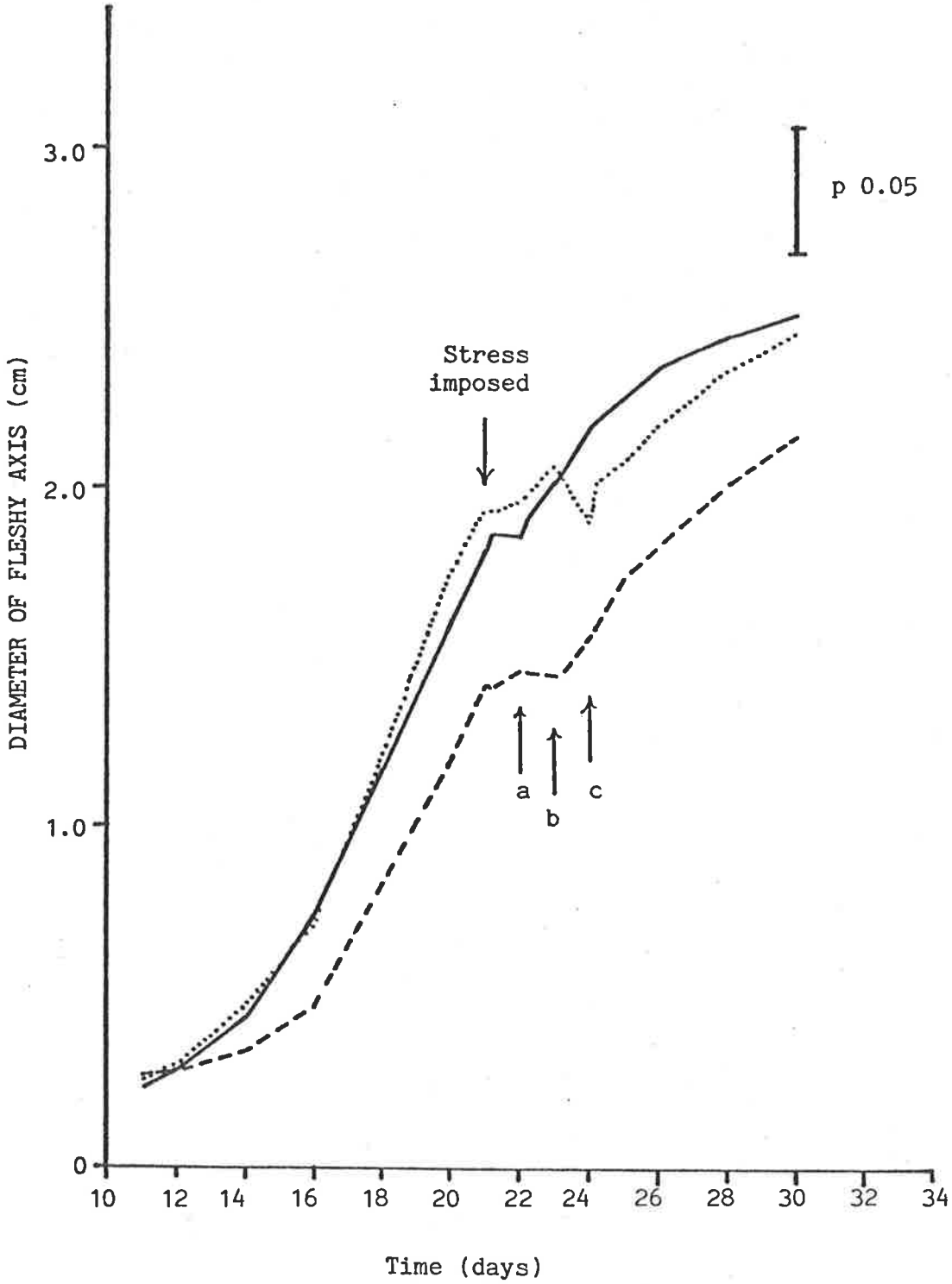
FIGURE 3.2.10

The effect of 24, 48 and 72 hours of cold stress (5°C) on Radish fleshy axis diameter growth.

———— 24 hours stress (a)

-------- 48 hours stress (b)

..... 72 hours stress (c)


3.0
2.0
1.0
0
DIAMETER OF FLESHY AXIS (cm)
p 0.05
Stress
imposed
a
b
c
10
12
14
16
18
20
22
24
26
28
30
32
34
Time (days)

possibly due to a higher evaporative demand of the growth cabinet environment; mean daily relative humidity of 50% as against a mean of 75% in the glasshouse. Leaf water potential at the 48th hour of stress was, however, similar in both experiments, viz. -19 and -21 bars respectively. None-the-less lower relative humidity may have resulted in more rapid reduction, and less rapid recovery, in leaf water potential and consequently reduced overall plant water status. This sensitivity of growth to evaporative demand has been emphasised in other crops. In well watered Kikuyu, for instance, growth is highly responsive to the alteration of evaporative demand and is considerably enhanced during cloudy periods when evapotranspiration is reduced (Murtagh, 1978). The possibility of other unidentified variables, however, cannot be excluded as causing this conflict in results. For example some factor related to growth rates may increase the susceptibility of plants to stress damage. In this comparison of experiments, the significant effect was associated with a substantially faster growth rate (figures 3.1.1 and 3.2.7), and certainly the relatively greater growth rate and the higher evaporative demand existing in this experiment would imply that the plants having yield significantly reduced by -10 bar PEG for 48 hours were exerting a greater demand on the substrate moisture supply.

The relative effects of the different types of stress imposed in this experiment have also been investigated in another Radish cultivar 'White Icicle', with similar stress levels and methods of imposition (Chu, 1974). All stress types were shown to completely inhibit shoot elongation but plant fresh and dry weight increases differed between various stresses. It was concluded that fresh weight and plant height were more sensitive to stress than was growth in dry weight, and that water stress produced a more profound effect than either salt or cold stress. Data showing osmotic adjustment during water stress, occurring

after a transient leaf water deficit, was also presented. Low temperature had no effect on plant water status. These findings, although obtained on physiologically younger plants, support the conclusions drawn from this present experiment.

It is apparent from the data on fleshy axis diameter that salt and cold stress caused far less reduction than the PEG treatments. The magnitude of fleshy axis contraction increased primarily with PEG concentration and to a proportionally lesser extent with duration of stress (figures 3.2.6, 3.2.7 and 3.2.8). Similarly, the extent of recovery during the initial rapid phase was decreased as the level and duration of stress were increased. Comparison of the pattern of diameter loss for the -10 bar PEG for 72 hours stress (figure 3.2.7) with the pattern of decline in leaf water potential (figure 3.2.11) obtained in an almost identical experiment (Chu, 1974) indicates that the reduced water status of shoot tissue is mirrored in the response of the fleshy axis. Work on Cotton and Red Pine has shown that changes in stem diameter during water stress follow changing leaf water potential closely (So, 1979), and supports this hypothesis. Further, it can be proposed that the initial rapid phase of recovery corresponds to the recovery of shoot water potential. As leaf area (figure 3.2.1) is increasingly reduced by more severe stress episodes, so too is the ability of the rapid recovery phase to result in the return of fleshy axis diameter to near the prestress level. The second, slow, phase of recovery probably relates to the degree of ultrastructural damage incurred as the severity of stress was increased, and possibly accounts for marked differences in the degree of response between the severe water stress treatments and the -5 bar water stress, the salt, and the cold treatments. A study on Sunflower leaf mesophyll cells during desiccation has shown that tonoplast and plasmalemma disruption commence at -15 bar leaf water potential (47% RWC) and continues to completion at -26 bars (28% RWC or less); tissue was

FIGURE 3.2.11

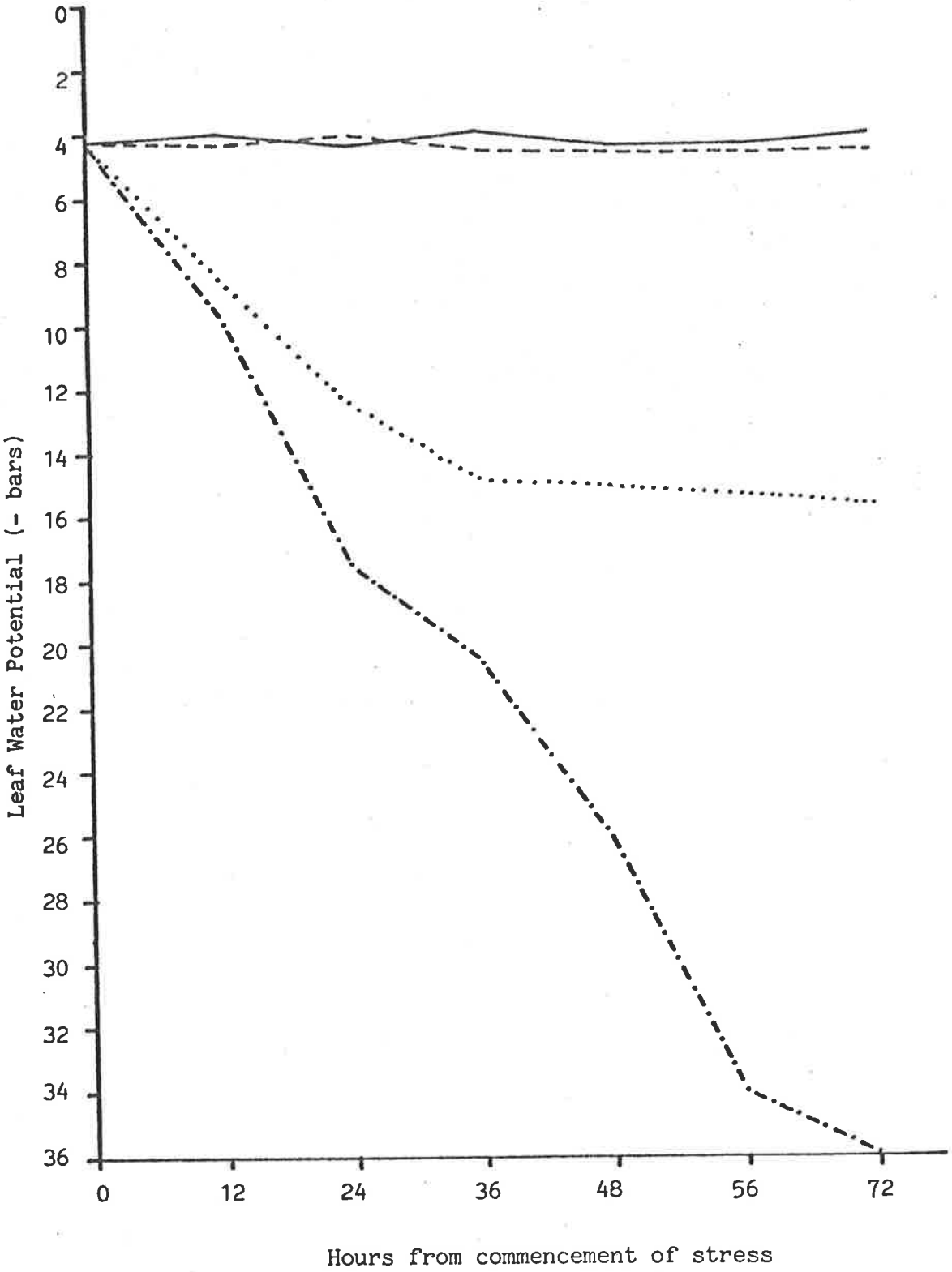
The water potential of the first leaf of Radish, cv. White Icicle, subjected to different environmental stresses (from; Chu, 1974).

———— Control

– – – – – – – – $5^{o}C$ stress

. . . . . -10 bar NaCl stress

-.-.-.-.- -10 bar PEG stress


Leaf Water Potential (- bars)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
0
12
24
36
48
56
72
Hours from commencement of stress

incapable of full rehydration at a water potential below -20 bars (Fellows and Boyer, 1978). This is relevent to both fleshy axis response and the reduction in leaf area, particularly the phenomenon of leaf margin necrosis. Leaf margin necrosis occurs on recently expanded healthy green leaves as well as on older mature leaves, which suggests that a gradient of leaf water potential decreasing towards the margin is generated during stress. Tissue death from desiccation then occurs at the margins independent of any overall senescence. A similar response has been reported as a consequence of atmospheric drought (Maximov, 1929).

3.3 Continuous Monitoring of Radish Fleshy Axis Diameter during Episodes of Water Stress

3.3.1 Introduction

As reported in the previous experiment (section 3.2) marked fluctuations in fleshy axis diameter occurred during and following episodes of water stress. The general response was a loss of diameter during stress followed by a rapid return towards the previous diameter on stress alleviation.

In order to obtain detailed information on the shrinkage and recovery process an experiment was designed to continuously monitor fleshy axis diameter during stress imposition and alleviation. So as to confine the study to realistic situations only a relatively mild and a relatively severe stress were imposed (ref. section 3.2).

3.3.2 Methods

Plants were grown in controlled environment under a 12 hr photoperiod. Ten Radish seeds, cv. 'Mars', were sown into each pot (top diameter 160 mm). A coarse sand rooting medium was used and pots watered daily with Hoagland's solution ($\frac{1}{2}$ strength). By day 12 the pots had been thinned (section 3.2) to 3 plants.

The experiment consisted of 3 treatments, control (no water stress), -5 bar PEG (mild stress) and -10 bar PEG (severe stress) each for 48 hr and was replicated 4 times. Stress was imposed by applying 600 ml of the appropriate solution to each pot and was alleviated by washing out the solution with 900 ml of nutrient solution. Unlike the previous experiments, the pots were supplied with a further 600 ml of the appropriate solution 24 hr after the initial application in an attempt to prevent undue concentration of the PEG solution following water loss through transpiration. Only one replication of each treatment was continuously monitored throughout stress. Single plants from each pot

were chosen with fleshy axis diameters closest to the mean diameter of all plants (3 cm).

Stress episode of the remaining 3 replications was commenced at 9.30 am on day 33 post sowing and maximum fleshy axis diameters were recorded at 9.30 am, 12.30 pm, 4.30 pm and 9.30 pm both during stress and on the day of stress alleviation. As only one transducer was available for continuous diameter monitoring the remaining replications of each of the three treatments were monitored over three separate intervals. The control was monitored on days 23 to 26, the mild stress on days 29 to 32 and the severe stress on days 26 to 29. Monitoring continued from 10.00 am on the first day up to 10.00 am the day following stress alleviation.

The transducer was extremely sensitive to plant movement and for this reason could not be used in the growth cabinet. The measurements were made in a room with restricted air movement but with the environment controlled to give similar conditions to those in the growth cabinet.

Relative water content was measured 48 hr after stress imposition on one plant from each of the pots remaining in the growth cabinet. Fleshy axis maximum diameter was recorded every second day during the growth of all plants in these 3 replications, commencing 10 days after sowing. All plants were harvested 10 days after stress alleviation i.e. day 45.

3.3.3 Results

Leaf relative water content fell during the period of stress, mild and severe stress resulted in 63.5% and 44.0% RWC respectively as against 86.6% RWC in control plants. Severe desiccation induced marginal leaf necrosis in the severely stressed plants (figure 3.3.1).

Maximum fleshy axis diameter, as in the previous experiment (section 3.2), was markedly reduced during stress (figure 3.3.2). The reduction in diameter was greatest under the severe stress episode, whilst

FIGURE 3.3.1

Comparison of control and plants water stressed for 48 hours with -5 bar PEG (centre) and -10 bar PEG (RHS).


IV.
2.
IV.
3.

FIGURE 3.3.2

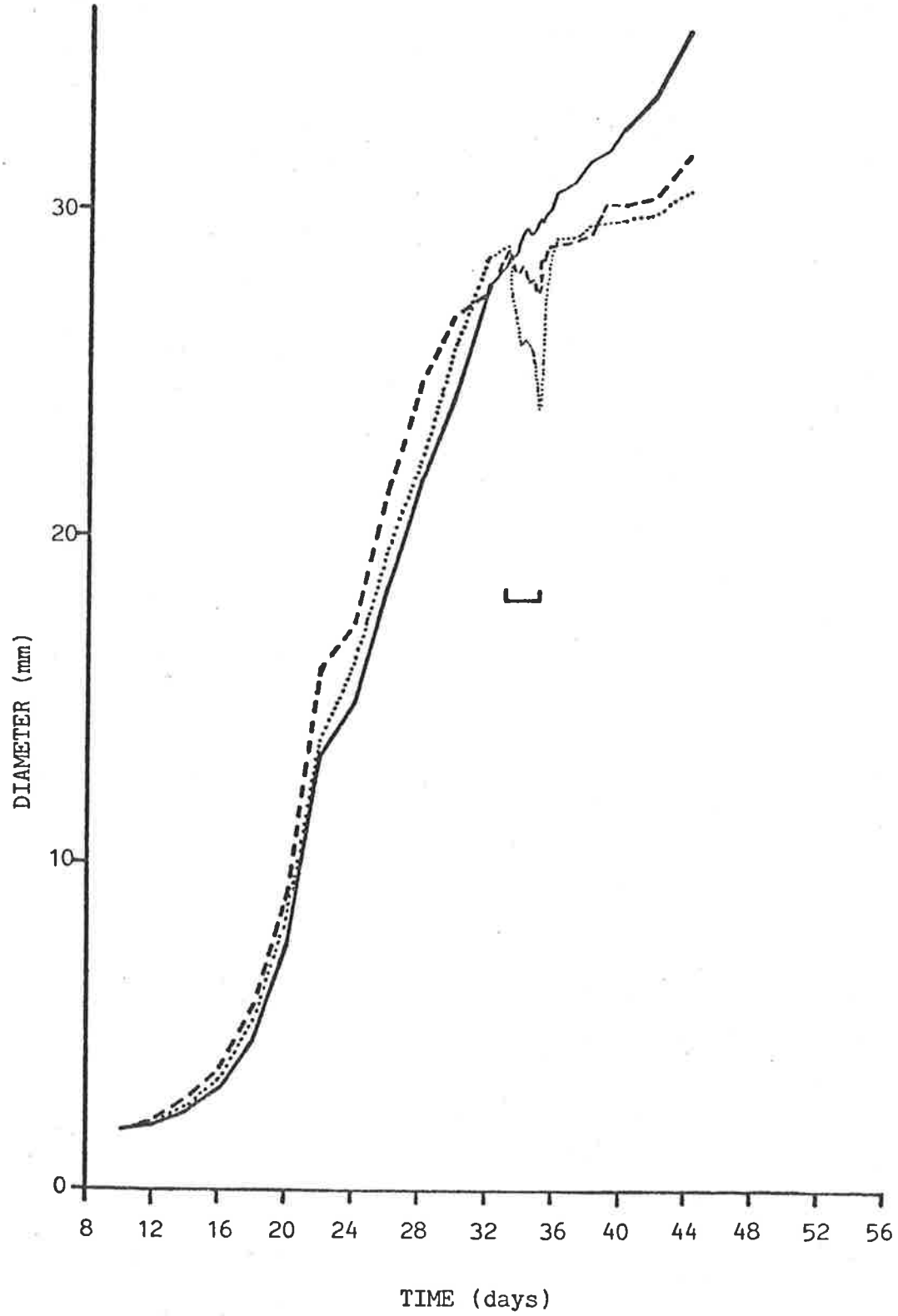
The effect of 48 hour episodes of -5 and -10 bar PEG-induced water stress on Radish fleshy axis diameter growth.

———— Control

– – – – – – – – -5 bar PEG

..... -10 bar PEG

└──┘ Stress period


30
20
10
0
DIAMETER (mm)
8
12
16
20
24
28
32
36
40
44
48
52
56
TIME (days)

control plants continued to grow and maintained this difference in diameter to the final harvest. Again, the fleshy axis diameter of stressed plants increased rapidly when stress was alleviated but thereafter mildly stressed plants continued to expand at a greater rate than those severely stressed. However, neither treatment attained the rate of expansion of control plants within the period of the experiment. When the second application of PEG was made after 24 hours stress the fleshy axis diameters increased briefly but thereafter continued to decrease (figures 3.3.2 and 3.3.3). Axis diameter was reduced at the end of the 48 hour period 4% by mild stress and 18% by severe stress over the immediate prestress diameter.

Similar responses were found with the single plants monitored continuously (figure 3.3.4), both the loss in diameter with the imposition of stress and the expansion which occurs upon immediate recovery follow a uniphasic pattern, with diameter asymptotically approaching a lower limit as stress continued. Following application of PEG, the rate of decrease in diameter (figure 3.3.5) was greater and continued for longer in the severely stressed plant. The diameter of the mildly stressed plants reached a lower limit 12 hours after stress was imposed and thereafter changed but little, whereas severely stressed plants continued to decrease in diameter throughout the 48 hours of stress. This loss in diameter was unaffected by the diurnal light cycle also, in contrast to the earlier data, the second application of PEG had little or no effect on diameter (figure 3.3.4). The immediate expansion upon re-watering was very rapid, being largely complete within the first hour following stress alleviation. The rate of expansion then fell to a rate almost indistinguishable from that in the control plants.

The data gathered at the final harvest (table 3.3.1) demonstrate that neither episode of water deficit had lasting effects on shoot fresh weight, shoot dry weight, fleshy axis dry weight or fleshy axis density

FIGURE 3.3.3

The effect of 48 hour episodes of -5 and -10 bar PEG-induced water stress, and subsequent alleviation, on Radish fleshy axis diameter.

———— Control

– – – – – – – – -5 bar PEG

. . . . . -10 bar PEG

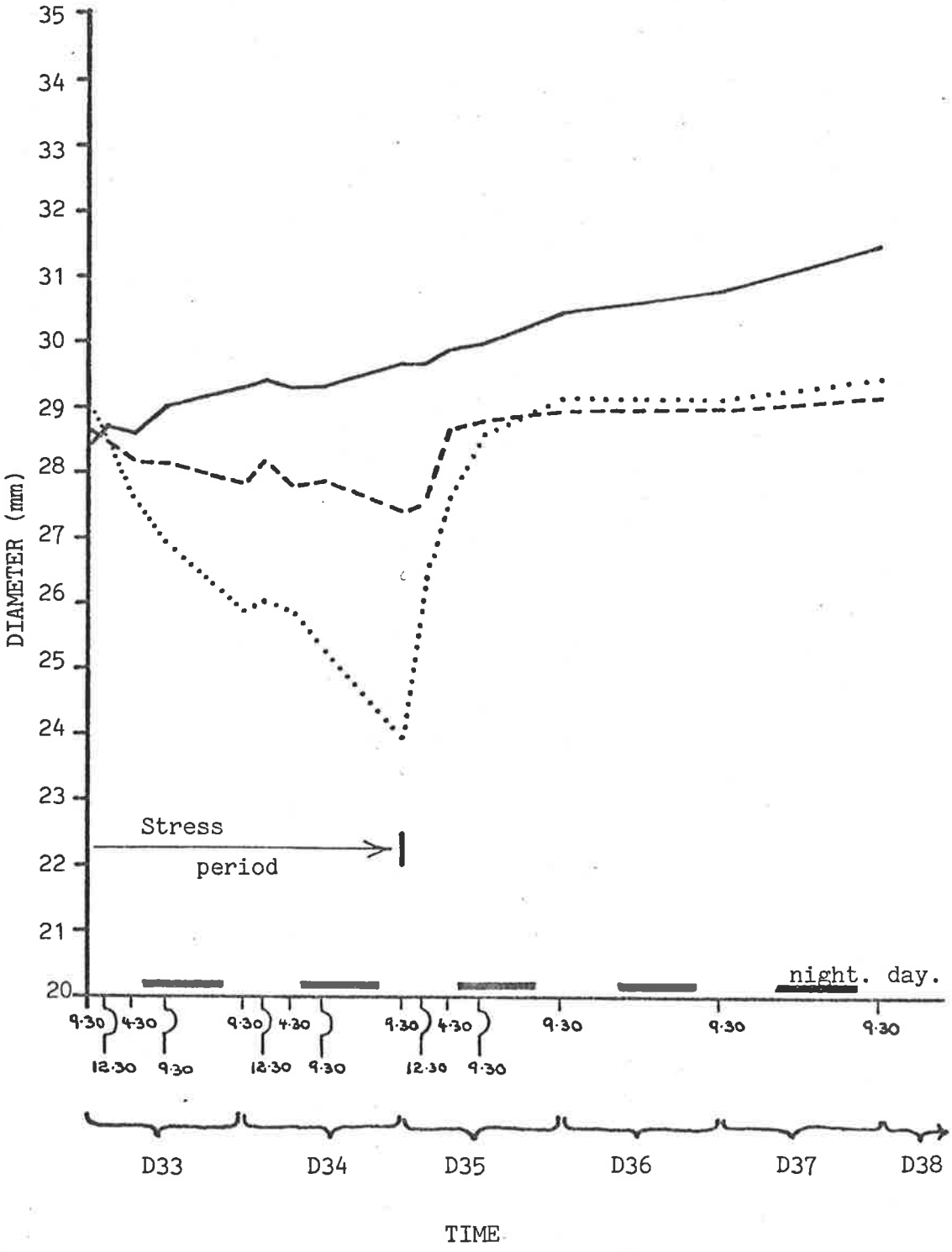

35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
DIAMETER (mm)
Stress
period
night. day.
9·30
4·30
12·30
9·30
D33
D34
D35
D36
D37
D38
TIME

FIGURE 3.3.4

Cumulative change in Radish fleshy axis diameter monitored continuously during, and immediately after, 48 hour episodes of -5 and -10 bar PEG-induced water stress.

———— Control

-------- -5 bar PEG

..... -10 bar PEG

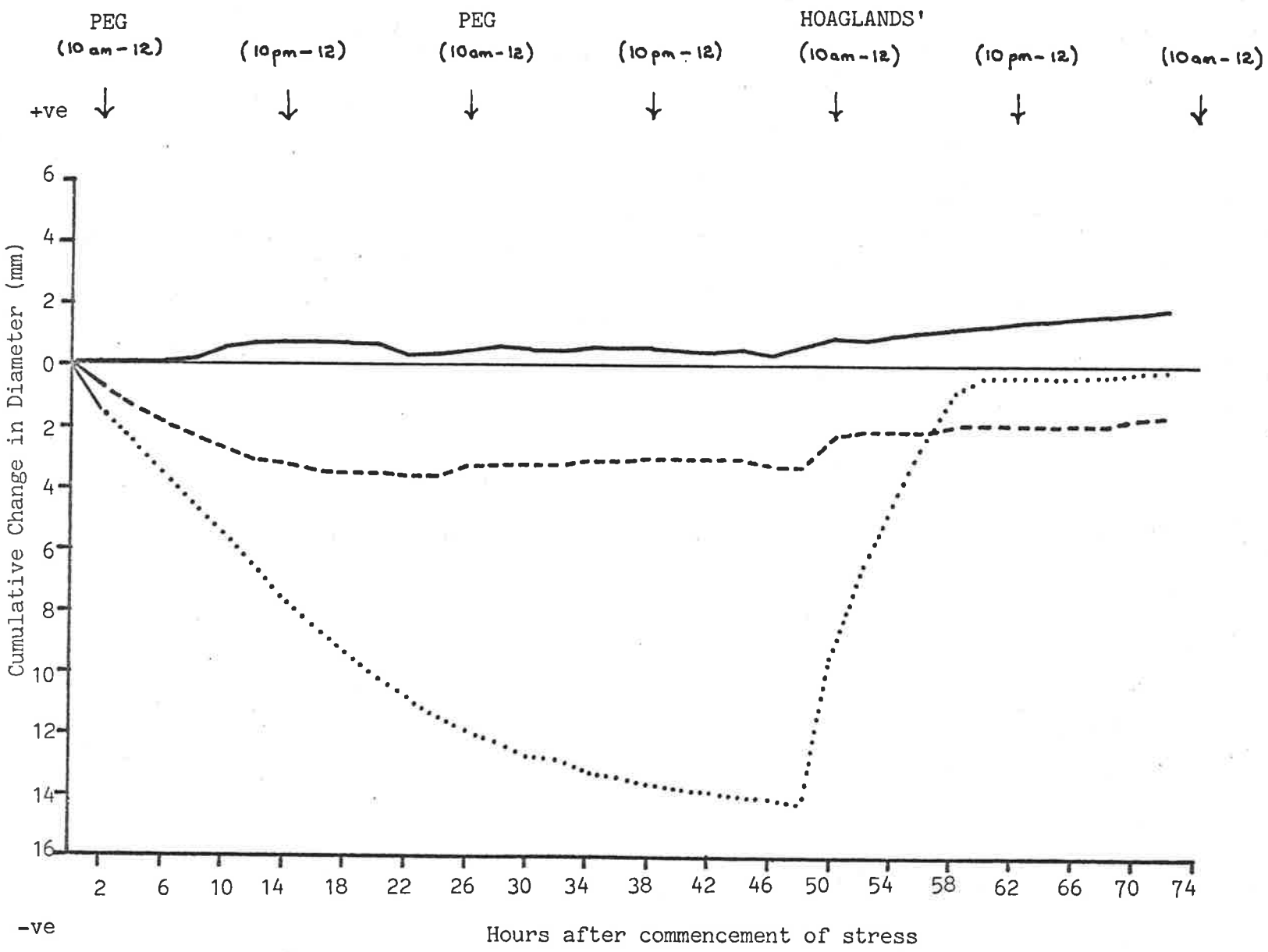

PEG
(10 am - 12)
(10pm - 12)
PEG
(10am - 12)
(10pm - 12)
HOAGLANDS'
(10am - 12)
(10 pm - 12)
(10am - 12)
+ve
-ve
Cumulative Change in Diameter (mm)
6
4
2
0
2
4
6
8
10
12
14
16
2
6
10
14
18
22
26
30
34
38
42
46
50
54
58
62
66
70
74
Hours after commencement of stress

FIGURE 3.3.5

Rate of change in diameter of Radish fleshy axes during 48 hour episodes of -5 and -10 bar PEG-induced water stress and subsequent alleviation.

———— Control

-------- -5 bar PEG

..... -10 bar PEG

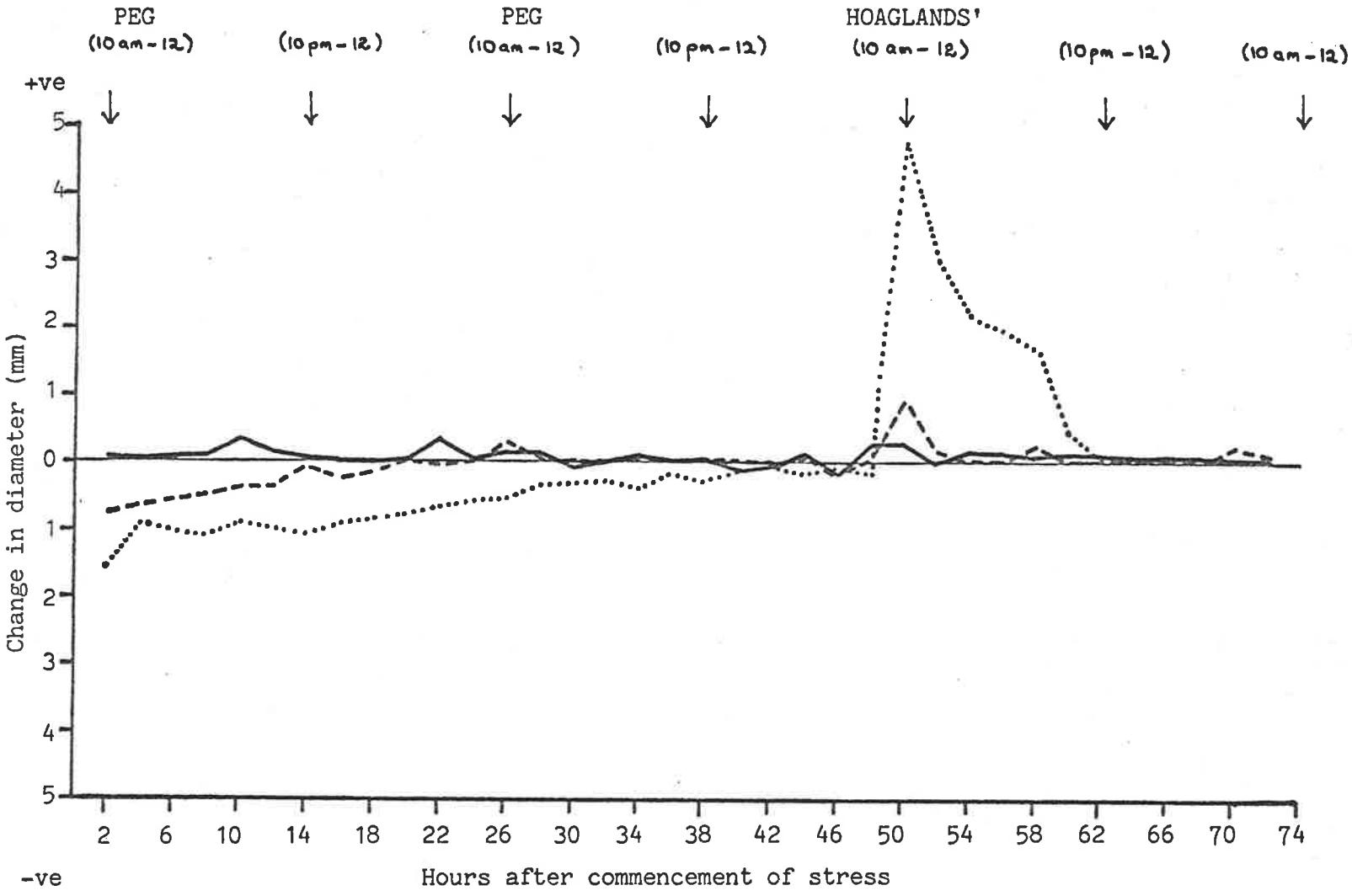

PEG
(10 am - 12)
(10 pm - 12)
PEG
(10 am - 12)
(10 pm - 12)
HOAGLANDS'
(10 am - 12)
(10 pm - 12)
(10 am - 12)
+ve
-ve
Change in diameter (mm)
5
4
3
2
1
0
1
2
3
4
5
2
6
10
14
18
22
26
30
34
38
42
46
50
54
58
62
66
70
74
Hours after commencement of stress

TABLE 3.3.1

Final harvest growth data collected after the imposition of 2 levels of PEG-induced water stress.

