THE EFFECT OF MILD WATER STRESS ON VEGETATIVE GROWTH IN TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) AND *PYRUS BETULAEFOLIA* BUNGE

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THIS THESIS IS DEDICATED TO
MY DEAREST WIFE
CHRISTINE

... blessed is the man that trusts in the Lord,
whose confidence is in him.
He will be like a tree planted by the water
that sends out its roots by the stream.
It does not fear when heat comes;
its leaves are always green.
It has no worries in a year of drought
and never fails to bear fruit.

Jeremiah 17:7-8.

ABSTRACT

The effects of mild water stress and physical root restriction on leaf parameters and assimilate partitioning have been studied in order to understand and refine orchard management techniques which utilise both of the above stress elements (e.g. regulated deficit irrigation (RDI)).

Initially, a system for applying a controlled level of water stress was developed and plant responses within this system defined. The system involved using aeroponic tanks with water stress generated by cycling the misting pumps on and off (intermittent misting). Similar systems have been used by other workers to stimulate hormonal changes but no work has been reported detailing vegetative growth responses. The intermittent misting technique was compared to polyethylene glycol (PEG) generated water stress, using tomato (*Lycopersicon esculentum* Mill. cv. Virosa F1) as a model plant.

Water stress studies were then carried out on *Pyrus betulaefolia* (an important root-stock for the asian pear (nashi) fruit crop (*Pyrus serotina*)) using intermittent misting. These results were compared to those from a root restriction trial involving *P. betulaefolia* in a circulating hydroponic system.

The performance of polyethylene glycol in the aeroponic system appeared to be better than in the various hydroponic systems which have been reported. Polyethylene glycol 4000 gave the best results (from PEG 1000, 4000 and 6000), in terms of in minimum level of phytotoxicity, up to a total nutrient solution water potential (Ψ_W) of around -6 bar.

Under an intermittent misting regime, tomato plants were subjected to a range of misting pump off-times up to 1.55 hours, with a constant on-time of 1 minute (to saturate the root system). It was found that important plant parameters could be related in a negative logarithmic fashion to misting pump off-time (e.g. leaf Ψ_W, plant part dry weights, allometric k value, net photosynthetic rate and stomatal conductance). For *P. betulaefolia* trees, exposed to misting pump off-times of up to two hours, plant parameters were also related to the negative logarithm of the misting pump off-time. This was despite the fact that the tomato seedlings grew approximately exponentially while *P. betulaefolia* plants grew in a more linear fashion. Hence it was concluded that intermittent misting was an ideal method for generating a controlled water stress (under which plant responses could be predicted) in both pure and applied experimental work.

Under physical root restriction (with water stress minimized), no significant differences were found in several important parameters, including net photosynthetic rate. Also, in contrast to the water stress response, assimilate partitioning to the shoot system increased (increase in the allometric k value). The relative increase in partitioning was greatest in the stem component, this being the plant part most severely affected by water stress.

Under both water stress and physical root restriction the allometric k value appeared to change rapidly, following application of the treatment, and then remain constant. This constancy was tested by using a previously unutilized plot involving the shoot/root ratio *versus* time linearized plant dry weight.

The results of plant responses to both water stress and physical root restriction are discussed in relation to vegetative growth control measures in fruit crops such as RDI. Consideration is also given to the overall mechanisms behind observed growth responses under the two stress regimes.

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"... in a technologically advanced society, the manipulation of stress offers a precise means of regulating plant growth for economic and aesthetic purposes." (Hanan, 1972)

1.1 THE IMPORTANCE OF UNDERSTANDING WATER STRESS

Effects of water stress on plant growth and development have been the subject of extensive study over the past century. This reflects the importance of water shortage over much of the world's productive land in reducing yields or causing total crop failures (Hsiao *et al.*, 1976b). Under field conditions, frequent and moderate soil drying is one of the most common stresses experienced by a plant during its life cycle (Zhang and Davies, 1989a).

Water stress is just one of a number of biological stresses which influence plant growth. Brown and Scott (1984) define the term biological stress as any external stimulus acting on a portion of the root or shoot system to the extent that normal growth is significantly reduced, modified or stopped. Hence water stress results from a change in the water status of the environment (above and/or below ground) which modifies normal growth.

The importance of water stress is reflected in the vast amount of relevant scientific research and literature. Plant responses to a water deficit were reviewed by Vaadia *et al.* (1961) and later by Hsiao (1973) in a key paper. Since that time numerous reviews have been written covering more specific areas (e.g. Begg and Turner, 1976; Hanson and Hitz, 1982; Turner, 1986b) as knowledge and information on the subject has grown. Turner (1986b) noted that about 15,000 articles on plant water relations were written in the ten years from 1976 to 1986, an average of four papers every day, so there is no shortage of reading material.

Nevertheless there is still lack of knowledge in key areas, such as the role of plant hormones. Before these substances came to prominence, understanding was centred around physical concepts. Soil water deficits were thought to be transmitted to the leaves simply by a water potential (Ψ_W) gradient. Now the involvement of plant hormones in all aspects of internal physiology is apparent, with water stress related interactions between the root and shoot system being no exception. However, the role

of physical gradients in water content and potential must not be totally neglected since they could still hold the key to unlocking the interactive complexities between a plant and its environment (e.g. see McIntyre, 1987).

The large amount of literature available on plant water relations, and in particular water stress, can lead to confusion and misunderstanding. It is therefore imperative that all relevant fundamental concepts be embodied in the discussion of experiment results in this area. Furthermore, discussion of water stress responses with respect to horticultural production requires the consideration of wider issues involving hormonal interactions and other environmental factors. This requires a thorough and comprehensive literature survey. In order to lay the foundation for discussing experimental work, a review of the literature dealing with water stress on plant physiology was carried out. The review was divided into four sections which cover elements of the SPAC (soil-plant-air continuum), biochemical processes, growth aspects and plant hormones respectively.

1.2 A BASIS FOR CONSIDERING WATER STRESS PHYSIOLOGY

A useful model to consider when exploring the complex area of plant water stress is a plant having a root system in soil and a shoot system in air. More generally one can think in terms of the root environment and the shoot environment (allowing for man-made systems such as hydroponics and aeroponics). A change in the water relations of either or both environments may lead to plant water stress. The various stress combinations are as follows;

i/ Moist shoot environment (small vapour pressure deficit (vpd)) + wet root environment (high soil water potential (Ψ_W(soil))).
ii/ Moist shoot environment (small vpd) + dry root environment (low Ψ_W(soil)).
iii/ Dry shoot environment (large vpd) + moist root environment (high Ψ_W(soil)).
iv/ Dry shoot environment (large vpd) + dry root environment (low Ψ_W(soil)).

Simple growth studies have shown how a plant responds to each combination (e.g. Nagarajah and Schulze (1983)). All four combinations can occur naturally but when considering water stress 'development' *per se*, doubt surrounds number ii above (Kramer, 1988; Boyer, 1989) because this can only develop after a period of evapotranspirational water loss which produces a drier soil.

Regardless of the changes in environmental water status, two points should always be remembered;

i/ Water enters a plant via the root system and is lost from the shoot system (limited water uptake is also possible via the leaves under humid conditions and water loss from the roots when Ψ_W(soil) is less than the root water potential (Ψ_W(root)) but these possibilities are seldom significant).

ii/ The water potential (Ψ_W) of the atmosphere is usually far lower than that of the soil.

1.3 WATER STRESS AND HORTICULTURAL PRODUCTION

In considering the effects of water stress one is thinking of a plant receiving 'suboptimal' levels of water. But what is optimal? The question may perhaps be resolved with respect to crops grown for vegetative parts but the situation for perennial fruit crops is very complex as noted by Hudson (1970) in relation to critical soil water deficits. In horticultural terms the water requirement for a crop was succinctly defined by Spomer (1985) as "the minimum amount of water required to provide optimal yield". Optimal yield could most usefully be defined in financial terms which are dependent firstly upon the biomass production *versus* harvest index relationship and secondly upon the quantity/quality price differential with all that this complex relationship entails. Huck (1984) points out that maximizing water flux through the plant is not the same as maximizing economic returns. This is because decreased photosynthesis may have little effect on economic yield. Potential yields are being constantly increased with the introduction of new technology and production techniques. Of particular significance here are those which increase yield per unit volume of water required such as regulated deficit irrigation (RDI) (Mitchell *et al.*, 1986) (see following section). Such techniques have highlighted the profound revelation that complete elimination of water deficits is not a universally desirable goal for horticulturists. This traditional view, however, still continues to flourish in the literature (e.g. Atkinson and Thomas, 1985).

The basis for techniques such as RDI was, in the first instance, the control of vegetative growth and thus improvement of the harvest index (marketable yield / total yield ratio). This is the fundamental, though frequently neglected, aim in a large amount of horticultural research. Passioura (1986) believes that the root systems of

many crop plants may be excessively large but notes that there is little information on the size needed to maximise yield.

There is an inherent limitation to increasing the harvest index beyond a certain point. This is apparent even in the vegetative phase as experimental work to follow will show. The plant, being a multicomponent organism, must retain a balance between components in order to function properly. Excess demand by one component leads to internal imbalance and consequently a reduction in gross productivity. For example, water stress is simply an imbalance between demand by the shoot and supply by the root.

It is true that plants show a high degree of flexibility in their morphology but this comes at a cost to both productivity and stress tolerance. If the root systems of crop plants are reduced, by whatever means, in order to increase the harvest index, management systems, especially with respect to water supply, will need to improve. Otherwise increased production will again be at the mercy of climatic whims.

Beevers (1985) states that "there are at present no powerful chemical treatments or cultural practices that ensure the superiority of one sink (that is, a carbohydrate importing organ) over another". From a physiological standpoint, one sink can never have continual superiority as this would inevitably lead to imbalance. Rather, it is a case of manipulating the ontogenic pattern of sink superiority to suit a specific crop. This goal is generally not well understood and if it is to be achieved, detailed knowledge is needed of the basis for sink superiority and the developmental pattern. One of the many avenues for investigation is to study plant responses to stress. Experimental work to follow embodies in part this philosophy.

From a horticultural standpoint, survival characteristics are generally of little importance because yields are irreversibly lost long before these take effect. Survival is favoured by high leaf resistance but this restricts photosynthesis and hence productivity (Jones, 1976). Nevertheless survival characteristics are still important in third world countries where subsistance horticulture must cope with few resources and often a very harsh environment. They are also a factor in marginal situations where adequate control of certain climatic and/or edaphic features may be either impossible or economically unviable. The economic environment of today is pushing more and more land into the marginal category thereby bringing back the issue of plant survival.

Today most horticultural crops, in developed countries at least, are of high value and hence considerable expense goes in to creating a favourable growing environment. This involves providing shelter, irrigation and fertilizers while eliminating pests and diseases. The crop and microclimate can now be accurately and

precisely monitored to allow accurate control by a wide range of methods. According to Hubick *et al.* (1986a) three options are available when water is limiting production:

i/ Irrigate.
ii/ Farm humid areas more intensively.
iii/ Select (breed) plants which need less water.

All three of these options are being pursued in order to increase horticultural production world wide. Irrigation provides the greatest boost to productivity even in areas receiving a large annual rainfall. However Goode (1972) points out that irrigation timing is critical in such climates to ensure economic benefits. Intensive farming of humid areas will continue to be a factor as people strive to maximise economic returns from a unit of land. If the cost of irrigation can be avoided then there is an immediate advantage, other things being equal.

Breeding plants for greater productivity is an important long term goal for many crops. It tends to be more important however for agronomic species which are annuals *versus* perennials and of low unit cost. With tree crops, breeding for greater water use efficiency is perhaps of marginal importance due to the crop value, economic viability of irrigation and time periods involved in a breeding program. The main role for plant breeding has been, and will continue to be, improving product quality.

1.4 VEGETATIVE GROWTH CONTROL IN HORTICULTURE

The importance of controlling vegetative growth in modern fruit production cannot be overemphasized. Economic factors, particularly the cost of land and labour, continually require more intensive production and increased mechanization. With increased tree density the normal growth habit of the tree must be severely curtailed. Pruning is wasteful in terms of both time and money and loss of assimilated dry matter to a nonproductive end. Hence management systems which reduce vegetative growth and increase productivity have been keenly sought.

Buttrose and Mullins (1968) established a relationship between shoot growth and root pruning. Since that time root pruning has been developed as a successful commercial technique for achieving the goals mentioned above (Ferree, 1989; Geisler and Ferree, 1984; Schupp and Ferree, 1988; 1989). It still suffers, to some extent,

from the drawbacks associated with shoot pruning but with correct timing and degree of root removal good results are obtained. Schupp and Ferree (1988) found that root pruning could reduce shoot growth without affecting the yield of apple trees. In addition, yield efficiency, fruit colour and soluble solids were increased with a decrease in pre-harvest drop.

Another new technique which shows more promise in many respects to that of root pruning involves subjecting trees to a controlled water stress for part of the growing season. This technique is popularly known as regulated deficit irrigation (RDI) and has been developed in Australia (Chalmers *et al.*, 1981; Chalmers *et al.*, 1984; Chalmers *et al.*, 1986; Mitchell and Chalmers, 1982; Mitchell *et al.*, 1986; Mitchell *et al.*, 1989; Proebsting *et al.*, 1989). An important factor is that vegetative and reproductive growth should be separated in time to some extent, usually with an initial flush of vegetative growth at the start of the season. Chalmers *et al.* (1984) have shown that only 25% and 27% of fruit growth in peach and pear respectively occurs while vegetative growth is more active. This will be true to some extent for most deciduous perennial fruit crops (Chalmers *et al.*, 1984). However in some species the separation may be too small to be utilised successfully. A positive factor is that there is a tendency for the separation to increase with tree age due to vegetative growth stopping earlier in the season (Chalmers and van den Ende, 1975) and it is in older trees where vegetative growth control is essential.

To summarize the RDI principle, trees are water stressed at the start of the season which inhibits vegetative growth. Just before rapid fruit growth begins full irrigation is restored. The result is that fruit growth can actually increase due to reduced competition from vegetative sinks (Chalmers *et al.*, 1981; Chalmers *et al.*, 1986; Mitchell and Chalmers, 1982). The water stress is imposed by initially withholding irrigation to develop a soil water deficit and then to apply some fraction of the amount being lost from the soil (e.g. 20% to 50%) (Chalmers *et al.*, 1986; Mitchell *et al.*, 1989).

It is interesting to note here that there are other examples of using an imposed water stress to modify plant growth which have been around for many years. These generally relate to stimulating and controlling flowering. In Italy the practice called 'Forzatura' is used to obtain summer fruit from lemon trees (Barbera *et al.*, 1985).

1.5 **BACKGROUND TO EXPERIMENTAL WORK**

This thesis covers only the plant responses to water stress in the vegetative phase. A full understanding of plant responses at this level leads naturally on to the fruiting stage where major physiological changes occur. Generative organs are strong metabolic sinks which monopolise plant hormone distribution by producing their own supply primarily via the seed (Salisbury and Ross, 1985). This has a large effect on the top-root relationship and the way both parts respond to environmental variables, including a lack of water (Avery, 1969; Erf and Proctor, 1987; Giulivo *et al.*, 1985; Jackson, 1984). Also, plants can be very sensitive to a deficit at one point in their reproductive cycle often due to reduced root growth in the transition to reproductive development (Bierhuizen, 1976), where a change from terminal to lateral root growth may occur (Brown and Scott, 1984).

This thesis was undertaken with both theoretical and practical plant physiological considerations in mind. A high value horticultural crop was used and hence the focus was upon mild water stress effects and not those associated with severe stress. In some situations there may be a need to investigate more severe stress even on fruiting trees (e.g. Fereres *et al.*, 1979) but these cases will not be considered in detail. Of particular significance are those physiological features which enable the plant to remain economically productive under increasing water stress or perhaps become even more productive.

Experiments were conducted over a three year period in accordance with the three aims given below:

i/ Develop an experimental system using aeroponic tanks to give controlled levels of water stress.

ii/ Investigate the differences between unconfounded water stress and physical root restriction with respect to leaf parameters (photosynthetic rate, stomatal conductance, leaf Ψ_W) and assimilate partitioning.

iii/ Investigate the relationship between root environment Ψ_W and vegetative growth parameters in tomato and *Pyrus betulaefolia.*

To facilitate work in relation to the first aim tomatoes were chosen as a model plant because of their rapid growth, ease of management and depth of knowledge with respect to their physiology.

An aeroponic system is similar to that of hydroponics except that the root system is either partially or completely suspended in air rather than water. It has been used in scientific investigations for many years (see Vyvyan and Trowell (1952) and references therein) but its full potential appears to have been neglected. In recent times it has been developed commercially in order to make 'liquid culture' installations simpler in construction, easier to run and more economical (Massantini, 1985; Nir, 1980), "permitting maximum control of the growth process" (Nir, 1980).

For scientific research, aeroponic systems present an ideal means of growing plants which have completely accessible and unrestricted roots. Accessibility means that any form of root manipulation, hormone treatment etc. can be carried out easily with minimal disturbance. Workers who have already utilised the aeroponic system include Hubick et al. (1982), Hubick and Reid (1988), Soffer and Burger (1988) and Thuantavee (1990).

In developing the aeroponic system at Massey University a simple yet flexible design was required. The final arrangement is described in detail in appendix 18.4. Experiments associated with aim (i) are given in sections 8, 9 and 10.

An experiment based on aim (ii) was conducted in a circulating hydroponic system (see section 11 and appendix 18.5). This system was designed and constructed by Mr B.R. McKay of the Horticultural Science Department at Massey University (Palmerston North, New Zealand) based on the deep flow technique (DFT) system of Willumsen (1983). The experiment was conducted using *Pyrus betulaefolia* plants so as to link in with aim (iii) which centres on the experiment given in section 10. *Pyrus betulaefolia* was used because it is a common rootstock for Asian pears (Nashi) in New Zealand and adapts well to non-ideal conditions (as described below). It was considered important to use a rootstock because the root system is of central importance with respect to plant water relations. Part of the focus of aim (iii) was directed towards the relationship between root and shoot subsystems. Turner (1986b) considered root-shoot interaction under water stress to be a key area for further study.

1.6 *PYRUS BETULAEFOLIA* AND THE NASHI

1.6.1 *THE NASHI*

The term 'nashi' now generally refers to cultivars derived from the Japanese pear species, *Pyrus serotina* Rehder var. *culta* Rehder (Buwalda and Meeking, 1989

unpublished) as opposed to the more general term 'asian pear' which includes the Chinese species *P. ussuriensis* Maxim. (Griggs and Iwakiri, 1982). Nashi fruit are round in shape with firm, crisp, juicy flesh and either smooth or russet skin (Griggs and Iwakiri, 1982). They have been grown extensively in Japan for a long time but were only introduced into New Zealand in 1977. The five major cultivars, Hosui, Kosui, Shinsui, Shinseiki and Nijisseiki were released from quarantine in 1980 (White, 1981). A variety of rootstocks have been used both in New Zealand and overseas including *P. serotina*, *P. calleryana*, *P. betulaefolia*, *P. communis* and quince (*Cydonia oblonga* Mill.) (Griggs and Iwakiri, 1982; Klinac and Pevreal, 1987).

Planting and production in New Zealand has increased rapidly over the past three years with growth forecast to continue into the 1990's (Wilton, 1987). Industry success will depend in part on the accumulation of crop knowledge related to New Zealand conditions. Buwalda and Meeking (1989, unpublished) note that general pipfruit recommendations have been used which are clearly not ideal in the long term. Development of a dwarfing rootstock would also be of great benefit (Klinac and Pevreal, 1987), as it has been to the apple industry.

1.6.2 *PYRUS BETULAEFOLIA*

Pyrus betulaefolia grows in a wild state over north and northwestern China where it is the major rootstock (Shen, 1980). It has become the preferred rootstock in the United States (replacing *P. communis* (Griggs and Iwakiri, 1982)) due in part to its greater resistance to pear decline (Loreti and Morini, 1977; Westwood and Lombard, 1977).

Trees on *P. betulaefolia* are more vigorous than on other rootstocks (Klinac and Pevreal, 1987; Larsen, 1982) and often exhibit poor yield efficiency (Larsen, 1982) though not always (Gur *et al.*, 1978). These are important disadvantages under modern high density growing systems. They also tend to be variable in performance and suffer from chlorosis (Gur *et al.*, 1978). Strong heterogeneity is exhibited by *P. betulaefolia* plants propagated from seed. For this reason clonal propagation has been investigated with hormonally treated softwood stem cuttings proving to be most successful (Loreti and Morini, 1977).

Trees on a *P. betulaefolia* rootstock however show very good adaptation to high clay and sand soils (because they tolerate both water logging and drought), while exhibiting increased resistance to certain pests and diseases (Andersen *et al.*, 1984;

Larsen, 1982; Loreti and Morini, 1977; Shen, 1980). They also tolerate low temperature and soils with a high pH (Shen, 1980). *P. betulaefolia* may be used where the physiological disorder 'Yuzuhada' is a problem (Kanato *et al.*, 1982) but are not very resistant to fire blight (Westwood and Lombard, 1977). For these reasons *P. betulaefolia* will continue to be widely used, especially where climatic and edaphic conditions are marginal.

The ability of *P. betulaefolia* to tolerate flooding was clearly shown by Andersen *et al.* (1984) who found that trees could survive 20 months of continuous root submergence. Andersen *et al.* (1985) found that pear roots were able to maintain high respiration rates under anaerobiosis and utilize oxygen efficiently under low oxygen concentrations. They did not find evidence of aerenchyma (parenchyma with large intercellular spaces) but suggested that anaerobic respiration may be important under flooding.

LITERATURE REVIEW

WATER STRESS ON VEGETATIVE GROWTH

2 WATER STRESS AND WATER IN THE SOIL-PLANT-AIR CONTINUUM

Water constitutes over 80% to 90% of plant tissue weight (Hsiao, 1973; Huck, 1984) and carries out the following functions (Spomer, 1985):

i/ Hydraulic agent.
ii/ Biochemical reagent.
iii/ Solvent.
iv/ Protoplastic structural agent.

Water in plants is linked to water in the soil via root epidermal cells and to water in the atmosphere predominantly via stomata. Thus the plant may be thought of as a conduit through which water travels from the soil to the atmosphere, forming the so called soil-plant-air continuum (abbreviated as SPAC).

Water in the soil, plant and air is influenced by different sets of factors and held by quite different combinations of physical forces. These interact through the plant, thus creating a complex system. In characterizing the water status of a plant, or in fact any point in the SPAC, two basic parameters are needed, the water content (WC) and Ψ_W i.e. how much water is present and how strongly it is held within the system. Measurement of the rate of movement may also be important (Spomer, 1985). The following discussion will deal with these topics, as well as stomata, which constitute the primary control point for water movement within the plant, transpiration, water use efficiency and translocation.

2.1 PLANT WATER POTENTIAL AND ITS COMPONENTS

Water potential is the chemical potential of water taken from a reference point of zero for pure free water at atmospheric pressure and at the temperature of the system being considered (Boyer, 1969). Introduction of the pressure chamber (Scholander *et al.*, 1964) has allowed quick and easy measurement of bulk tissue Ψ_W within the plant. Unlike other plant parameters such as stomatal conductance, plant water potential (Ψ_W(plant)) is comparatively constant within a given plant part (e.g. leaves) and so only a few measurements are needed to obtain a reliable estimate (Jones and Cummings, 1984). Furthermore, morphology and physiology of the sample have relatively little effect (Goode and Higgs, 1973). For these reasons Ψ_W(plant) has been quoted extensively in recent literature, at times without proper regard to its significance.

It has been recognized for several years that in terms of physiological significance it is not total water potential *per se* which is important but rather its components, pressure and osmotic potential (Schulze, 1986b). According to Krizek (1985) "there is no proof that total water potential has any direct effect on physiological processes". Even in the case of pressure and osmotic potential, there are only limited examples of a link to physiological processes. Sinclair and Ludlow (1985) state that the only clear example of a relationship between the thermodynamic state of water and physiological performance is that between pressure potential (Ψ_P) and abscisic acid (ABA) accumulation.

Water potential is frequently stated as being important because water moves down Ψ_W gradients (Salisbury and Ross, 1985). However this may not be the case if solutes are present (Sinclair and Ludlow, 1985), as in the phloem, where water can flow anti-parallel to the Ψ_W gradient because of a significant solute concentration (Passioura, 1984). Furthermore, Jones and Higgs (1989) found that leaf water potential (Ψ_W(leaf)) was of little value in predicting the stomatal conductance of apple trees when compared with the environmental variables vpd, shortwave radiation and air temperature. Questions have also been raised as to the use of Ψ_W as a water stress indicator. Garnier and Berger (1985) tested all basic parameters and found that the commonly measured Ψ_W(leaf) was not a very good indicator but that stem water potential (Ψ_W(stem)) and $d\Psi_W$ (Ψ_W(stem) - Ψ_W(leaf)) were sensitive to changes in Ψ_W(soil).