PEG Treatment for 48 hr	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Leaf Area (cm^2)	Fleshy Axis Fresh Weight (g)	Fleshy Axis Dry Weight (g)	Fleshy Axis Volume (cc)	Fleshy Axis Diameter (cm)	Fleshy Axis Density (g/cc)
-5 bar	9.34	1.10	199.2	17.17	1.01	15.9	3.35	1.06
-10 bar	10.36	1.03	207.5	16.13	0.96	14.8	3.10	1.09
Control	10.72	1.25	255.4	25.33	1.30	20.6	3.56	1.23
L.S.D. (0.05)	/	/	34.3	5.17	/	3.4	0.21	/

(g. fresh weight/volume in c.c). Interplant variability within treatments was high however and this may have decreased the likelihood of discerning statistically significant differences between treatments. Nevertheless, plant leaf area was significantly decreased, the 2 stress treatments producing a similar reduction in comparison to the control. A similar response was obtained with fleshy axis fresh weight and volume. Only the severe stress treatment, however, was significantly different from the control in the case of fleshy axis diameter.

During the course of the stress episodes diffusive resistance measurements, at irregular intervals, were made on the last fully expanded leaf of one of the plants from each pot treated with -5 bar PEG in the 3 replications remaining in the growth cabinet. Diffusive resistance increased rapidly after the onset of -5 bar PEG stress (figure 3.3.6) in a manner similar to the loss of diameter (figure 3.3.3), and, after the initial increase over 6-8 hr little increase in diffusive resistance or further decrease in fleshy axis diameter had occurred by the 24th hour of stress. However, a marked increase in diffusive resistance, not reflected in a diameter response (figure 3.3.4, continuously monitored diameter), was shown to occur during the night cycle.

3.3.4 Discussion

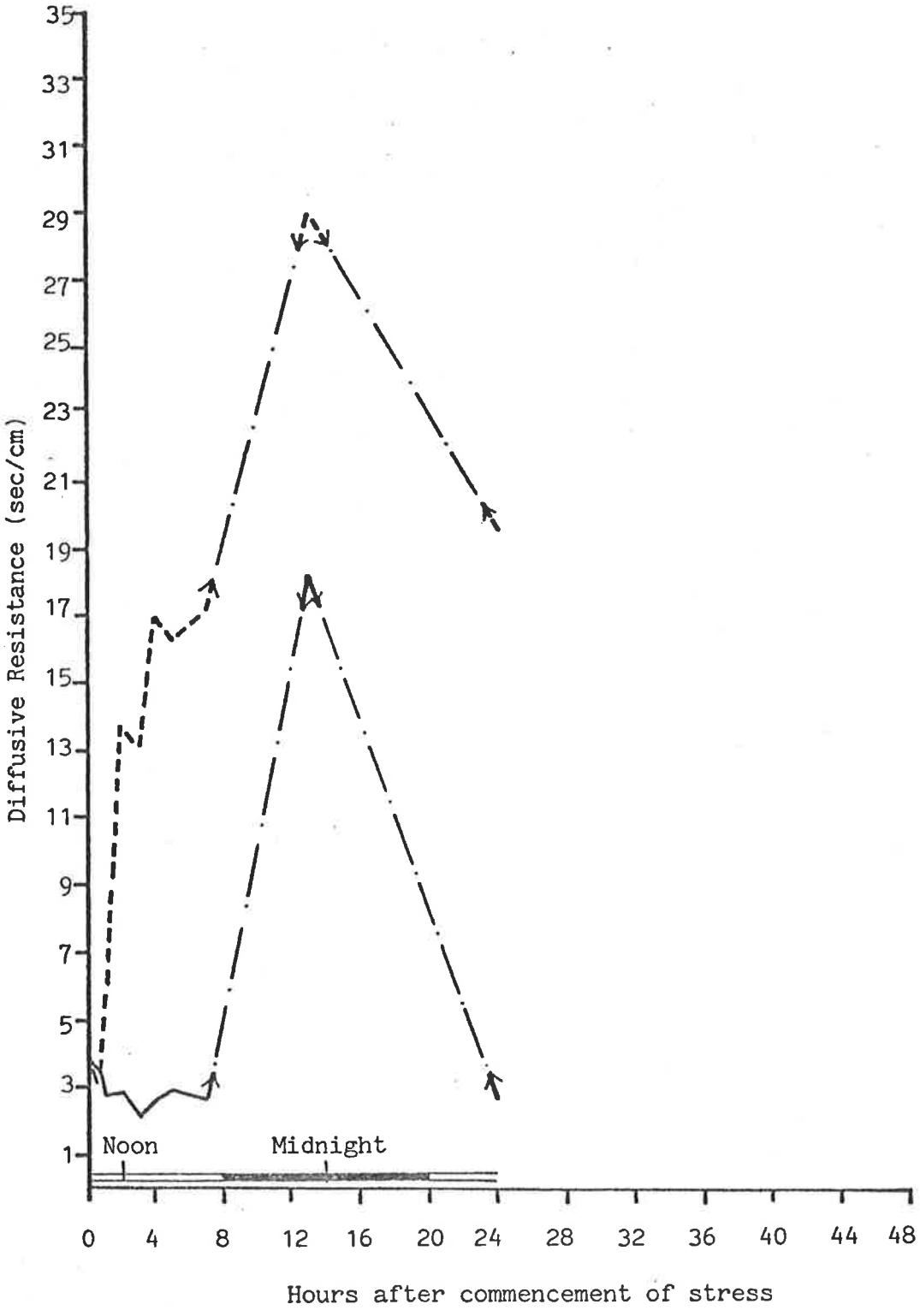
The results indicate that exposure of Radish plants to short stress episodes can cause a significant reduction in yield, and this finding supports the previous experiment (section 3.2). However, in contrast to the previous experiment with similar stress treatments, only fleshy axis fresh weight and volume and leaf area were significantly reduced. An overall conclusion can be drawn that although short episodes of stress can reduce all growth attributes, fresh weight gain and perhaps expansion growth (as marginal leaf necrosis also affects leaf area) appear to be the most sensitive processes. A similar study on Radish (Chu, 1974) also reported greater sensitivity of fresh weight and expansion.

FIGURE 3.3.6

Change in diffusive resistance of Radish plants stressed with -5 bar PEG for 24 hours.

———— Control

-------- -5 bar PEG


Diffusive Resistance (sec/cm)
35
33
31
29
27
25
23
21
19
17
15
13
11
9
7
5
3
1
Noon
Midnight
0
4
8
12
16
20
24
28
32
36
40
44
48
Hours after commencement of stress

Continuous monitoring revealed no short term fluctuations in diameter, such as might be anticipated during the night cycle, and produced diameter curves corresponding fairly closely to those presented in the previous experiment (section 3.2).

Diffusive resistance results, which reveal a more complete closure of stomata at night in stressed plants (-5 bars PEG), when considered in comparison with the diameter results show that complete stomatal closure does not result in improved water status of the fleshy axis. It is also apparent that stressed plants had a higher maximum diffusive resistance than unstressed plants. This result conflicts with data relating Cotton stem diameter to leaf water potential, relative water content and diffusive resistance. Cotton stem diameter during a stress episode increased during the night period closely in line with increasing leaf water potential and diffusive resistance and with a less close correlation to increasing relative water content (Jordan and Ritchie, 1971). The method of stress induction may cause this apparent conflict; whereas the night diffusive resistance and stem diameter of the water deficit droughted Cotton are closely allied, it may be that, where a relatively constant osmotic solution bathes the root system, water availability from the medium does not increase as evaporative demand decreases.

in an attempt to ensure that plants were not, at any time, exposed to abundant water. In view of the short equilibration time between water applications and the continual loss of water through the plants, the water content would not have equilibrated throughout the mass of soil in the time available. As water was applied to the soil surface, it is also possible that root growth in the upper layers of soil was stimulated. The description of the treatment in terms of field capacity is given, therefore, to define the amount of water supplied, not the water status of the soil. Nevertheless, it is anticipated that this treatment effectively reduced soil water availability.

3.4 Soil Water Deficit Induced Stress effects on the Pattern of Fleshy Axis Development

3.4.1 Introduction

This experiment was designed to study the effect of a water deficit imposed by allowing plants to deplete a limited water store. Different regimes were established by allowing the soil water content to fall to predetermined percentages of field capacity before rewatering. These cyclic stress treatments were continued for 7 weeks and the effects on yield and fleshy axis anatomy were measured.

3.4.2 Methods

Two types of stress regime were imposed on the plants. In the first the soil water content was allowed to fall to a predetermined level and was then returned to field capacity. In the second the soil water content was cycled between 2 levels, both below field capacity.* Figures 3.4.1 and 3.4.2 illustrate these 2 regimes. The treatments chosen for the experiment were:

Regime 1 : Field capacity (F.C.) to 35% F.C.

Regime 2 : F.C. to 25% F.C.

Regime 3 : F.C. to 15% F.C.

Regime 4 : 35% F.C. to 15% F.C.

A 1 part coarse river sand : 1 part black clay loam rooting medium was used in this experiment, and pots (20 cm top diameter) of plants were maintained within regime limits by the gravimetric method employing a 15 kg clockface balance. Field capacity (100%) was defined as the water content (15%) after 7 days free drainage from saturation. Other determinations of field capacity (Hausenbuiller, 1978) gave soil water content values of 24% (48 hr free drainage from saturation) and 17% (-0.1 bar capillary equilibrium method). Soil dry weight was determined following oven drying (105^{o}C) for 24 hr. No attempt was made to limit

FIGURE 3.4.1

Water use pattern, monitored by the gravimetric method, for pots of Radish (10 plants) cycled between 100 and 35% of field capacity.

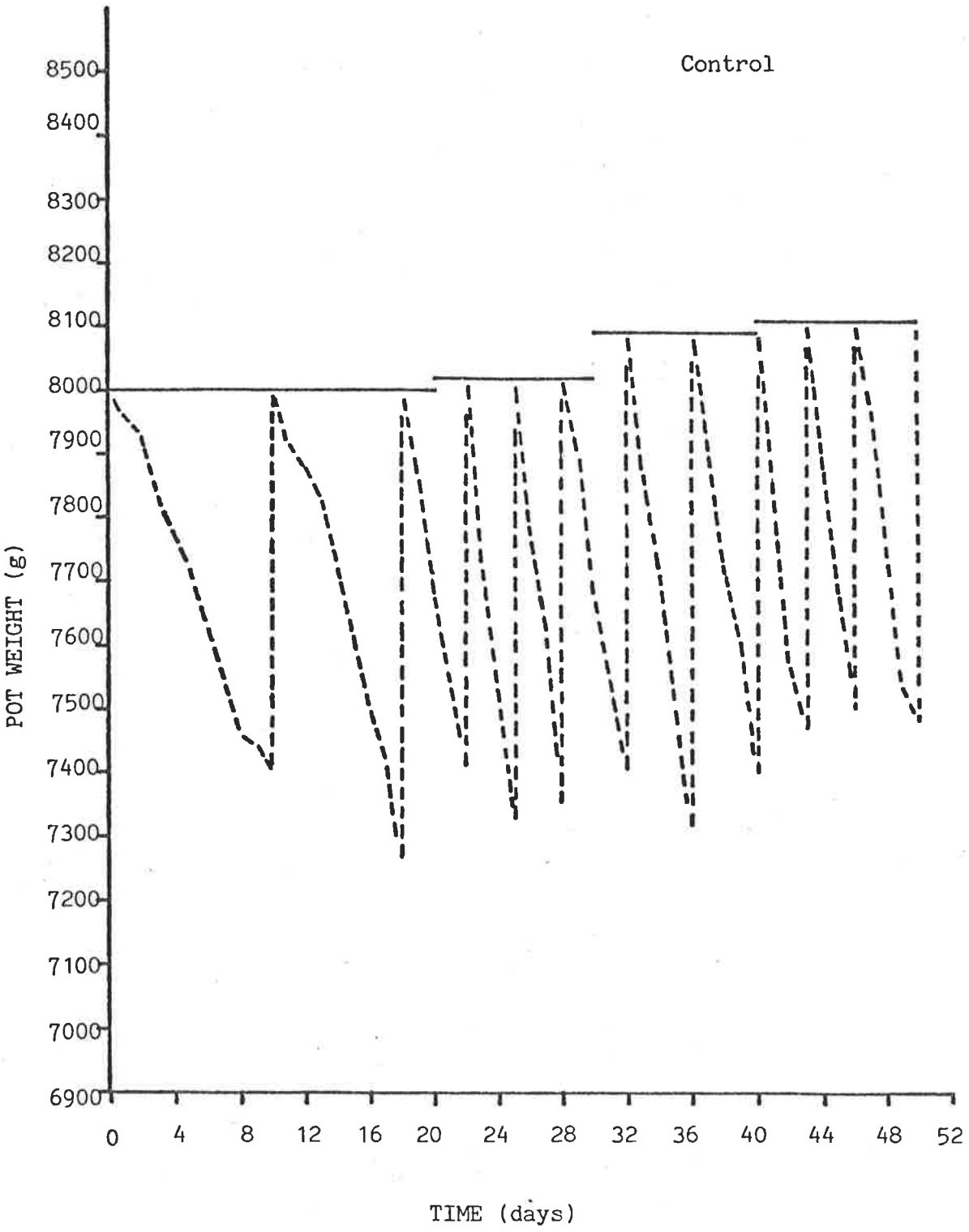
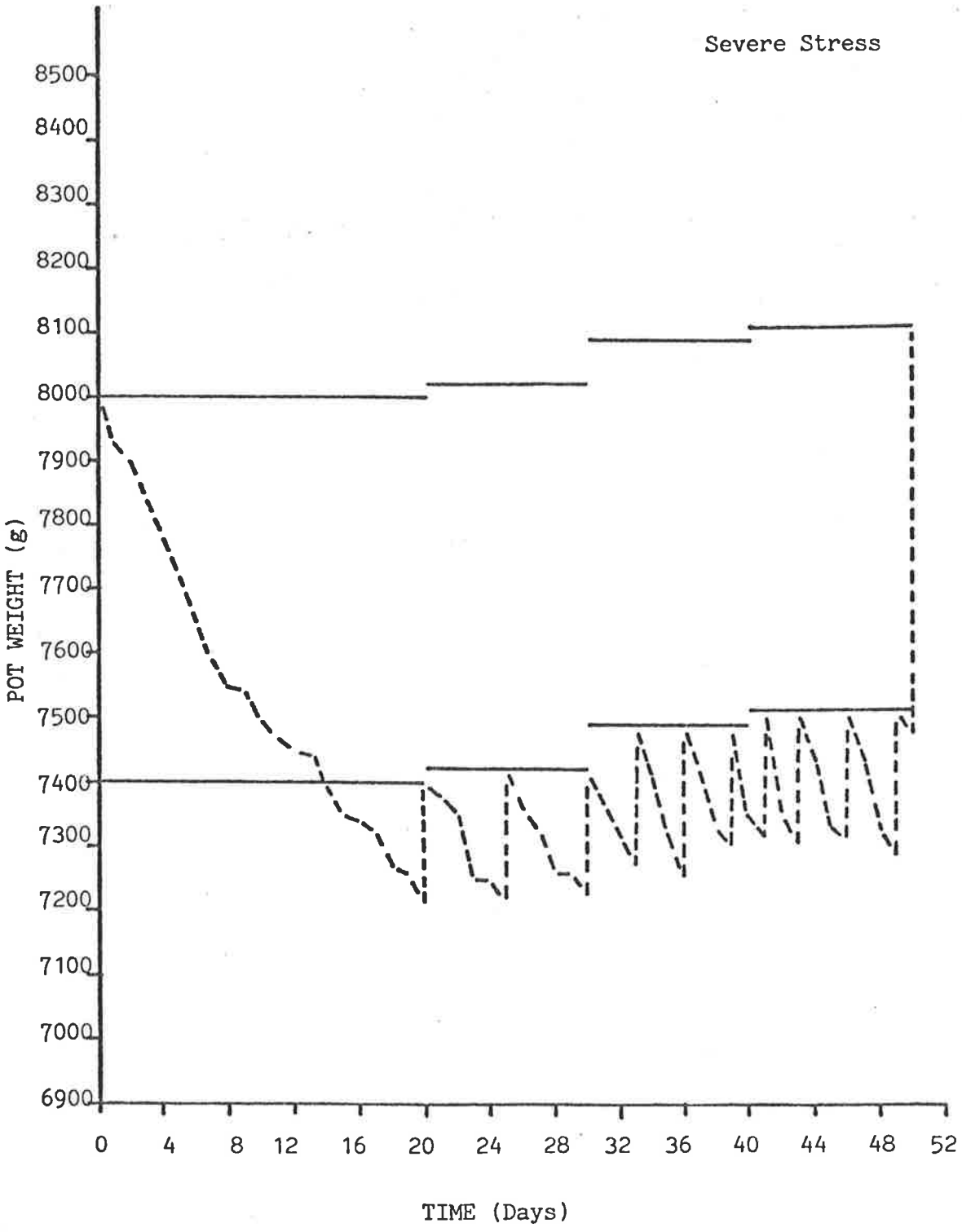

Control
8500
8400
8300
8200
8100
8000
7900
7800
7700
7600
7500
7400
7300
7200
7100
7000
6900
POT WEIGHT (g)
0
4
8
12
16
20
24
28
32
36
40
44
48
52
TIME (days)

FIGURE 3.4.2

Water use pattern, monitored by the gravimetric method, for pots of Radish (10 plants) cycled between 35 and 15% of field capacity.


Severe Stress
8500
8400
8300
8200
8100
8000
7900
7800
7700
7600
7500
7400
7300
7200
7100
7000
6900
POT WEIGHT (g)
0
4
8
12
16
20
24
28
32
36
40
44
48
52
TIME (Days)

surface evaporation during the growth period, however, a compensation was made for the increase in pot weight attributable to plant growth. Three additional pots (regime 1) were harvested at 10 day intervals commencing on day 20 and plant weight estimated from these samples was taken into account in the subsequent additions of water (table 3.4.1). Pot weights were recorded at 10 am daily to indicate the need for rewatering and to provide data on water usage.

Water was applied simply via top watering, and to ascertain whether moisture was evenly distributed down the soil profile in the pot, 2 additional pots were grown (one maintained under regime 2 and the other regime 4). When the plants were well established, the pots were harvested on the day following a return to the maximum water content appropriate to their respective regimes. Soil from each pot was divided into fractions from 4 depth zones and the moisture content of each was determined. The absence of any systematic or marked differences in water content of the soil from different levels (table 3.4.2) indicated that water was reasonably evenly distributed throughout the profile, even where pots were rewatered to a water content less than that at field capacity.

An attempt was made to establish the relationship between soil water content and soil suction potential (figure 3.4.3). Pressure membrane apparatus (Winter, 1974) was used to establish soil suctions of -1, -3, -10 and -15 bars, and the capillary method was used to establish -0.1 bar. After allowing 7 days for equilibration, soil moisture contents were determined and the relationship plotted from the means of 2 replicates. Unexpectedly, beyond -3 bar the soil moisture content was not reduced at lower suction potential. This apparent anomaly may have been due to the characteristics of the rooting medium:- mechanical analysis (Hausenbuiller, 1978) showed 73% of the soil particles to be greater than 50μ in size and 21% to be less than 2μ in size (table 3.4.3). This would suggest that most of the soil water was held loosely but that the remainder was tightly

TABLE 3.4.1

Details of water regimes imposed for four treatment levels of water availability.

Regimes	Regime Pot Weight Limits	Adjustments for Increasing Plant Weight		
	Day 1	Day 20	Day 30	Day 50
(% of field capacity)	(g)	(g)	(g)	(g)
100-35	8000-7398	20	70	22
100-25	8000-7307	20	70	22
100-15	8000-7216	20	70	22
35-15	7398-7216	20	70	22

TABLE 3.4.2

Soil water content with depth in pots (20 cm top diameter) containing 50:50 sand/soil rooting medium.

Level	Treatment	
	Regime 1 (100-35% F.C.)	Regime 4 (35-15% F.C.)
(cm)	(%)	(%)
0-4	13.8	3.1
4-8	10.1	4.5
8-12	12.6	5.2
12-16	11.1	5.1

± SD ?

FIGURE 3.4.3

Soil-water retention curve for 50% coarse river sand: 50% black clay loam rooting medium used for water regime experiments.

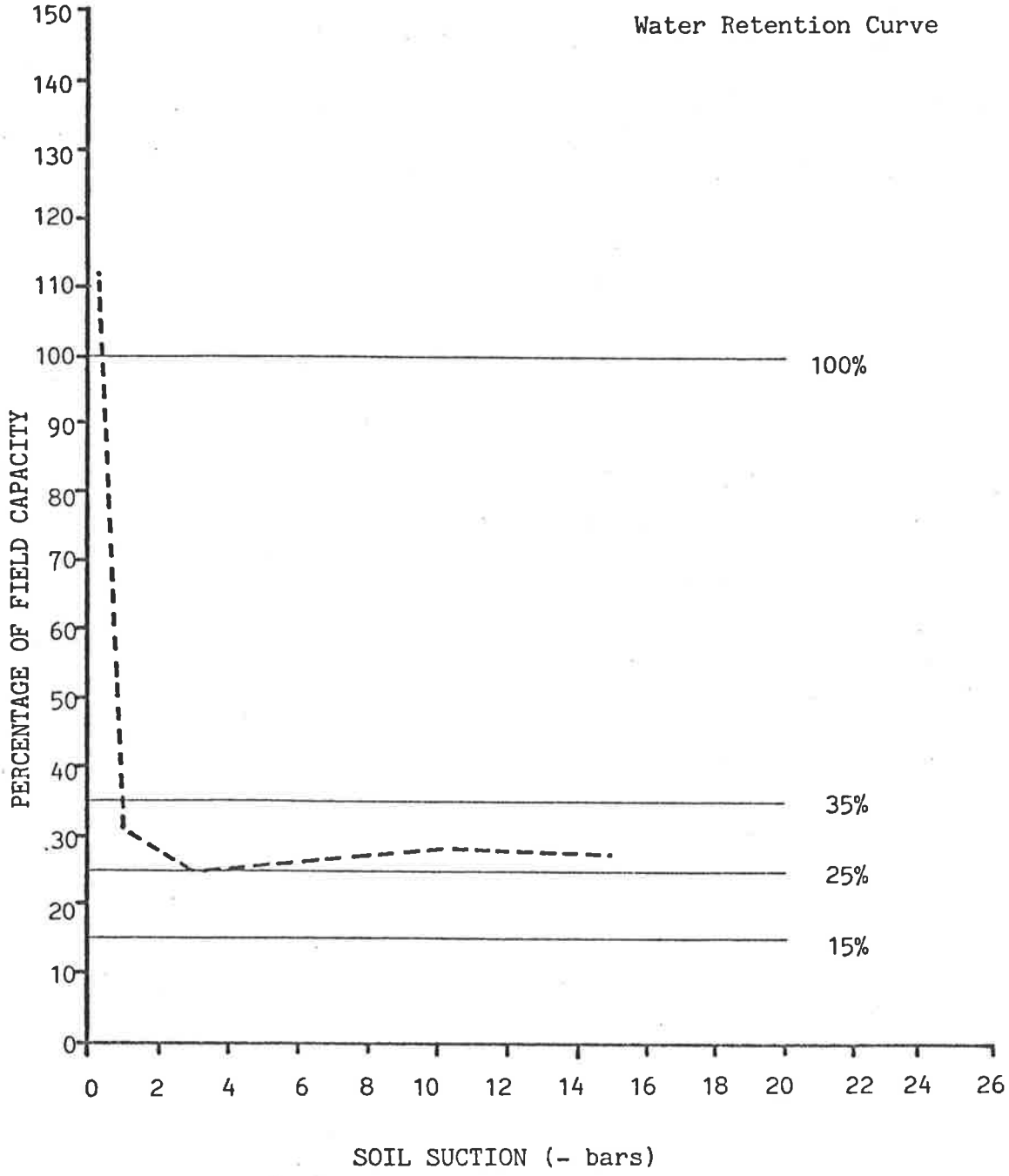

Water Retention Curve
PERCENTAGE OF FIELD CAPACITY
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
100%
35%
25%
15%
0
2
4
6
8
10
12
14
16
18
20
22
24
26
SOIL SUCTION (- bars)

TABLE 3.4.3

Characteristics of the 50:50 sand/soil rooting medium used in water regime experiments.

Characteristic	Category	Value
Bulk density	/	1.47
Specific gravity	/	2.50
Porosity (%)	/	41.2
Mechanical Analysis (%) (particle size)	<2μ	21.1
	2-20	3.0
	20-53	1.7
	53-500	54.5
	>500μ	19.7

held and may have been only expressable from the soil at pressures beyond -15 bars. Soil porosity estimation supported this conclusion with a value of 41%.

The experiment was grown in the glasshouse under late summer conditions with the photoperiod extended to 14 hours with artificial lighting. During the growth period the average daily mean relative humidity (monitored by hygrograph) was 75%. The 4 water regimes were replicated 5 times.

The pots, containing 7 kg of soil mix, were each sown to a depth of 1 cm with 10 pairs of Radish seed (cv. 'Mars'). On day 7 the least uniform seedling in every pair was removed. Nutrition was supplied by slow release fertilizer (3 g Osmocote^{R}, 3 month formulation) mixed evenly in the top 10 cm of the potting mix. At the sowing date (day 1) all pots were at field capacity.

When the plants were harvested on day 50, those from each pot were divided into pairs with similar fleshy axis diameter. One plant of each pair was chosen at random for anatomical studies and the other for dry weight determination. Five pairs of plants were selected from each pot in this way, but only plants from 3 of the 5 replicates were used for anatomical studies. Transverse sections of the fleshy axes were prepared from the point of maximum diameter for the collection of anatomical data. All non-destructive measurements, e.g. fresh weight, were collected from all 10 plants in each pot.

3.4.3 Results

The harsher water regimes (3 and 4) caused significant reductions in fleshy axis yield (table 3.4.4) and fleshy axis diameter, volume, fresh weight and dry weight were significantly reduced. This reduction appeared to be related to the volume of water supplied to the plants, as both of the harsher stress regimes caused similar reductions in yield and were

TABLE 3.4.4

Harvest yield data from 4 water regime treatments offering decreasing levels of water availability.

Treatment	Regime Limits (%F.C.)	Total Water Applied (cc)	Total Plant Fresh Weight (g)	Water Use Efficiency (g/kg)	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Leaf Area (cm^2)	Leaf Number	Fleshy Axis Fresh Weight (g)	Fleshy Axis Dry Weight (g)	Fleshy Axis Diameter (cm)	Fleshy Axis Volume (cc)	Fleshy Axis Density (g/cc)	Shoot Moisture Content (%)	Fleshy Axis Moisture Content (%)
Regime 1	100-35	6621	22.14	1.72	10.75	0.77	199.7	6.5	11.39	1.06	2.39	11.92	0.96	92.8	92.5
Regime 2	100-25	6000	18.17	1.52	8.92	0.66	176.2	6.8	9.25	0.97	2.16	9.51	0.97	92.6	91.6
Regime 3	100-15	4936	13.53	0.92	8.99	0.69	182.8	5.8	4.54	0.39	1.61	4.99	0.91	92.3	91.2
Regime 4	35-15	4341	12.96	0.96	8.87	0.68	171.9	6.1	4.09	0.48	1.56	4.52	0.92	92.3	90.2
L.S.D.	/	623	2.89	0.25	(N.S.)	(N.S.)	(N.S.)	0.5	1.89	0.14	0.28	2.08	(N.S.)	(N.S.)	(N.S.)

supplied with similar total quantities of water (table 3.4.4). The water use efficiency of the plants in these regimes was considerably lower (0.9) than that of the more liberal water treatments. As the water use efficiency was reduced 44% by the harsh treatments, whilst the volume of water supplied was reduced by only 27%, something more than a simple volume relationship caused the differences in yields (which suffered a 34% reduction on a total plant fresh weight basis).

Differences in shoot growth did not reach statistical significance (fresh weight and leaf area) with the exception of leaf number which was significantly reduced by regime 3. The reduction in leaf number was due to increased leaf death (senescence) apparently associated with plants cycling over a large range of soil water status (100 to 15% F.C.). The greater apparent sensitivity of the growth of the fleshy axis to differences in water supply was sufficient to result in significant effects on total plant fresh weight despite the lack of shoot response.

Plants wilted severely as they approached the lower limits of regimes 3 and 4, and to a lesser extent 2. This wilting appeared to be more intense as the plants aged.

Differential effects of the various regimes on fleshy axis diameter occurred early (figure 3.4.4) and continued through to the end of the experiment. By day 18 regimes 1 and 2 clearly diverged from, and maintained faster growth than, regimes 3 and 4. Regimes 1 and 2 had been rewatered for the first time before day 18 but regimes 3 and 4 were first rewatered after this time. The diameter of the fleshy axis of plants subjected to regime 1 increased at a greater rate than that of plants in regime 2, but the diameters of plants in regimes 3 and 4 were similar and considerably less than the other two.

Anatomical studies of the fleshy axes suggested that the effect of stress on fleshy axis diameter was due to a significant reduction in ray parenchyma cell size, and consequently to reduced total ray area

FIGURE 3.4.4

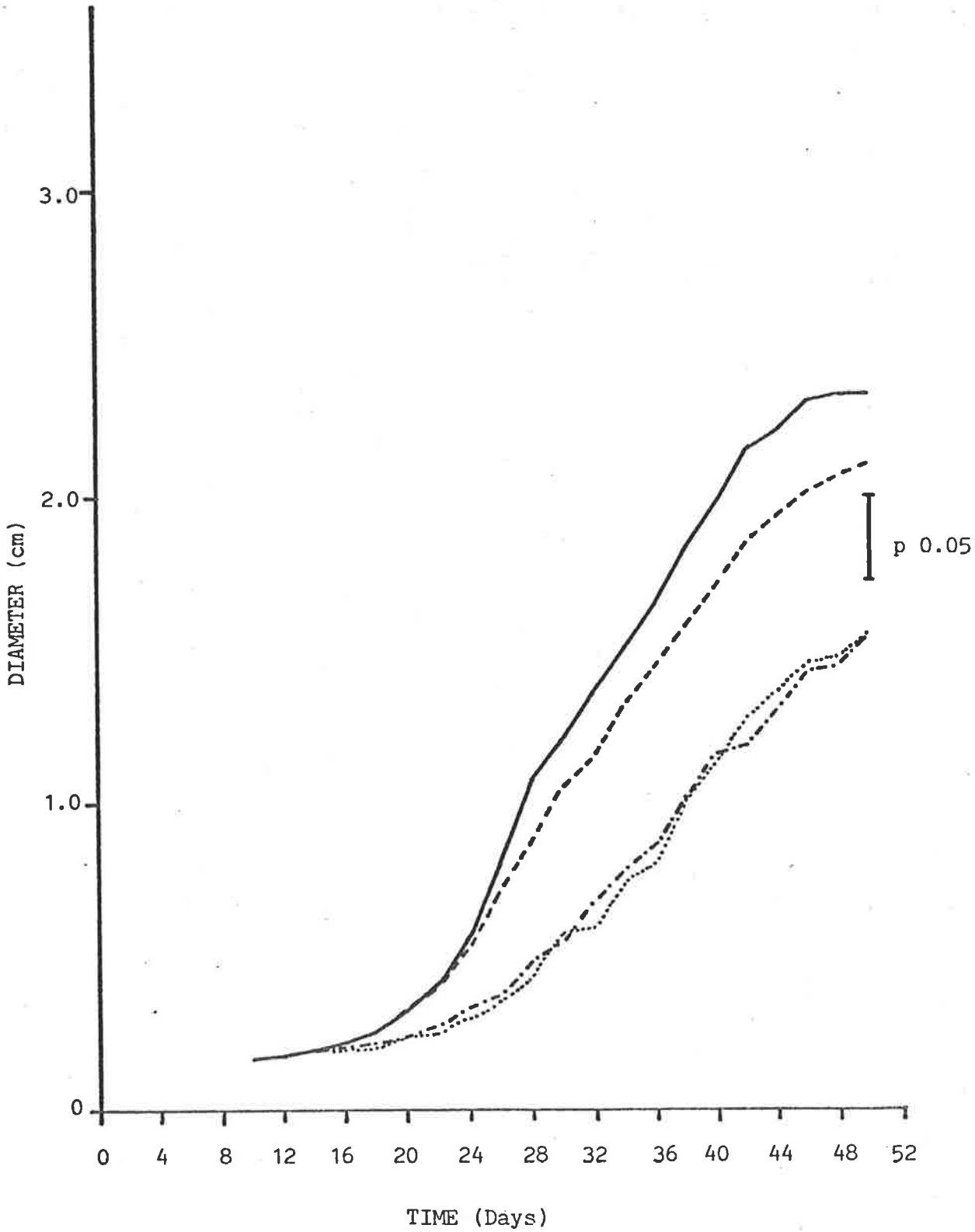
The effect of different water regimes (4), offering decreasing water availability, on the diameter growth of the Radish fleshy axis.