It has been suggested that relative water content (RWC) may be better than Ψ_W(plant) for explaining physiological changes (Ritchie and Jordan, 1972; Schulze, 1986b; Sinclair and Ludlow, 1985). It is a stable yet dynamic parameter which may integrate the plant water balance over several days (Sinclair and Ludlow, 1985). Measurements can be made simply using the technique of Barrs and Weatherley (1962) or with a β-ray gauge (Boyer, 1969). However RWC does use turgidity as its reference point and Boyer (1989) questioned whether leaves are ever fully turgid *in situ*. Thus Ψ_W still stands as the best single measure of plant water status (Richter, 1976; Spomer, 1985) with an important role in experimental comparison (Boyer, 1989), although its use in indicating a particular stress level is dubious (Davies and Zhang, 1991). The key is to use it with discretion and in full awareness of its possible inadequacies.

Plant Ψ_W is composed of component water potentials as follows:

$$\Psi_W = \Psi_P + \Psi_S + \Psi_M + \Psi_g \qquad 2.1$$

Where: Ψ_P = pressure potential; Ψ_S = osmotic potential; Ψ_M = matric potential; Ψ_g = gravitational potential.

The magnitude of each component may vary significantly between different plant tissues (Scott Russell, 1982).

Plant water stress results from a change in the water status of the biotic environment. Theoretically this could involve a change in either the water content or water potential of the environment. However for a closed system, allowing only water exchange, the two are related (though not always in a simple fashion because of hysteresis in both soil and plant tissue). Hence water stress may be defined in terms of a change in Ψ_W(plant), although this may be misleading for two reasons. Firstly it has been noted that during the day, Ψ_W(plant) is influenced largely by atmospheric factors (Rudich *et al.*, 1981) whereas the water stress may be due to a decrease in Ψ_W(soil). Secondly, the components of Ψ_W (*viz.* pressure and osmotic potentials) can be masked by, or themselves mask, changes in Ψ_W(plant). For example, Ψ_S often decreases under water stress (osmotic adjustment) thereby reducing or eliminating loss of turgor (Ψ_P) while Ψ_W(plant) is falling.

If Ψ_W(leaf) is measured at dawn this generally correlates well with Ψ_W(soil) (Garnier and Berger, 1985; Natali *et al.*, 1985a, b, c; Rutter and Sands, 1958) because plant and soil water potentials tend to equilibrate during the dark period (Klepper, 1968; Natali *et al.*, 1985a). Plant Ψ_W diverges from soil Ψ_W during daylight hours under the influence of atmospheric factors (Rudich *et al.*, 1981).

2.1.1 *PRESSURE POTENTIAL*

Pressure potential, which in the cell is positive and constitutes turgor pressure, has received much attention over the years (see Zimmermann, 1978) because it has been considered as the physical link between plant water status and growth processes (Zur and Jones, 1981). It is a function of both the osmotic potential of the vacuole and elastic properties of the cell wall (Oertli, 1976), the latter being a function of cell size, osmotic volume and cell wall thickness. Turgor maintenance is favoured by small, highly elastic cells (Turner and Jones, 1980).

Turgor pressure is believed to control the electrochemical properties of the membrane and membrane transport along with many aspects of cell metabolism which stem from these changes (Zimmermann, 1978) and has been given a central role in the physiochemical transduction of plant water status (Hsiao *et al.*, 1976a; Turner and

Jones, 1980). It is thought to be required for general membrane functioning and membrane integrity throughout the plant (Zimmermann, 1978). A number of observations have indicated that turgor changes cause rapid alteration in gene expression. Synthesis of ABA for example requires translation of nuclear genes (Guerrero and Mullet, 1988). In *Escherichia coli* turgor changes alter expression of the kdp operon which is involved in K^+ transport through cell membranes. In wilted plants several poly $(A)^+$RNAs increase in concentration but this cannot be induced by heat shock or exogenous ABA (Guerrero and Mullet, 1988).

However, in recent times, the role of turgor pressure in controlling cell expansion and stomatal conductance (g_s) has been thrown into doubt (Munns, 1988) and new evidence supports this. Kuang *et al.* (1990) found that maintaining high xylem Ψ_W by pressurization could not prevent decreased cell elongation or osmotic adjustment (see section 2.1.2), the two being linearly related. Such results suggests that leaf turgor controls neither elongation nor osmotic adjustment but that root produced hormones may be involved (Kuang *et al.*, 1990). This is a very important aspect which needs a strong research emphasis in the next few years.

Rapid changes in Ψ_W(leaf) result principally from changes in Ψ_P (Meidner, 1983). However when Ψ_W is high, the correlation between Ψ_W and Ψ_P tends to be poor due to osmotic adjustment (Davies and Lakso, 1978). Leaf pressure potential (Ψ_P(leaf)) can be influenced by both Ψ_W(soil) and atmospheric evaporative demand (Gavande and Taylor, 1967). If Ψ_W(leaf) is high, Ψ_P(leaf) changes rapidly with small changes in leaf water content. Hence Ψ_P(leaf) is highly unstable (Sinclair and Ludlow, 1985).

The plant may maintain leaf turgor by maintaining water uptake, osmoregulating or reducing water loss. The latter is achieved by reducing leaf area and stomatal conductance as well as leaf rolling, leaf movement and increasing leaf reflectivity (Turner, 1986). Pressure potential is sensitive to a change in cell water content (cell volume) near full turgidity because Ψ_P and cell volume are nonlinearly related. The actual relationship is unknown but has been represented by various exponential or power functions (Schulte and Hinckley, 1985).

2.1.2 *OSMOTIC POTENTIAL*

The abundance of differentially permeable membranes in the plant system enables Ψ_S to be significantly different within tissues and cells. Xylem sap is very dilute (Nonami and Boyer, 1987) with a Ψ_S generally below -0.5 bar (Ritchie and

Hinckley, 1975). In contrast, Ψ_S of a cell varies considerably and changes with age and environmental conditions. These changes are of two types:

i/ Hydro-passive changes - concentration and dilution by change in cell volume.
ii/ Hydroactive changes - net change in solute levels by transport through membrane or biochemical alteration of cell material.

It is the second type of change which may be called osmotic adjustment (erroneously called osmoregulation (Munns, 1988)).

When a plant experiences a water stress (or several of the other biological stresses e.g. low temperature (Augé and Stodola, 1989)), cellular Ψ_S is often shown to decrease and this decrease reduces or eliminates loss of Ψ_P (turgor). A large amount of literature has been produced in recent years on osmotic potential changes (see reviews of Morgan (1984) and Munns (1988)) but as Munns (1988) points out, our knowledge on the subject has not increased in similar fashion. It is important to appreciate that osmotic adjustment cannot in itself promote growth, because the accumulating solutes must be derived from essential processes, but it is one of the elements in the physiological adjustment to stress (Munns, 1988).

Osmotic adjustment has been shown to occur in both roots and shoots (e.g. Turner *et al.*, (1987)), commonly with a larger absolute change in leaves but a larger percentage change in roots (Oosterhuis and Wullschleger, 1987). With young seedlings most osmotic adjustment occurs in the root system (Hsiao *et al.*, 1976a). Roots in fact may be capable of complete osmotic adjustment (turgor maintenance) while shoots may not, and this can be used to explain changes in the S/R ratio under stress (Schildwacht, 1989). In fact, osmotic adjustment can have a multitude of effects throughout the plant as indicated by Turner (1986).

Osmotic adjustment in leaves is associated with a decrease in starch and increase in glucose plus fructose (Düring, 1985). However numerous other substances are believed to be involved including inorganic ions (K^+, Cl^-, NO_3^-), organic acids, amino acids (eg. proline (Hsiao, 1973)) and quarternary ammonium compounds (Hanson and Hitz, 1982). Often it has been difficult to locate the substances influencing Ψ_S, for example Conroy *et al.* (1988) found that increases in soluble sugar and potassium ion concentration could not account for the level of osmotic adjustment. An explanation for this may relate in part to the complex involvement of other factors such as changes in cell size and cell wall thickness (Cutler and Rains, 1978). There are also significant technical problems due to the range of possible osmotica and cellular compartmentalization. Other substances to be implicated in osmotic adjustment are

proline, betaine (glycinebetaine) and the polyamine putrescine. Accumulation of proline betaine in the cytoplasm is needed to balance the osmotic potentials between cytoplasm and vacuole and to protect the cell membranes (Hanson and Grumet, 1985; Lösch, 1984). This hardening effect may be more important (Kluge, 1976) than the decrease in Ψ_S. Putrescine may have a role in regulating cellular pH which tends to decrease under stress conditions (Flores *et al.*, 1985).

The degree of osmotic adjustment is related to the duration and severity of stress (Oosterhuis and Wullschleger, 1987; Turner *et al.*, 1987), rate of stress development (Hanson and Hitz, 1982; Turner and Jones, 1980; Turner *et al.*, 1987), genotype and organ type (Düring, 1985; Morgan, 1977, 1980), organ age (Düring, 1985; Morgan, 1984) and preconditioning (Morgan, 1984). Düring (1985) found that potential for osmotic adjustment declined with age while Henson (1982) found increased osmotic adjustment in expanding leaves. Exposure to stress conditions (stress hardening/ preconditioning) has generally been found to increase a plants capacity for osmotic adjustment (Conroy *et al.*, 1988; Cutler and Rains, 1977; Morgan, 1984) especially in later developing leaves (Morgan, 1984). Some results do not fit this pattern (Henson, 1982) but may be the result of other changes occurring during preconditioning such as a reduction in tissue elasticity (Jones and Turner, 1978).

It has been found that the degree of osmotic adjustment is negatively related to the speed of water stress development (Turner and Jones, 1980) such that no adjustment may occur at rates of stress development above 10 bar day^{-1} (Hanson and Hitz, 1982). This may explain the tendency of Ψ_S changes to persist longer in field grown plants (Oosterhuis and Wullschleger, 1987). Generally Ψ_S will return to pre-stress levels, after the elimination of water stress, within a relatively short period of time. An investigation of cotton plants by Oosterhuis and Wullschleger (1987) revealed that half the adjustment was lost after three days without stress.

Osmotic adjustment is positively related to the ambient CO_2 concentration (Conroy *et al.*, 1988) and negatively to the degree of shading (Acevedo *et al.*, 1979). This is not surprising considering that most of the osmotically active solutes are formed via the photosynthetic pathway (Morgan, 1984). In fact osmotic adjustment can be stimulated simply by a change in photoperiod (Augé and Stodola, 1989).

2.2 PLANT RESISTANCES

Flux of water through any part of the plant is inversely proportional to the resistance at that point. This Ohms law analogue for plants is known as the Huber - Gradmann - van den Honert model:

$$Q = d\Psi_W/R \qquad 2.2$$

Where: Q = flux; R = resistance.

The largest resistance in the SPAC occurs at the the leaf-air interface (Jarvis, 1985) where potential differences may reach 1000 bar (Scott Russell, 1982).

Although the simple and appealing relationship has often been proved wrong, it is still useful in developing understanding and should not be discarded (Passioura, 1984). Generally it is not expected that flow will be proportional to the water potential difference unless (Passioura, 1984):

i/ Flow is liquid only.
ii/ The system is isothermal.
iii/ Conductivity is independent of water potential.
iv/ No solutes are present.

Many workers have found that plant resistances, especially root resistance, are dependent on the rate of flux (Aston and Lawlor, 1979; Scott Russell, 1982; Tinklin and Weatherley, 1966). In fact all plant resistances may be a function of flow (Davies, 1986; Weatherley, 1976) and so should be treated as differential rather than simple. It has been suggested by Passioura (1984) that nonlinearity between Ψ_W and R results from the involvement of solutes in the flow path. Passioura and Munns (1984) investigated the relationship using soil, sand and hydroponically grown plants. They found, using a root pressurization technique, that Ψ_W was linearly related to Q only for soil and sand grown plants. Hydroponically grown plants showed nonlinearity and hysteresis. The value of the Y-intercept, for pressure chamber balance pressure *versus* Q, changed diurnally and was often much larger than $d\Psi_S$. This difference could not be explained but possibilities put forward were an unknown solute or some minimum pressure required to open plasmodesmatal valves. A correlation was found between nonlinearity in hydroponic plants and a decrease in root air spaces (Passioura and Munns, 1984).

Meron *et al.* (1989) investigated resistances in the shoot system using covered and uncovered leaves. It was estimated that 40% of the shoot resistance resided in the stem and 60% in the leaf (petiole + lamina). In general the stem component of total plant resistance is small but gradients as high as 5 bar per metre of stem may occur under high transpiration rates (Hellkvist *et al.*, 1974). Roots

contribute the greatest resistance (Aston and Lawlor, 1979; Davies, 1986; Garnier, *et al.*, 1986; Weatherley, 1976), with values being two to ten times higher than for leaves and stems (Davies, 1986).

Root resistance decreases in the presence of root growth due to increased root permeability (Teskey and Hinckley, 1981). Resistance in the leaf increases with age (Ritchie and Hinckley, 1975), perhaps mainly due to a decrease in the hydraulic conductivity of the petiole (Patrick, 1988), as does the root upon suberisation (Syvertsen, 1985a). In the long term growth of lateral roots and an increase in stem diameter reduce whole plant resistance as flux increases resulting in a morphological homeostasis (Richter, 1976).

With water stress, flux through the plant decreases and resistance to water flow increases due primarily to an increase in root resistance (Camacho-B *et al.*, 1974a; Syvertsen, 1985a). Various hypotheses have been put forward to explain variable root resistance with varying flux. Erbe in 1933 used an immiscible liquid in pores model (Tinklin and Weatherley, 1966) while Tinklin and Weatherley (1966) used a purely physical model based on the rotameter principle. Cavazza *et al.*, (1985) have attributed the reversible increase in root resistance to expansion of air bubbles in the xylem as the Ψ_W gradient (i.e. xylem tension) increases, while Davies (1986) put forward the possibility of a change in the pathway of water movement. The change in water uptake pattern with E may also be important. At low E most water is taken up at the root tips whereas under high E the basal region is the main absorbing zone (Weatherley, 1979). This region is suberised and hence has a higher resistance (Syvertsen, 1985a). Short term changes in whole plant resistance calculated from the transpirational flux and Ψ_W changes may in fact be incorrect because storage water is not accounted, since, Lösch (1984) reported that calculations which included storage water changes showed a constant resistance.

When xylem resistance reaches a certain value, air comes out of solution and the water column breaks resulting in embolisms. These may be irreversible in woody plants where there is no evidence of root pressure (Tyree and Sperry, 1988). In the model of plant water movement developed by Tyree and Sperry (1988) it is predicted that all plants operate near the point of catastrophic xylem failure under dynamic water stress. Dynamic water stress refers to that which arises from variation in flow rate through the plant and not transpiration rate *per se*. Such a model is in support of Zimmermanns plant segmentation hypothesis where plants sacrifice vulnerable minor branches in order to reduce water stress.

2.3 STOMATA

2.3.1 *STOMATA AND PLANT WATER LOSS*

The part of the plant which has received the most attention with respect to plant-water relations has undoubtably been the stomata. They are the primary control point in the SPAC for regulating water loss (Kaufmann and Fiscus, 1985; Quarrie and Jones, 1979) although their principle function is to allow uptake of CO_2 for photosynthesis. Stomata also allow O_2 into the leaf for dark respiration (Levitt, 1976). Davies *et al.* (1981) postulated that stomata act in conjunction with solute regulation to maintain a minimum Ψ_P, rather than acting independently to maintain some arbitrary Ψ_W(leaf). However the dominance of a CO_2 uptake role has been substantiated by measurement of the substomatal cavity. This indicated that dimensions of the cavity optimise mesophyll CO_2 uptake rather than minimize water loss (Lösch, 1984).

Stomatal aperture is not uniform over the leaf even under non-stress conditions. There are gradients in aperture over the lamina surface with conductance decreasing towards the tip, base and margin of the leaf (Alvino *et al.*, 1985) where guard cells tend to be smaller and more frequent (Smith *et al.*, 1989; Spence, 1987). Gradients may be as high as 1 $\mu m\ mm^{-1}$ (Smith *et al.*, 1989). There is also variation in any given portion of lamina. Laisk *et al.* (1980) represented this variation by a symmetrical bell shaped function for *Vicia faba* leaves and suggested that the underlying controlling factor may be turgor.

Two phases of stomatal opening have been suggested, the spannungsphase and the motor phase (Stålfeldt, 1924). In the spannungsphase it appears that changes occur within guard cells, such as an increase in ion concentration, without a change in aperture size. Once aperture begins to increase, guard cells have entered the motor phase. Laisk *et al.* (1980) found that a bell shaped distribution curve of stomatal aperture shifted as stomata opened but the shape of the distribution curve was unaffected. This implies that change in aperture is independent of aperture size and that the driving force is distributed evenly over the leaf surface. In this situation the spannungsphase would result from the driving force varying with wider limits than stomatal aperture (Laisk *et al.*, 1980).

Results from Downton *et al.* (1988a, b) support those of Laisk *et al.* (1980) with respect to the large distribution in stomatal apertures. They specifically noted however that stomata closed in patches over the leaf and not randomly. Spence (1987) suggested that stomata are functionally separate entities which respond to the same set

of environmental factors but as individuals. More work is needed on gradients in environmental and physiological factors over and through individual leaves to further test this hypothesis.

The response of stomata to water stress has often been considered as a response to a threshold, where closure begins at a certain Ψ_W(leaf). Values for the threshold level in tomato have been quoted between -7 and -9 bar (Rudich and Luchinsky, 1986; Tan and Buttery, 1982a) while in other crops they can be much higher e.g. citrus, -30 bar (Syvertsen, 1982); blueberry, -22 bar (Davies and Johnson, 1982). The common range is -2 to -10 bar (Hanson and Hitz, 1982). These conclusions, however, may simply be a manifestation of using resistance and not conductance (Hall *et al.*, 1976; Tan and Buttery, 1982a). The relationship between resistance and Ψ_W(leaf) is hyperbolic and so gives the impression of a threshold response. The same data plotted using conductance usually gives a linear relationship showing a gradual change in stomatal aperture (Biscoe *et al.*, 1976; Dettori, 1985) although this is not always so (Turner *et al.*, 1978). Hence conductance is the better parameter to use although Burrows and Milthorpe (1976) still place emphasis on the resistance relationship in terms of large changes in r_s at low Ψ_W(leaf). They also suggest that resistances should be used when several components are being considered (multiple resistances). It should be noted that the common units of cm s^{-1} are theoretically incorrect as a vapour pressure gradient is the actual driving force rather than absolute humidity. Hence units such as mol m^{-2} s^{-1} are suitable (Hall *et al.*, 1976). In certain cases a threshold like response does appear to operate, especially under high rates of stress (Jones and Rawson, 1979). The stomata of onion are a notable example as they exhibit almost on-off behaviour, operating in a narrow Ψ_W range (- 3 to -7 bar) (Miller *et al.*, 1971). 'Threshold' values may have use in indicating water stress sensitivity (Tan and Buttery, 1982a).

Stress preconditioning lowers the Ψ_W(leaf) needed to close stomata (Brown *et al.*, 1976; Hanson and Hitz, 1982; Lakso, 1979; McCree, 1974). Thomas *et al.* (1976) report adjustments in the critical Ψ_W(leaf) of up to 6 to 8 bar, hence caution is needed in interpreting the gs-Ψ_W(leaf) relationship from plants with an unknown stress history. This is an important factor when selecting model systems to study water stress effects. For example, plants grown in containers can readily suffer water deficits due to the limited root volume. If they are not managed carefully while growing to experimental size their stress history may be highly variable.

Having said this, stomatal response is still a useful characteristic for identifying plants capable of high yields under soil moisture limitations (Quarrie and Jones, 1979). Stomatal conductance may be used to predict growth effects under water

stress (Venezian *et al.*, 1985) as long as caution prevails and careful measurements are taken due to its high variability. Jones and Cummings (1984) found that leaf to leaf variation was 25% greater than that between trees suggesting the need for several samples per plant.

With adequate soil moisture, changes in irradiance and vpd can account for at least 80% of the variation in g_s (Whitehead *et al.*, 1981). However, once soil moisture becomes limiting the situation becomes considerably more complicated for several reasons. First, Ψ_W(leaf) is very dependent on atmospheric conditions during the day (Rudich *et al.*, 1981), changing independently of Ψ_W(soil) in response to changing transpiration rates (Schulze, 1986b). Second, root derived chemical signals may have an effect on leaf physiology which is not reflected in Ψ_W(leaf) changes (Blackman and Davies, 1985a, b). Some years ago, Rutter and Sands (1958) noted reduced numbers of open stomata, and time remaining open, with decreasing Ψ_W(soil). Cohen and Cohen (1983) found that stomatal conductance was predominantly controlled by Ψ_W(soil) if it was less than -0.2 bar. When Ψ_W(soil) was high there was a negative correlation between midday g_s and vpd. Davies *et al.* (1987b) obtained a good correlation between g_s and Ψ_W(root). On the other hand it has often been found that Ψ_W(leaf) is unsuitable to estimate soil water content except under very dry conditions (Rossi Pisa and Bigaran, 1985; Syvertsen, 1985a). The critical factor is that plants tend to equilibrate with the wettest part of the rootzone (Garnier and Berger, 1987) and furthermore that stomata appear to respond to conditions at or near the root rather than bulk Ψ_W(leaf) (Aston and Lawlor, 1979; Brinckmann *et al.*, 1984).

2.3.2 *HORMONAL INVOLVEMENT IN STOMATAL OPERATION*

Abscisic acid has been considered important in stomatal movement since its ability to effect stomatal aperture was discovered (Hiron and Wright, 1973). Levels of ABA required to cause stomatal closing are very low, below the levels normally occurring in unstressed leaves (Cowan *et al.*, 1982). Hence g_s is not always correlated with leaf ABA levels (Raschke, 1982) although linear relationships have been found (Itai and Benzioni, 1976). Reduced g_s often precedes an increase in endogenous ABA (Davies *et al.*, 1987a). This apparent anomaly was resolved by studying the distribution of ABA in a leaf. It has been found that the protonated form of ABA (ABAH) can diffuse through membranes without a carrier whereas the deprotonated form cannot. Therefore, ABA is distributed according to pH gradients, with

concentration increasing as pH increases (Zhang and Davies, 1987). The chloroplast stroma has a high pH and so acts as an alkali trap (Cowan *et al.*, 1982), sequestering up to 80% of leaf ABA (Hartung *et al.*, 1982). Irradiance causes an increase in chloroplast pH and so increases the amount of entrapped ABA (Cowan *et al.*, 1982).

The other important anomaly, that of stomatal recovery while leaf ABA concentrations remain high, can also be explained by compartmentalisation if ABA does not have access to sites of action. It could also depend on the rate of arrival and removal from these sites but not on metabolism, which for ABA is too slow to be significant (Raschke and Hedrick, 1985).

Stomatal response to ABA and other growth substances has been reported to be proportional to the log of their concentration (Davies *et al.*, 1987b). A log relationship was found for endogenous ABA concentration in the xylem sap (Zhang and Davies, 1989a) but not for exogenous ABA, where the relationship was linear (Atkinson *et al.*, 1989; Bunce, 1987a). The response of stomata to ABA decreases with leaf age and with successive ABA exposures (Atkinson *et al.*, 1989). There is also considerable species variation, for example, ABA level appears to be unimportant in apricot stomatal control but very important for grape species (Loveys *et al.*, 1987).

It appears that epidermal cells are unable to synthesize ABA so that increases in that tissue are due to mesophyll produced ABA being released into the apoplast (Raschke, 1982). It is interesting to note that stomata do not respond to ABA unless living epidermal cells are present and yet guard cell protoplasts do (Walton, 1980). This important anomaly needs to be clarified. Stomata probably only respond to the + enantiomer of ABA (Raschke, 1982). It is now believed that the site of action of ABA on stomata is the apoplastic side of the guard cell plasmalemma (Atkinson *et al.*, 1989; Davies *et al.*, 1987b). Consequently, high levels of ABA in the chloroplasts or cytoplasm will not be effective. ABA inhibits the flux of K^+ into guard cells (Itai and Benzioni, 1976; Mansfield, 1976) and increases chloroplast starch levels (Mansfield, 1976). Calcium ions may be involved as secondary messengers in conjunction with calmodulin. De Silva *et al.* (1985) found that free passage of Ca^{2+} into the cytosol was needed for an ABA effect.

Both auxin and cytokinin also appear important in stomatal movement, increasing g_s, while little significance has been placed on giberellin levels (although GA_3 can increase stomatal aperture) (Livne and Vaadia, 1972). Auxin promotes K^+ absorption by coupling it to proton extrusion (Levitt, 1976). Root produced CK is central to shoot physiology as will be discussed later. The CK/ABA ratio may be more significant than the individual hormone levels (Blackman and Davies, 1985a). Under water stress, reduced uptake of N and P alters the sensitivity of stomata to ABA via an

effect on CK production in the root (Turner, 1986b). Stomata close in response to the ABA metabolite phaseic acid (PA) and also certain short chain fatty acids (e.g. decanoic, undecanoic acid) (Walton, 1980). Some of these also interfere with the Hill reaction (Vieira da Silva, 1976) which may be a factor. Auxin antagonizes ABA action and reduces the ability of stomata to respond to CO_2 (Snaith and Mansfield, 1982). Snaith and Mansfield (1982) believed that stomatal action could be attributed to a critical balance between these two hormones. Auxin also establishes the abaxial to adaxial g_s ratio (Cox *et al.*, 1985) which is linear (Rossi Pisa and Bigaran, 1985). The difference in adaxial-abaxial response is due to different K^+ permeabilities, with reduced K^+ flux on the adaxial side (Lösch, 1984).