———— Regime 1 (F.C. to 35% F.C.)

-------- Regime 2 (F.C. to 25% F.C.)

-.-.-.-. Regime 3 (F.C. to 15% F.C.)

..... Regime 4 (35% F.C. to 15% F.C.)


3.0
2.0
1.0
0
DIAMETER (cm)
p 0.05
0
4
8
12
16
20
24
28
32
36
40
44
48
52
TIME (Days)

(table 3.4.5). As a result, the band of pericycle surrounding the ray region occupied a large percentage of the cross sectional area of stressed (regimes 3 and 4) fleshy axes, but a lesser proportion of control sections. The data also reveals trends towards reduced cell size in the pericycle, and reduced cell number in both pericycle and ray tissues of stressed fleshy axes, however, these differences did not reach the level of statistical significance.

When parenchyma cell size (figures 3.4.5 and 3.4.6) is plotted against diameter for all treatments, the data ~~is~~ are found to fit a common curve. This indicates that stress caused a general decrease in parenchyma cell size but did not interfere with the pattern of development within this tissue type. This is particularly important when the pattern of fleshy axis development is considered, as the sequence of initial slow hypocotyl development followed by initiation of rapid cell expansion is not altered by stress. Thus it appears that a reduced rate of cell expansion was the cause of smaller diameters associated with regimes 3 and 4. A similar relationship is found for pericycle tissue (figure 3.4.7). In this tissue cells are divided from the cambium and expand until the fleshy axis approaches a diameter of 8-12 mm at which time periclinal cell division is initiated and pericycle growth in the anticlinal plane to a large extent ceases. This pattern of pericycle development accomodates the rapid expansion of the parenchyma tissue. Again, as data from all treatments fit the general curve it can be concluded that stress was without differential effect on the pattern of development, but had simultaneous and similar effects on cell expansion and cell division. The relationship between pericycle width and cell size and number (figures 3.4.8 and 3.4.9) was linear and supports this conclusion as both cell division and cell expansion data from all treatments was fitted by the same regression line. Further, the fact that no malformation of the fleshy axis nor increased frequency of splitting was observed in

TABLE 3.4.5

The effect of different regimes (4) offering decreasing water availability on Radish fleshy axis anatomy at harvest.

Treatment	Regime Limits	Average Individual Ray Parenchyma Cell Area	Calculated Ray Parenchyma Area	Calculated Number of Ray Parenchyma Cells to Occupy Ray Parenchyma Area	Average Individual Pericycle Cell Area	Calculated Pericycle Area	Calculated Number of Pericycle Cells to Occupy Pericycle Area	Percentage of T.S. Area Occupied by Pericycle	Pericycle Width	Number of Cells Across Pericycle	Calculated Fleshy Axis T.S. Area	Fleshy Axis Diameter	Fleshy Axis Volume
	(%F.C.)	(μ^2)	(mm^2)	($x10^4$)	(μ^2)	(mm^2)	(10^3)	(%)	(μ)		(mm^2)	(mm)	(cc)
Regime 1	100-35	2016	452	28.4	331	26.8	73.6	6.2	333	31.0	477	23.6	10.3
Regime 2	100-25	1501	389	30.6	276	21.9	73.5	6.3	316	30.7	411	21.5	9.8
Regime 3	100-15	990	192	23.4	296	16.0	46.5	9.1	303	27.8	209	14.4	4.2
Regime 4	35-15	1016	190	18.3	285	14.5	46.2	9.4	293	26.8	205	14.1	3.7
L.S.D.	/	569	123	(N.S.)	(N.S.)	(N.S.)	(N.S.)	1.0	(N.S.)	(N.S.)	128	4.5	2.3

FIGURE 3.4.5

The effect of decreased water availability on the relationship between Radish fleshy axis diameter and the number of ray parenchyma cells per unit area (cell size index).

▲ Regime 1 (F.C.-35% F.C.)

△ Regime 2 (F.C.-25% F.C.)

○ Regime 3 (F.C.-15% F.C.)

● Regime 4 (35% F.C.-15% F.C.)

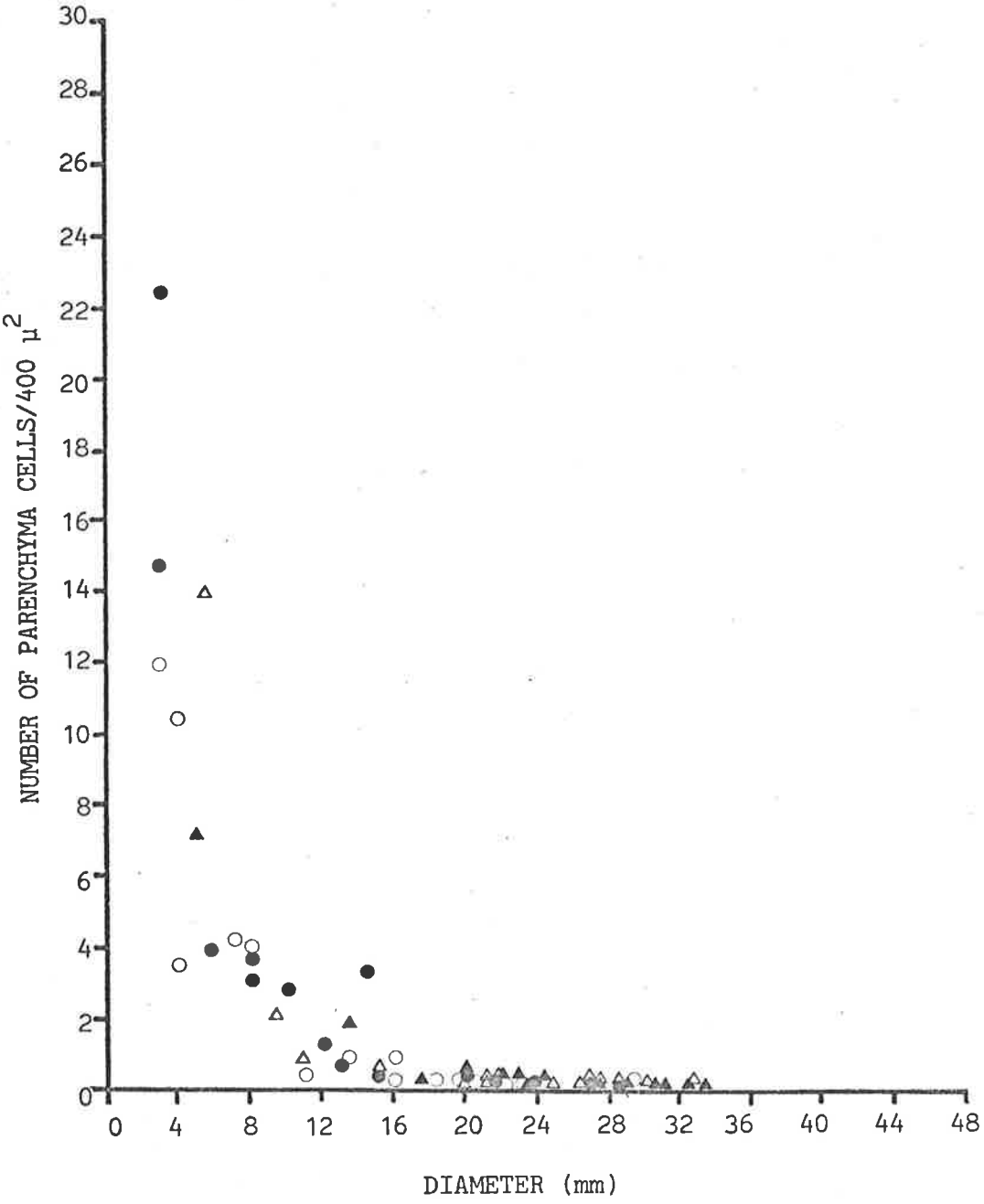

NUMBER OF PARENCHYMA CELLS/400 μ2
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
4
8
12
16
20
24
28
32
36
40
44
48
DIAMETER (mm)

FIGURE 3.4.6

The effect of decreased water availability on the relationship between Radish fleshy axis diameter and average ray parenchyma cell area.

▲ Regime 1 (F.C.-35% F.C.)

△ Regime 2 (F.C.-25% F.C.)

○ Regime 3 (F.C.-15% F.C.)

● Regime 4 (35% F.C.-15% F.C.)

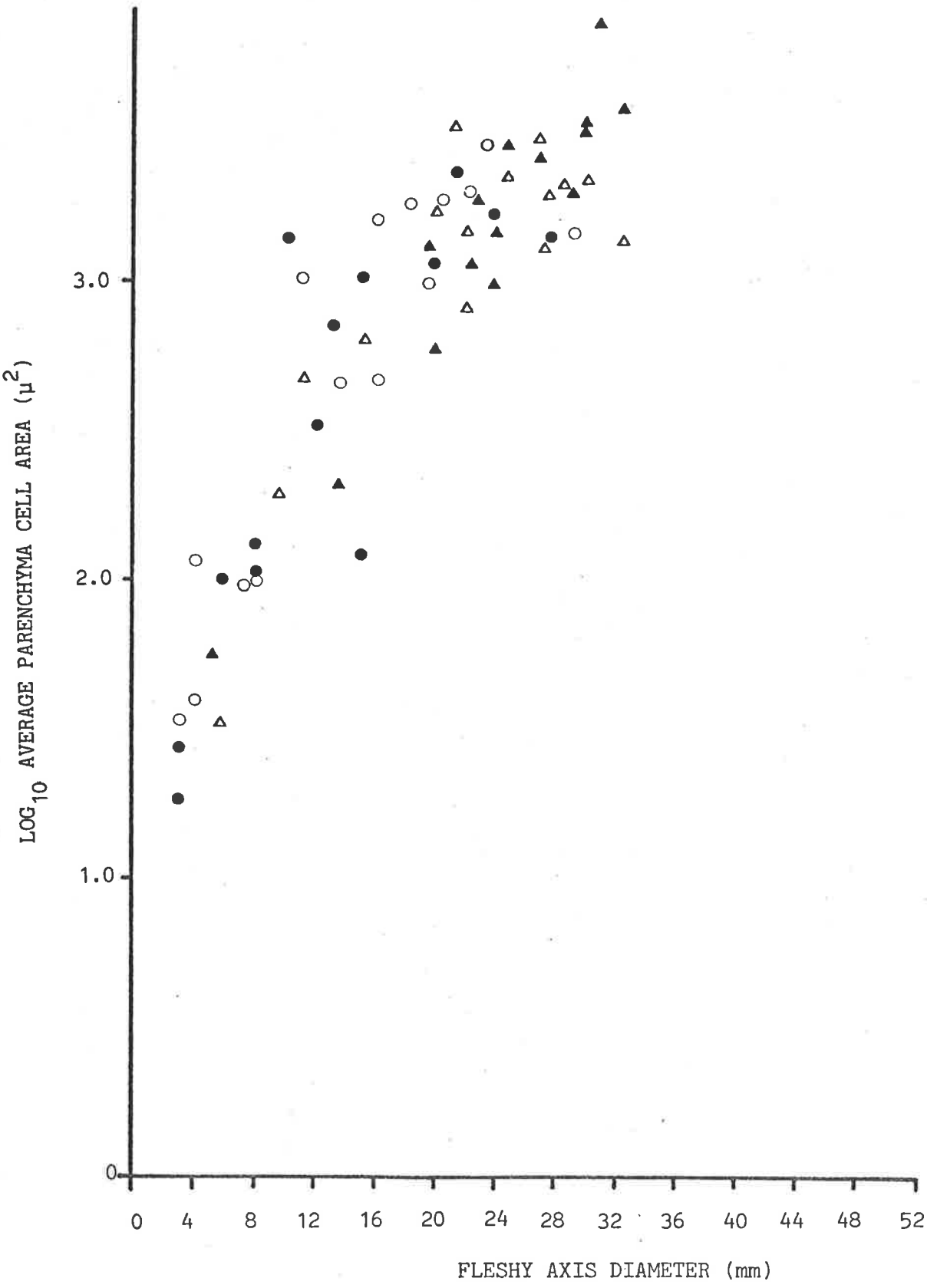

LOG10 AVERAGE PARENCHYMA CELL AREA (μ2)
3.0
2.0
1.0
0
0
4
8
12
16
20
24
28
32
36
40
44
48
52
FLESHY AXIS DIAMETER (mm)

FIGURE 3.4.7

The effect of decreased water availability on the relationship between fleshy axis diameter and average pericycle cell size.

▲ Regime 1 (F.C.-35% F.C.)

△ Regime 2 (F.C.-25% F.C.)

○ Regime 3 (F.C.-15% F.C.)

● Regime 4 (35% F.C.-15% F.C.)

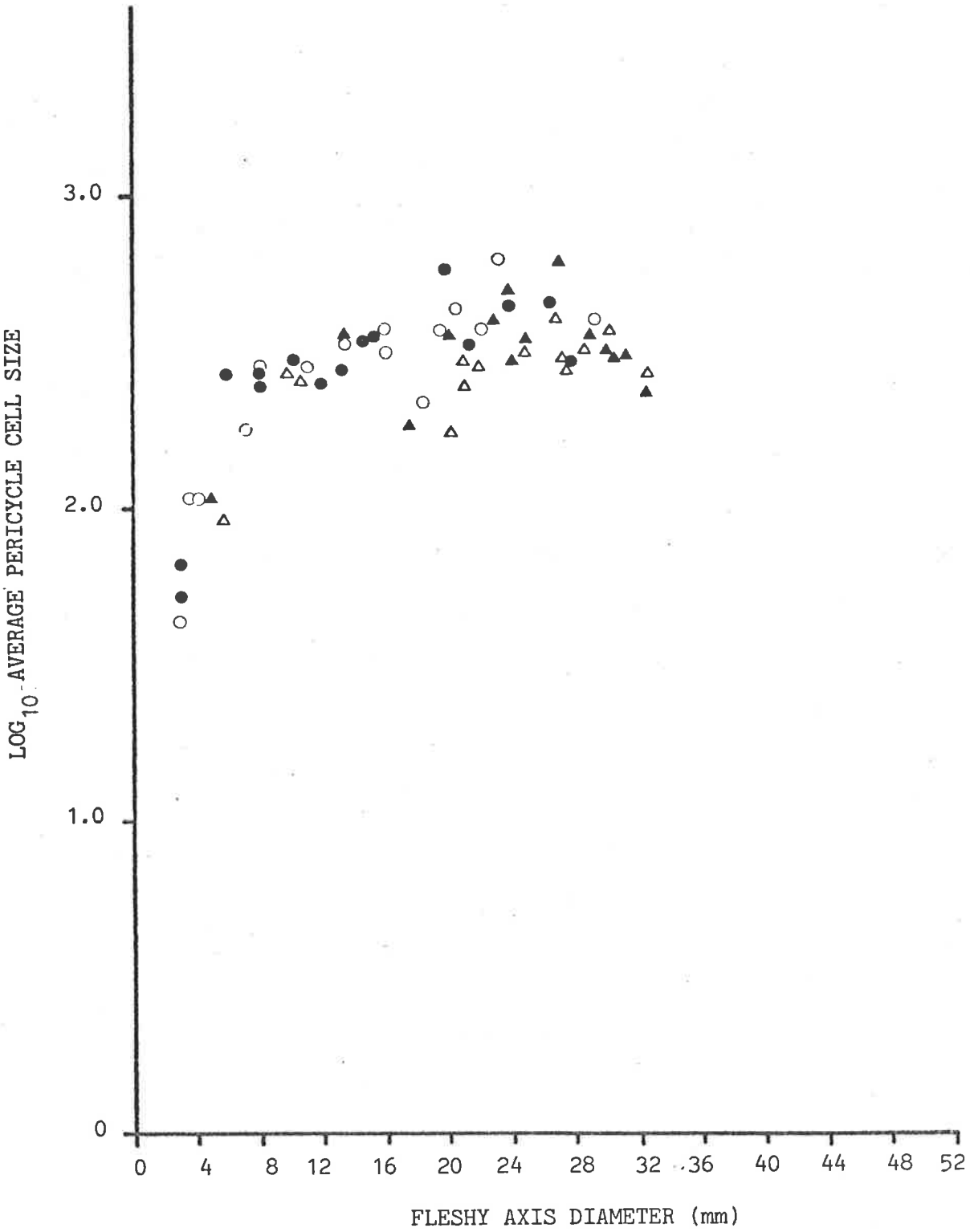

3.0
2.0
1.0
0
LOG10 AVERAGE PERICYCLE CELL SIZE
0
4
8
12
16
20
24
28
32
36
40
44
48
52
FLESHY AXIS DIAMETER (mm)

FIGURE 3.4.8

The relationship between pericycle width and average cell area in Radish fleshy axes subjected to a range of soil moisture regimes : (y = 0.87.x + 51.5; L.R. = 0.862).

▲ Regime 1 (F.C.-35% F.C.)

△ Regime 2 (F.C.-25% F.C.)

○ Regime 3 (F.C.-15% F.C.)

● Regime 4 (35% F.C.-15% F.C.)

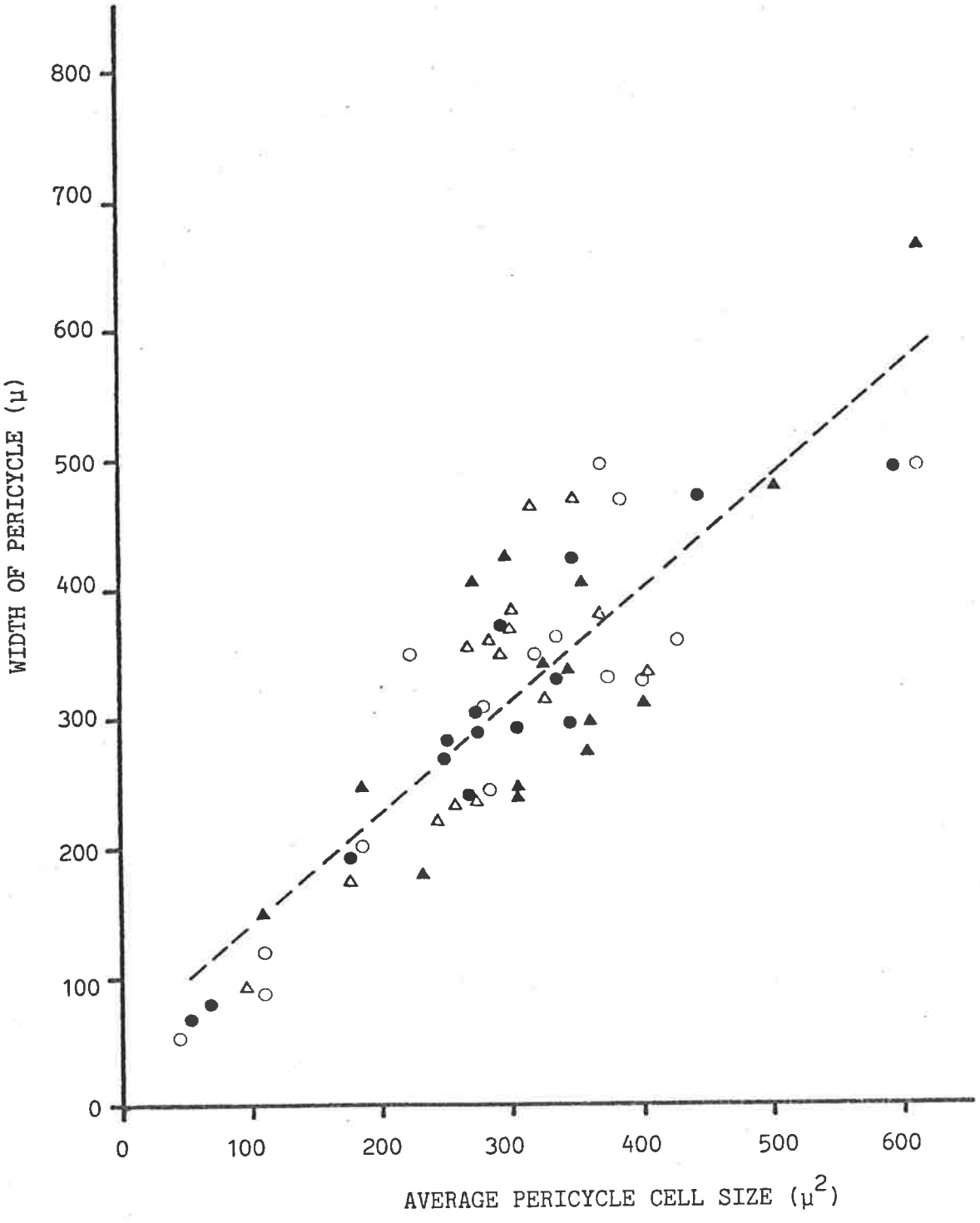

WIDTH OF PERICYCLE (μ)
800
700
600
500
400
300
200
100
0
0
100
200
300
400
500
600
AVERAGE PERICYCLE CELL SIZE (μ²)

FIGURE 3.4.9

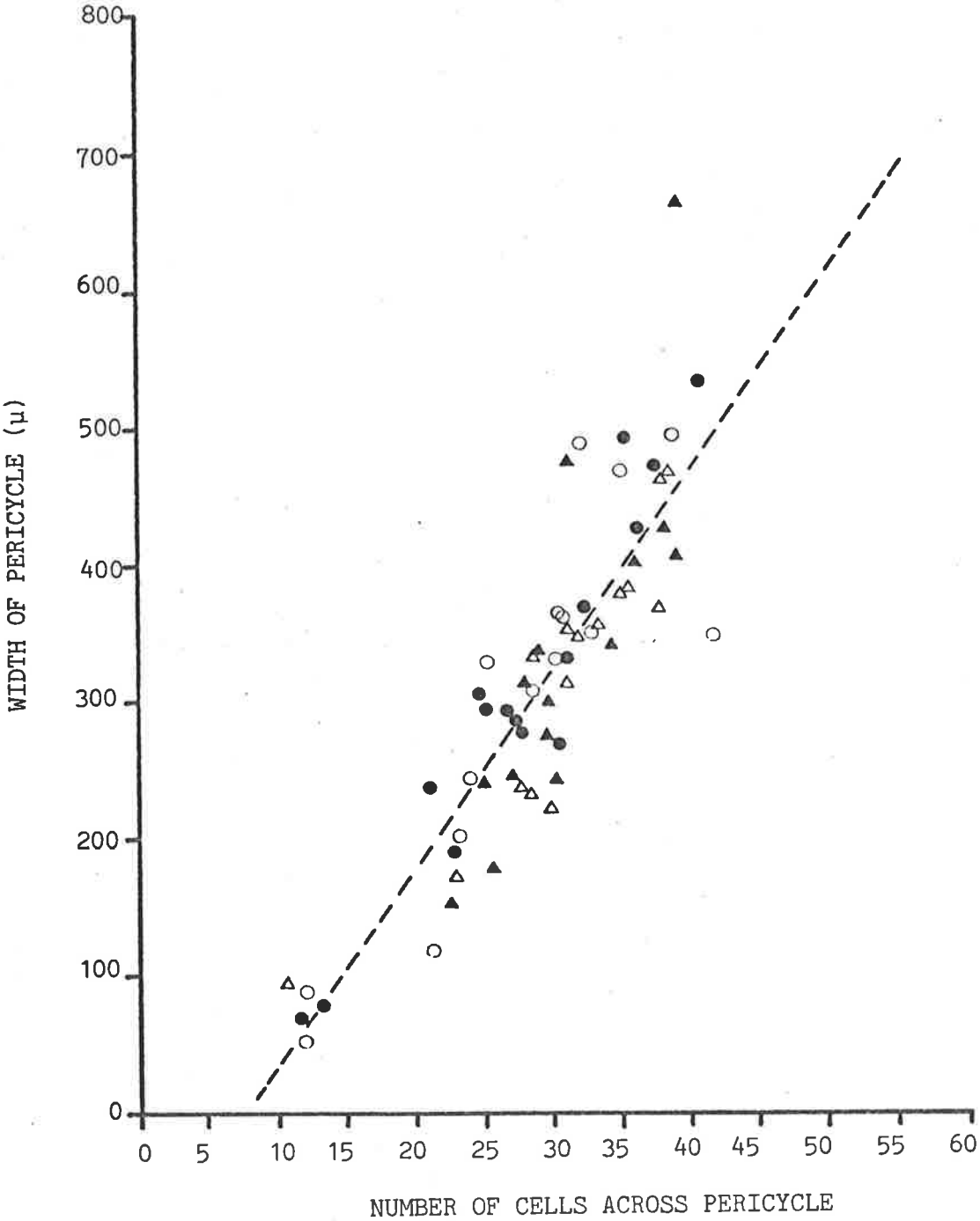
The relationship between pericycle width and the number of cells across the pericycle of Radish fleshy axes subjected to a range of soil moisture regimes:
($y = 14.7.x + [-115.8]$; L.R. = 0.876)

▲ Regime 1 (F.C.-35% F.C.)

△ Regime 2 (F.C.-25% F.C.)

○ Regime 3 (F.C.-15% F.C.)

● Regime 4 (35% F.C.-15% F.C.)


800
700
600
500
400
300
200
100
0
WIDTH OF PERICYCLE (μ)
0
5
10
15
20
25
30
35
40
45
50
55
60
NUMBER OF CELLS ACROSS PERICYCLE

any treatment including that cycled between 100 and 15% of field capacity supports the conclusion that the pericycle and ray parenchyma tissues maintain their general growth patterns relative to each other despite water stress.

Individual measurements of growth and fleshy axis anatomy only differed significantly in certain comparisons between the two harsh regimes and those which were less severe. There was a general trend for a greater severity of effect from regime 1 to regime 4, however. In the light of this trend, regime 1 can be considered to have been amply supplied with water, regime 2 to confer a mild stress, and regimes 3 and 4 to exert successively more severe stress. Individual differences were frequently obscured by considerable interplant variability, but the general conclusion is clear.

3.4.4 Discussion

It is apparent that reduced yield was due to water stress at lower limits of regimes 3 and 4 rather than being directly related to the total volume of water supplied. Regimes 1 to 4 were watered, respectively, 10, 8, 6 and 16 times each (table 3.4.6). The harsher regimes experienced either longer (regime 3) or more frequent (regime 4) exposure to very low soil moisture than regimes 1 and 2. The water use efficiency of plants grown under the harsh regimes was reduced to only slightly above half that of the more liberal regimes by the very low soil moisture and indicates poor adaptation to these conditions. Similar results have been obtained with Peas (Manning *et al.*, 1977) where water use efficiency dropped substantially under harsh conditions. It was suggested that variation in water use efficiency, which is not constant within species or between plants grown at different moisture levels, provides a tool for improving crops both genetically and physiologically. Water use efficiency is generally considered to be

TABLE 3.4.6

Water used and number of rewaterings for individual pots constituting a glasshouse water regime experiment offering increasing levels of water stress.

Treatment	Regime Limits	Replication I		Replication II		Replication III		Replication IV		Replication V	
		Water Used	Number of Rewaterings	Water Used	Number of Rewaterings	Water Used	Number of Rewaterings	Water Used	Number of Rewaterings	Water Used	Number of Rewaterings
	(%F.C.)	(cc)		(cc)		(cc)		(cc)		(cc)	
Regime 1	100-35	6429	10	6759	11	6342	10	6644	9	6929	9
Regime 2	100-25	5832	7	5362	7	6114	8	6159	8	6532	8
Regime 3	100-15	6277	6	4879	6	3994	5	4884	6	4647	5
Regime 4	35-15	4669	17	5031	17	3750	14	3520	13	4736	17

primarily related to stomatal control and improved water use efficiency of plants growing under arid conditions has been reported several times (Lange and Losch, 1980). The low diffusive resistance (section 3.3) in Radish during stress when compared to that at night, supports the proposal that Radish does not exert efficient stomatal control. As stomatal response is small it is not surprising that yield is not directly proportional to the volume of water applied.

Although the relationship between the processes of cell expansion and cell division requires further investigation it is clear that cell size was generally reduced across the 2 major tissue types, the pericycle and the ray parenchyma. Stress did not prevent the fleshy axes from developing according to the normal pattern of growth. This supports the conclusion that this crop, and other biennial 'root' crops (Salter and Goode, 1967), have no stage of growth particularly sensitive to water stress in the first season. Three growth stages were identified from the anatomical observations (pre-initiation of axis swelling, initiation of axis swelling and post-initiation of fleshy axis swelling) but none showed greater or lesser sensitivity as judged by deviation from the diameter curve pattern of well watered plants. In Turnip, sensitive growth stages have been identified by a technique of improving the soil water status and measuring the resultant effects on final yield (Stanhill, 1958). Although these stages were identified in relation to their sensitivity to water supply, they were derived from the response to wet conditions and not to drought. Improved water availability during the seedling stage resulted in increased plant yield at harvest. A wet period during early 'root' swelling depressed final yield however, but this was attributed to changes in nitrogen utilization. The greatest increase in final yield was recorded when the wet treatment was imposed closest to the harvest date. This experiment demonstrates the effects of improved water status at times differing in the relationship between

plant growth potential and evaporative demand. It does not identify stages particularly sensitive to water stress however, when this is judged by the extent to which a stress episode will result in an irreversible depression of fleshy axis size.

The consequence of reduced cell size as a response to water stress was illustrated many decades ago (Maximov, 1929). In Beet it was established that those plants within a sample population which had experienced drought, and which had the smallest cells, were the plants producing the 'finest' (i.e. largest) fleshy axes (table 3.4.7). Conversely, for plants grown under favourable conditions, the greatest yield was associated with the largest cell size. Thus it can be concluded that smaller cell size as a consequence of stress is also of adaptive importance in withstanding a stress period. It can be speculated that, if Radish had been bred and selected for yield under non-irrigated rather than irrigated conditions, Radishes with relatively improved water use efficiency and larger numbers of smaller cells would have been produced under regimes 3 and 4.

TABLE 3.4.7

Correlation between cell size and 'root' development of Beet grown under dry and humid conditions respectively (*).

Group	Diameter of Parenchymatous Cells	Average Weight of Roots (g)	
	(μ)	Dry Year (1905)	Humid (1906)
I	8-11	271.7	-
II	11-14	172.7	64.3
III	14-17	67.0	139.2
IV	>17	33.3	207.6

* Maximov, 1929 (After Kolkunov)

3.5 The effect of Severe Soil Water Deficit, and its alleviation, on the development of the Fleshy Axis

3.5.1 Introduction

The previous experiment (section 3.4) prompted a more detailed examination of the anatomy of the fleshy axis during water stress, with the aim being to clearly delineate stress effects between cell expansion and division processes. For this reason a regime harsher than those used in the previous experiment was compared with a well watered regime to ensure the greater likelihood of significant differences. In order to further extend the understanding of Radish fleshy axis development in relation to water stress the experiment was designed such that after a considerable period of severe water limitation, stressed plants were rewatered to the control regime and the recovery followed by observation of the anatomy of the fleshy axis and of the growth of the plant.

3.5.2 Methods

The procedures for pot preparation and water regime maintenance were similar to those described for the previous experiment; except that in the present experiment mineral nutrients were supplied in the watering solution ($\frac{1}{2}$-strength Hoagland's solution) and no attempt was made to adjust for the increase in plant weight over time. The 2 regimes selected were:

1. Field Capacity (F.C.) to 35% F.C. [Control]
2. 25% F.C. to 15% F.C. [Severe stress]

Three harvests were carried out (30, 35 and 45 days post-sowing), with a subharvest being taken on day 40. By day 30 the stressed pots had experienced the lower limit of their regime at least once following the slow drying down from field capacity (day 10). On day 36 the severe stress treatment was converted to the control regime.

The experiment was conducted in a growth cabinet under a 12 hr photoperiod. Daily mean relative humidity was approximately 50%. The treatments were replicated 4 times and the experiment was statistically analysed as a factorial design.

Plant establishment proved difficult and 3 attempts to populate the pots with uniform seedlings failed. The first attempt repeated the methods used in the previous experiment, but here emergence was poor. The second attempt involved sowing the seeds just below the surface, but also failed to produce acceptable emergence. It was then decided to use transplanted seedlings grown in a coarse-sand filled seedling flat sown to a depth of 1 cm. Erratic and poor emergence remained a problem with this system also. Finally, surface sterilized 'Mars' seed was spread onto water-soaked vermiculite in seedling flats. The seed here was covered to 0.5 cm with wet vermiculite and the flats were covered with glass sheets. Emergence was rapid and uniform; on day 8 the glass was removed and the seedlings were transplanted 2 days later. Ten seedlings were transplanted into each pot. All pots were at field capacity initially and the water deficit treatment commenced from transplanting (day 10). The poor emergence of the seedlings in the earlier attempts was probably due to excess soil water and consequent reduced partial pressure of oxygen around the seeds (MacDonald and Gordon, 1978).

Prior to each harvest, the diameter of the fleshy axis was measured on each plant and the plants (10) in every pot were labelled in order of increasing diameter. Plants 1, 5 and 10 were then harvested on days 30 and 35 for the assessment of water status, counting of cell numbers in leaf discs and measurement of fleshy axis anatomy. The remaining 7 plants in each pot were used to assess plant growth. After stress was alleviated the procedure was altered; on day 40 (4 days

after stress alleviation) plants 4 and 7 in the remaining pots were harvested for leaf-disc cell counts and to provide data on the fleshy axis anatomy. On day 45, plants 5 and 8 from the same pots were harvested and used to assess these parameters. The 6 remaining plants were used, on day 45, to provide growth data. In addition to the collection of anatomical data the response of the fleshy axis was recorded photographically on sections prepared by the JB4 Method (See Materials and Methods).

3.5.3 Results

Severe stress caused a marked reduction in plant growth, with significant effects on all aspects measured with the exception of cotyledon fresh weight, cotyledon moisture content and cotyledon number (table 3.5.1). The reduction in shoot fresh weight was reflected in shoot dry weight and was due to a reduction in both leaf area and leaf number. Fleshy axis diameter, fresh weight and dry weight were similarly reduced. The data also indicated that growth in length of the fleshy axis was inhibited by stress. Although growth recovered markedly following stress alleviation, by 9 days after rewatering differences between control and stressed plant growth parameters were still highly significant.

Stress reduced leaf relative water content by ca. 6% and also resulted in a significantly decreased leaf osmotic potential. Correspondingly, both leaf and hypocotyl water content were reduced by stress.

The major effect of stress occurred before the first harvest, and in the intervening period between the first harvest (day 30) and the second (day 35) small increases in growth measurements were recorded. For instance, leaf area increased marginally between days 30 and 35 (figure 3.5.1). Upon stress alleviation (day 36), leaf area increased at a rate comparable with that of control plants. Similar responses were

TABLE 3.5.1

Harvest yields and water relations for stressed, stress alleviated (day 36) and well watered Radishes harvested at 4 times during the growth period.

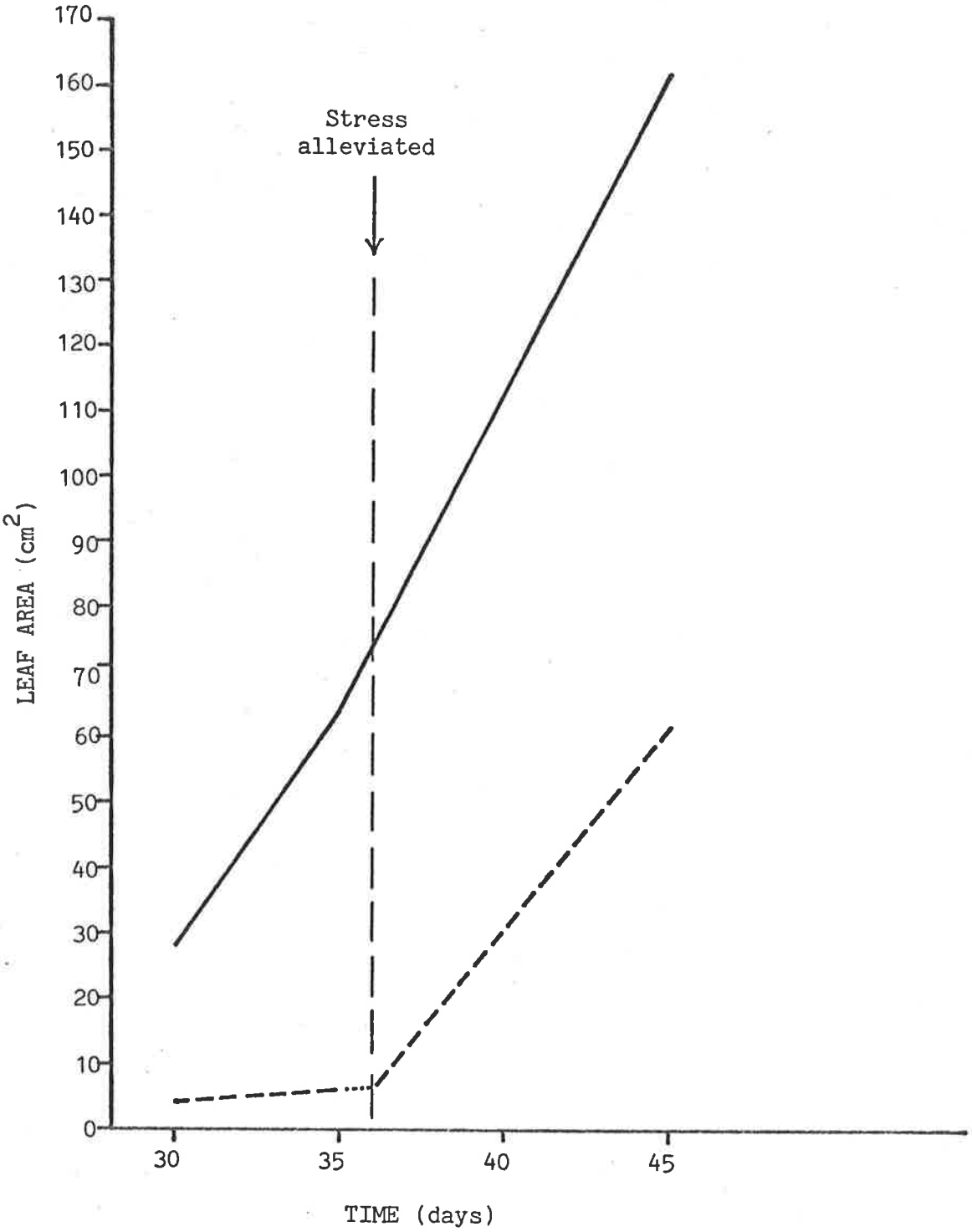
Harvest Day	Treatment	Shoot Fresh Weight (g)	Fleshy Axis Fresh Weight (g)	Cotyledon Fresh Weight (g)	Leaf Area (cm^2)	Leaf Number	Cotyledon Area (cm^2)	Cotyledon Number	Number of Cells per 1.1 cm Leaf Disc ($x10^3$)	Fleshy Axis Diameter (mm)	Fleshy Axis Length (mm)	Fleshy Axis Volume (cc)	Shoot Dry Weight (g)	Fleshy Axis Dry Weight (g)	Cotyledon Dry Weight (g)	Water Used (cc)	Osmotic Potential (-bars)	Relative Water Content (%)	Shoot Moisture Content (%)	Fleshy Axis Moisture Content (%)	Cotyledon Moisture Content (%)
30	Control	0.99	0.13	0.22	27.9	3.5	3.8	2.0	43.4	2.7	30.9	0.08	0.08	0.012	0.014	1392	5.8	95.6	92	92	94
	Stressed	0.14	0.04	0.11	4.2	2.2	2.2	1.7	94.5	1.7	28.6	0.03	0.02	0.007	0.009	973	7.0	88.9	84	84	91
35	Control	2.50	0.83	0.24	63.8	4.3	4.3	1.6	22.9	6.9	33.6	0.87	0.17	0.059	0.015	2476	6.1	95.6	93	93	94
	Stressed	0.23	0.05	0.26	6.1	2.4	2.0	1.4	72.1	1.9	28.8	0.03	0.03	0.007	0.008	1115	8.0	89.0	86	85	94
40	Control	/	/	/	/	/	/	/	22.1	15.0	/	/	/	/	/	/	/	/	/	/	/
	Stressed	/	/	/	/	/	/	/	29.9	2.5	/	/	/	/	/	/	/	/	/	/	/
45	Control	6.68	11.27	0.10	161.8	6.1	1.8	0.7	19.1	21.1	41.0	13.98	0.50	0.598	0.006	5215	/	/	93	95	94
	Stressed	2.46	0.95	0.11	61.7	5.0	2.2	1.1	23.9	7.7	30.0	1.12	0.19	0.068	0.007	2400	/	/	92	92	94
Treat.x Harvest		*	*	(NS)	*	(NS)	*	(NS)	*	*	(NS)	*	*	*	*	*	(NS)	(NS)	*	*	(NS)
Harvest						*		*			*						*	(NS)			(NS)
Treatment						*		(NS)			*						*	*			(NS)

FIGURE 3.5.1

The effect of severe water stress and its alleviation on the development of Radish leaf area.

_____ Control

- - - - - - - - Stressed


170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
LEAF AREA (cm^2)
Stress
alleviated
30
35
40
45
TIME (days)

measured for both shoot fresh and dry weight. The formation of new leaves was also virtually halted during the stress period, but subsequently proceeded at a rate exceeding the control rate (figure 3.5.2). The mean number of cells in an excised leaf disc, which is an index of cell size, recovered during stress to decrease at a rate similar to that of the control. On stress alleviation leaf cell size recovered at a rate exceeding that of the control (figure 3.5.3), finally yielding cells of a similar size by 9 days after stress alleviation. The difference between mean cell size and leaf area, which was significantly different to the control at the final harvest, was due to both a reduction in individual leaf size and reduced leaf number. Both these effects were presumably due to a reduction in cell division during stress as the cell size index shows that leaf cells expand rapidly on stress alleviation to very near the control cell size. The rate of leaf appearance exceeded the control rate following stress alleviation, but, as leaf number did not fully recover to the control level in the same period that full recovery in leaf cell size occurred, it appears that leaf primordium initiation was reduced during stress.

Shoot and fleshy axis fresh weight increased after alleviation of stress at slightly faster rates than the increases in dry weight (figures 3.5.4 and 3.5.5). This was due to the restoration of high tissue water status. Whereas shoot tissue showed some increase in both fresh and dry weight during stress, no such increase in these growth attributes was measured in the fleshy axis tissue. This indicates a greater relative sensitivity to water stress of fleshy axis growth, as compared to shoot growth.

Fleshy axis diameter, volume and length were affected by stress and its alleviation in a fashion almost identical to that already reported for growth in weight of this organ (figure 3.5.5). However, as 'Mars'

FIGURE 3.5.2

The effect of severe water stress and its alleviation on Radish plant leaf number.

———— Control

-------- Stressed

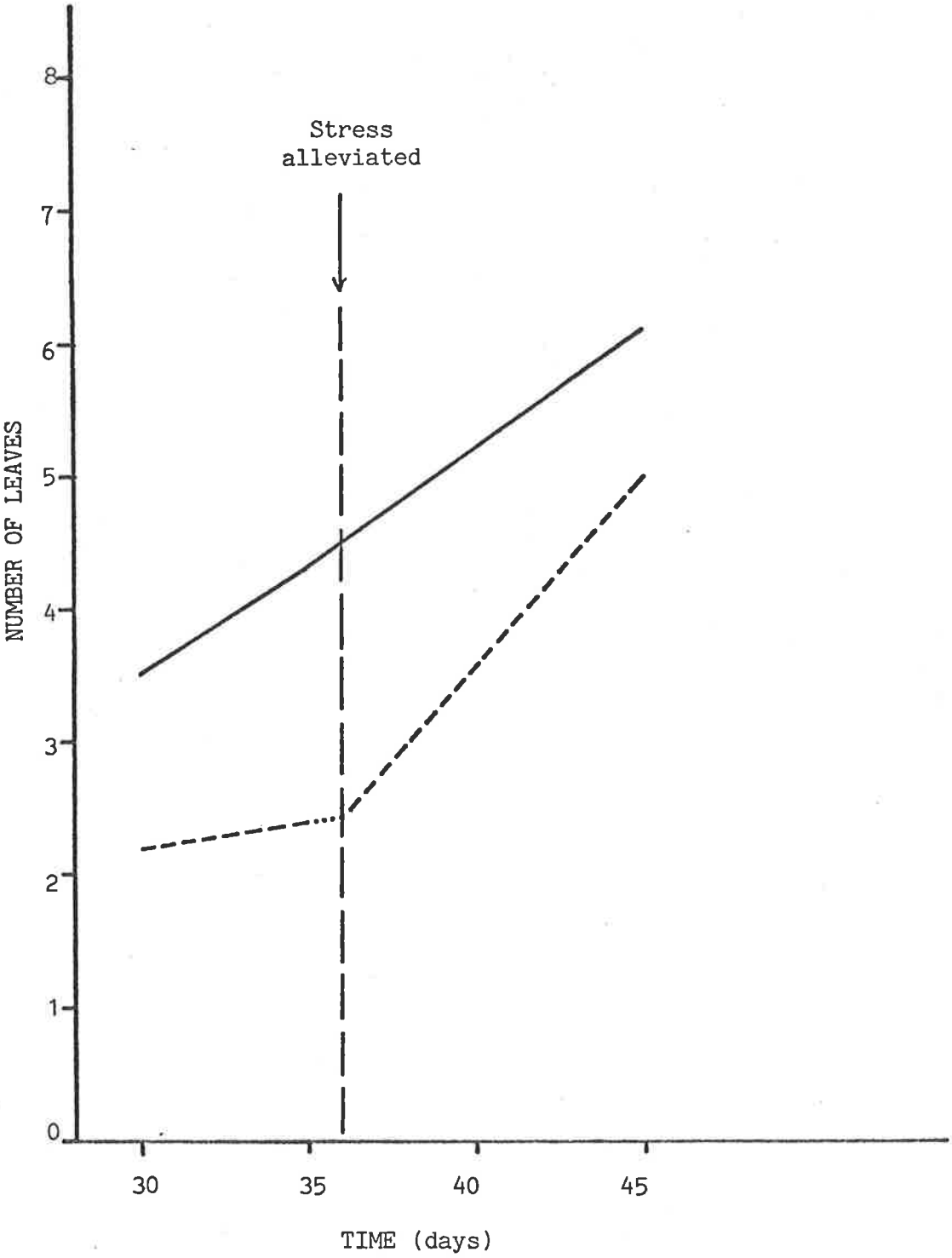

Stress
alleviated
NUMBER OF LEAVES
0
1
2
3
4
5
6
7
8
30
35
40
45
TIME (days)

FIGURE 3.5.3

The effect of severe water stress and its alleviation on the number of cells in excised 1.1 cm leaf discs.

———— Control

-------- Stressed

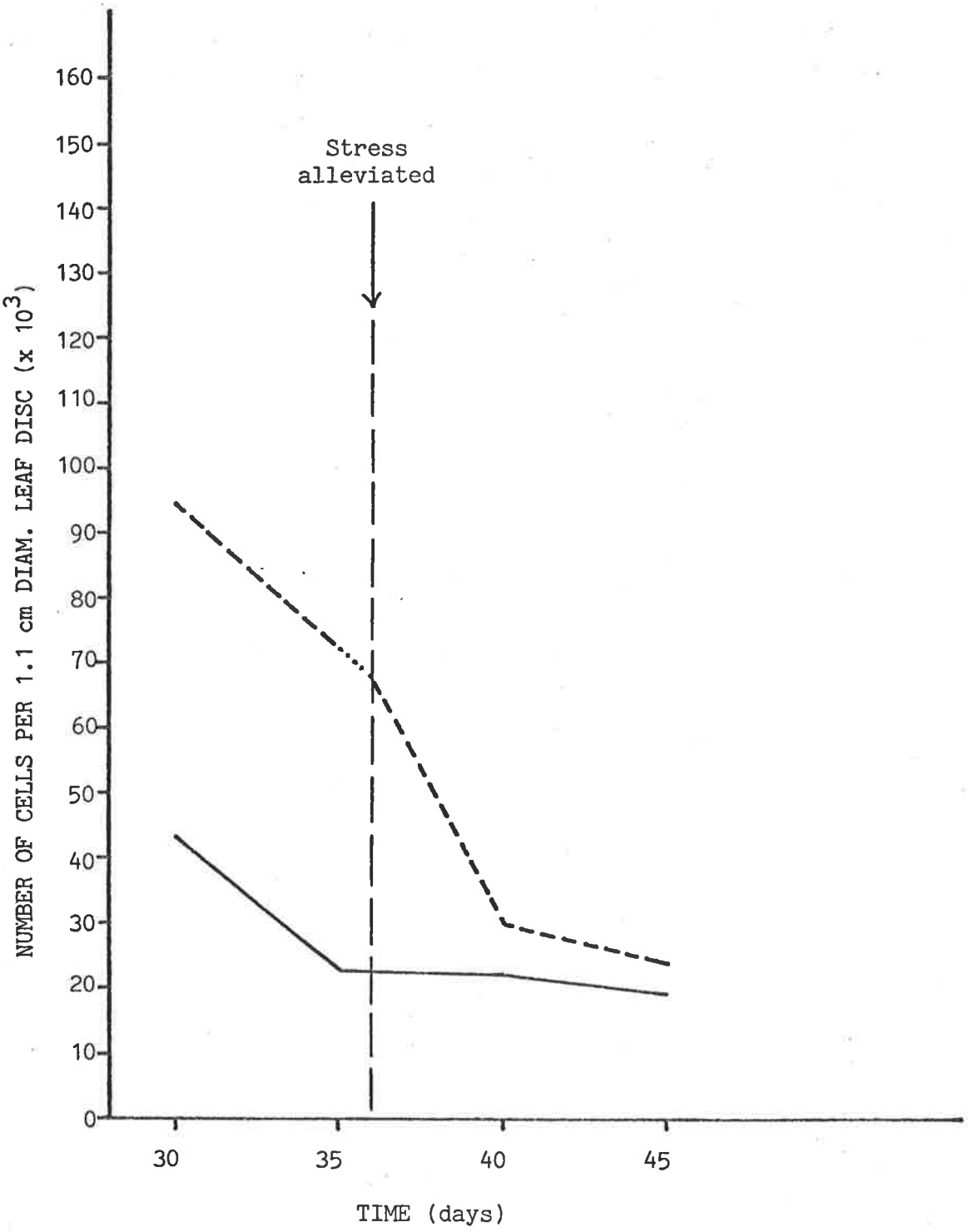

Stress
alleviated
NUMBER OF CELLS PER 1.1 cm DIAM. LEAF DISC (x 10^3)
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
30
35
40
45
TIME (days)

FIGURE 3.5.4

The effect of severe water stress and its alleviation on the fresh and dry weights of Radish shoot tissue.

———— Control

-------- Stressed

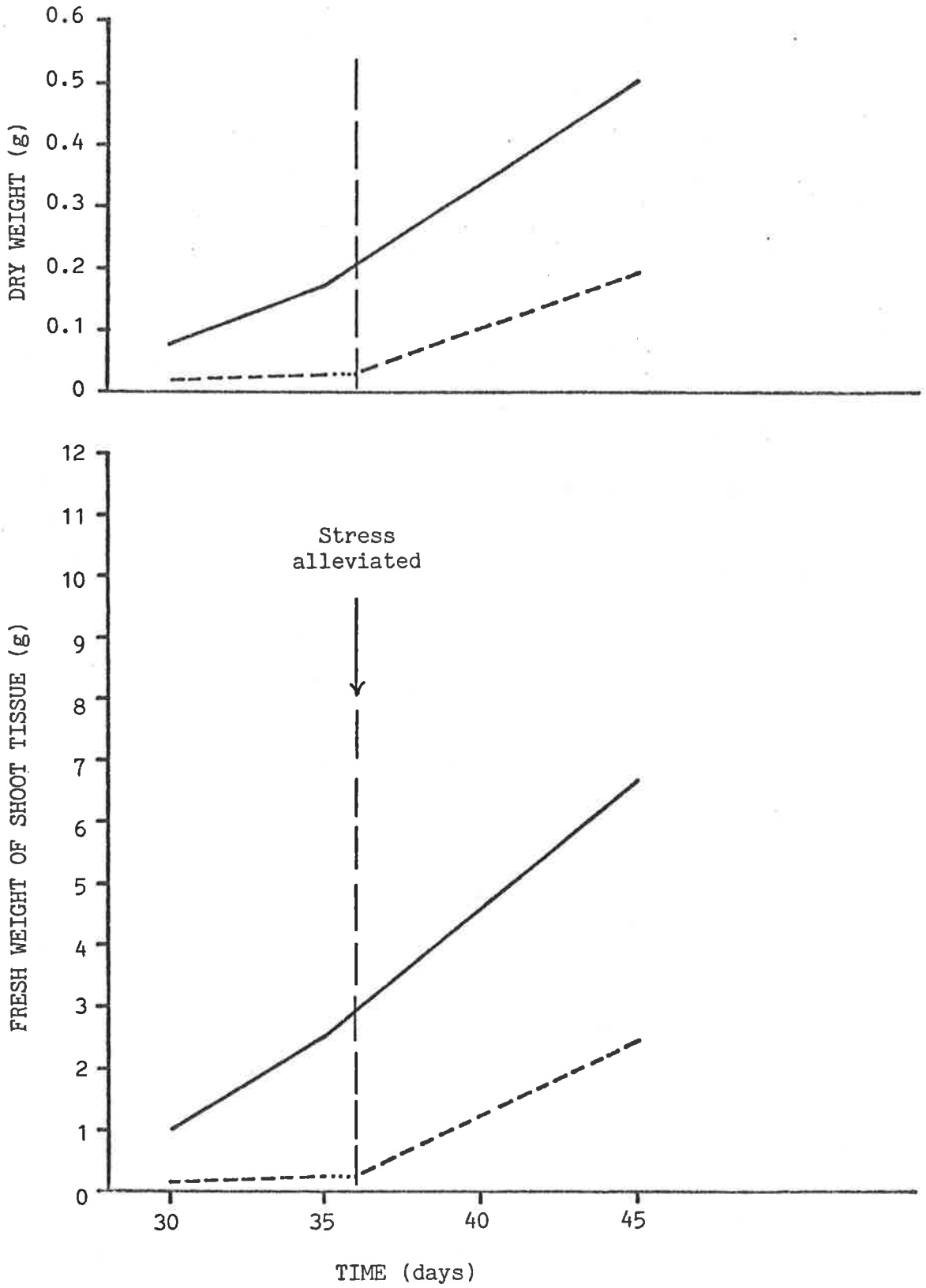
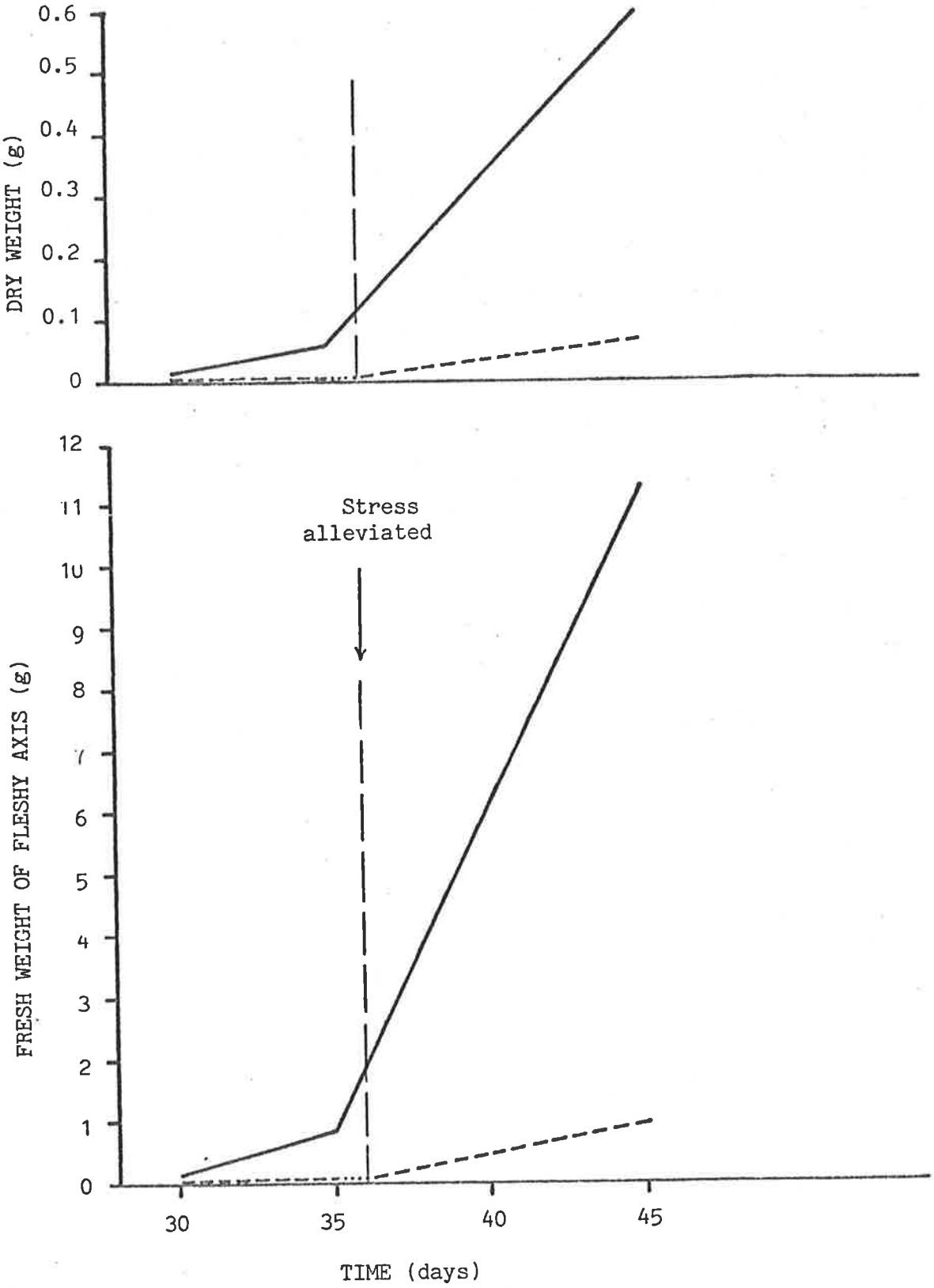

DRY WEIGHT (g)
0.6
0.5
0.4
0.3
0.2
0.1
0
FRESH WEIGHT OF SHOOT TISSUE (g)
12
11
10
9
8
7
6
5
4
3
2
1
0
Stress
alleviated
30
35
40
45
TIME (days)

FIGURE 3.5.5

The effect of severe water stress and its alleviation on the fresh and dry weights of the Radish fleshy axis organ.

———— Control

-------- Stressed


DRY WEIGHT (g)
0.6
0.5
0.4
0.3
0.2
0.1
0
FRESH WEIGHT OF FLESHY AXIS (g)
12
11
10
9
8
7
6
5
4
3
2
1
0
Stress
alleviated
30
35
40
45
TIME (days)

is a globe-type Radish, the effects were most pronounced on diameter and to a slightly lesser extent on volume (table 3.5.1).

In contrast to the immediate increase in cell expansion in shoot tissue upon rewatering (figure 3.5.3), fleshy axis recovery commenced only after a lag period following stress alleviation (figure 3.5.6).

At the anatomical level stress caused significant reductions in cell size in cortex, pericycle, cambium and ray parenchyma tissues (table 3.5.2, see also figure 3.5.7). Similarly cell number was reduced in all tissue types except cortex; as the cortex was formed prior to stress this exception was expected.

Pericycle tissue was considered in greatest detail as it constitutes the most homogeneous, persistent tissue type in the fleshy axis. Cell division in the pericycle was not reduced to the same extent, during the last 5 days of stress, as cell expansion which was virtually halted (figure 3.5.8). The number of cells in the anticlinal plane (i.e. across the pericycle, see figure 3.5.11) stabilized following 40 days of growth without water stress, whereas calculated pericycle cell width on the anticlinal plane continued to increase linearly with time. Stressed fleshy axes developed too slowly to attain the plateau in cell number along the anticlinal plane. This plateau occurs around the time of rapid periclinal cell division in the pericycle, and is probably closely involved with the rapid expansion and division of the ray parenchyma core tissues. After stress was alleviated, cells in the pericycle expanded slowly during a 4 day lag phase and then more rapidly and attained similar cell widths in the anticlinal plane as control pericycle cells. Cell number recovered in a similar fashion but did

FIGURE 3.5.6

The effect of severe water stress and its alleviation on the diameter of the developing Radish fleshy axis.

———— Control

- - - - - - - - - Stressed

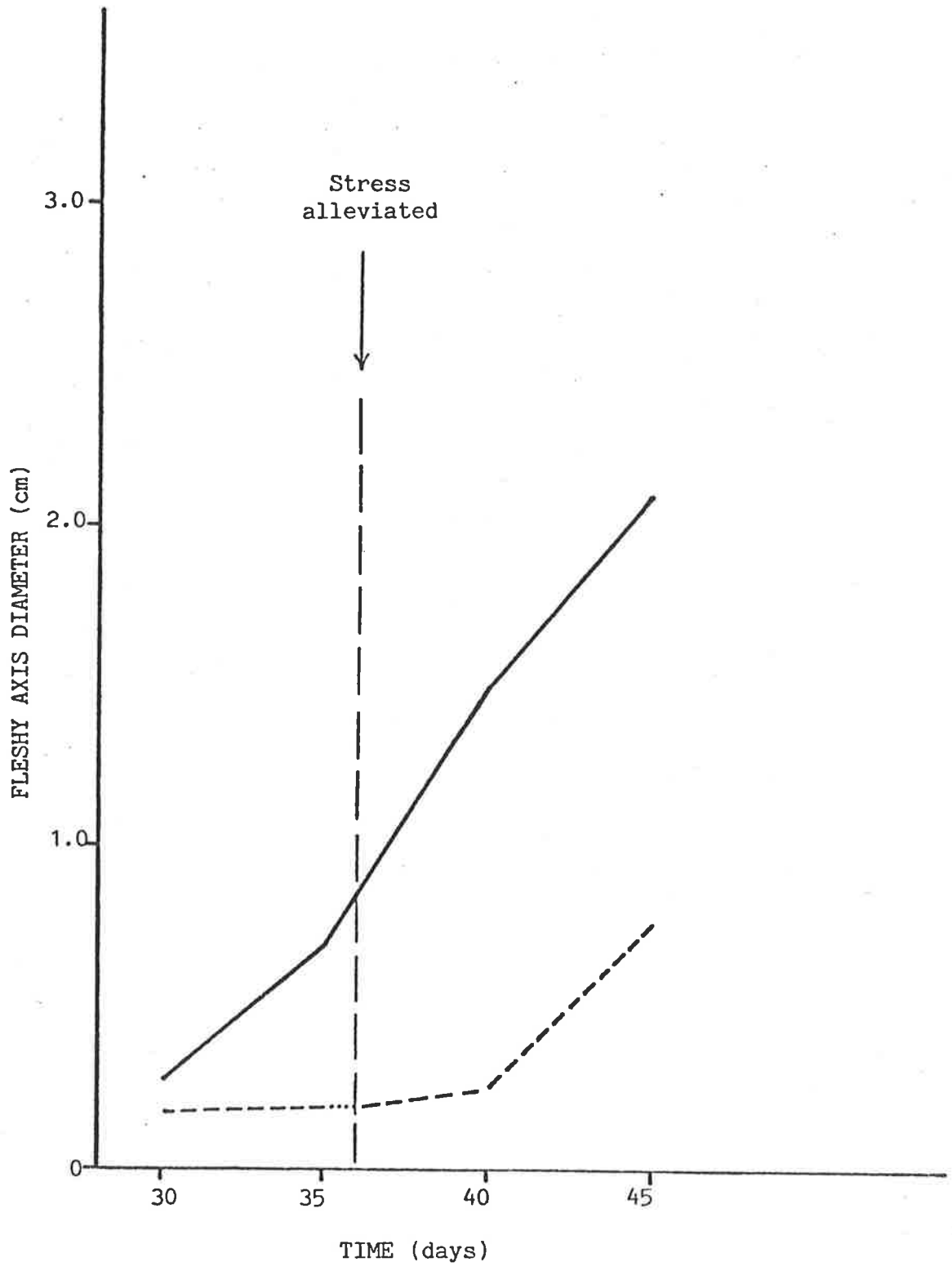

Stress
alleviated
3.0
2.0
1.0
0
FLESHY AXIS DIAMETER (cm)
30
35
40
45
TIME (days)

TABLE 3.5.2

Fleshy axis anatomical data at harvest of stressed, stress alleviated (day 36) and unstressed Radishes sampled at 4 times during the growth period.

Harvest Day	Treatment	Cortex Width (μ)	Number of Cells across Cortex	Calculated Individual Cortex Cell Width (μ)	Pericycle Width (μ)	Number of Cells across Pericycle	Calculated Individual Pericycle Cell Width (μ)	Cambium Width (μ)	Number of Cells across Cambium	Calculated Individual Cambial Cell Width (μ)	Fleshy Axis Diameter (mm)	Number of Cells per 400 μ^2 of Pericycle	Calculated Individual Pericycle Cell Area (μ^2)	Number of Cells per 400 μ^2 of Ray Parenchyma Tissue	Calculated Individual Ray Parenchyma Cell Area (μ^2)	Distance from Endodermis to Centre (μ)	Number of Cells from Endodermis to Centre	Calculated Average Individual Cell Width (μ)
30	Control	186	12	16	100	14	7	27	9	3	3.0	6.8	59	7.0	57	326	52	6
	Stressed	149	11	14	33	7	5	18	7	3	1.9	12.0	33	15.0	27	141	32	4
35	Control	/	/	/	196	26	8	29	8	4	7.3	3.3	121	1.3	308	1228	102	12
	Stressed	/	/	/	54	12	5	15	6	3	1.8	9.0	44	14.9	27	192	42	5
40	Control	/	/	/	314	34	9	27	8	4	16.5	2.1	191	0.6	667	/	/	/
	Stressed	/	/	/	95	17	6	23	8	3	2.2	8.3	48	9.4	43	327	57	6
45	Control	/	/	/	329	32	10	30	7	5	22.8	2.0	200	0.3	1333	/	/	/
	Stressed	/	/	/	271	27	10	30	6	6	9.7	2.8	143	1.0	400	1685	114	15
Treat.x Harvest					*	*	(NS)	(NS)	(NS)	(NS)	*	*	*	*	*	*	*	*
Harvest							*	(NS)	(NS)	*								
Treatment		*	(NS)	*			*	*	*	*								

FIGURE 3.5.7

A comparison of transverse sections (x 85) through the fleshy axes of a Radish severely droughted over 20 days and an unstressed control.