In recent years the role of root produced ABA has risen to prominence (Davies *et al.*, 1987a, b) through the midst of uncertainty. Torrey (1976) concluded that there was no good evidence for root ABA increasing leaf levels and favoured local ABA changes. Root exported ABA was often dismissed due to the low concentration involved but may none-the-less be important because of the site of action on guard cells and ABA compartmentalisation. Low concentrations of ABA moving from the root to shoot via the xylem would have direct access to the apoplast around guard cells even with high cell concentrations present (Atkinson *et al.*, 1989; Davies *et al.*, 1987b; Davies and Zhang, 1991). This could explain the observations of decreasing g_s preceding increases in leaf ABA (Davies *et al.*, 1987a) and the response of stomata to conditions in the root compared with bulk Ψ_W(leaf) (Aston and Lawlor, 1979). Stomata begin to open when ABA levels in the transpiration stream fall despite leaf levels remaining high (Walton, 1980).

Zhang and Davies (1989a) found no change in leaf ABA concentration during a soil drying cycle but a significant change in xylem ABA, which varied with soil water status. They suggested that ABA from roots and older leaves may delay the onset of water stress development in young leaves. The wilting of older leaves would not only increase xylem ABA but also decrease transpirational area (Zhang and Davies, 1989a). Support was given for Zimmermann's hydraulic segmentation hypothesis which involves the hydraulic isolation of parts of the plant in order to reduce water stress. On the other hand, even though the xylem of older leaves may block they are still capable of contributing ABA via the phloem (Zhang and Davies, 1989a).

The response of guard cells to ABA decreases with leaf age. This means that older leaves will sense a water stress before young leaves thus protecting them (Atkinson *et al.*, 1989). Stomatal movement also slows down as the leaf ages and through the growing season with a lowering of the Ψ_W(leaf) causing closure (Lakso,

1979). This has been put down to a decrease in Ψ_S (Lakso, 1979; Syvertsen *et al.*, 1981). Along with declining ABA response, stomatal sensitivity to internal CO_2 concentration (C_i) also decreases with age. The link between these two responses may be through photosynthetic modulation of guard cell pH, with its influence on the distribution of ABA (Atkinson *et al.*, 1989).

2.4 **TRANSPIRATION**

Transpiration may be considered as "an inescapable physical consequence of the development of terrestrial vegetation" (Scott Russell, 1982). Using the Ohms Law analogue for plants (Huber - Gradmann - van den Honert theory), photosynthesis and transpiration for a leaf are represented as follows;

$$Pn = dc / (r_a' + r_s' + r_i') \qquad 2.3$$

$$E = dw / (r_a + r_s) \qquad 2.4$$

Where: Pn = net photosynthesis; E = transpiration; r_a = boundary layer resistance; r_s = stomatal resistance; r_i = intracellular resistance (liquid phase resistance); ' indicates a CO_2 resistance; c = mole fraction of CO_2; w = mole fraction of water.

Hence E is more sensitive to stomatal movement than photosynthesis (Lakso, 1985) because of the extra 'resistance' in the CO_2 pathway *viz.* the internal resistance (r_i'). There is an almost linear relationship between g_s and E (Raschke, 1976). Transpiration can be adequately estimated from the ratio of vpd to r_s provided the leaf-air temperature difference is small (Camacho-B *et al.*, 1974b).

With regard to the transpiration stream attention has generally been directed towards movement through the root. Recently Canny (1990) has reviewed knowledge on the transpiration stream in the leaf. It appears that water enters the leaf symplast close to the tracheary elements, over small areas of membrane. This gives rise to high concentrations of certain solutes in the apoplast. Specialized scavenger cells which possess H^+-ATPase porter systems scrub selected solutes from this apoplastic stream (Canny, 1990).

Transpiration rate will of course decrease under water stress due to both stomatal closure and a falling soil water content. In a moderate climate, water stress

will result from a period of rapid transpiration, driven by a high radiation and temperature load. An exposed leaf on a bright day can receive, process and release as vapour water equivalent to its own fresh weight every 20 minutes or so (Canny, 1990).

Water use on a ground area basis (eg. m^3/Ha) is directly related to total leaf area while water use on a leaf area basis is inversely related to ground area (Natali *et al.*, 1985d). This means that small canopies lose relatively more water, and that the relationship between water use and canopy area is nonlinear (Renquist, 1987) (although for small plants a good linear relationship may exist between E and leaf dry weight (Cheeseman, 1984)). There is an inverse relationship between root length and uptake per unit length of root. This means that Ψ_W(leaf) can be the same regardless of the leaf area to root length ratio under the same soil water contents (Eavis and Taylor, 1979).

Efficiency of the water transport system is very important in determining how a plant responds to a water stress, along with the ability to control water loss (Camacho-B *et al.*, 1974a). A high resistance to water flow will be advantageous in dry environments. Camacho-B *et al.* (1974a) have identified three types of plants:

i) Combine strong regulation by stomata with low efficiency of water transport e.g. citrus and pear.
ii) Strong regulation with more efficient transport e.g. pepper.
iii) Little stomatal regulation plus a highly efficient transport system e.g. sunflower.

Ranking plants according to their hydraulic conductivities reflects plant vigour (Syvertsen and Graham, 1985).

2.5 **WATER USE EFFICIENCY**

Water use efficiency (WUE) is a key parameter, considering that water supply limits production on most of the world's arable land. Water use efficiency has been reviewed in detail by Fischer and Turner (1978). It is the ratio of dry matter produced to amount of water transpired over a certain period and is expressed in units such as mg d. wt g^{-1} H_2O. The close link between photosynthesis and transpiration means that dry weight increase is related to water use (Giulivo *et al.*, 1985; Rudich and Luchinsky, 1986) and that, other things being equal, increased transpiration means increased productivity (Fischer and Turner, 1978). Tedeschi and Zerbi (1985) report a linear relationship between yield and actual evapotranspiration. Productivity may be

assessed in terms of CO_2 uptake rather than dry matter i.e. WUE in mg CO_2 g^{-1} H_2O (Fischer and Turner, 1978; Hubick *et al.*, 1986a). This is useful because it removes variation in the carbon content of dry matter (Hubick *et al.*, 1986a). The ratio of dry matter to CO_2 is in the range 0.61 to 0.68 g (Fischer and Turner, 1978).

The inverse of WUE is commonly quoted, this being the transpiration ratio, which varies between 100 and 1000 (Bierhuizen, 1976). Turner (1986b) has suggested that the term WUE be used for dry matter production per unit of evapotranspiration and transpiration efficiency for dry matter production per unit of transpiration. This would appear to be a logical and useful distinction but one which has not yet been adopted.

Water use efficiency may be described by the equation (Turner, 1986b):

$$WUE = dC \times Dc \times (r_a + r_s) / (de \times De \times (r_a' + r_s' + r_i')) \qquad 2.5$$

Where: dC = leaf-air concentration difference for CO_2; Dc = diffusivity for CO_2; de = leaf-air concentration difference for H_2O; De = diffusivity for H_2O.

Water use efficiency is often calculated from instantaneous measurements of CO_2 uptake and transpiration (μmol CO_2 $mmol^{-1}$ H_2O). However, this has been shown to be poorly correlated with season long WUE (Martin and Thorstenson, 1988). Internal plant characteristics have much more impact on WUE than soil factors or cropping practices (Stanhill, 1986).

An interesting indirect way of calculating WUE which has recently been developed uses the ratio $^{13}C/^{12}C$ (Francey and Farquar, 1982; Hubick *et al.*, 1986a). This ratio is correlated with season long WUE (Martin and Thorstenson, 1988) in a negative manner (Farquar and Richards, 1984). The ratio arises mainly from enzymatic discrimination by Rubisco.

Factors which cause partial stomatal closure will increase WUE e.g. mild water stress (Bierhuizen, 1976; Fischer and Turner, 1978; Gates, 1955a; Jones, 1976; Mansfield, 1976). The ideal factor is in fact CO_2 because high CO_2 will lead to stomatal closure while promoting photosynthesis. Abscisic acid also approaches the ideal antitranspirant as it increases stomatal sensitivity to CO_2 (Mansfield, 1976). A stable Ψ_W(soil) is conducive to high WUE which may be the reason for increased WUE under trickle irrigation (Proffitt *et al.*, 1985). Changes in stomatal response characteristices will alter the effect of water stress on WUE. Lankes (1985), who worked with apple trees, found that WUE increased with water stress early in the season but the trend later reversed due to insensitive stomatal regulation.

Water use efficiency is very dependent on vpd, having an inverse linear relationship with vapour pressure, and also on the characteristics of Rubisco. Increasing temperature decreases WUE unless temperature is not optimal for photosynthesis. Although this factor will oppose increased WUE under water stress it is generally overshadowed by the r_s effect. Increasing r_s increases WUE, except when r_i to r_a is less than a critical value (Fischer and Turner, 1978), as does increasing $r_{cuticle}$ (Jones, 1976). Water use efficiency also increases as r_a decreases, except under low irradiance with high temperatures and high vpd (Jones, 1976), and as r_i decreases (Fischer and Turner, 1978). When r_i is low, A will be high along with WUE (Bierhuizen, 1976). Stomatal oscillations can increase WUE (Jones, 1976).

2.6 **TRANSLOCATION**

Transport in both the xylem and phloem may be considered in terms of mass flow (Patrick, 1987);

$$R = V \times A \times C \qquad 2.6$$

Where: R = rate of solute flow; V = flow velocity; A = cross-sectional area; C = concentration of solute.

$$Jv = Lp \times d\Psi_P(sieve) \qquad 2.7$$

Where: Jv = volume flux; Lp = hydraulic conductivity.

Note that $\Psi_P(sieve) = \Psi_W(apoplast) - \Psi_S(sieve)$ if the water potentials in the sieve tubes and apoplast are equal (Lang and Thorpe, 1986). Under the Munch hypothesis, sieve tube osmotic potentials dominate. This need not be the case however, and in fact if the $\Psi_P(apoplast)$ gradient is greater than the $\Psi_S(seive)$ gradient, translocation will be in the opposite direction (Lang and Thorpe, 1986).

The importance of hormones in assimilate transport is now well established. Removal of root or shoot growing regions results in decreased transport in that direction with normal transport partially re-established following application of auxin and cytokinin. Recently Gersani *et al.* (1980a, b) carried out a detailed analysis of various translocated metabolites and clearly identified the specific interactions between these two hormones. Basic patterns of translocation have been studied using ^{14}C

labelled compounds and manipulation of source/sink tissue (e.g. Khan and Sagar, 1966; Khan and Sagar, 1969a, b).

Atkinson and Farrar (1983), using carbon flux analysis on two grass species, found that translocation to the root was twice the rate to shoot structural growth, and maximum translocation coincided with maximum nitrate uptake. This may reflect the dependence of both processes on adenosine diphosphate (ADP) (Pearson, 1979). The rate of translocation may be affected by partitioning between storage (e.g. starch) and translocated (e.g. sucrose) forms (Huber *et al.*, 1985). Sucrose is the most common form of translocated assimilate (Huber *et al.*, 1985) and is the first free sugar formed from photosynthesis (Wyse *et al.*, 1985).

Under water stress, partitioning towards sucrose increases along with the breakdown of starch (see review of Chaves (1991)). The enzymes fructose-1,6-bisphosphate-1,phosphatase (FbPase) and sucrose phosphate synthase (SPSase) are key enzymes in sucrose synthesis while Fructose-2,6-bisphosphate (Fru-2,6-P_2) is a key regulatory metabolite (Huber *et al.*, 1985). Huber *et al.* (1985) postulate that SPSase and Fru-2,6-P_2 control the rate of carbon flux into sucrose. They show opposing diurnal rhythms which appear to be controlled by an endogenous clock. Recently however, Vassey *et al.* (1991) have concluded that the effect of water stress on SPSase activity is indirect via reduced photosynthesis resulting from stomatal closure.