1. Unstressed
2. Stressed

A. Cortex
B. Endodermis
C. Pericycle
D. Cambium
E. Parenchyma tissue
F. Xylem Elements

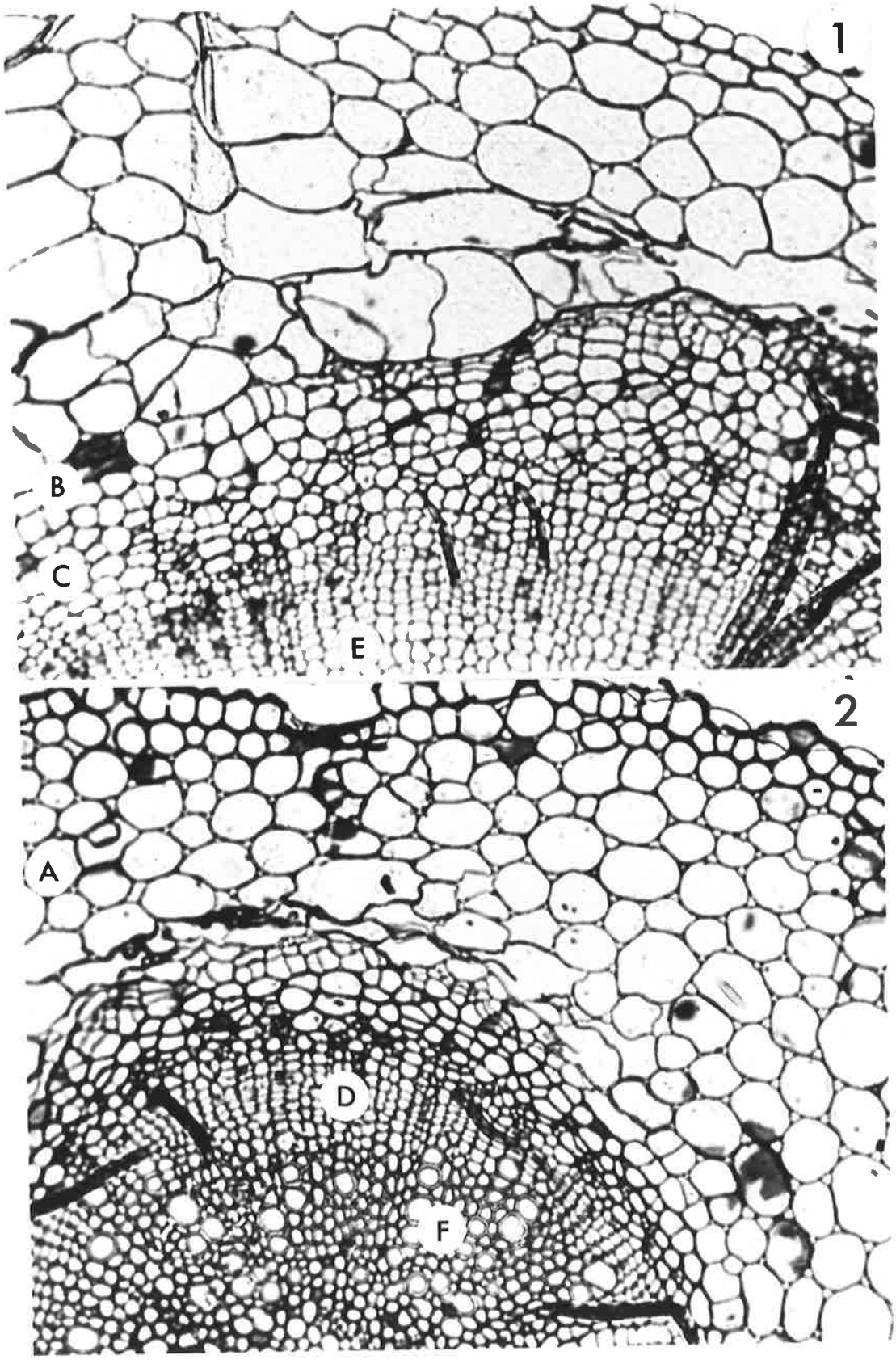
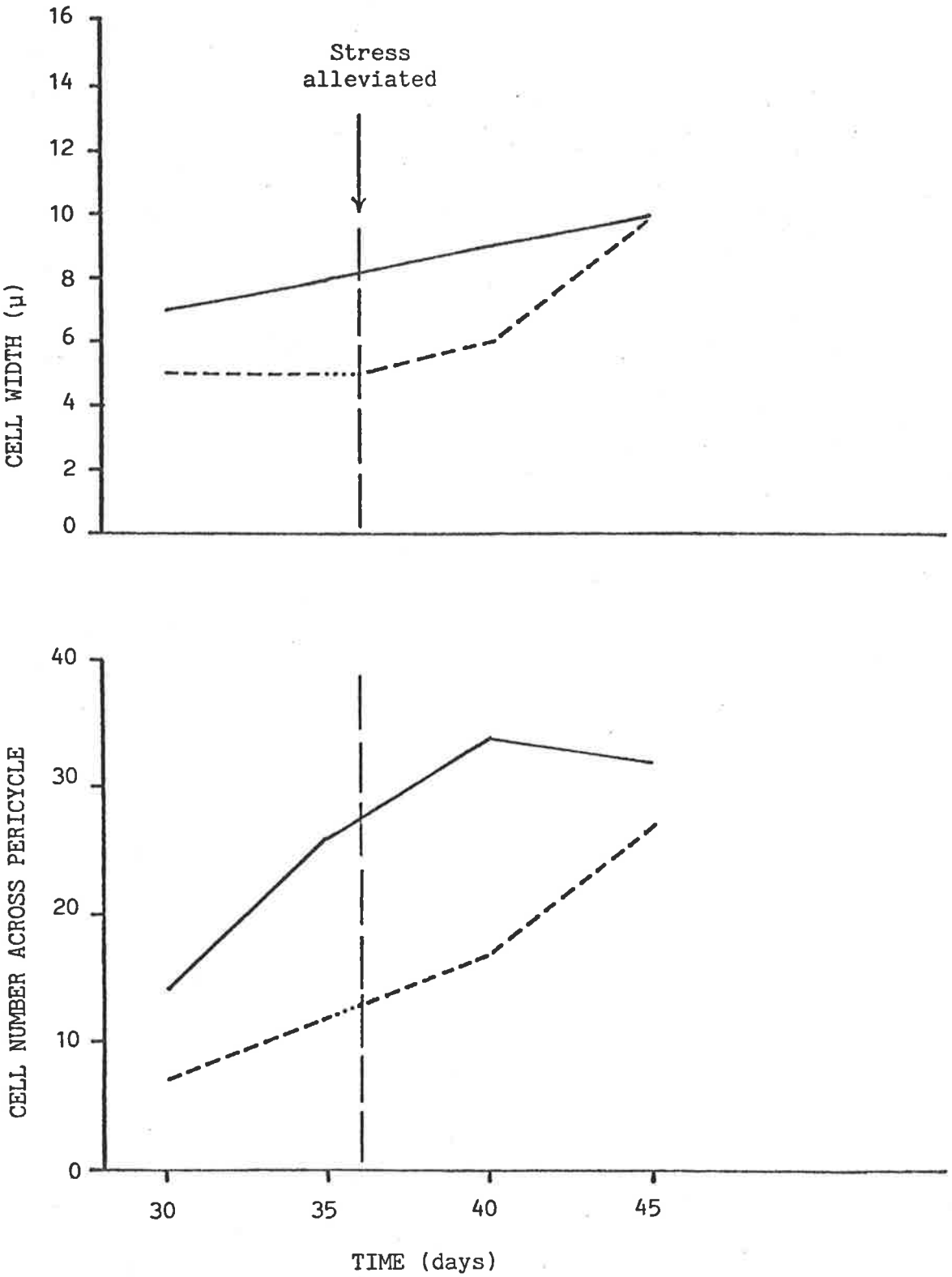

1
B
C
E
2
A
D
F

FIGURE 3.5.8

The effect of severe water stress and its alleviation on Radish fleshy axis average pericycle cell width (anticlinal plane) and the number of cells across the pericycle.

———— Control

--------- Stressed


Stress
alleviated
CELL WIDTH (μ)
16
14
12
10
8
6
4
2
0
CELL NUMBER ACROSS PERICYCLE
40
30
20
10
0
30
35
40
45
TIME (days)

not fully recover to control levels during the period of the experiment. Hence, reduced cell division was largely responsible for the difference between control and stress pericycle widths (anticlinal plane) recorded 9 days after stress alleviation (figure 3.5.9). Use of anticlinal pericycle cell width as an indicator of cell expansion (figure 3.5.8) was valid as the mean pericycle cell area, estimated from cell counts per unit area (400 μ^2), was almost identical (figure 3.5.10). However, unlike pericycle cell width, cell area was not shown to recover completely to the control level in the 9 days after stress alleviation. This difference is attributable to an unrealised potential for further expansion in the periclinal plane.

The ray tissues occupy the major portion of the cross sectional area of the expanding Radish axis and the proportion occupied increases with size. Data collected on transects from the axis centre to the endodermis represented primarily this predominant ray tissue. In comparison to control, stress reduced both cell size and cell number (figure 3.5.11) but these were shown to increase at a slow rate during the late 5 days of stress (figure 3.5.12). After stress alleviation, as with the pericycle tissue, a lag phase ensued during which little recovery occurred (figure 3.5.13), to be followed by rapid recovery in the following 5 days (figure 3.5.14). Ray parenchyma cell size in stressed plants did not equal that of the control plants during the period investigated (figure 3.5.15), however.

3.5.4 Discussion

Radish plants subjected to an extended period of severe stress which was then alleviated for 9 days yielded less than 10% of the control fleshy axis fresh weight and less than 40% of the control shoot fresh weight. The overall plant yield reduction was ca. 80% of the control

FIGURE 3.5.9

The effect of severe water stress and its alleviation on Radish fleshy axis pericycle width.

———— Control

-------- Stressed

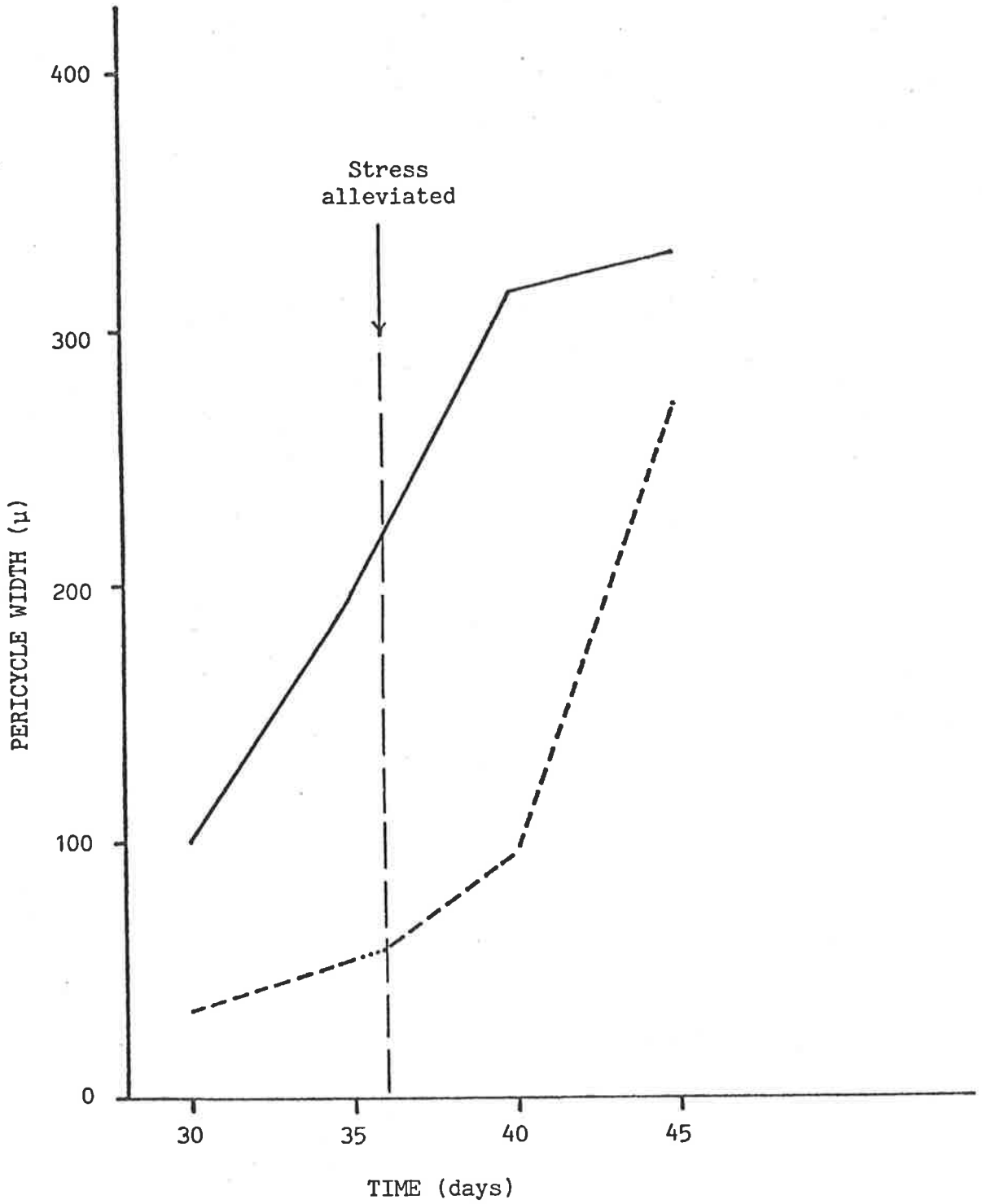
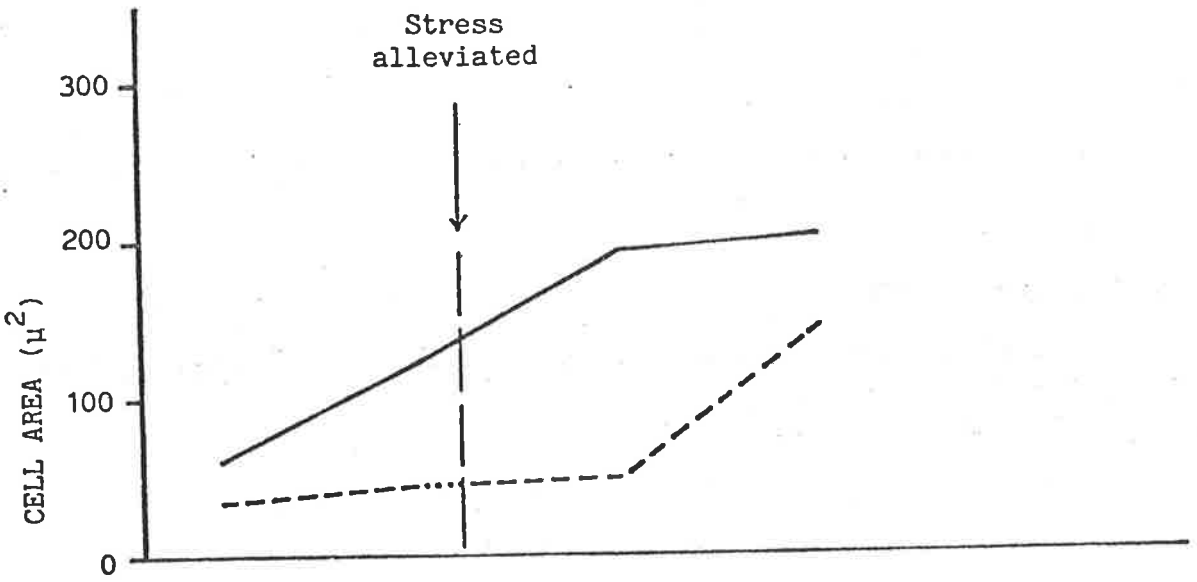

400
300
200
100
0
PERICYCLE WIDTH (μ)
Stress
alleviated
30
35
40
45
TIME (days)

FIGURE 3.5.10

The effect of severe water stress and its alleviation on the number of pericycle cells per unit area and the average pericycle cell area in the pericycle of the Radish fleshy axis.

———— Control

-------- Stressed


Stress
alleviated
300
200
100
0
CELL AREA (μ²)

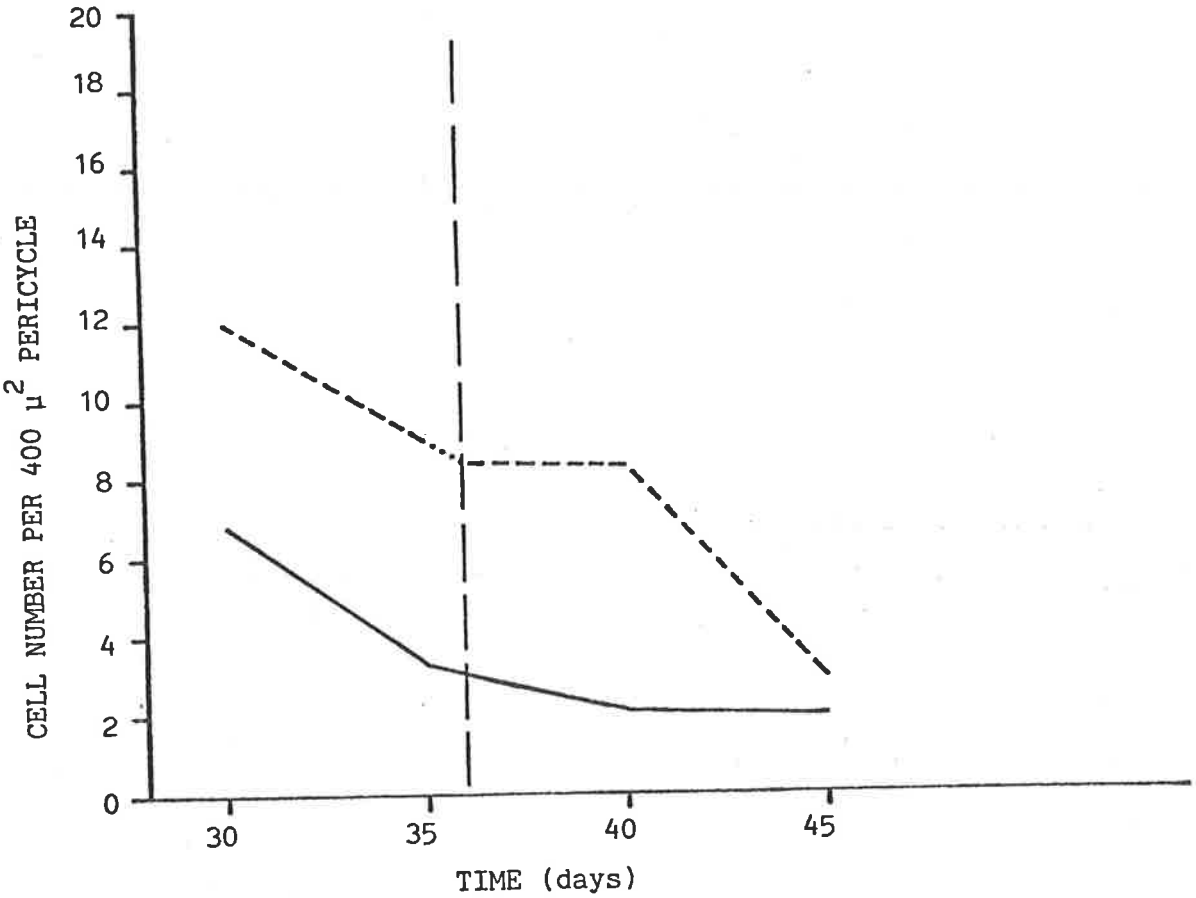

20
18
16
14
12
10
8
6
4
2
0
CELL NUMBER PER 400 μ² PERICYCLE
30
35
40
45
TIME (days)

FIGURE 3.5.11

A comparison of transverse sections (x 85) through the fleshy axes of a Radish severely droughted over 25 days and an unstressed control.

1. Unstressed
2. Stressed

A. Anticlinal plane
B. Periclinal plane

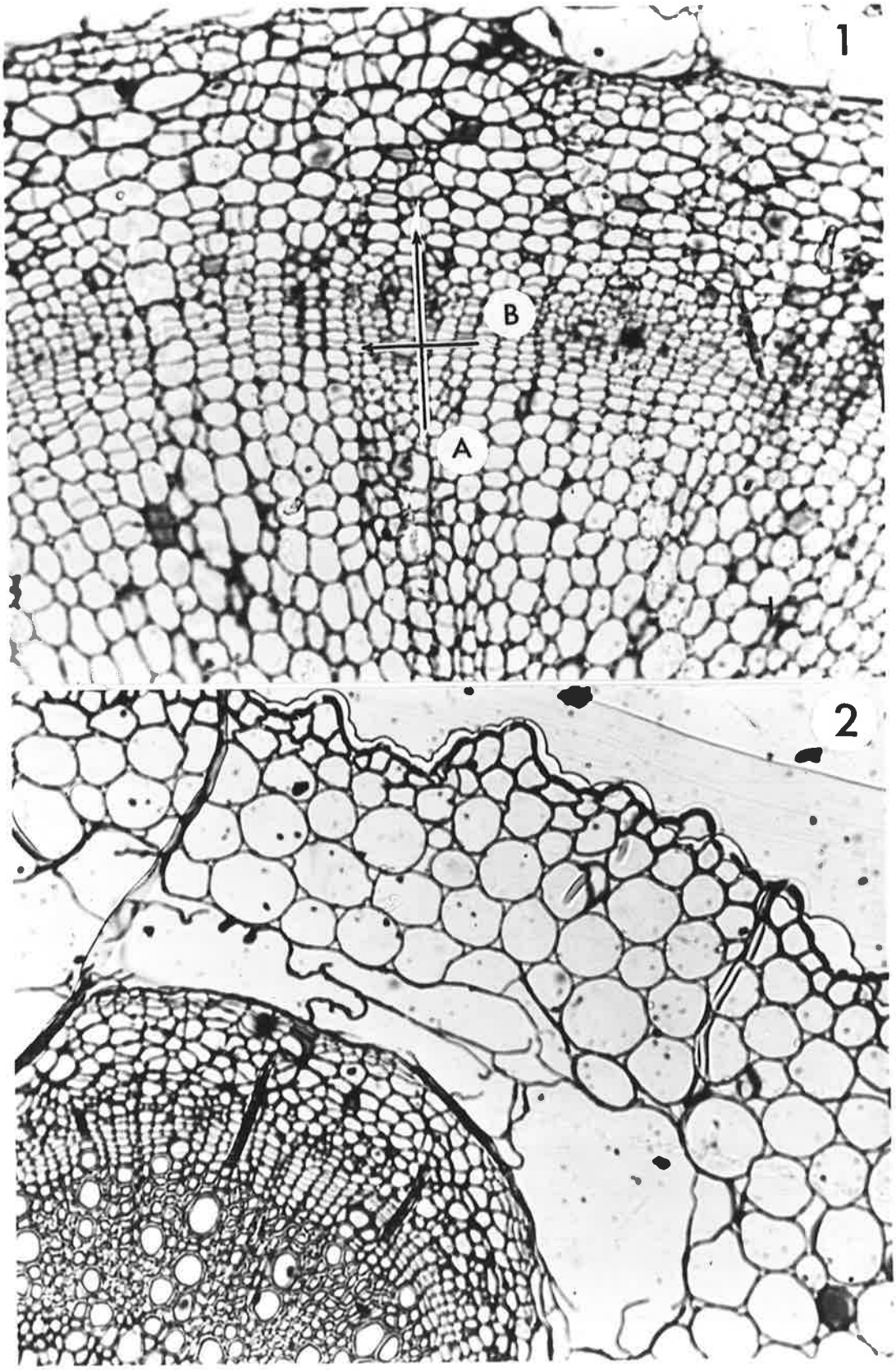

1
B
A
2

FIGURE 3.5.12

The effect of severe water stress and its alleviation on the distance, the cell number, and the average cell width along a transect from the centre of the Radish fleshy axis to its "endodermis".

———— Control

– – – – – – – – Stressed

• Distance from centre to "endodermis" (μ)

x Average cell width (μ)

⊙ Number of cells from centre to endodermis

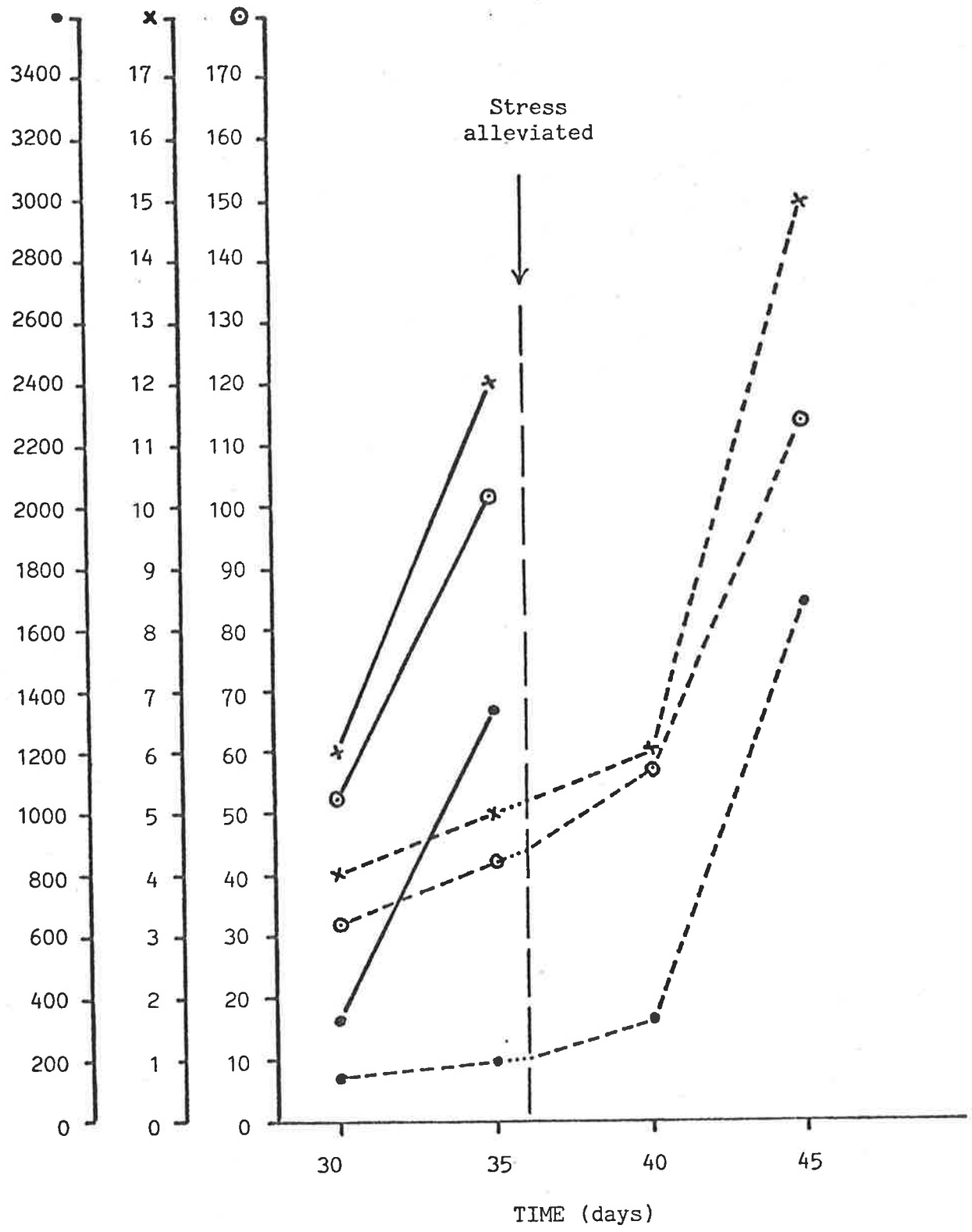
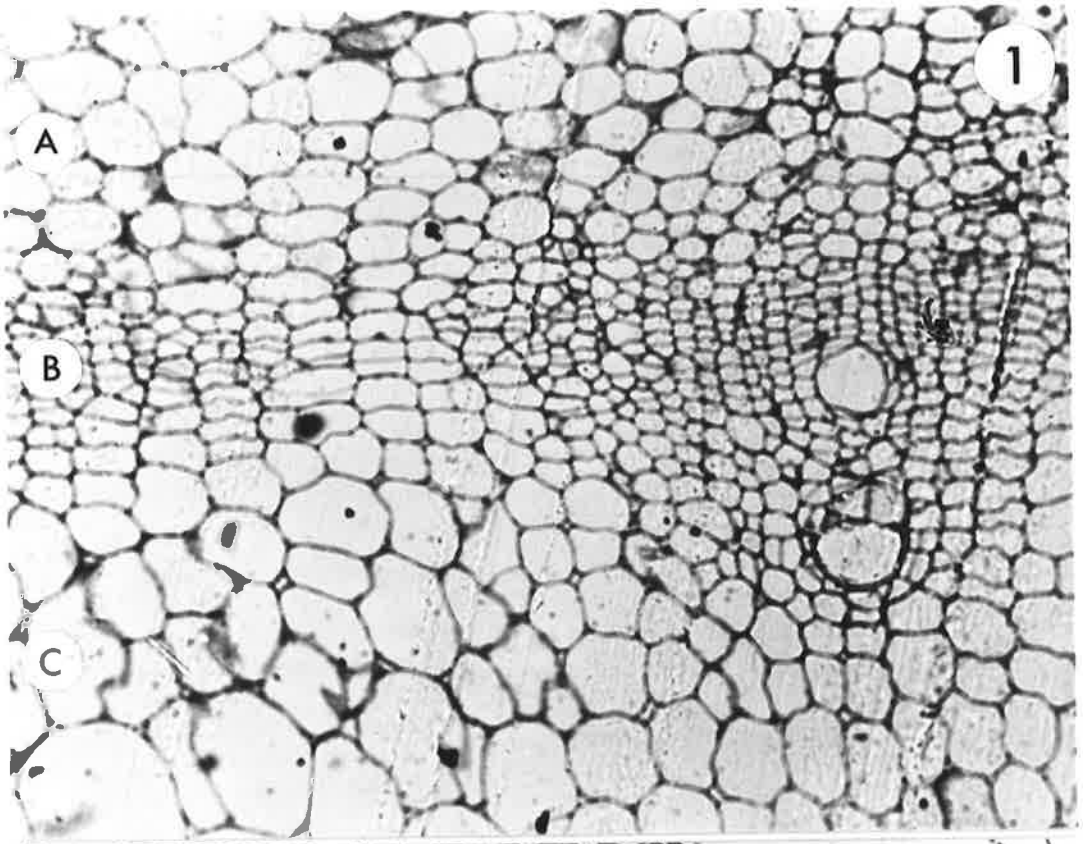

3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Stress
alleviated
30
35
40
45
TIME (days)

FIGURE 3.5.13

A comparison of transverse sections (x 85) through the fleshy axes of a Radish alleviated for 4 days after 36 days of drought and an unstressed control.

1. Unstressed
2. Stressed

A. Pericycle
B. Cambium
C. Parenchyma (Ray)


1
A
B
C

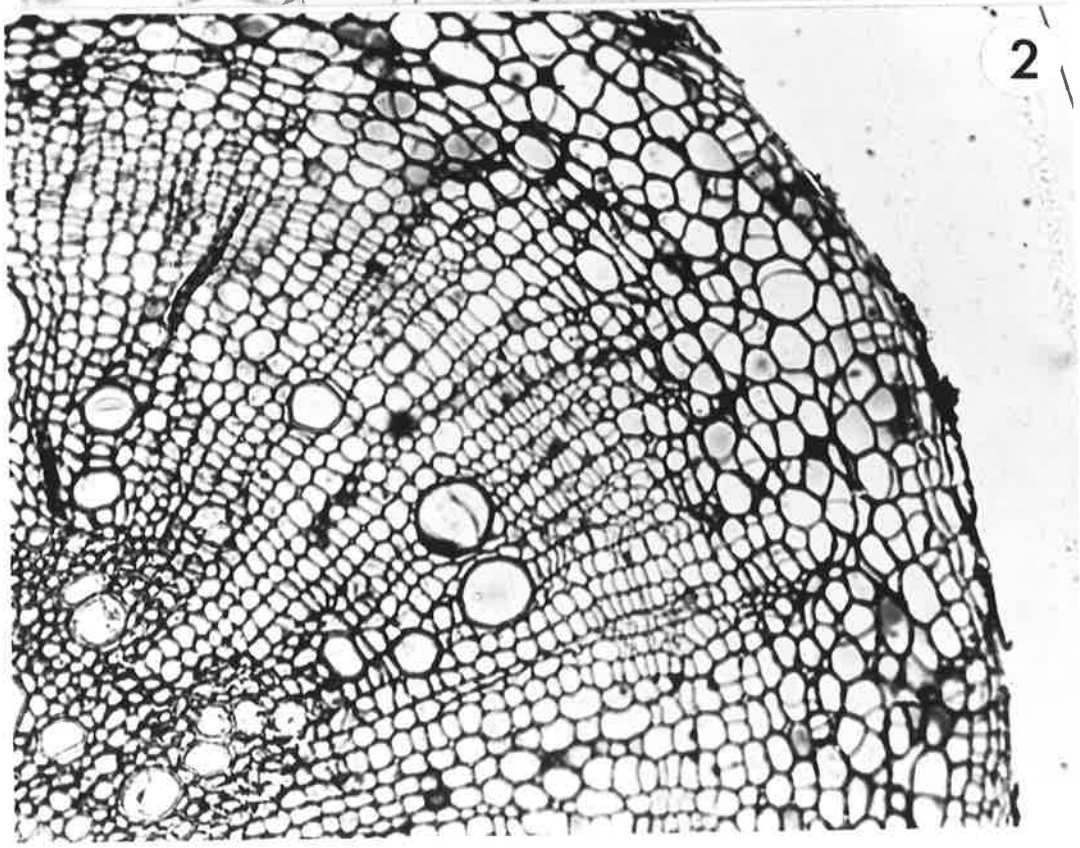
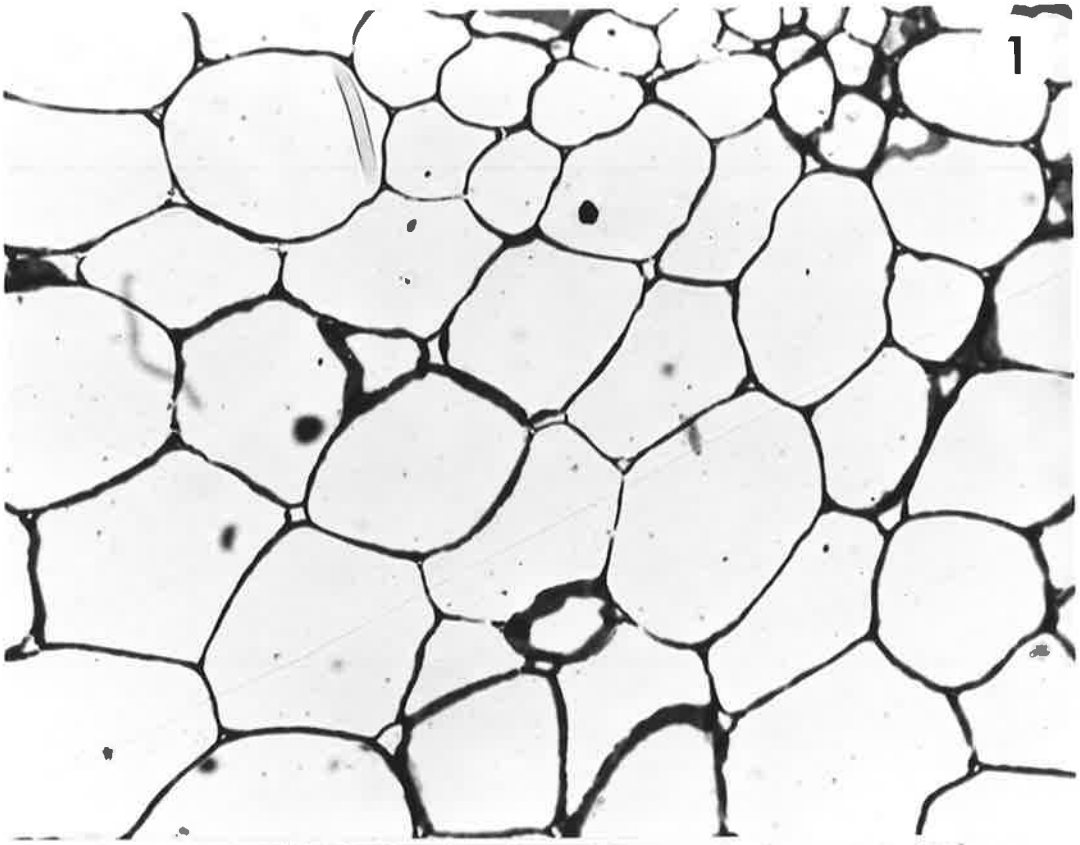

2

FIGURE 3.5.14

A comparison of ray parenchyma tissues (x 85) from the fleshy axes of a Radish alleviated for 9 days after 36 days of drought and an unstressed control.

1. Unstressed
2. Stressed


1

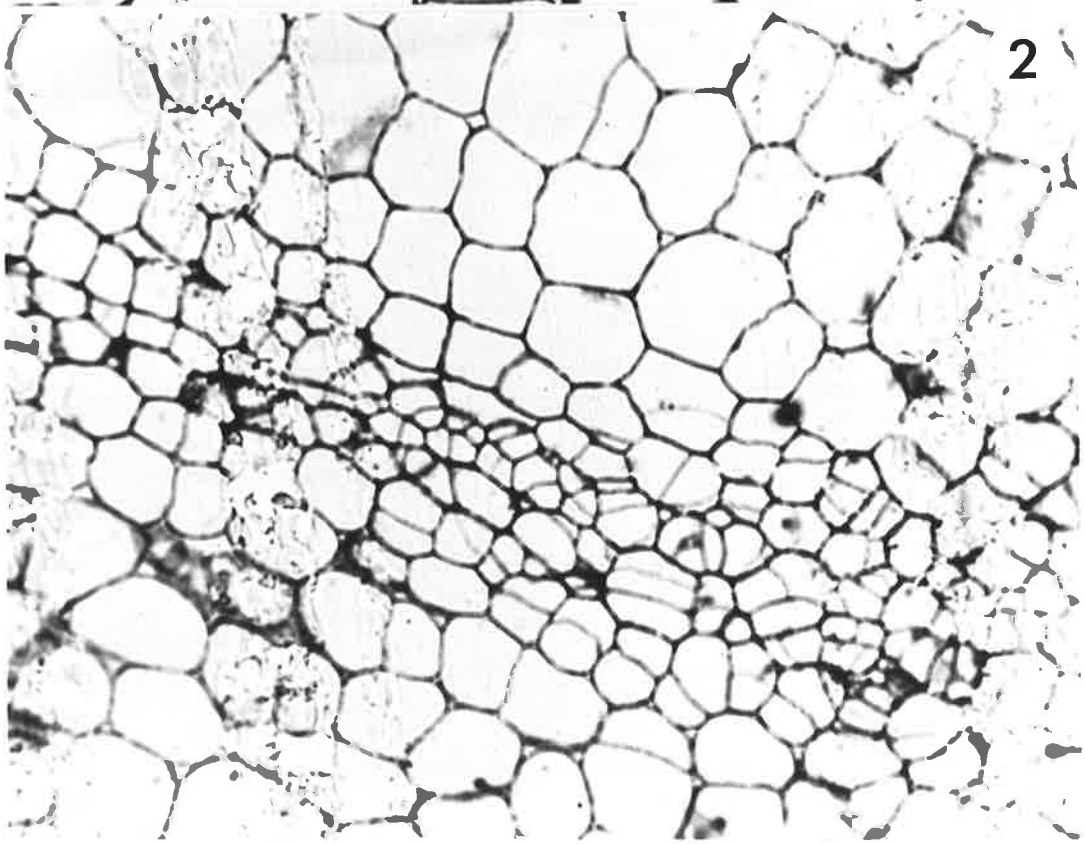
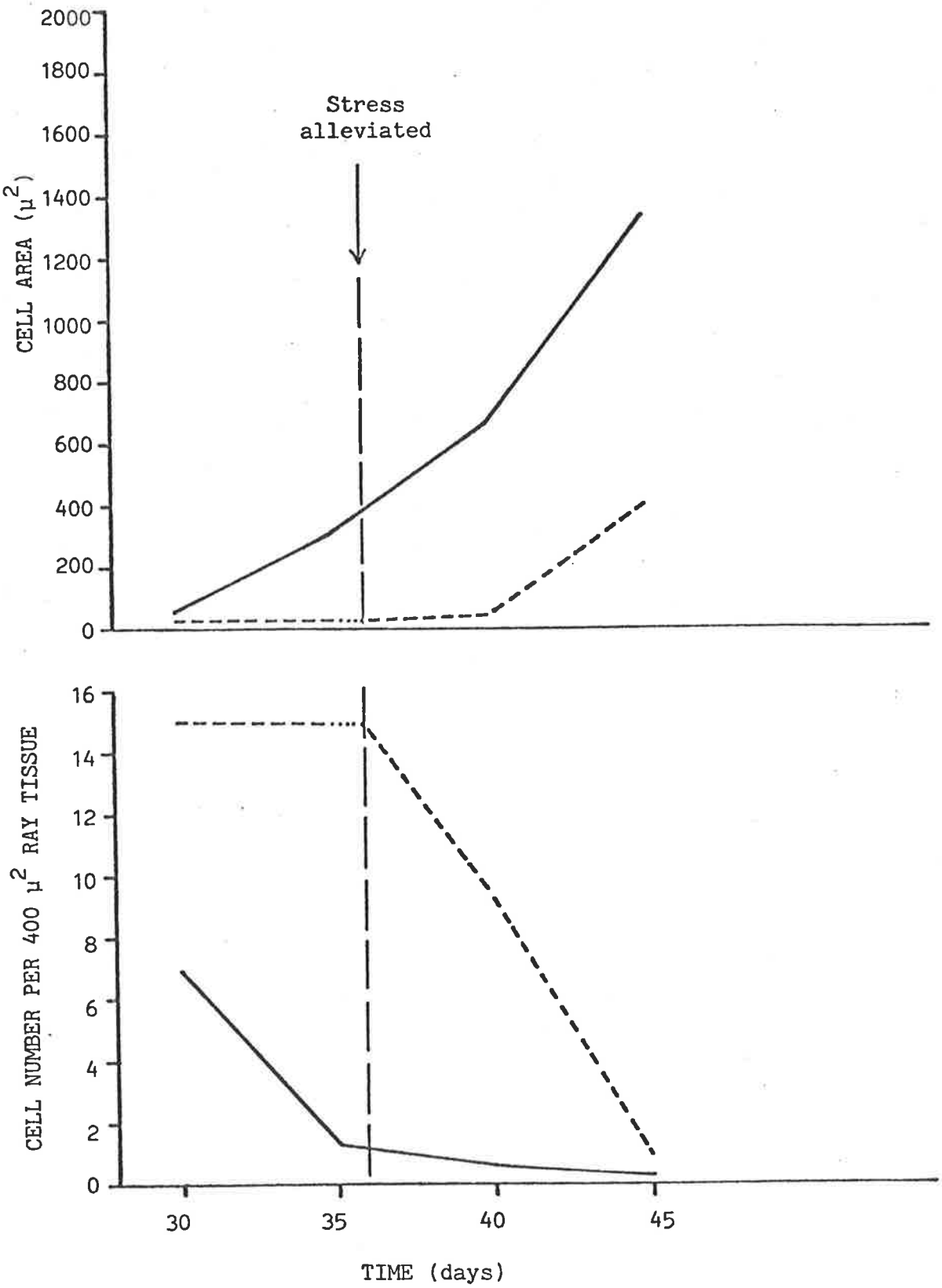

2

FIGURE 3.5.15

The effect of severe water stress and its alleviation on the number of ray parenchyma cells per unit area and the average parenchyma cell area in ray tissue of the Radish fleshy axis.

———— Control

-------- Stressed


2000
1800
1600
1400
1200
1000
800
600
400
200
0
CELL AREA (μ^2)
Stress
alleviated
16
14
12
10
8
6
4
2
0
CELL NUMBER PER 400 μ^2 RAY TISSUE
30
35
40
45
TIME (days)

yield. The long term reduction in shoot yield was apparently centred on cell division, but cell expansion was also reduced. Cell division, although proceeding at a slow rate during the last stages of stress, was reduced sufficiently to cause a reduction in the number of leaf primordia which were present and expanded following stress alleviation. In a like manner the number of cells within any leaf was reduced. Cell expansion in the leaves, recorded during the last 5 days of stress, occurred at a rate similar to the control rate. Prior to this, however, stress had effected a large reduction in cell size. On stress alleviation cell expansion was rapid, and leaf area expansion and leaf production (through the expansion of existing primordia) occurred at rates exceeding those of the control plants. A similar response to stress and its release has been proposed to explain the increase in leaf elongation rate above control levels following stress alleviation in *Panicum* (Ludlow and Ng, 1977). Incomplete length recovery in comparison with controls was attributed to the reduced rates of cell division during stress. The leaf area of Field Beans subjected to drought has been shown to be reduced mainly by mechanisms determining leaf size rather than those associated with leaf production or unfolding (Karamanos, 1978). This conclusion can also be drawn from the present experiment. During stress Radish leaf area was reduced to 10% of the control and after stress alleviation increased, but to only 40% of the control. In comparison, leaf number was reduced to only 55% of the control during stress, and, more importantly, recovered to 80% of the control after stress alleviation. Unfortunately, in the Field Bean study no attempt was made to describe the results in terms of cell expansion and division. However, it has been generally acknowledged that, during stress, cell expansion is the most sensitive leaf growth process as well as being the process most responsive to improvement of plant water status (Acevado *et al.*, 1971; Sepaskhah and Boersma, 1979; Barlow and Boersma, 1972).

The fact that leaf cell size during stress, increased during the later stages of stress at a rate similar to the control, indicates adaptation to the stress environment. An increase in the osmotic potential of expressed sap was noted during the stress period and implied a degree of osmotic adjustment. In a similar vein, it was reported in the Field Bean study (discussed above) that threshold values of leaf water potential at which the rates of leaf production and unfolding declined were more negative in a drier environment. In view of the preceding discussion it can be concluded that short term reduction in plant leaf area is attributable to the extreme sensitivity of cell expansion to water stress. The long term and largely irreversible reduction in leaf area is due primarily to the effect of stress on cell division.

The effect of stress on cell division and cell expansion processes in the fleshy axis is more complicated than in shoot growth due to the physical constraints on the growth of various constituent tissue types. This may in part explain the apparently greater sensitivity to stress of fleshy axis growth, compared with shoot growth. Stress reduced both cell expansion and cell division in the major fleshy axis tissues (pericycle and parenchyma). In the pericycle on the anticlinal plane, cell expansion was more sensitive to stress than cell division, but, as was the case for shoot growth, reduced cell division during stress appeared to offer the primary limitation to fleshy axis expansion. As growth in the ray parenchyma region is largely dependant upon the growth of the pericycle to provide 'room' for development these differences between division and expansion were not so obvious. During the last 5 days of stress cell width and cell number, in the anticlinal plane of the ray parenchyma region, both increased by ca. 20% indicating similar effects of stress on both processes. On the basis of these data it is

concluded that in the Radish fleshy axis, and the shoot, cell expansion is comparatively more sensitive to stress and more responsive to stress alleviation than cell division. Reduction in the latter process, however, is primarily responsible for long term yield reductions following drought periods.

3.6 Distribution of assimilated ^{14}C and the Sugar content of Stressed and Unstressed Radish plants

3.6.1 Introduction

The previous experiment demonstrated that young Radish plants were able to survive prolonged, severe water stress and to recover apparently undamaged. Growth was effectively suspended during the period of severe stress and it was of interest to examine the carbon balance of plants during that period, the distribution of any assimilated carbon among the plant organs and to ascertain whether metabolites accumulated and resulted in osmotic regulation in the stressed tissues.

3.6.2 Methods

A system of plant culture was required in which many plants could be grown which were similar to those investigated in the previous experiment (Section 3.5). Preliminary investigation led to the adoption of a culture system in which plants were grown in vermiculite and allowed to dry out over a number of days.

The experiment was conducted in a controlled environment. Temperature was maintained at $20^{o}C$, photoperiod 12 hr and light flux density 380 $\mu ein/cm^{2}/sec$ at canopy level. Relative humidity, monitored by hygrograph, had a daily mean of ca. 60%.

On day 1 surface sterilized medium grade seeds, cv. 'Mars', were placed into petri dishes lined with filter paper soaked in distilled water. These were wrapped in Alfoil and placed in the growth cabinet for 48 hr. These pregerminated seeds were then sown at a depth of 1 cm and a rate of 40 seedlings per pot (15 cm square surface area, 11.5 cm deep plastic pot) in vermiculite. The seedlings were thinned on day 10 to retain the 30 most uniform. The two treatments (control and stressed) were replicated 4 times and the whole experiment was duplicated to give a total of 16 pots. Up to day 10, all the pots were stood in aluminium

trays holding 2 pots (the duplicate pair) and containing half-strength Hoagland's solution to a depth of 2 cm. The solution was topped up daily and renewed weekly. Black plastic was fitted around the pots over the exposed solution surface to reduce evaporation and prevent algal growth. The pots which were to be subjected to water stress were removed from the trays on day 10 and allowed to dry down for 20 days. As a result, these plants had experienced a considerable water deficit by harvest (day 30) although they did not wilt markedly.

Forty-eight hours before the plants were harvested (day 28) the pots in one duplicate set were removed to an identical environment. Two replicates each of control and stressed pots (4 in all) at a time were then placed into a 100 l perspex chamber (CO_2 concentration of 64 mg) and exposed to 500 µci of $^{14}CO_2$ ($^{14}CO_2$ concentration of 0.395 mg) for 1 hr. The pots were then returned to their original environment until harvest.

On harvest, plants from $^{14}CO_2$ treated pots were divided into leaves, cotyledons, hypocotyls and roots. The aggregated material for each organ was immediately frozen in liquid nitrogen and then stored at -20°C. When the harvest was completed the frozen tissue was freeze dried for 72 hours and returned to -20°C for storage. Plants from each pot (30 per pot) were divided into groups of 10 and separated into leaves, cotyledons and hypocotyls. One group was used to assess growth and the other 2 groups treated as for the plants harvested from the $^{14}CO_2$ treated pots.

The plants used to measure growth were divided into 6 plants for weight and area data and 1 plant for each of leaf and cotyledon relative water content, leaf osmotic potential, leaf and cotyledon cell counts, and slide preparation for the collection of fleshy axis anatomical data.

The stored tissues from the $^{14}CO_2$ treated set were later finely ground and a 20 mg sub-sample from each tissue was used for the estimation of ^{14}C content and sugar concentration. These samples were used to measure radioactivity (c.p.m.) in crude ethanol extracts and the ethanol insoluble residues, the total soluble sugar concentration, and radioactivity in sucrose, glucose and fructose fractions together with their concentrations.

Free proline was estimated with material drawn from the set not treated with $^{14}CO_2$.

3.6.3 Results

As before, water stress resulted in a considerable reduction in growth (figure 3.6.1). The most noticeable reduction was that of leaf fresh weight, which was reduced by around 80% (table 3.6.1). Leaf dry weight was similarly reduced by stress, however, no significant reductions in cotyledon and hypocotyl dry weights were observed (tables 3.6.1 and 3.6.4) despite indications of a reduction in their fresh weight (table 3.6.1). This perhaps indicates that there was a trend to decreased fresh : dry weight ratios in these organs. Leaf relative water content was significantly reduced by stress, but only by 5%. There was a small but non-significant increase in leaf cell sap osmotic potential, but there were significant increases in leaf sugar concentration (see later). Although the total photosynthetic area was significantly reduced by stress, lack of significant difference in leaf and cotyledon numbers indicated that the reduction in individual leaf area was the primary stress effect. This area reduction was due largely to reduced cell size in the leaves (table 3.6.1).

Water stress did not cause a significant decrease in either cell size or cell number in the Radish hypocotyl (table 3.6.2). However, a consistent trend for a reduction in both these parameters was indicated

FIGURE 3.6.1

Comparison of Radish plants grown under control conditions (LHS) with plants having experienced a 20 day 'soil'-water depletion cycle (RHS).

TABLE 3.6.1

The effect of a 20 day 'soil'-water depletion cycle on the growth and water relations of Radish.

Organ	Fresh Weight (g)		Dry Weight (mg)		Moisture Content (%)		Photosynthetic Area (cm^2)		Number		Relative Turgidity (%)		Osmotic Potential (-bars)		Number of Cells/1.1 cm disc (x 10^3)	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Leaves	1.28	0.24	95	32	93	87	33.8	7.3	3.3	2.5	92	87	7.4	8.5	31.7	60.5
Cotyledons	0.37	0.20	17	17	96	93	7.3	4.2	2.0	1.7	96	93	/	/	12.3	16.1
Hypocotyl	0.18	0.10	13	10	93	88	/	/	/	/	/	/	/	/	/	/
(*) LSD	0.894		7		0.73		27		(NS)		1.7		(NS)		4.5	

TABLE 3.6.2

The effect of a 20 day 'soil'-water depletion cycle on Radish hypocotyl (fleshy axis) anatomy.

Treatment	Cortex Width (μ)	Cell Number across Cortex	Pericycle Width (μ)	Cell Number across Pericycle	Cambium Width (μ)	Cell Number across Cambium	Hypocotyl Diameter (mm)	Number of Pericycle Cells/ 400 μ^2	Number of Parenchyma Cells/ 400 μ^2	Distance from Endodermis to Centre (μ)	Number of Cells from Endodermis to Centre
Control	175	8.5	82.5	13.6	30.6	9.3	3.2	7.5	6.5	423	64.6
Stress	155	9.6	51.9	10.5	13.1	6.0	2.0	15.0	17.8	206	43.1
(*) LSD	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)

by the data collected from each of the tissue types comprising the young fleshy axis. Stress resulted in thickening of the cell walls of the hypocotyl parenchyma cells (table 3.6.3). This effect is clearly demonstrated in figure 3.6.2, where stressed tissue stained with toluidine blue gave a blue reaction in comparison to purple in the same region of unstressed tissue. Toluidine blue reacts with lignin to yield a blue color.

The data discussed thus far has demonstrated that the growth and morphology of the plants were affected by the water stress imposed in this experiment, and that the responses closely resembled those observed in the previous experiment (Section 3.5) where stress was imposed continuously over an extended period. Data obtained from supplying plants with $^{14}CO_2$ in the present experiment can therefore be assumed to typify this extended stress situation.

The major difference between control and stressed plants lay in the reduced leaf dry weight (table 3.6.4). Root dry weight could not be accurately measured because of binding of small roots to the vermiculite medium. The root systems of the stressed plants were observed to be more extensive, and individual roots thinner, than those of control plants. Assuming that the control and stressed roots accumulated similar total amounts of radioactivity, the relative photosynthetic rate on a leaf area basis can be calculated for the two treatments. Control and stressed plants had photosynthetic areas of 41 and 12 cm^2 respectively (table 3.6.1) and had total ^{14}C counts (excluding roots, table 3.6.4) of 3521 and 1297; thus, respectively they fixed 86 and 108 c.p.m. per cm^2. It would appear, from this calculation, that the rate of photosynthesis per unit leaf area (even allowing for possible greater total counts in the roots of control plants) was relatively unaffected by stress; assimilation therefore being restricted by the reduced leaf expansion in stressed plants.

TABLE 3.6.3

Ray parenchyma cell wall thickness in the hypocotyl (fleshy axis) of control and water stressed Radish.

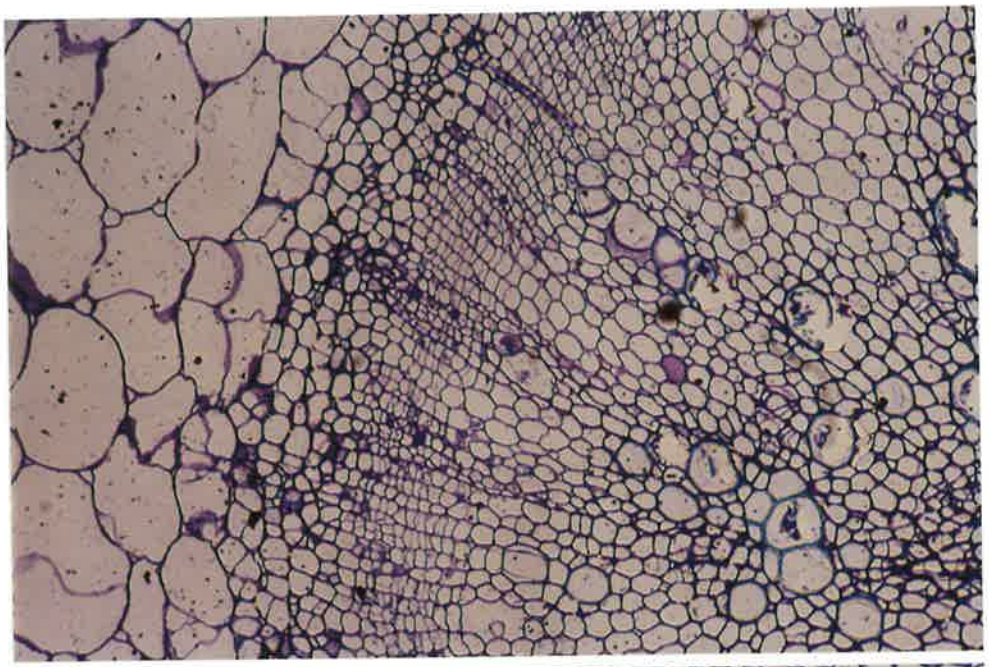
Replication	Cell wall thickness (μ)	
	Control	Stressed
I	0.625	0.675
II	0.600	0.788
III	0.488	0.713
IV	0.588	0.763
Mean (*)	0.575	0.735

FIGURE 3.6.2

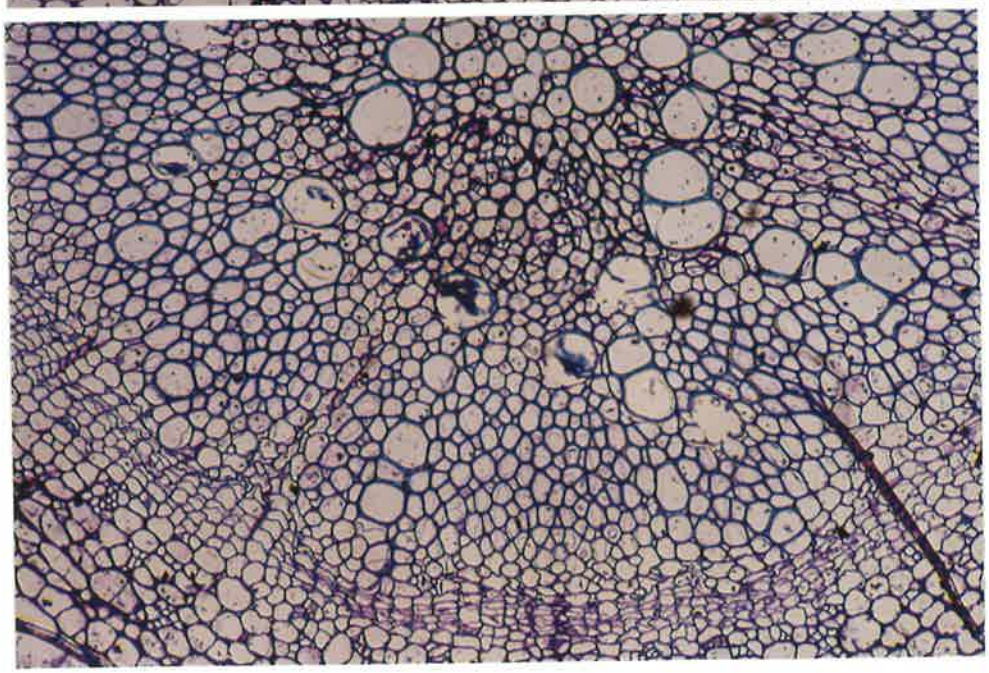
Comparison of transverse sections (X 85) through Radish fleshy axes, of similar diameter, from a control plant and a plant having experienced a 20 day 'soil'-water depletion cycle.

A. Control

B. Stressed



A



B

Analysis of the proportioning of radioactivity between alcohol soluble and alcohol insoluble fractions of the various plant organs (table 3.6.4) revealed that control and stressed tissues had a similar distribution (24-26% in the soluble fraction of leaves for example). This shows that a major imbalance, as may be expected through massive accumulation of organic solutes, did not occur under water stress at least during the 48 hours prior to harvest.

If the total radioactivity in the leaves, cotyledons and hypocotyls is made equal to 100% (table 3.6.5), the ^{14}C distribution between these organs can be assessed. Stress led to significantly higher portion of the total radioactivity being located in the cotyledons and hypocotyls of plants. This trend was similar for both the soluble and insoluble fractions, and, resulted in a lower portion of the total plant radioactivity being found in the leaves of stressed plants than in those of control plants. These differences could reflect differences in translocation or in metabolism. Assimilated ^{14}C was necessarily used for leaf growth in the control plants, whilst the observation of a higher proportion of total plant ^{14}C in the cotyledons and hypocotyls of stressed plants suggests the assimilated carbon is retained in the former (which have largely ceased expansion growth by this age) and transported into the latter.

Both in the alcohol soluble and insoluble fractions radioactivity on a dry weight basis was higher in the leaves of control than in stressed plants (table 3.6.6). In the remaining organs the concentration of radioactivity per unit dry weight was comparable for the soluble fractions. However, the concentration in the insoluble fraction deviated from this pattern as control plants showed a significantly higher level.

The concentration of sugar was higher in the hypocotyl than in the other tissues in both treatments (table 3.6.6) and stress resulted in a significant increase in sugar concentration in all organs.

TABLE 3.6.4

Dry weight yield, and ^{14}C counts in the alcohol soluble and insoluble fractions, of the various organs of $^{14}CO_2$ treated control and stressed Radish plants.

Organ	Plant organ dry weight (mg)		Plant organ total c.p.m. (X 10^3)		Plant organ c.p.m. in the soluble fraction (X 10^3)		Plant organ c.p.m. in the insoluble fraction (X 10^3)		Soluble fraction counts as a percentage of total organ counts (%)	
	Control	Stressed	Control	Stressed	Control	Stressed	Control	Stressed	Control	Stressed
Leaves	87	39	3092	814	730	208	2363	606	24	26
Cotyledons	19	20	187	236	81	79	107	157	43	35
Hypocotyl	11	12	242	247	53	58	290	189	22	24
Roots	/	/	/	/	/	/	/	/	/	/
(*) LSD	5		257		60		212			

TABLE 3.6.5

Percentage distribution of ^{14}C counts amongst organs of $^{14}CO_2$ treated control and stressed Radish plants.

Organ	Percentage of total counts (%)		Percentage of counts in alcohol soluble fraction (%)		Percentage of counts in alcohol insoluble fraction (%)	
	Control	Stressed	Control	Stressed	Control	Stressed
Leaves	88	63	85	60	89	64
Cotyledons	6	18	9	24	4	17
Hypocotyl	7	19	6	17	8	20
Total (+)	100	100	100	100	100	100
(*) LSD	5		5		6	

+; excluding roots

TABLE 3.6.6

^{14}C counts and sugar concentrations (on a mg dry weight basis) in organs of control and water stressed Radish plants.

Organ	Counts per minute in alcohol soluble extract from one milligram dry weight (cpm)		Counts per minute in alcohol insoluble extract from one milligram dry weight (cpm)		Sugar concentration in one milligram dry weight (µg)	
	Control	Stressed	Control	Stressed	Control	Stressed
Leaves	8391	5425	27095	15894	38.9	55.0
Cotyledons	4125	3985	5491	7556	21.6	74.0
Hypocotyl	4489	4928	16220	16169	75.1	94.6
Roots	2540	2741	14308	7230	24.4	46.8
* LSD	1132		6391		19.5	

The bulk of this increase was attributable to an increased sucrose concentration (table 3.6.7), although increases in both glucose and fructose concentration also occurred. These increases in sugar concentration were accompanied by increased specific activity (table 3.6.7). The only exception was the leaf tissue where the specific activity was not increased by stress. Thus it would seem evident that tissues importing sucrose showed higher specific activities whilst those exporting sucrose (i.e. leaves) were unchanged.

Proline did not accumulate under the level of stress which was developed. Table 3.6.8 compares the amount of proline accumulated in Radish tissues stressed in this situation and that accumulated by excised tissue (of the same age) stressed by floating on PEG (-15 bar for 24 hr). The relative water contents of the excised tissues were far lower than those of plants stressed in the vermiculite system.

Thus, in summary, although Radish plants were experiencing a severe 'soil'-water deficit, as evidenced by significant growth reductions, they adapted to some extent to avoid visible wilting. The direction of metabolism was somewhat altered by stress; for instance the marked lignification of hypocotyl cells. Similarly, sugar concentrations in all organs rose during stress. However, the photosynthetic rate appeared unaltered by stress, with the real limit on carbon fixation thus being the reduced photosynthetic area. The leaves of stressed plants exported a larger portion of fixed $^{14}CO_2$ to the hypocotyl than control plants. They consequently had reduced c.p.m. on a unit dry weight basis for both alcohol soluble and alcohol insoluble fractions.

Discussion

The growth reduction, due to water stress, reported in this experiment was similar to that determined in the previous experiment

TABLE 3.6.7

Concentrations of sucrose, glucose and fructose, and their specific activities, in the various organs of $^{14}CO_2$ treated control and stressed Radish plants.

Organ	Sugar	Concentration in one milligram dry weight of tissue (µg)		Specific activity in one milligram dry weight of tissue (cpm/µg)	
		Control	Stressed	Control	Stressed
Leaves	Sucrose	4.2	7.6	29.0	20.1
	Glucose	12.4	15.6	31.7	32.7
	Fructose	10.5	9.3	22.2	28.6
Cotyledons	Sucrose	4.7	8.6	12.4	17.1
	Glucose	7.1	18.4	29.4	48.2
	Fructose	6.8	11.2	19.9	27.1
Hypocotyl	Sucrose	7.6	15.3	14.1	33.3
	Glucose	16.5	17.5	38.7	47.0
	Fructose	10.3	10.1	25.9	35.3
Roots	Sucrose	5.1	14.8	12.8	40.7
	Glucose	10.7	10.6	18.3	18.3
	Fructose	8.0	10.1	9.3	14.8
(*) LSD		6.5		11.3	

TABLE 3.6.8

A comparison between proline accumulated in Radish subjected to slow substrate moisture depletion stress and the potential proline accumulation caused by PEG-induced water stress.