An important factor in the rate of translocation is the depression of Ψ_P at the sink end (Patrick, 1987) and elevation of Ψ_P at the source end (Fellows and Geiger, 1974; Lucas, 1985). Loading of sugar into the phloem of minor veins represents an important ontogenic event in leaves with the switch from net import to net export (Fellows and Geiger, 1974; Turgeon, 1989). Depression of Ψ_P at the sink depends on phloem unloading. The common route of unloading was generally considered to be the apoplast (Wyse *et al.*, 1985), but now the symplast is given major importance especially in meristematic sinks (Ho, 1988; Patrick, 1987). It is likely that hydraulic conductivity of the symplast is a limiting factor for translocation (Patrick, 1987). At the cellular level it has been found that the proton-motive force (PMF) across the plasma membrane is from outside to inside, while at the tonoplast it is in the opposite direction (Wyse *et al.*, 1985). Wyse *et al.* (1985) conclude that sucrose loading into vacuoles occurs via a proton-sucrose antiport system. At the plasma membrane a proton/sucrose co-transport system has been envisaged (Lucus, 1985; Wyse *et al.*, 1985) although as Lucus (1985) points out, this cannot be clearly established in light of the observation that all cell types can take up sucrose.

Winneberger (1958) postulated that transpiration was the energy source for both organic and inorganic solute translocation but this is now known to be generally

incorrect. Mass flow circulation within the xylem and phloem may be sufficient for nutrient uptake and transport without any transpiration stream. Mortensen (1986) measured large increases in growth at very high relative humidites (90% to 95%) for a wide range of species. Gisleröd *et al.* (1987) observed a decrease in macro-nutrient content with increasing relative humidity (r.h.) (i.e. decreasing transpiration) but this was not detrimental to growth. However, calcium deficiency may affect both reproductive (Tromp and Oele, 1972) and vegetative growth (Holder and Cockshull, 1990) under a very high r.h..

Water stresses which reduce photosynthesis may not affect translocation rates. This is because temporary buffering can occur from stored compounds (Dale and Sutcliffe,, 1986). Under low rates of Pn, translocation solutes are mainly the early starch breakdown products rather than the fixed sugars themselves (Ho, 1979). Greater effects on translocation than photosynthesis have been reported (Huber *et al.*, 1984; Rudich and Luchinsky, 1986). Translocation is unlikely to be affected by plant water status *per se* but rather by Ψ_W gradients, with low Ψ_W regions attracting relatively more assimilate (Lang and Thorpe, 1986). Decreased translocation under water stress will occur through reduced growth lowering sink strength (assimilate demand) and from reduced Pn. There may also be rapid water stress effects on the phloem itself (Dale and Sutcliffe, 1986).

3 WATER STRESS ON BIOCHEMICAL PROCESSES

3.1 PHOTOSYNTHESIS

The complex process of photosynthesis centres around a reaction where CO_2 is fixed into a biochemical substance. The enzyme associated with this reaction is ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco), which constitutes the major leaf protein, having a molecular weight of approximately 550,000 and eight catalytic sites per molecule (Jensen and Bahr, 1977).

3.1.1 *PHOTOSYNTHESIS AND PLANT HORMONES*

Hormones are now implicated in the regulation of photosynthesis rather than simple feedback hypotheses (Starck *et al.*, 1979). Both GA and CK increase the activity of Rubisco in fully expanded leaves but not young leaves (which have naturally high levels of GA) (Wareing, 1979). This occurs through enzyme activation rather than synthesis although synthesis of the minor subunit may occur. Enhancement of photosynthesis by GA application could also be due to changes in membrane permeability enabling more rapid mobilization of carbohydrate (Avery *et al.*, 1979). The positive effects of CK suggest that a continuous supply from the roots may be necessary to maintain photosynthetic capacity (Bradford, 1982).

Photosynthesis has been found to decrease in proportion to the amount of ABA supplied (Bunce, 1987a; Ward and Bunce, 1987). Abscisic acid can have the following effects on the photosynthetic system:

i/ Decrease carboxylation efficiency (initial slope of Pn-C_c curve) (Bunce, 1987a; Raschke and Hedrick, 1985).

ii/ Decrease CO_2 saturated photosynthesis (ribulose-1,5-bisphosphate (Ru-1,5-BP) regeneration capacity) (Raschke and Hedrick, 1985).

iii/ Decrease CO_2 limited and light saturated photosynthesis (Ward and Bunce, 1987).

iv/ Increase CO_2 compensation point (Raschke and Hedrick, 1985) (not found by Ward and Bunce (1987)).

v/ Decrease quantum yield (Ward and Bunce, 1987).

However the significance of these direct ABA effects on the photosynthetic apparatus are now strongly disputed. The decrease in photosynthetic rate correlated with increasing levels of ABA is being put down to a decrease in stomatal conductance (Downton *et al.*, 1988a, b; Loveys, 1984; Sharkey and Seemann, 1989; Terashima *et al.*, 1988), as is the decrease in Pn associated with high salt levels (Downton *et al.*, 1990, Brugnoli and Lauteri, 1991).

Abscisic acid, as well as causing stomatal closure, increases the sensitivity of stomata to CO_2 (Lakso, 1985; Raschke and Hedrick, 1985) so enabling the plant to maximize photosynthesis while subjected to a decreasing water supply. It should be noted here that although the percentage of ABA in the chloroplast (relative to total cellular ABA) decreases during water stress, actual levels increase (Loveys, 1977; Walton, 1980). For example under mild stress, chloroplast levels may increase two times while non-chloroplast levels increase ten times (Loveys, 1977). Increase in chloroplast permeability to ABA may be the result of farnesol production (Walton, 1980).

It has already been noted that GA and CK increase the activity of Rubisco in fully expanded leaves (Wareing, 1979). It is possible that reduced levels of CK and GA in the leaves under water stress (e.g. from reduced root translocation) could also be involved in limiting photosynthesis. This has not been investigated in detail.

3.1.2 *PHOTOSYNTHESIS AND CARBON DIOXIDE MOVEMENT*

When the CO_2 concentration at the site of carboxylation (C_c) is plotted against assimilation rate (A), the graph is at first linear and then at some point asymptotes parallel to the X-axis. The slope of the linear portion is correlated with maximum Rubisco activity (carboxylation efficiency (Farquar and Sharkey, 1982; Nicolodi *et al.*, 1988)) while the maximum photosynthetic rate (assyptote value) is correlated with electron transport rate i.e. Ru-1,5-BP regeneration capacity (von Caemmerer and Farquar, 1981) (and not irradiation (Farquar *et al.*, 1980)). In this region, rates of election transport and adenosine triphosphate (ATP) synthesis are independent of C_c while regeneration of nicotinamide adenine dinucleotide phosphate (NADPH) and Ru-1,5-BP are also virtually independent. Assimilation rate still increases slightly with increasing C_c due to diversion of Ru-1,5-BP from oxygenation to carboxylation (Farquar and Sharkey, 1982). Under saturating CO_2 conditions, O_2

evolution is limited by the regeneration of Ru-1,5-BP (Nicolodi *et al.*, 1988). It has been found that nonlinearity occurs at a C_c of approximately 230 ppm corresponding to CO_2(air) of 330 ppm i.e. ambient CO_2 (Farquar and Sharkey, 1982; Nicolodi *et al.*, 1988). Hence plants are usually operating at the transition between Rubisco activity limitation and Ru-1,5-BP regeneration limitation. Here a small change in gs causes a large change in dE/dA and hence water use is optimized (von Caemmerer and Farquar, 1981). It has been shown that optimal conductance occurs when dE/dA remains constant in time and space. This necessitates a feedforward response to both light intensity and r.h. while indicating parallel changes in photosynthetic capacity and g_s (Farquar and Sharkey, 1982).

At this point it is appropriate to clarify the resistance terminology used with respect to the leaf. In the analysis of water and CO_2 movement using resistances an internal resistance (r_i') is included in the CO_2 pathway to account for the biochemistry of CO_2 fixation ($(Pk)^{-1}$) and also resistance to movement within the mesophyll cells (r_m') (Jones and Slatyer, 1972b). Unfortunately r_m has often been used in place of r_i causing considerable confusion even in recent times (e.g. Lakso, 1985). The carboxylation resistance has in the past been given the symbol r_e' while r_m' has been termed the transport resistance (r_t') (Jones and Slatyer, 1972b; Jones, 1973b). From the way in which r_i' has been calculated it has also been called r_r', the residual resistance (e.g. Gifford and Musgrave, 1972).

Internal resistance is usually calculated as the remainder after boundary layer (r_a') and stomatal (r_s') resistances have been subtracted from the total resistance (Salisbury and Ross, 1985). It may also be calculated from the slope of the Pn - C_i response curve (Kriedemann and Canterford, 1971) (c.f. the slope of the Pn - C_c curve which equals the constant k (von Caemmerer and Farquar, 1981)).

The difference between C_i and C_c is now thought to be small, that is, the resistance to diffusion in the liquid phase (through the mesophyll cells) (r_m') is low. It has been shown that in turgid tissue r_m' is unimportant and independent of the rate of evaporation (Jones and Higgs, 1980). This contrasts with the original hypothesis of Gaastra (1959) who considered that C_c was close to zero (i.e. r_m' was large) due to a lack of knowledge about the enzyme kinetics of Rubisco (Farquar and Sharkey, 1982). It also contrasts with initial attempts to partition r_i' using oversimplified models.

The linear portion of the A - C_c relationship may be represented as follows (Farquar and Sharkey, 1982):

$$A = (CO_2(air) - CO_2(\Gamma)) / (r_a' + r_s' + r_m' + (Pk)^{-1})P \qquad 3.1$$

Where: $r_m' + (Pk)^{-1} = r_i'$.

The $(Pk)^{-1}$ term in Eqn. 3.1 represents what may be called the carboxylation resistance although, as Farquar and Sharkey (1982) point out, there is no real analogy between resistances and enzyme kinetics. Early attempts to partition out the 'carboxylation resistance' should be viewed with caution (e.g. Jones, 1973b).

Use of Eqn. 3.1 has led to several important incorrect conclusions as Farquar and Sharkey (1982) clearly point out. The two which should be noted here are:

i/ Stomatal resistance contributes the main limitation to photosynthesis (e.g. Gifford and Musgrave, 1972).
ii/ Stomatal limitation increases with water stress.

The point to remember is that Eqn. 3.1 is only valid in the linear portion of the curve whereas under natural conditions and many experimental conditions the plant is operating under Ru-1,5-BP regeneration limitation. Recently even these provisos have been brought into question with the discovery that C_i measured with an infra-red gas analyzer (IRGA) may not be representative of photosynthesizing leaf tissue (Downton *et al.*, 1988a, b). This point will be discussed in detail in a later section.

Due to the increase which is occurring in CO_2(air), and to investigate the potential for improving yield, a significant amount of work has been done on photosynthesis and growth at high CO_2 concentrations. High CO_2(air) tends to lessen the effect of biological stresses (e.g. water stress (Huber *et al.*, 1984)), but not nutrient deficiency (Tolbert, 1984), and gives rise to an increase in WUE (Du Cloux *et al.*, 1984; Huber *et al.*, 1984; Tolbert, 1984). The increase in dry matter under high CO_2(air) is not as great as would be expected, due to a decrease in photosynthesis per unit LA (Du Cloux *et al.*, 1984). This results from a reduction in both Rubisco activity and the Hill reaction (Kriedemann and Wong, 1984). Ludwig and Withers (1984) observed a faster decline in Pn and Rubisco activity with leaf age under high CO_2(air).

3.1.3 *PHOTOSYNTHESIS AND THE PLANT WATER BALANCE*

Carbon dioxide fixation rate is generally observed to decrease with water stress, as first reported by Kreusler in 1885 (Boyer, 1976b), although a small increase

may be found under slight dehydration (Vieira da Silva *et al.*, 1984). This decrease is due to both decreased activity per unit leaf area and reduced leaf area (decreased production and increased abscission) (Boyer, 1976b). Maximum photosynthetic rate (CO_2 and light saturated) is reduced under severe water stress (Ben *et al.*, 1987; Bunce, 1986) and may be the most stress sensitive photosynthetic parameter (Ben *et al.*, 1987). Quantum yield (moles O_2 per mole photons) is also reduced (Ben *et al.*, 1987; Bois *et al.*, 1985; Jones, 1973b). Severe and mild stress are arbitrary terms but may be defined using a critical Ψ_W of -15 bar, e.g. see Ben *et al.* (1987). Note that the maximum rate of photosynthesis is determined initially by the source-sink relationship during cell expansion (Bunce and Ward, 1986) and subsequently by the rate of Ru-1,5-BP regeneration (Lange *et al.*, 1987). Mild water stress has no effect on quantum yield or photosynthetic parameters in general if it is CO_2 or light limited (Ben *et al.*, 1987). Any decrease in photosynthesis must eventually be related to a decrease in Rubisco reaction rate as this is the primary carboxylating enzyme (Mott *et al.*, 1986) although it should be remembered that in some species the CO_2 compensation point increases with stress (Bunce, 1986; Tenhunen *et al.*, 1984).