Organ	Slow depletion of substrate moisture (20 days)				-15 bar PEG for 24 hr. (excised tissue)			
	Proline concentration (µg/mg d.w.)		Relative water content (%)		Proline concentration (µg/mg d.w.)		Relative water content (%)	
	Control	Stressed	Control	Stressed	Control	Stressed	Control	Stressed
Leaves	0.57	1.58	92	87	0.42	15.30	98	45
Cotyledons	0.54	0.47	96	93	0.56	3.20	99	57
Hypocotyl	0.48	1.73	/	/	0.31	1.66	/	/
Roots	/	/	/	/	0.23	0.09	/	/
(*) LSD	(NS)				2.02			

(section 3.5); in which cell expansion and division were reduced both in shoot and fleshy axis tissues by a slowly developed water stress. That the level of stress developed in the present experiment did not significantly disrupt the photosynthetic rate per unit leaf area indicates that neither a significant increase in stomatal/mesophyll resistance, nor a decrease in chloroplast activity (Pospisilova *et al.*, 1978) was caused by the severe restriction of water availability. Thus, at least where stress develops slowly, the early reduction in total photosynthesis in the young Radish is due to a reduced production of new leaf area. That is, primarily through limitation of cell expansion. Similarly, for Corn, Soybean and Sunflower subjected to water stress there is an earlier, and more severe, inhibition of leaf enlargement than of photosynthesis per unit leaf area (Boyer, 1970).

The lignification of hypocotyl parenchyma cell walls may have been a specific response to water stress, and, both smaller cells and thicker cell walls have been characterised as mechanisms conferring greater drought tolerence (Iljin, 1957; Cutler *et al.*, 1977).

The observation of increased sugar concentration in all organs of Radish during water stress has also been made in Cotton (Eaton and Ergle, 1948), in which sugars doubled in the leaves, stems and roots. The disappearance of leaf starch during water stress has been widely reported, and subsequent carbohydrate transport to other plant parts (e.g. roots) has been suggested (Iljin, 1957). These reported responses are compatible with the present observations in Radish where there was reduced ^{14}C c.p.m./g d.w. in the leaves of stressed plants, and the lower organs contained a greater proportion of the assimilated ^{14}C.

3.7 Comparison of the effects of Soil Water Deficit and of PEG Induced Water Stress

3.7.1 Introduction

Previous work (section 3.2) has indicated that short episodes of PEG-induced water stress have traumatic but relatively short term effects on growth provided that the stress does not kill large portions of the plant. On the other hand, long episodes of soil water deficit have less traumatic but longer term effects on growth (section 3.4). This experiment was designed to compare these two methods of inducing stress. From those earlier experiments it was also apparent that young plants may be less sensitive to stress than older plants in terms of tissue water deficit and consequent yield reductions. In order to test these hypotheses the water relations of Radish plants stressed by both techniques were estimated and yield data collected as plants experienced a series of stress episodes.

2.7.2 Methods

The experiment was conducted in a growth cabinet (12 hr photoperiod) and mineral nutrients were supplied by watering with Hoagland's solution ($\frac{1}{2}$ -strength). Relative humidity had a daily mean of 55%. Both control plants and those subjected to water deficit stress were grown in a 1:1 mixture of sand and loam ; those subjected to PEG stress were grown in coarse river sand alone. Seed, cv. 'Mars', was surface sterilized and grown for 9 days on nutrient solution in filter paper lined petri dishes. Six uniform seedlings were transplanted on day 10 into each pot (160 mm top diameter), and these were reduced to 5 on day 15 through the removal of the least uniform plant. Replication was fourfold yielding with 3 treatments and 4 harvests a total of 48 pots.

Harvests were made at 10 day intervals commencing 20 days after sowing. PEG stress was imposed by flooding the pots with 500 ml of solution (-10 bars PEG in nutrient solution) on days 19, 29, 39 and 49, and was relieved 24 hours later on each occasion with 1000 ml of nutrient solution. Water deficit stress involved cycling the pots (figure 3.7.1) between field capacity and wilting (ca. 15% F.C.). Field capacity in the pots, whose soil capacity (at field capacity) was 2 kg, was defined as the water content following 48 hr drainage from saturation. No adjustment was made for increase in plant size and surface evaporation was not prevented. Control plants were watered daily with 100 ml of nutrient solution to day 30, 150 ml to day 40 and then 200 ml until day 50.

Plant water status was assessed for all treatments, prior to alleviation of PEG stress, on a plant selected randomly from each pot scheduled to be harvested. For harvests on days 20, 30 and 40, water potential (pressure bomb) was measured on the first formed true leaf. This same leaf was then used to estimate relative water content. On day 30 osmotic potential was measured (microosmometer) on the second formed leaf following restoration of full turgor by floating it for 4 hr on distilled water. As the oldest leaves were chosen for these measurements, effects of ageing could also be assessed. On day 50 water potential and relative water content were estimated for all the leaves of the plant. The remaining 4 plants in each pot were allowed to recover fresh weight for 6 hours before being harvested for growth assessment.

3.7.3 Results

The soil water deficit imposed entailed 8 rewaterings from wilting (15% F.C.) as compared with 4 episodes of PEG stress (figure 3.7.1). The degree of wilting of the leaves produced by PEG (figure 3.7.2) during an episode of stress was also attained in plants subjected

FIGURE 3.7.1

Treatments imposed to compare PEG and soil water deficit techniques as methods of inducing plant water stress.

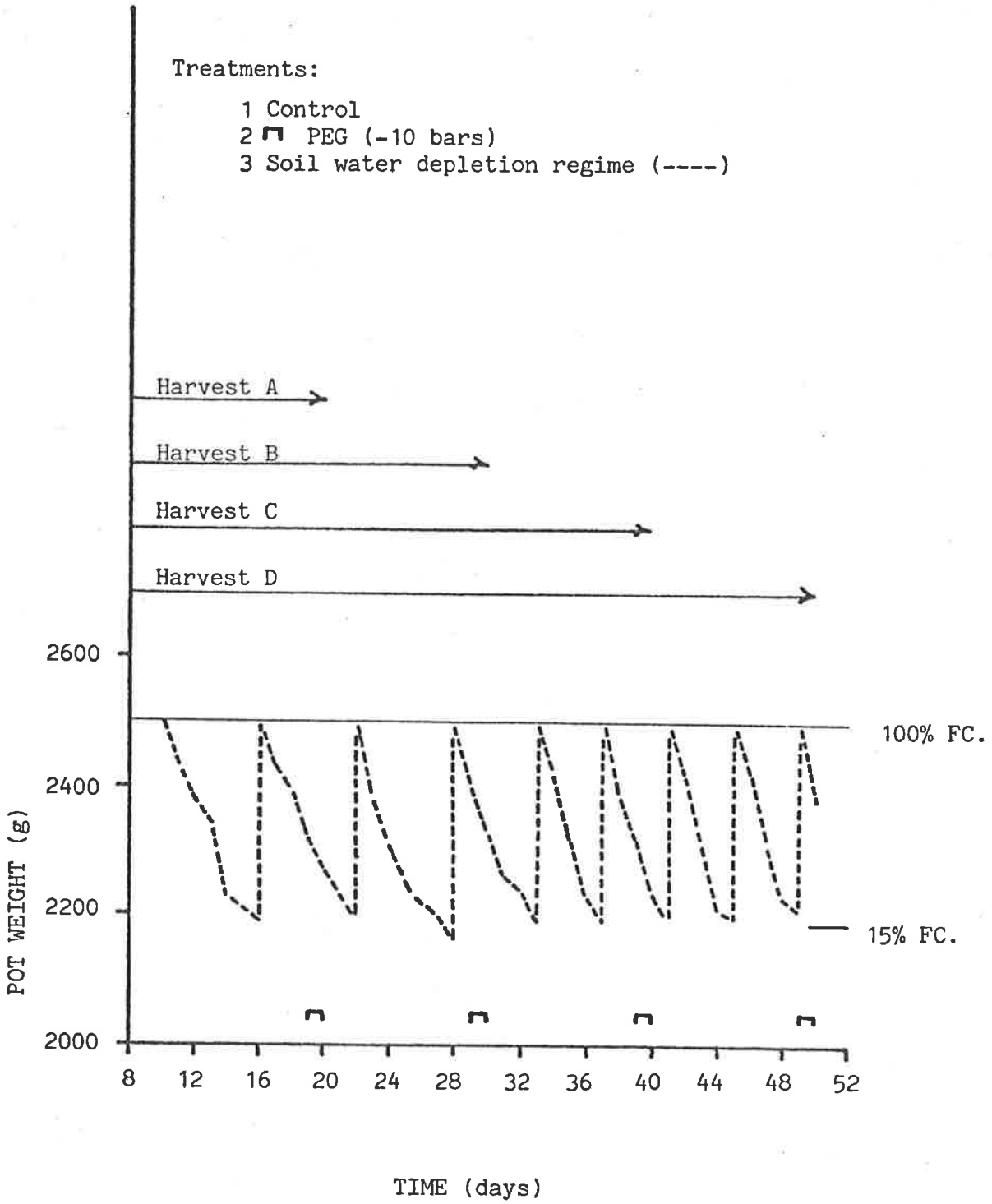

Treatments:
1 Control
2 PEG (-10 bars)
3 Soil water depletion regime (----)
Harvest A
Harvest B
Harvest C
Harvest D
100% FC.
15% FC.
POT WEIGHT (g)
2600
2400
2200
2000
8
12
16
20
24
28
32
36
40
44
48
52
TIME (days)

FIGURE 3.7.2

A comparison of the effect of -10 bar PEG stress, a well watered control (LHS), and, Radish plants previously subjected to repeated soil water deficits (RHS).

to a soil water deficit. As individual replicates in any single treatment lost water at different rates, this apparent similarity of plant water status in the two treatments is not obvious in the water relations measurements when averaged across replications (table 3.7.1). When this data is considered for the individual pots, however, similar low levels of water status in the 2 treatments (+) occur, particularly where individual pots have lost water to the lowest level of soil water depletion (table 3.7.2).

Exposure to PEG reduced relative water content by approximately 10% and water potential by -7 bars on day 20 in comparison to watered plants (table 3.7.1). As time progressed, however, the relative water content of PEG treated plants fell further, to about 50% of that of controls on day 50. In contrast, leaf water potential appeared to increase over this period. This lack of agreement between the 2 estimates of tissue water status may be attributed to the onset of senescence and to stress induced leaf margin necrosis in PEG stressed plants (figure 3.7.2). This may well have exposed the xylem to the atmosphere and allowed the ingress of air leading to unrealistically high water potentials being recorded with the pressure bomb. This interpretation is supported by the relatively consistent water potentials obtained with time in the water deficit treatment where no marginal necrosis was observed. Relative water content measurements, which are not susceptible to this error, are thus considered to provide a more meaningful account of leaf water status for this treatment, although these will also be subject to the errors associated with the infiltration of water into necrotic tissue. The osmotic potentials of the sap expressed from leaf tissue on day 30 only, indicate that higher concentrations of cell solutes occurred in the stressed tissues (table 3.7.1). As the PEG-stress tissue was wilted at the time of harvest and then returned to full turgor prior to determination of osmotic potential the change in osmotic potential must

TABLE 3.7.1

Growth and water relations data for comparison of PEG and water deficit induced stress with a well watered control over a series of 4 harvests.

Harvest Day	Treatment	Water Potential (-bars)	Relative Water Content (%)	Osmotic Potential (-bars)	Shoot Fresh Weight (g)	Fleshy Axis Fresh Weight (g)	Leaf Area (cm^2)	Coty-ledon Area (cm^2)	Leaf Number	Coty-ledon Number	Shoot Dry Weight (g)	Fleshy Axis Dry Weight (g)	Fleshy Axis Maxi-mum Diameter (cm)	Fleshy Axis Volume (cc)
20	Control	4.8	81.8	/	0.37	0.03	5.2	3.5	1.9	2.0	0.029	0.003	0.14	/
	-10 PEG	12.0	67.8	/	0.30	0.04	5.0	4.4	2.3	2.1	0.033	0.003	0.15	/
	Regime	7.1	82.3	/	0.28	0.03	3.9	3.5	2.1	2.0	0.027	0.002	0.12	/
30	Control	3.6	90.7	7.8	3.47	1.34	87.1	7.3	4.9	1.9	0.254	0.079	0.94	1.36
	-10 PEG	11.3	55.3	16.0	1.55	0.22	36.5	4.1	4.1	1.4	0.149	0.018	0.40	0.18
	Regime	4.2	90.4	11.1	1.47	0.11	37.7	3.7	4.4	1.6	0.125	0.009	0.28	0.12
40	Control	3.1	94.4	/	6.31	6.37	157.6	4.8	6.6	1.8	0.415	0.358	1.96	6.23
	-10 PEG	6.4	48.7	/	3.25	0.81	82.0	0.6	5.6	0.2	0.306	0.060	0.77	0.81
	Regime	5.7	72.4	/	3.27	1.07	86.4	1.4	5.7	0.5	0.282	0.091	0.80	1.02
50	Control	2.2	92.2	/	9.24	15.29	233.8	2.7	7.3	0.6	0.645	0.839	2.89	15.92
	-10 PEG	3.8	43.8	/	4.61	3.01	94.3	0.0	4.2	0.0	0.457	0.195	1.38	2.96
	REgime	5.2	78.9	/	4.60	2.43	118.1	0.5	6.8	0.2	0.407	0.197	1.32	2.61
Interaction		*		*	*	*	*	*	*	*	*	*	*	*
Treatment			*											
LSD		2.4	7.6	1.8	0.84	1.69	20.5	1.4	0.8	0.2	0.056	0.287	1.02	2.03

TABLE 3.7.2

Water status of first formed leaves of individual plants growing under control, -10 bar PEG-stress (24 hr), and, soil water deficit regime stress, and harvested at 10 day intervals.

Plant Identification	Treatment	Day 20		Day 30		Day 40		Day 50	
		Water Potential (- bars)	Relative Water Content (%)	Water Potential (- bars)	Relative Water Content (%)	Water Potential (- bars)	Relative Water Content (%)	Water Potential (- bars)	Relative Water Content (%)
Replication I	Control	5.0	78	4.2	87	5.2	90	3.5	91
	PEG	11.8	76	9.4	52	4.2	44 (+)	2.6	28
	Regime	5.2	82	4.5	87	5.7	49 (+)	1.4	94
Replication II	Control	6.8	84	4.5	93	1.9	98	2.0	94
	PEG	12.5	65	11.5	53	7.4	47	1.3	58
	Regime	7.9	80	6.3	91	6.3	91	2.2	94
Replication III	Control	3.5	82	2.9	92	1.6	94	1.9	93
	PEG	10.0	60	12.8	61	9.5	54 (+)	6.5	47
	Regime	6.5	88	2.5	92	7.7	52 (+)	8.9	75
Replication IV	Control	3.9	84	2.9	91	3.6	96	1.2	90
	PEG	13.5	70	11.3	56	4.3	50	4.8	42 (+)
	Regime	8.9	80	3.6	92	2.9	97	8.1	53 (+)

have been due to solute accumulation.

Both stress treatments reduced all growth parameters, e.g. fresh weight, by approximately equal amounts (table 3.7.1) and, as confirmed by significant harvest x treatment interactions, the difference between control and stressed plants increased with time. The reductions in shoot fresh and dry weights due to stress (figure 3.7.3) were far less than the reductions in fleshy axis fresh and dry weights (figure 3.7.4). The only aspect of plant growth which was affected differently by the 2 types of stress treatments was leaf growth, where PEG-induced water stress resulted in significantly fewer leaves, and consequently reduced plant leaf area, than did the soil water deficit treatment. This difference became apparent during the later stages of the experiment (figure 3.7.5) and was due to the accelerated senescence of older leaves. Marginal leaf necrosis, which was observed in some instances to leave only a small tissue area around the midrib also contributed to the reduction in leaf area.

On day 50 (final harvest) all of the leaves of each plant selected for the measurement of water status were assessed separately so as to provide data to examine the relationship between water potential and relative water content. The PEG stressed plants differed from the others in that the relative water content was low, but water potential varied over a wide range (figure 3.7.6). The probable reasons for this have already been discussed. In an effort to compare all the water relations data collected and to verify the suspected effect of tissue age and damage on the relationship between relative water content and water potential linear regressions were calculated as indices of water stress effects. A change in variance was interpreted to be a measure of necrosis effects. On the pooled data, the same excluding PEG data, and, the control plus PEG data alone, regression coefficients were -0.529, -0.620 and -0.505 respectively. These results indicate PEG data increased

FIGURE 3.7.3

The effect of sequential PEG and soil deficit induced water stress treatments on Radish shoot fresh and dry weight.

———— Control

-------- -10 bar PEG (4 x)

..... Water regime (100-15% FC)

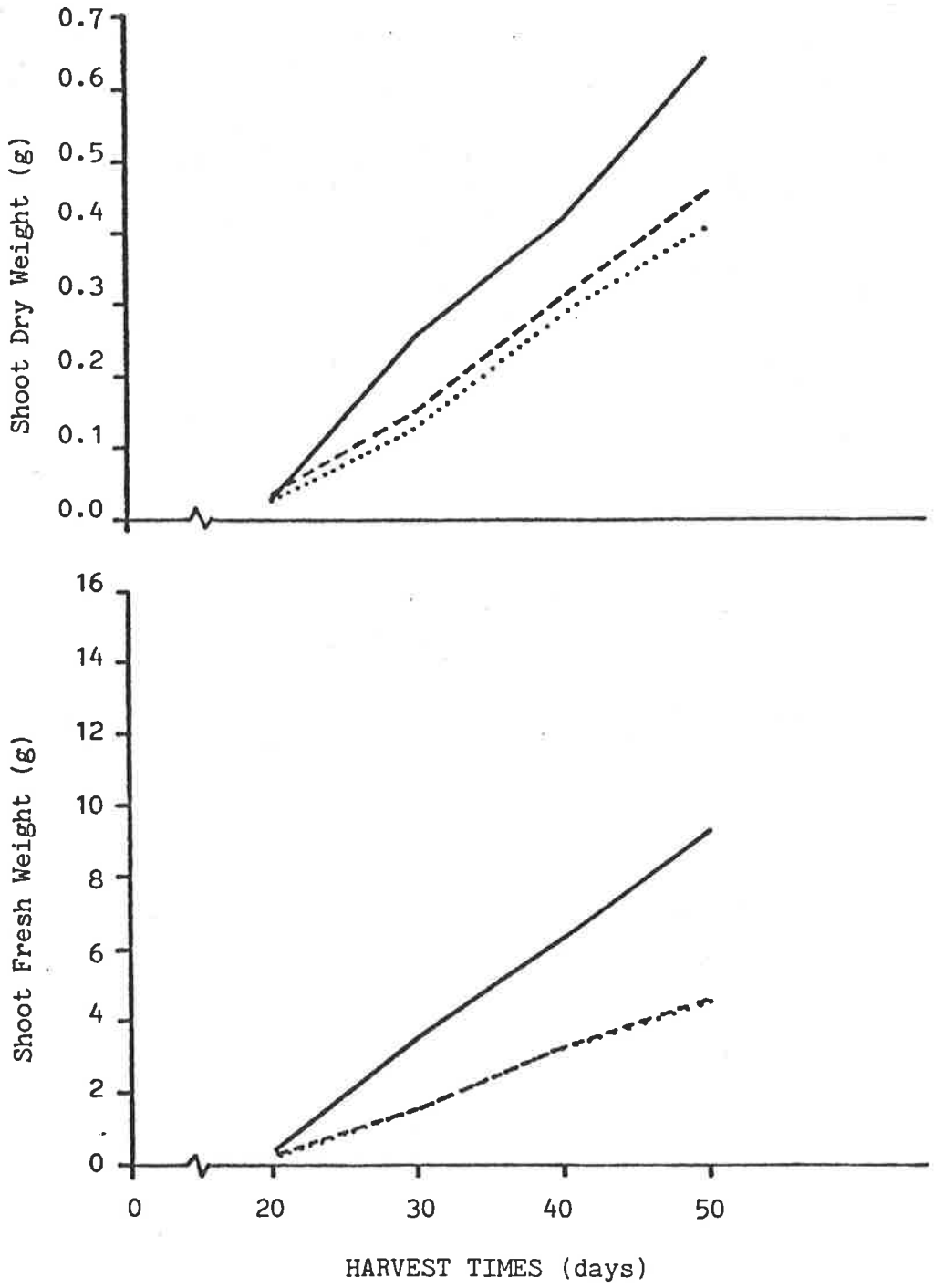

Shoot Dry Weight (g)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Shoot Fresh Weight (g)
16
14
12
10
8
6
4
2
0
0
20
30
40
50
HARVEST TIMES (days)

FIGURE 3.7.4

The effect of sequential PEG and soil water deficit induced water stress treatments on Radish fleshy axis fresh and dry weights.

———— Control

-------- -10 bar PEG (4 x)

..... Water Regime (100-15% FC)

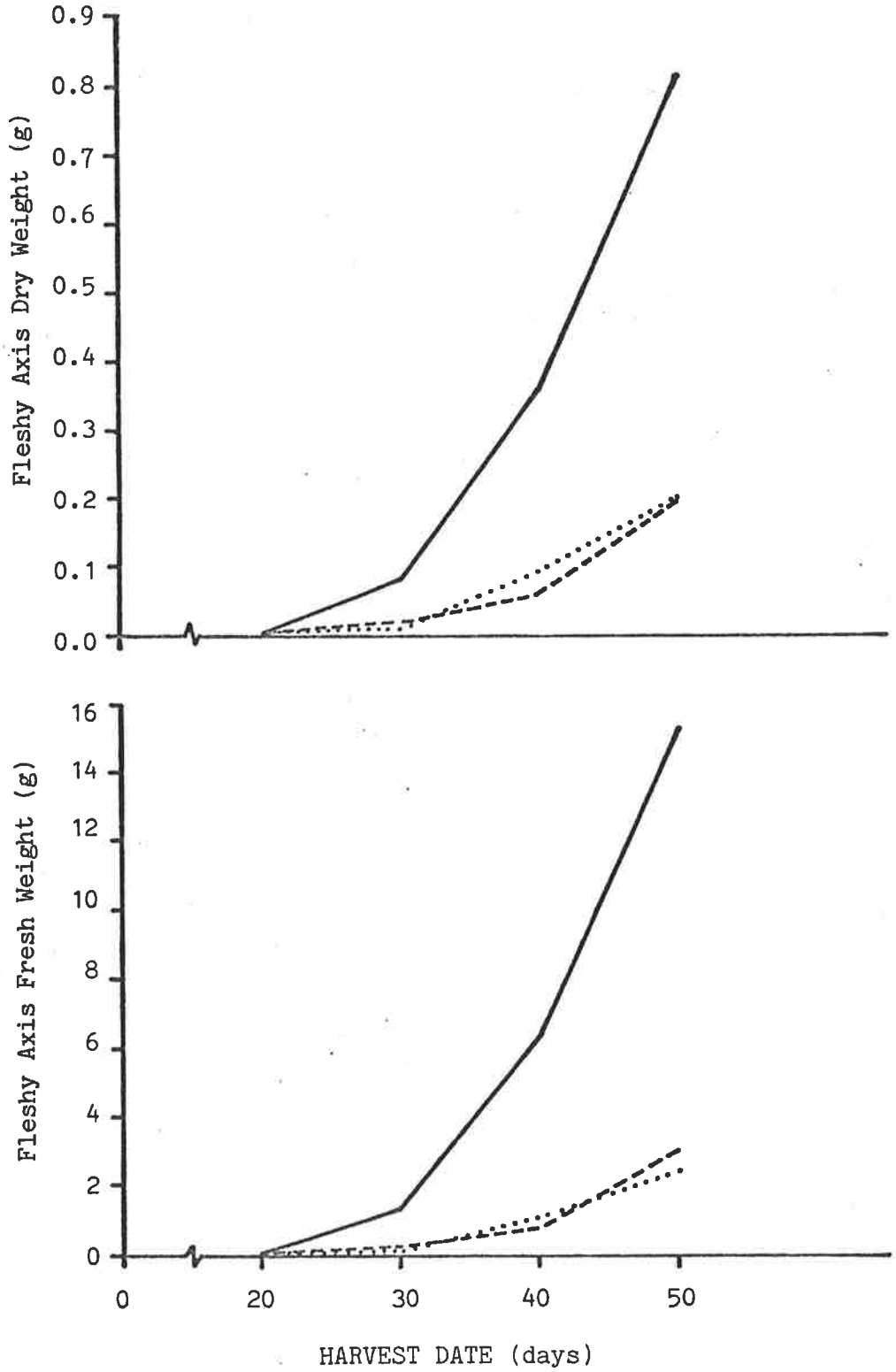

Fleshy Axis Dry Weight (g)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Fleshy Axis Fresh Weight (g)
16
14
12
10
8
6
4
2
0
0
20
30
40
50
HARVEST DATE (days)

FIGURE 3.7.5

The effect of sequential PEG and soil water deficit induced water stress treatments on Radish leaf area.

———— Control

-------- -10 bar PEG (4 x)

..... Water regime (100-15% FC)

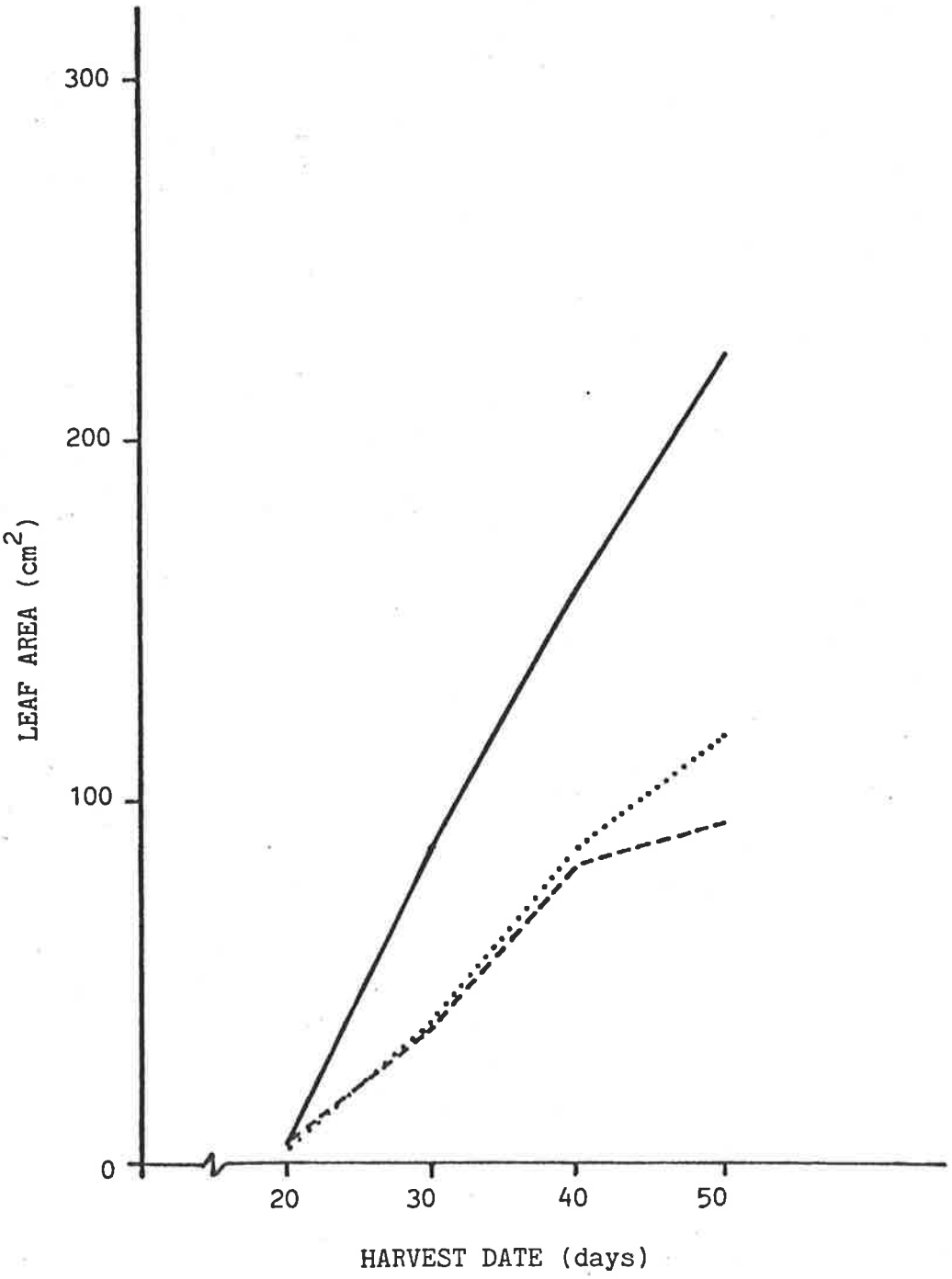

300
200
100
0
LEAF AREA (cm^2)
20
30
40
50
HARVEST DATE (days)

FIGURE 3.7.6

The relationship between water potential and relative water content measured on Radish leaves taken from control, PEG stress and soil water deficit stress conditions.

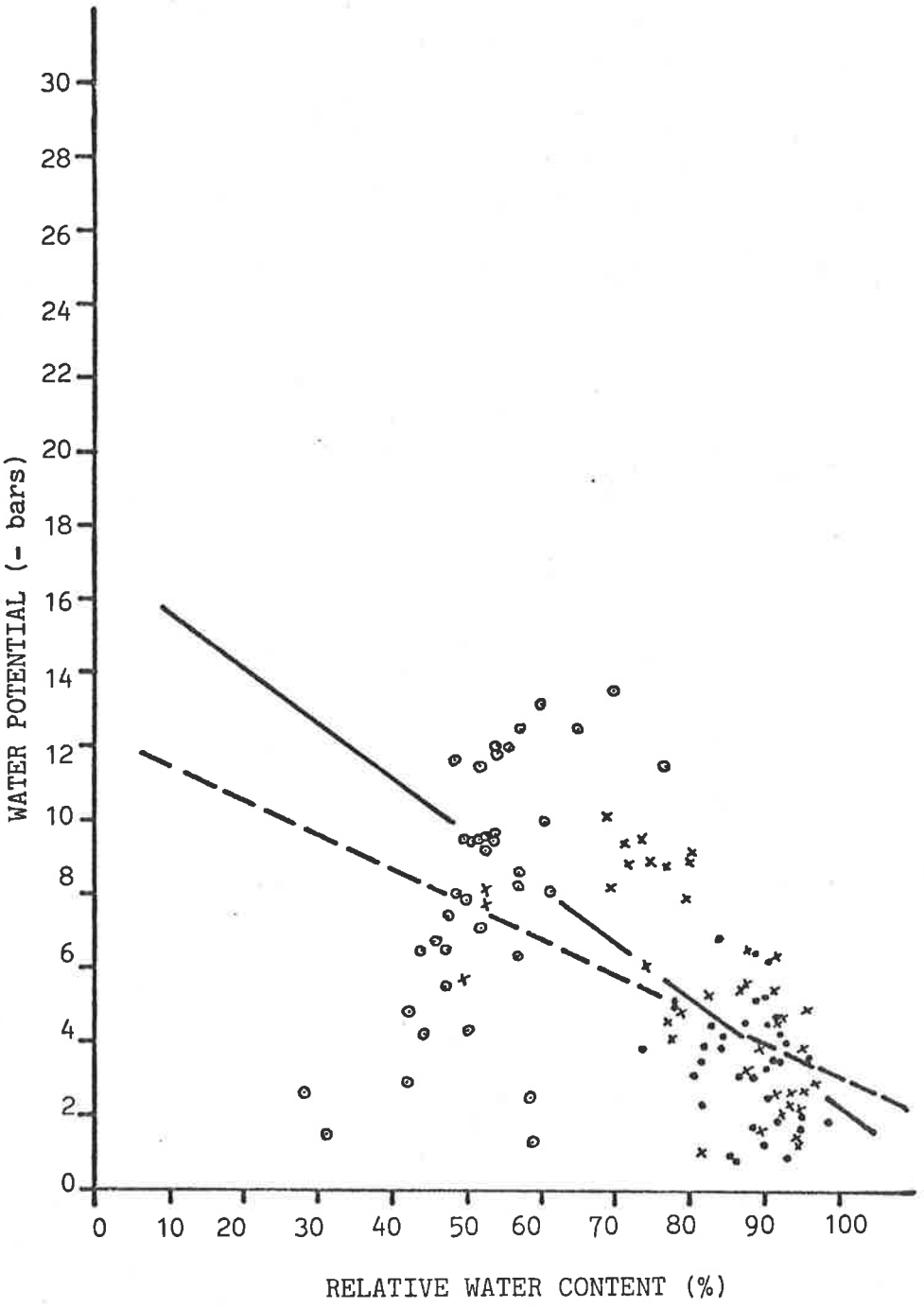
• Control

⊙ PEG (-10 bars)

x Water Regime (100-15% FC)

———— Regime + Control
(-0.6197, y= -0.1487.x+ 17.0431)

--------- PEG + Control
(-0.5050, y= -0.0932.x+ 12.3953)


WATER POTENTIAL (- bars)
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
10
20
30
40
50
60
70
80
90
100
RELATIVE WATER CONTENT (%)

the variance and thus that necrosis introduced appreciable errors into water potential estimation. Regressions calculated on data from each of the first and second harvests separately and the pooled data from these 2 harvests yielded coefficients of -0.7119, -0.9199 and -0.8130 respectively. These show, when compared with the regression coefficient on the data from the final harvest, that variance increased with time. The slope of the regressions were -0.2189 for the pooled data from the first 2 harvests, and -0.0930 for the data for the final harvest. This marked change in slope with time (figure 3.7.7) may indicate a change in tissue characteristics (e.g. turgor maintenance) as leaves mature and age. Thus, as leaves age they become more susceptible to PEG-induced water stress damage, and, under both types of water stress, are less able to maintain a favourable water balance. Accelerated senescence, discussed earlier, may be a consequence of this inability.

3.7.4 Discussion

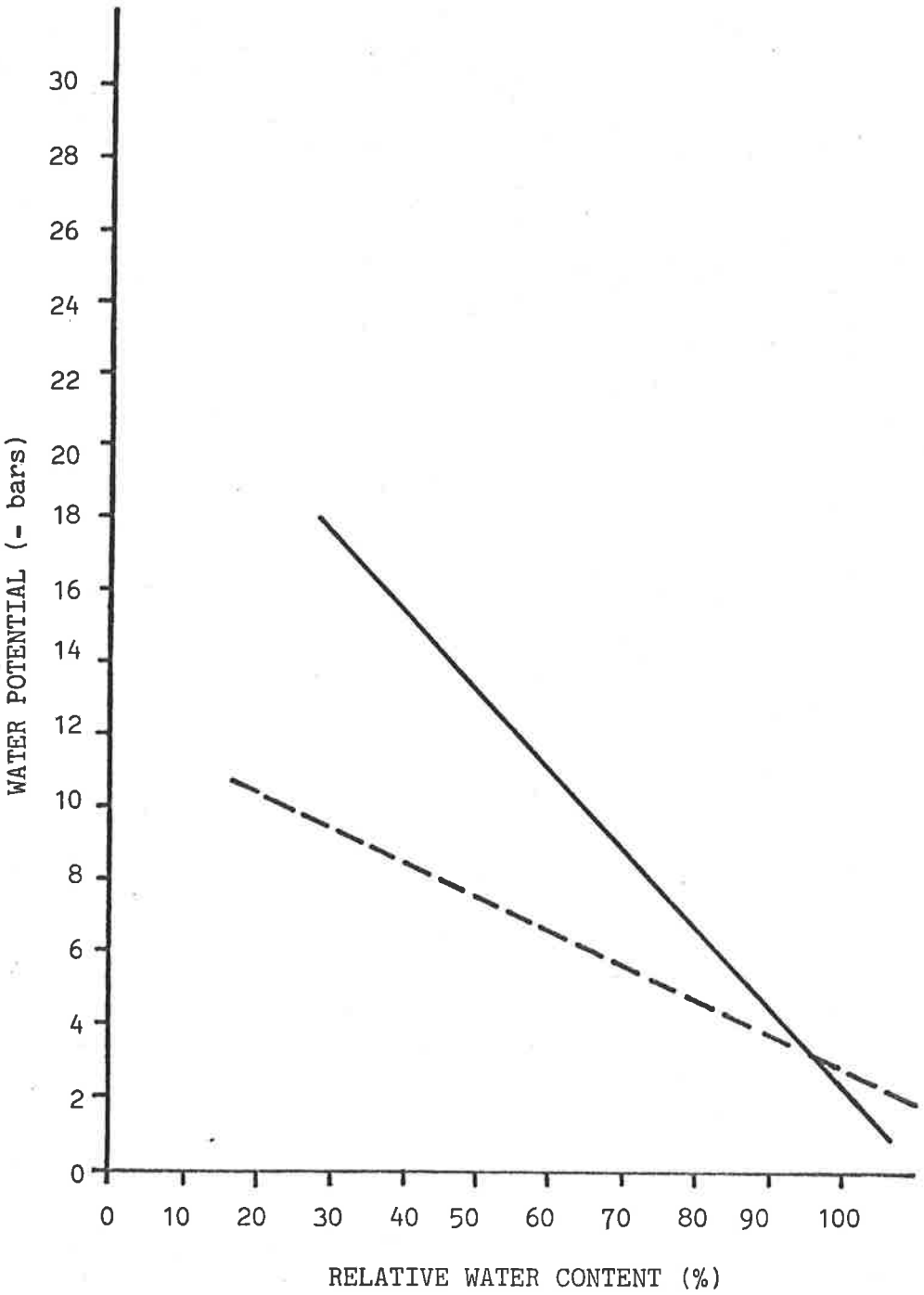
The greater sensitivity to stress of fleshy axis growth, compared with shoot growth, appears to be maintained whether stress is short and abrupt (PEG) or involves relatively long drying periods (water regime). However, as both stress types caused similar yield reductions in both shoot and fleshy axis tissue, whilst only 4 episodes of PEG-stress were imposed as against 8 episodes of water deficit, it may be concluded that PEG had an effect in excess of that caused by a slower reduction in water status. This can be termed a 'shock' effect, and is reflected in the significantly greater reduction in leaf area and leaf number caused by PEG as compared to water deficit. The possibility of PEG-uptake by the plant cannot be excluded, however, and has been associated with leaf margin necrosis in dicotyledons (Lawlor, 1970). Its proposed action is to block the transpiration pathway within the leaves.

FIGURE 3.7.7

The relationship between relative water content and water potential in Radish leaves from old plants (50 days) compared with the relationship for young plants (20-30 days).

_____ Young leaves (-0.8131; y=-0.2189.x + 24.2363)

_ _ _ _ _ Old leaves (-0.5327; y=-0.0930.x + 12.1574)


WATER POTENTIAL (- bars)
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
10
20
30
40
50
60
70
80
90
100
RELATIVE WATER CONTENT (%)

In the present experiment leaves of PEG-stressed plants had a significantly lower sap osmotic potential (table 3.7.1) than water deficit treated plants. This has also been reported in Beans (Pospisilova, 1977), in which water potential is also lower. Due to leaf margin necrosis the water potential of PEG-stressed Radishes could not be accurately measured. Although no explanation for the differential treatment effects was tendered for Beans, it was stressed that PEG-induced physiological responses may differ from those in wilted plants. Extrapolating from the previous experiment (section 3.6), massive proline accumulation under PEG-stress, as against little accumulation in droughted plants, may example one such physiological difference.

Accelerated senescence of mature leaves was recorded in the present study, whilst in a previous experiment (section 3.5) a reduction in leaf formation was recorded. The difference is due to plant age, with senescence the primary feature of old plants and reduced leaf number the primary feature of young plants. Similarly, Soybean and Sorghum plants stressed before flowering suffer less irreversible leaf injury than older plants (Sionit and Kramer, 1976). As a general rule, young actively growing tissues suffer the greatest check in growth, but are more tolerant of water deficit than older tissues which undergo senescence (Begg and Turner, 1976). In *Panicum*, ageing of younger tissues is actually suspended whilst death of older leaves proceeds progressively from the oldest towards the youngest (Ludlow, 1975). Older Radish leaves are less able to maintain a favourable water balance (figure 3.7.7), a finding also reported for Kale (Pospisilova, 1974); this suggests younger tissues have a greater capacity to osmoregulate to some degree.

Accelerated senescence and leaf margin necrosis appear to be different processes. The oldest leaves show marginal necrosis and also go yellow and die, whereas 'middle-aged' leaves which have suffered

marginal necrosis retain considerable areas of healthy tissue. It is suggested that senescence occurs through altered metabolism (e.g. irreversible hydrolysis) and that marginal necrosis is due to the extreme 'low' of a water potential gradient declining toward the margin (i.e. irreversible ultrastructural damage).

CHAPTER 4

GENERAL DISCUSSION

Short episodes of water stress while the Radish plant is growing rapidly cause a significant growth setback which persists for some time (figure 3.2.7). In contrast, stress imposed during slow growth has little persistent effect (figure 3.1.1). Repeated episodes of short-term stress result in highly significant yield reduction (figure 3.7.4). These marked effects of water stress on Radish water status and yield are apparently attributable to poor stomatal limitation of water loss during the light period (figure 3.3.5). Support for this general conclusion comes from the low values of water use efficiency observed during drought (table 3.4.4), the continual water loss which is revealed by monitoring fleshy axis diameter (Jordan and Ritche, 1971; Rooke *et al.*, 1977) during short stress episodes (figure 3.3.4), and the leaf margin necrosis (figure 3.3.1) which appears to be due to marked gradients of water potential across the leaf blade (Manohar, 1977). Osmotic adjustment during short episodes of stress was recorded (table 3.7.1) but was of insufficient magnitude to restore turgor. In another study on Radish (Kibreab and Danielson, 1977) the fleshy axis sap osmotic potential was found to alter over a number of days (10) to maintain some fleshy axis expansion against applied external pressure of up to 5 bar. The increased atmospheric pressure about the fleshy axis was used to simulate a reduction in turgor pressure such as would be a consequence of mild water stress, and thus the osmotic adjustment recorded indicates that Radish may be able to effectively osmoregulate to maintain turgor under analogous water stress conditions.

The general conclusion may be drawn that short episodes of water stress can cause a significant reduction in plant yield if either they are repeated (section 3.7) or are of sufficient intensity (section

3.2) as to result in large scale plant damage. Otherwise, such episodes have only a transient effect, with the recovery time decreasing as the level and duration of stress is decreased (section 3.2). In this case the Radish plant continues to grow and shows no significant reduction in yield at harvest (section 3.1).

A long term reduction in water availability initiates a distinctly different response. The growth of plants at successively more severe water regimes results in progressively lower yields, with fleshy axis yield being reduced proportionally more than that of the shoot (table 3.4.4). Further, if severe stress is alleviated to a favourable regime a lag period between fleshy axis recovery (figure 3.5.10) and the more immediate shoot recovery (figure 3.5.3) is apparent. The possibility that this differential effect is hormone mediated (e.g. interrupted cytokinin synthesis and transport; Radin and Loomis, 1971) warrants investigation.

Cell size in both the shoot (table 3.5.1) and the fleshy axis (table 3.4.5) was reduced by stress, and under severe water stress reduced cell division was also recorded (table 3.5.2). As cell size readily recovered towards the control level following stress alleviation it was concluded that reduced rates of cell division during stress were primarily responsible for the more persistent reduction in leaf area. Reduced leaf cell number as a result of stress has also been reported in Sugar Beet (Morton and Watson, 1948). However, in contrast to the present results with Radish (section 3.7), the rates of leaf production and of leaf death in Sugar Beet were unaffected by stress. This discrepancy may have been due to differences in the severity of stress in the two experiments. As with Radish, Sugar Beet was reported to both wilt and recover readily.

Hence, as a general rule, cell division in both the Radish shoot and the fleshy axis continues at a reduced rate during stress, whilst cell expansion is halted. The overall pattern of cellular differentiation is otherwise unaffected by stress, and the anatomy of the fleshy axis of a stressed plant is indistinguishable from that of a well-watered plant of similar diameter.

A clear delineation between cell division and cell expansion with respect to their sensitivity to water stress cannot be drawn. For instance, there may be an interrelationship between the two processes such that cell expansion of daughter cells could be a prerequisite for subsequent division (Doley and Leyton, 1968). During stress, the accumulation of organic solutes in the cell allow cell expansion by decreasing intracellular osmotic potential which in turn may result in increased turgor. Also, in the short term, changes in cell wall and membrane properties (e.g. cell wall extensibility, elastic modulus and hydraulic conductivity) can allow cell expansion at a much reduced turgor (Wyn Jones *et al.*, 1979). The cell expansion capacity of small daughter cells is not the sole limit to cell division during stress. In *Vicia faba* roots, for example, cell expansion is reduced by stress whereas cell division is much less affected (Murin, 1979). Cell division in this case is most limited in the S-phase, the duration of which is extended. Similarly, extension of the duration of the mitotic cycle has been found in Barley and Sunflower shoot meristems as a result of salinity stress (Gaidamakina, 1967). Small cells have been found to require less turgor for expansion than do large cells, which require a higher turgor pressure to stretch the cell wall and initiate extension growth (Steudle *et al.*, 1977). Thus in view of the reports of greater sensitivity of cell division to stress in comparison to cell expansion (e.g. Wheat grains, Brocklehurst *et al.*, 1978; Sugar Beet leaves, Terry *et al.*, 1971; Radish cotyledons, Kirkham *et al.*, 1972), and considering the apparently contrary

evidence (Murin, 1979), it can be concluded that cell division is initially reduced relatively more than cell expansion by mild stress. Then, as stress intensity increases cell expansion may be virtually halted whilst a reduced rate of cell division continues. The early adaptation of cell expansion may be attributable to a reduction to a lower turgor pressure requirement for expansion.

In short, cell division is more sensitive to stress than cell expansion but continues at reduced rates even during severe water deficit. Cell expansion on the other hand is less resilient in its response and under water stress, particularly in species which do not readily osmoregulate, is the most obviously affected process. If stress is alleviated the cell division effect is the primary persistent limitation to recovery. For example, although cell elongation is highly responsive to moderate daytime water deficit in Soybean leaves, this parameter does not limit leaf area development (Wenkert *et al.*, 1978). This type of relationship probably accounts for the finding (see above) that stress effects on cell division limit Sugar Beet leaf area.

The Radish plant has the ability to withstand very severe drought conditions (section 3.5) and even to maintain slow growth under such conditions (figures 3.5.1 and 3.5.6). This ability was apparently associated with tissue age, with young plants demonstrating this potential. Older plants subjected to similar degrees of stress wilted more readily and leaf senescence commenced. Cell size may be of fundamental importance, especially with regard to the extent of vacuolation (Iljin, 1957). Reduced cell and vacuolar size may effect protection through lessening susceptibility to mechanical damage on shrinkage and through a lower osmotic potential, especially in plants with a poor ability to osmoregulate (Cutler *et al.*, 1977). Young severely droughted Radishes accumulated higher tissue sugar concentrations than control plants

(table 3.6.6) and PEG-stressed Radishes accumulated massive quantities of the imino acid proline. Both these responses may be related to osmoregulation. The accumulation of free proline in Radish has been shown to be linearly related to the shoot water status beyond ca -5 bars leaf water potential (Chu, 1974). Accumulation of these solutes, and others (e.g. amino acids, K^+), has been associated with the survival of younger tissues of Wheat plants (Munns *et al.*, 1979). Sugar and amino acids were the major components of organic osmotica, but as drought was extended and K^+ concentration exceeded 250 mM the proline concentration increased greatly until it exceeded the concentration of all amino acids. The exposed Wheat leaves had little capacity for osmoregulation, and failed to survive the drought. The differential response between tissues of different ages, both in Radish and Wheat, thus appears to be related to cell size and osmotic adjustment.

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APPENDIX I

1.1 Anatomical changes occuring in the developing Radish fleshy axis.

Transverse sections of the fleshy axes of 10, 24, 37 and 48 day old plants were prepared in order to follow changes in anatomical structure as this organ developed (section 3.1).

Figure 1 relates the maximum diameter of these plants to the time at which they were harvested. Slow early growth is followed by a rapid increase in fleshy axis size. Diameters marked A. to D. directly relate to the following 4 figures (figures 2 - 5).

Figures 2 and 3 are similar and show that little anatomical change occurred between days 10 and 24. At this age the organ consists of a compact 'core' of vascular tissue surrounded by a thin band of pericycle and encased in the prominant cortex tissue type. During this 12 day period pericycle cells enlarge slightly and xylem elements in the vascular core are displaced, apparently yielding to some larger and many smaller parenchyma cells.

By day 37 (figure 4) the cortex is being sloughed off, and the central 'core' has expanded considerably through an increase in both cell size and cell number. Large xylem elements, a large-parenchyma cell core, distinct cambium and pericycle bands, and radiating xylem arms characterise this period. In the following 11 days rapid growth resulted in a well-developed fleshy axis in which ray parenchyma cells constituted the major tissue type (figure 5).

FIGURE 1

The increase in Radish fleshy axis diameter over time.

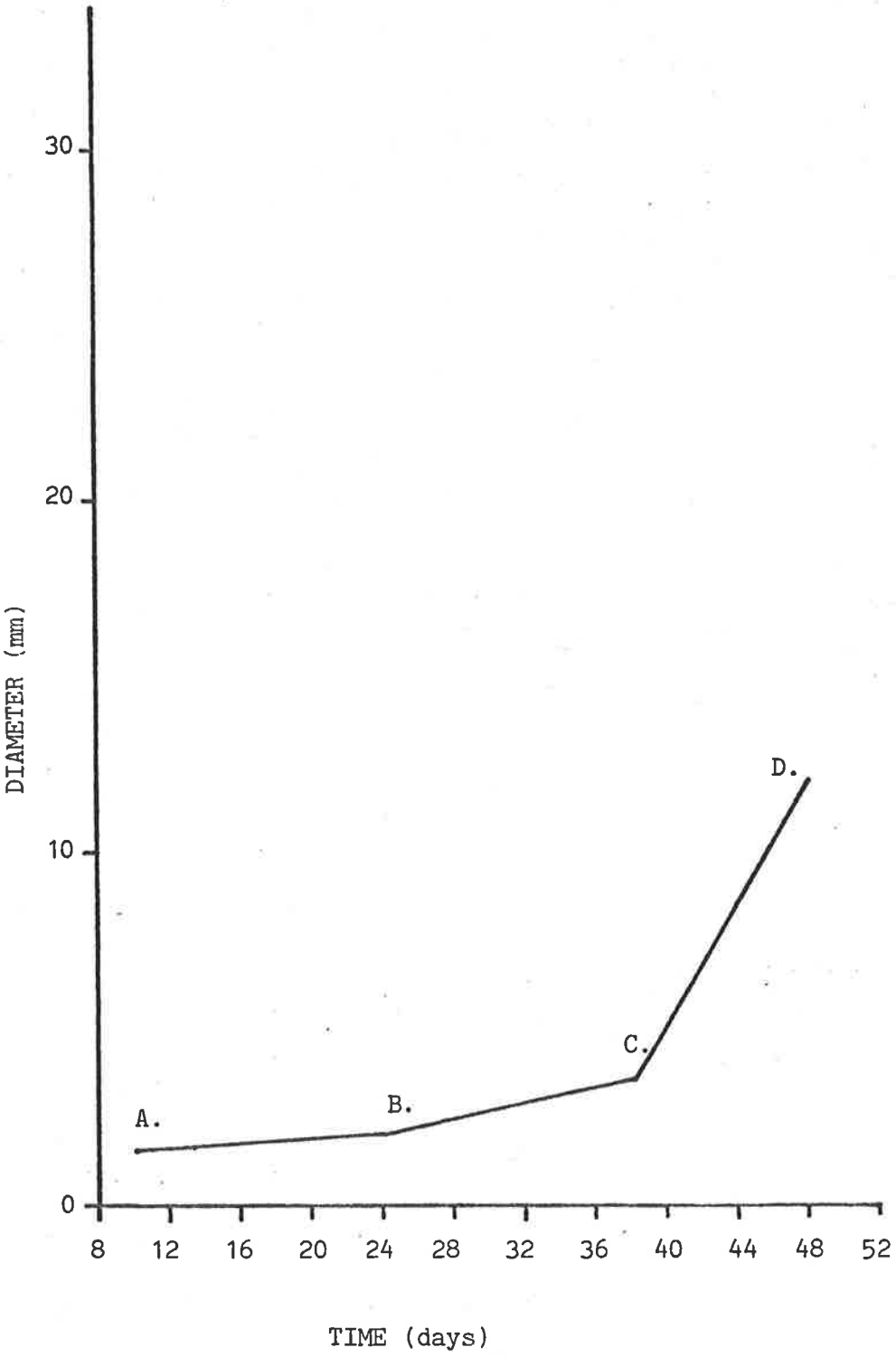
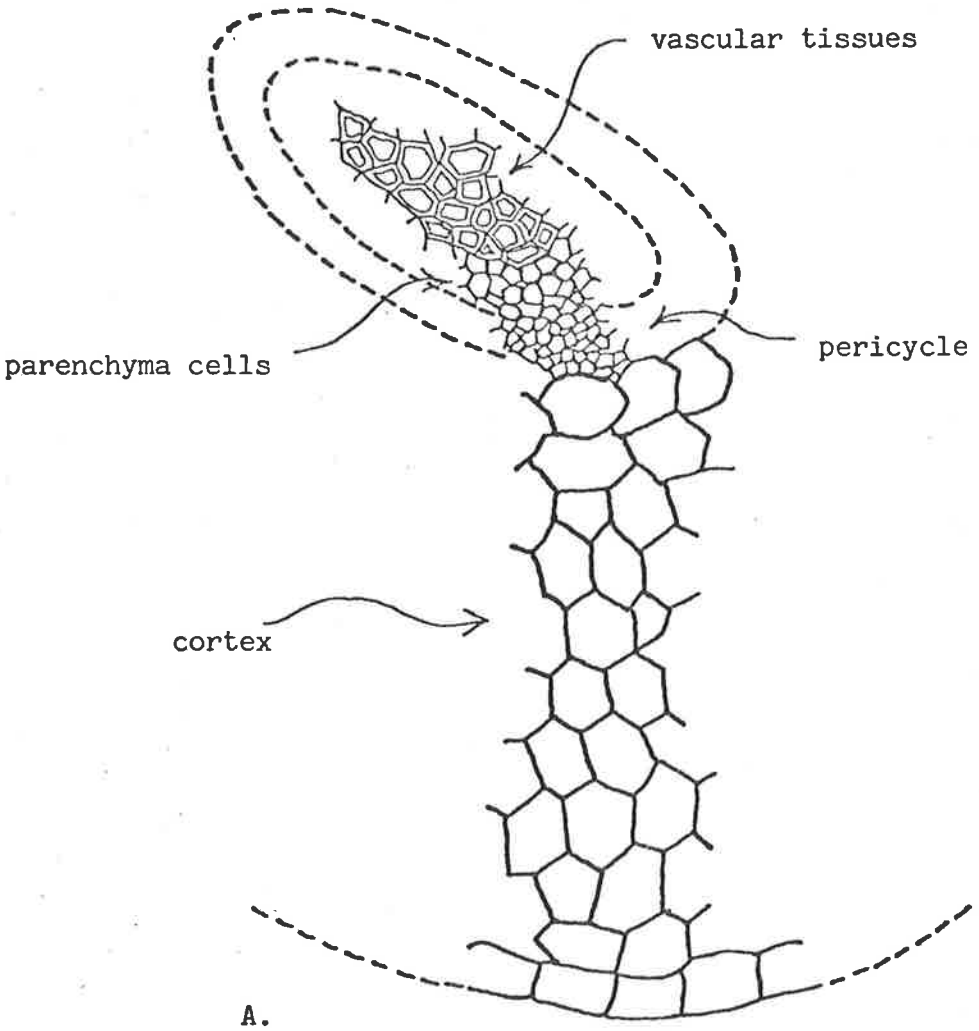

30
20
10
0
DIAMETER (mm)
A.
B.
C.
D.
8
12
16
20
24
28
32
36
40
44
48
52
TIME (days)

FIGURE 2

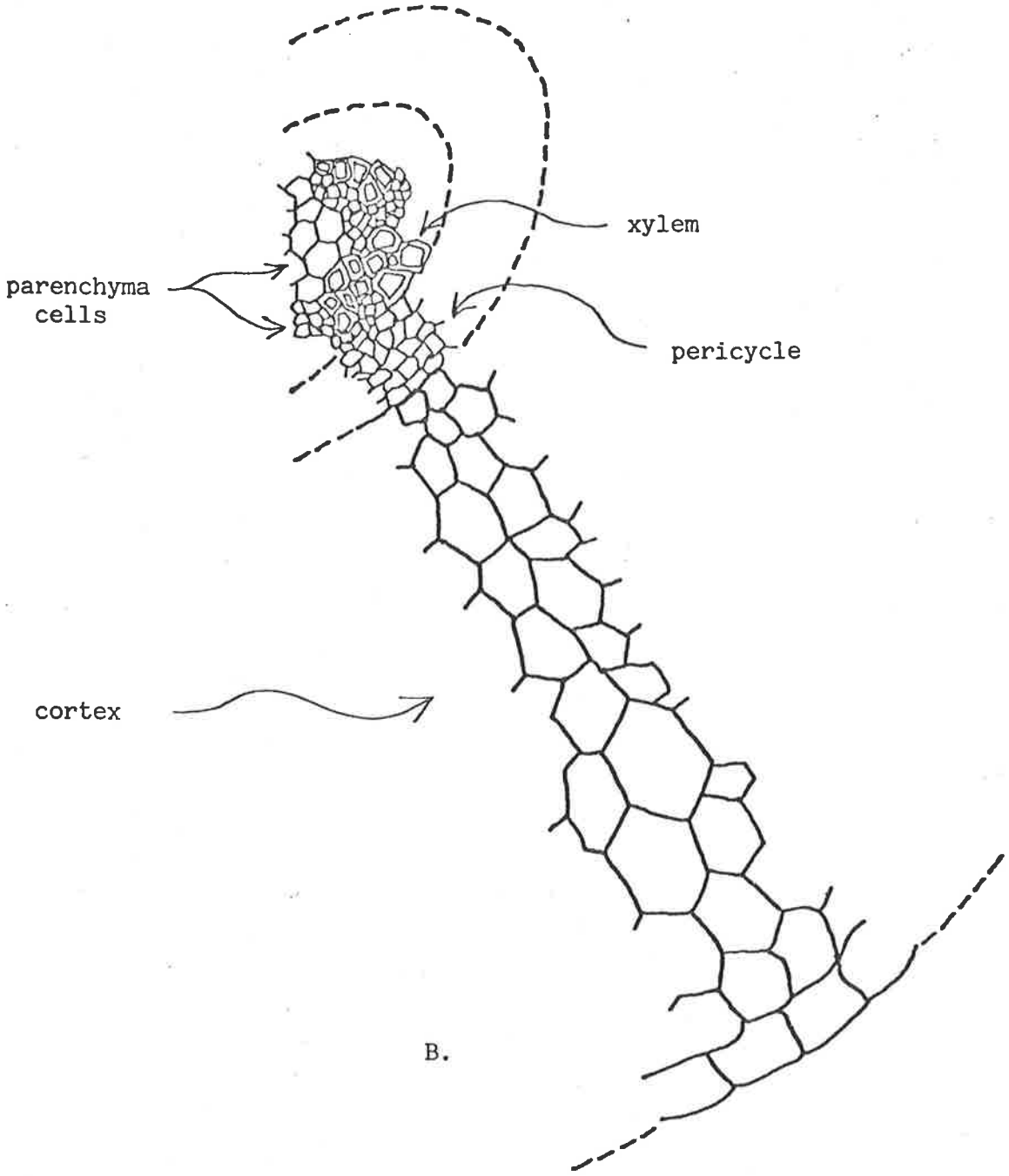
Diagrammatic representation of the transverse section through a 10 day old Radish fleshy axis.



A.

FIGURE 3

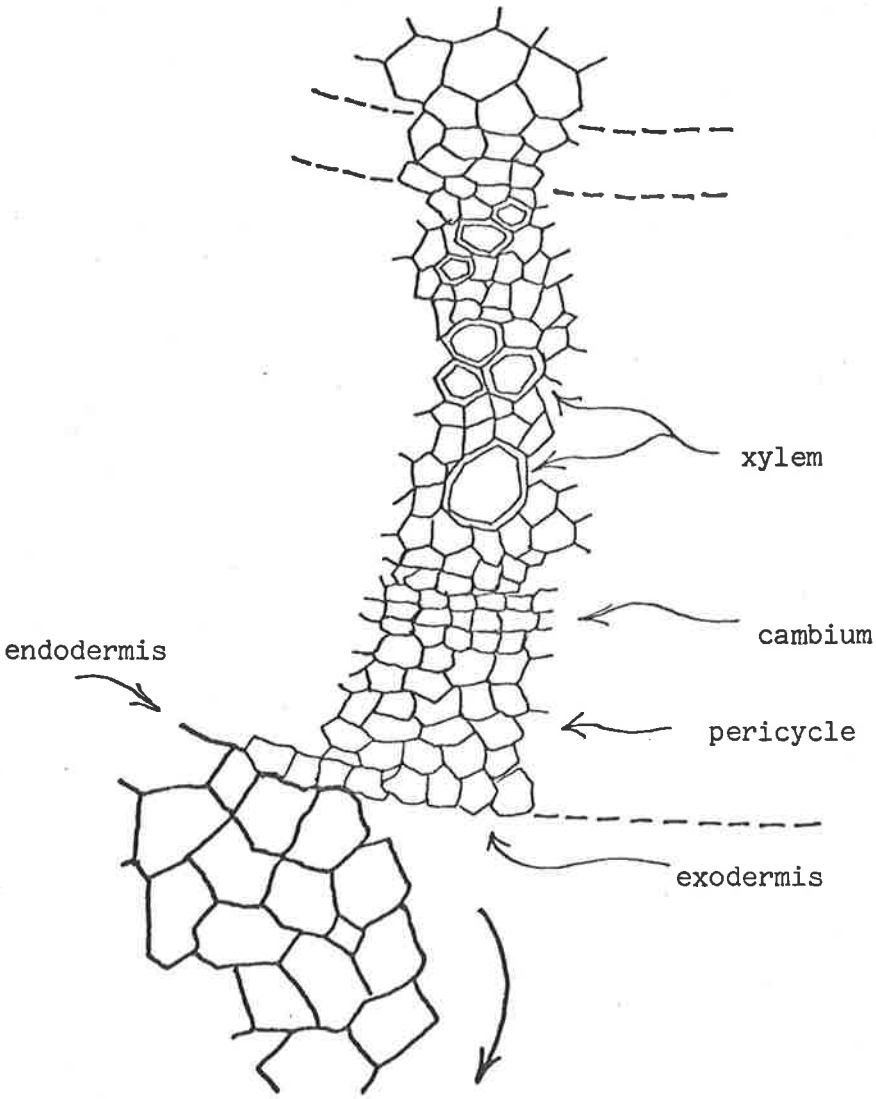
Diagrammatic representation of the transverse section through a 24 day old Radish fleshy axis.



B.

FIGURE 4

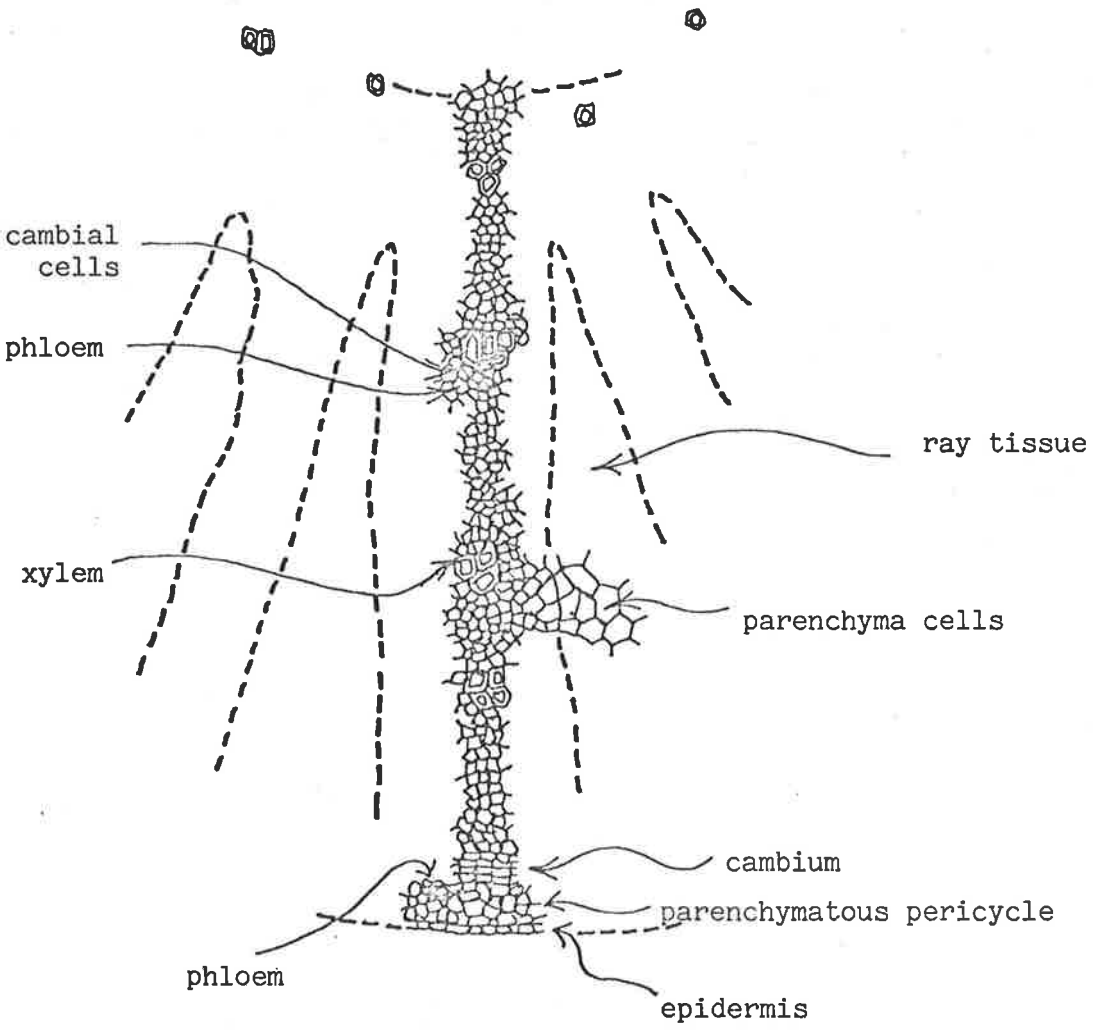
Diagrammatic representation of the transverse section through a 37 day old Radish fleshy axis.



C.

FIGURE 5

Diagrammatic representation of the transverse section through a 48 day old Radish fleshy axis.


cambial cells
phloem
ray tissue
xylem
parenchyma cells
cambium
parenchymatous pericycle
phloem
epidermis

D.