Kirschbaum (1988) found a bi-phasic pattern to the recovery of photosynthesis following water stress. The first phase was rapid while the second slower phase could be improved by darkness. Water stress was proposed to have two independent and parallel effects, the first being simply reversible and the second involving damage to the photosynthetic apparatus. Damage may result from high temperature disruption to the photosynthetic apparatus and assimilating enzymes (Byörkman, 1981) following reduced transpiration as stomata close (Glenn *et al*,. 1989; Idso, 1982).

The decrease in Rubisco activity with water stress has usually been correlated with stomatal conductance resulting in a strong linear (Flore *et al.*, 1985; Munger *et al.*, 1987) or hyperbolic (Jarvis, 1976; Munger *et al.*, 1987) relationship although there are exceptions (Alvino *et al.*, 1985). Such correlations have led to the conclusion that photosynthesis declines because of reduced g_s limiting diffusion of CO_2 into the leaf (Brix, 1962; Jones, 1973b; Lakso, 1979; Rudich and Luchinsky, 1986; Tan and Buttery, 1982a) as Schneider and Childers suggested back in 1941 (Boyer, 1976b). However many workers have disputed this in recent times, suggesting that nonstomatal factors are very important (e.g. Sharp and Boyer, 1986). Wong *et al.* (1979) turned the A - gs relationship right around by stating that g_s was determined by the capacity of mesophyll tissue to fix carbon.

Responses of photosynthesis and g_s to water stress have been found to differ. Jones and Rawson (1979) showed that photosynthesis was more sensitive than g_s to

rate of stress induction between 0.15 to 0.7 MPa day^{-1}. Hand *et al.* (1982) correlated g_s with Ψ_W(leaf) and Pn but Pn did not recover as quickly from severe stress. During the development of citrus leaves, changes in Pn are more strongly correlated with mesophyll rather than stomatal conductance (Syvertsen, 1985b). In Peach, g_s reaches a maximum in expanding leaves before A (Anderson and Brodbeck, 1988).

The stomatal - nonstomatal limitation argument has continued to rage through the midst of uncertainty. Over the years it has changed from solely stomatal (Gaastra, 1959), to only partly stomatal from the 1970's (e.g. Hansen, 1971) to the early 1980's (e.g. Farquar and Sharkey, 1982) and now back to predominantly stomatal (Chaves, 1991; Downton *et al.*, 1988a; Downton *et al.*, 1990; Brugnoli and Lauteri, 1991; Farquhar, 1989; Farquhar *et al.*, 1989; Taylor and Dobson, 1989; Terashima *et al.*, 1988; Vassey *et al.*, 1991). Vassey *et al.* (1991) have concluded that "there is no effect of water stress on photosynthesis except by way of stomatal closure until relatively severe water stress occurs". Further refinements to our understanding will no doubt occur in the future as results from the above workers are critically explored using different techniques and experimental systems such as chlorophyll fluorescence spectroscopy (e.g. Daley *et al.*, 1989).

In recent times two important interrelated factors have contributed to this uncertainty, namely the calculation of internal CO_2 concentration and stomatal limitation. Before considering these, it is relevant to note that the nature of the system used by researchers also needs to be given more consideration. As a system becomes more complex (e.g. cell, tissue, organ, organism, community), control mechanisms also become more complex due to interactive processes. Extrapolation of stomatal responses in isolated epidermal strips to natural canopy systems is at best, approximate, and at worst, totally misleading.

Jones (1973a) was the first to indentify an important problem which arises when calculating stomatal limitations to photosynthesis. Values are very dependent on whether stomatal resistance or carboxylation resistance is considered to change first. That is, the calculation is path dependent. Despite this revelation ambiguous values have continued to be quoted and the inherent differences highlighted. For example, Assmann (1988) investigating radiation stress calculated only a 5% stomatal limitation for the stomata first option while for the mesophyll option this increased to 23%. To overcome the problem of path dependency Jones (1973a) recommended use of the state function approach involving what he termed as "limitations". The "relative limitation" due to two processes was defined as the ratio of the change in concentration drop across each component for a small change in overall system rate.

Path dependent methods have led to calculated nonstomatal limitation values being very high. Nicolodi *et al.* (1988) calculated that nonstomatal factors accounted for 50% of the reduction in CO_2 assimilation rate by assuming that g_s declined first. The limitation may appear even higher if in fact mesophyllic photosynthesis decreases first (Prioul *et al.*, 1984) and has been shown to increase with increasing stress (Farquar and Sharkey, 1982). Matthews and Boyer (1984) concluded that Pn at low Ψ_W was limited more by reduced chloroplast activity than g_s. With respect to Pn acclimation they found that nonstomatal factors were the major contributors. Cornic *et al.* (1983) found that the nonstomatal component was dependent on CO_2 partial pressure. However if water stress was rapid the nonstomatal component accounted for nearly all of the decrease in Pn at all partial pressures.

These results infer a poor correlation between g_s and C_i. Sharkey *et al.* (1982) showed that C_i tended to be nearly constant at irradiances above 10% full sunlight. Water stress therefore was thought to decrease 'both' A and g_s and not A through g_s (Wong *et al.*, 1979). The feedback loop was proposed to operate not only through CO_2 but also other metabolites of photosynthesis (e.g. ATP, NADPH, Ru-1,5-BP) because C_i increases with CO_2(air). These metabolites would be transported to the guard cells from the mesophyll (Wong *et al.*, 1979).

The internal CO_2 concentration has in the past been calculated using the fluxes of CO_2 and H_2O (Farquar and Sharkey, 1982):

$$C_i = ((g_s - E/2) \times CO_2(air)) - A) / (g_s + E/2) \qquad 3.2$$

Hence the assumption is made that the CO_2 and H_2O paths are identical and so C_i is the "average partial pressure of CO_2 at the sites of evaporation" (Sharkey *et al.*, 1982). The above assumption appears valid (Sharkey *et al.*, 1982) although others on occasions have suggested otherwise (Jones and Slatyer, 1972a).

Internal CO_2 provides the basis for determining stomatal and nonstomatal effects. If g_s is limiting Pn, then C_i will decrease along with g_s (Sharkey *et al.*, 1982). If C_i does not decrease, then other factors limiting Pn are suggested. A number of workers have observed C_i to be constant (Raschke and Resemann, 1986; Smith and Ager, 1988; Tenhunen *et al.*, 1984). Smith and Ager (1988) were able to correlate g_s with A for flooding treatments but did not detect any decline in C_i.

The above method sounds all too clear and simple and with the increase in availability of photosynthesis measuring equipment (e.g. the LI-6200 Photosynthesis System), C_i calculations have flourished. Unfortunately it would now appear that this

calculation, along with the stomatal limitation calculation, has lead to a large overestimation of the nonstomatal component. This is not to say that a constant C_i does not indicate a large nonstomatal effect but rather that current techniques for measuring C_i and g_s may be inaccurate. It has been shown that C_i measured using an IRGA does not equal the actual average C_i for the leaf (Downton *et al.*, 1988a, b; Laisk, 1983). This is because stomatal conductance becomes increasingly non-uniform under stress, the stomata being observed to close in patches (Downton *et al.*, 1988a), although perhaps not fully (Bunce (1987b). In fact IRGA C_i will be in error, whenever there is significant diffusive resistance between stomata, and large variation in stomatal aperture (Laisk, 1983). Difference in stomatal variability exists between species and groups of plants. Laisk (1983) found that the number of closed stomata for a given set of conditions was greater in monocotyledons than dicotyledons. Note that calculated C_i also does not take cuticular transpiration into account (Terashima *et al.*, 1988).

Chlorophyll fluorescence spectroscopy is now used to calculate, what are believed to be, accurate average C_i values for a leaf. This is done using non-photochemical quenching (q_E) which is correlated with the build-up of a transthylakoid proton gradient and hence is very responsive to a decrease in C_i (Daley *et al.*, 1989; Downton *et al.*, 1988a). The real accuracy of chlorophyll fluorescence spectroscopy requires further verification so that it may be used confidently as a standard method.

With IRGA C_i representing the CO_2 concentration in patches where stomata are open, and C_i approaching the CO_2 compensation point (Γ) in patches where stomata are closed, the following correction has been proposed and shown to be valid (Downton *et al.*, 1988a):

$$\text{Corrected } C_i = R \times (\text{IRGA}(C_i) + ((1 - R) \times \Gamma) \qquad 3.3$$

Where:

$$R = A(\text{stress}) / A(\text{control}) \qquad 3.4$$

A thorough treatment of the calculation of C_i under patchy stomatal aperture conditions has been given by Farquhar (1989).

Using chlorophyll fluorescence quenching all of the decrease in photosynthesis by ABA (and hence water stress) can now be accounted for by stomatal

closure (Downton *et al.*, 1988a) (this had previously been concluded for xylem supplied ABA by Loveys (1984)). Terashima *et al.* (1988) found the same result using a leaf disc O_2 electrode system at high CO_2 which also overcomes the patchiness problem. They suggested that stomata vary intrinsically in their response to ABA. Sharkey and Seemann (1989) showed that photosynthesis under mild water stress occurred in patches. This corresponded with patchy stomatal closure. They concluded that the major effect of mild water stress on CO_2 assimilation was through reduced g_s and not a decrease in the capacity for photosynthesis. Current techniques for measuring A, C_i and g_s are generally incapable of detecting this patchy behaviour. They provide some sort of average figure which hides the true physiological state.

From the foregoing discussion it would now appear that nonstomatal effects of water stress on photosynthesis are generally insignificant when compared with the stomatal limitation. For this reason, detailed work which has been carried out on the photosynthetic apparatus and associated biochemical pathways will not be discussed. Interested readers are referred to the following papers,

Ben *et al.*, 1987; Berkowitz and Gibbs, 1984; Boyer and Potter, 1973; Boyer and Younis, 1984; Bunce, 1986; Downton and Millhouse, 1984; Farquar and Sharkey, 1982; Farquar *et al.*, 1980; Govindjee *et al.*, 1981; Hand *et al.*, 1982; Havaux *et al.*, 1986a, b; Huber *et al.*, 1984; Jones, 1973c; Kaiser, 1982, 1987; Lawlor and Khanna-Chopra, 1984; Lee-Stadelmann and Stadelmann, 1976; Lösch, 1984; Mott *et al.*, 1986; Mougou *et al.*, 1984; Nicolodi *et al.*, 1988; Pham Thi *et al.*, 1984; Rudich and Luchinsky, 1986; Schulze, 1986b; Schwab and Heber, 1984; Sesták *et al.*, 1984; Sharkey and Seemann, 1989; Sharp and Boyer, 1985, 1986; Sivak and Walker, 1986; Syvertsen, 1985a; Terry, 1984; Vassey and Sharkey, 1989; Vieira da Silva, 1976; Vu and Yelenosky, 1988; Wolfe *et al.*, 1988.

Related to the 'non-stomatal effects' question, is that of the role of ABA, whose effects on leaf physiology are similar to those of water stress (Sharkey and Seemann, 1989). In fact Bunce (1987a) concluded that effects were identical and suggested that water stress effects were mediated by ABA. Associated with such hypotheses is the suggestion that ABA acts directly on the photosynthetic apparatus. Cornic and Miginiac (1983) have shown that ABA decreases the carboxylation capacity of leaves. No effect of ABA, however, was observed on isolated chloroplasts. This could suggest that ABA acts via the plasmolemma (and cytoplasm) (Raschke, 1982). It is now believed, however, that the effects of ABA are due solely to its effect on stomatal aperture, with no direct effect on chloroplasts (Downton *et al.*, 1988a, b; Loveys, 1984; Sharkey and Seemann, 1989; Terashima *et al.*, 1988). Much more basic research is needed to adequately evaluate the validity of this view.

3.2 **RESPIRATION**

Any factor which decreases photosynthesis should decrease respiration assuming that the two respiration coefficients remain constant (Amthor, 1989). According to Amthor (1989) stress may involve "qualitative and quantitative changes in most or all functional components of respiration". Evidence has been conflicting however. Sometimes dark respiration is said to increase with water stress (Iljin, 1957; Lawlor and Khanna-Chopra, 1984; Mougou *et al.*, 1984), sometimes decrease (Jorba *et al.*, 1985; Syvertsen, 1985b), while at other times it is found to be insensitive (Lakso, 1985). Change in the respiration coefficients may be involved in these differences (Amthor, 1989) along with other factors such as the plant material used, age, severity of stress and rate of induction (Boyer, 1976b; Vieira da Silva, 1976). Accumulation of organic substances associated with osmoregulation will tend to increase respiration (Amthor, 1989). Respiration is still high when photosynthesis has been reduced to zero and this gives rise to an increase in the CO_2 compensation point (Boyer, 1976b).

It is known that the soluble fraction of mitochondria are only moderately sensitive to water stress whereas reaction rates for membrane bound enzymes can be sharply reduced (Lösch, 1984). This may relate to a drop in the number of cristae which results from an increase in acid lipase activity (Vieira da Silva, 1976). With an increase in dark respiration there is a rise in the NADH content and substrate comes from stored compounds because of reduced Pn (Lawlor and Khanna-Chopra, 1984). Oxidative phosphorylation becomes progressively uncoupled (Vieira da Silva, 1976).

Respiration losses may be separated into growth and maintenance components. These are directly proportional to growth rate and total dry matter respectively (Fischer and Turner, 1978). The growth component will be rapidly reduced under water stress. Daily losses of dry matter from respiration can be in the order of 2 to 10% (Iljin, 1957). Photorespiration is reduced under water stress (Vieira da Silva *et al.*, 1984). This may be due to a decrease in decarboxylation of glycine in the mitochondria (through lipase activity) rather than an effect on glycolate oxidase (Vieira da Silva, 1976).

For further discussion on respiration, water stress and partitioning of respiratory components see section 15.

4 WATER STRESS ON PLANT DEVELOPMENT

4.1 CELL DIVISION

Cell division does not appear as sensitive to water deficits as cell expansion and in fact decreased rates may be due to the decreased rate of expansion itself (Hsiao, 1973). While cell expansion is affected by short term water deficits, long term deficits appear necessary to reduce cell division (Hsiao, 1973). This may help to explain elevated growth rates after a short period of stress with the existence of a population of unexpanded cells. Leaf initiation and early growth which are primarily due to cell division behave as though limited by mineral supply (Patrick, 1988), this supply is reduced under water stress.

4.2 CELL EXPANSION

Cell expansion is the outward manifestation of plant growth, which may be represented as follows (Greacen and Oh, 1972; Hsiao, 1973; Taylor and Davies, 1985):

$$\text{Growth Rate} = \sum g \times (\Psi_P - \Psi_P(th)) \qquad 4.1$$

Where: $\sum g$ = gross extensibility; Ψ_P = pressure potential(turgor pressure); $\Psi_P(th)$ = threshold pressure potential.

The principle behind this model is that growth can not occur unless turgor is above a certain value, turgor being the force causing cell wall enlargement (stretching the cell wall and breaking chemical bonds). Turgor pressure results from water uptake and for this uptake to occur substantial Ψ_W gradients are needed. This is because water must pass through several cell layers in the expanding region. In this region continued cell expansion inhibits the development of maximum turgor (Boyer and Westgate, 1984; Nonami and Boyer, 1987) and the elongating tissues have a high resistance to water movement (Nonami and Boyer, 1987). Cell volume enlargement is eventually limited by resistance of the wall to further expansion (Zimmermann, 1978) ie. the coefficient of elasticity becomes too high (where the coefficient of elasticity is related to 1/Eg).

Representing growth by a simple linear relationship to turgor is in fact incorrect as it has been widely shown that the relationship between Ψ_P and cell volume is exponential. This was first clearly defined by Tyree and Hammell (1972) in their analysis of the pressure-volume curve technique, using the foundation laid by Gardner and Ehlig (1965). Hsiao *et al.* (1976a) concluded that turgor has a critical role in cell growth but that physical models linking the two may be misleading due to the metabolic and regulatory responses involved. This was reiterated by Kramer (1988) who noted that cell enlargement and turgor are not always correlated.

The role of hormones in growth is still being elucidated. Auxins which are produced primarily in apical meristems were identified early as having a key role. The acid growth hypothesis was developed in which growth is encouraged by cell wall loosening caused by acidification of the apoplast from auxin stimulated H^+ secretion (Taylor and Davies, 1985). This efflux is mediated by ATPases which appear to be located in the plasma membrane. Auxin does not activate the ATPase directly but may modify the concentration of an ATPase effecter (Cleland, 1982). Evans and Mulkey (1982) present evidence for the same mechanism operating in roots. ABA decreases the capacity of cell walls to respond to acidity. This can be compared to the effect of water stress *per se* which decreases a cells ability to acidify the apoplast in the first place (Davies *et al.*, 1987a).

The relationship between growth rate and soil water content depends on the physiology of the plant and the prevailing environmental conditions (Krizek, 1985). Cell expansion is believed to be the most sensitive physiological process to a water deficit (Hsiao, 1973) and hence is the first to slow down or stop. It is also very temperature dependent (Monteith *et al.*, 1981). Mild water stress can reduce shoot and leaf expansion without any effect on g_s or A (Andersen and Brodbeck, 1988). Following more severe stress, tissue growth may be slow to return to normal levels relative to other parameters such as stomatal conductance (Kim and Lee-Stadelmann, 1984). From equation 4.1 the sensitivty of cell expansion to a loss of turgor pressure is evident. Expansion ceases once Ψ_P reaches a threshold value while other processes may be affected only when turgor is lost (Ψ_P = 0 bar) (Hsiao, 1973). Threshold Ψ_P for root cells has been estimated at about 3.5 bar whereas for shoot cells it is in the order of 6.5 bar (Greacen and Oh, 1972). Hsiao (1973) reports that both $\sum g$ and $\Psi_P(th)$ can change with stress and that they are probably linked to metabolism. Acevedo *et al.* (1971) found that $\sum g$ always increased during water stress, that is, growth was maintained at a lower Ψ_P. This could also occur via a lowering of $\Psi_P(th)$.

For irreversible cell expansion to occur there must be a production of new wall materials and this synthesis is also highly sensitive to water deficits (Hsiao, 1973).

Hsiao (1973) suggests that synthesis is reduced because of the reduction in cell elongation, rather than elongation being limited by a lack of new wall materials. Some form of feedback suppression of cell wall polymer synthesis would be expected if cellular levels of precursors and monomers increased.

Acevedo *et al.* (1971) found that leaf elongation was dynamically dependent on soil water supply. They concluded that decreased growth was likely to be the direct result of a lack of turgor. On the other hand, it has since been shown that the situation is not so simple, as expansive growth can stop even though turgor is maintained (Morgan, 1984). Nonami and Boyer (1987) concluded that Ψ_W(xylem) could decrease and inhibit enlargement without the loss of normal turgor levels in the expanding cells. This was clearly demonstrated by Nonami and Boyer (1989). Within enlarging cells, maximum turgor is inhibited due to continued cell expansion and this creates a Ψ_W gradient between the expanding region and nearby xylem vessels which is essential for continued expansion (movement of water into the expanding cell). Hence a small decrease in Ψ_W(xylem) will result in loss of the Ψ_W gradient without any change in Ψ_W of the expanding region (Nonami and Boyer, 1989).

The biochemistry of cell expansion is complex and allows for a multitude of limiting factors. Cell expansion may be limited by mineral ion supply, especially nitrogen (Patrick, 1987). With adequate soil water and nitrate available, flux of nitrate to the shoot is independent of transpiration rate. When soil water content is low however, nitrate levels in the xylem decrease with transpiration (Hansen and Hitz, 1982) through a dilution effect and nitrate reductase is inhibited (Rudich and Luchinsky, 1986; Wolfe *et al.*, 1988). Therefore leaf nitrate levels may be limiting under water stress conditions.

4.3 **THE SHOOT SYSTEM**

Conflicting responses of leaf production under water stress have been noted from the literature (Steinberg *et al.*, 1990). This may simply reflect differences in stress level, plant material and experimental conditions. Generally water stress would be expected to markedly reduce leaf area development due to the sensitivity of cell expansion (Hsiao, 1973). The position of the growing points in dicotyledons at the edge of the canopy is an accentuating factor (Steinberg *et al.*, 1990). Reductions in leaf size may be positively correlated with productivity and yield during and after water stress (Rawson and Turner, 1982). Steinberg *et al.* (1990) found that the

decrease in dry matter production under water stress for peach seedlings was due principally to a decrease in shoot growth and leaf production.

Smaller leaf area may result from slower growth (initiation and expansion) and/or leaf abscission. Under water stress leaves possess smaller epidermal and mesophyll cells (Rudich and Luchinsky, 1986). Leaf initiation is usually constant with time but is reduced by small water deficits (Clough and Milthorpe, 1975).

4.4 **THE ROOT SYSTEM**

4.4.1 *ROOT GROWTH AND WATER UPTAKE*

Due to the difficulties of studying the root system of field grown and potted plants, understanding of root physiological responses has lagged behind that of above ground plant parts. However when one considers that 50% of the primary production in a forest and 75% in a grassland goes into new root formation (Caldwell, 1976) the importance of a good understanding is obvious. Roots, along with the shoot system, follow a pattern of seasonal growth even under continually favourable conditions (Cockroft and Olsson, 1972). Brown and Scott (1984) have related root growth and distribution to the following:

i/ Depth of root zone.
ii/ Characteristics of root zone; soil density, aeration, water movement, nutrients, pH, toxic elements.
iii/ Intra and interspecific root competition.

Plant species differ markedly in their sensitivity to oxygen deficiency. This is affected by other factors including mineral nutrition (especially nitrate) which can increase tolerance (Veen, 1989). Of the three plants investigated by Veen (1989) (cucumber, maize, tomato), tomato was found to be most sensitive to lack of oxygen in the root zone.

Theoretical modelling of water uptake by roots has proceeded to quite an advanced level (e.g. Slack *et al.*, 1977) although for most purposes a simple model is adequate. For example the equivalent single membrane model (Dainty, 1985) can be defined by the basic flow equation. Some models of root uptake have focused on the

effect of soil properties (Gardner, 1960) while others have considered roots as the primary control point for uptake of water and minerals (Dalton *et al.*, 1974). In all cases only liquid water uptake has been considered. Recent work by Dalton (1989) has shown the possibility of a vapour component to water uptake which may increase with the rate of transpiration.

Much debate has occurred over the pathway for water from root surface to xylem. Three possible pathways exist (Newman, 1976):

i/ Symplast.
ii/ Apoplast.
iii/ Vacuole-to-vacuole.

Recent research favours cell to cell movement, that is, the symplastic pathway (Dainty, 1985; Jeschke and Steudle, 1984) as described by Newman (1976). Roots constitute the greatest resistance to water flow within a plant, as previously noted, accounting for half the total plant resistance (Meidner, 1983). This resistance is almost solely in the radial direction from epidermis to xylem (Lösch, 1984; Steudle, 1989) as longitudinal resistance in both the root and shoot is small (Lösch, 1984). The major point of resistance is located at the endodermis where all solution is forced to pass through the endodermal cytoplasm which contains a lower Ψ_S and hence greater turgor than surrounding root cells (preventing sap leakage from the xylem) (Meidner, 1983).

Root permeability may follow an autonomous rhythm, being several times higher during the day (Newman, 1976). Permeability increases with temperature and upon root death, while decreasing in the presence of metabolic inhibitors and a low O_2/CO_2 ratio. An average value for root resistance would be 1.9×10^5 bar kg^{-1} m^{-2} s^{-1} (Hopmans *et al.*, 1985). Permeability is highest in young roots (root tip portions) under low flux but in older roots (basal portions) under a high flux (Weatherley, 1976; Newman, 1976). Generally though, uptake per unit length is greatest in unsuberised regions (Dwyer and Stewart, 1985; Scott Russell, 1982) (permeability 10 times greater (Caldwell, 1976)) with maximum absorption beginning about 5 mm back from the root tip (Greacen *et al.*, 1976).

Interaction occurs between plant nutrients and root uptake. Allen and Raven (1984) found that water uptake was higher for plants receiving NH_4^+-nitrogen compared with NO_3^--nitrogen.

Scott Russell (1982) gives three important root factors for plant survival and growth under a water stress. These are, in order of importance:

i/ Rapid elongation of the root axis into wet zones.
ii/ Extensive branching.
iii/ Rapid development of new roots after the stress has been relieved.

Distribution of roots in the soil has been related to Ψ_W(soil), aeration and mechanical resistance (Richards and Cockroft, 1974) along with soil chemical and microbiological properties, plant genetic potential and climate (Brown and Scott, 1984). Root distribution under various conditions, and in particular different levels of inter-plant competition (which results in sharp gradients in Ψ_W(soil)), has been graphically illustrated by large scale excavation procedures (Atkinson and Wilson, 1980; Atkinson *et al.*, 1976; Caldwell, 1987; Rogers and Head, 1969).

Decreasing soil resistance may in fact be more important than increasing Ψ_W(soil) in causing proliferation of roots into wetter zones (Greacen and Oh, 1972; Hoogenboom *et al.*, 1987). New root growth is dependent on localized water supply because of an immature vascular system (Sharp and Davies, 1979) with root tips taking most of their water directly from the soil (Huck, 1984). Teskey and Hinckley (1981) found that root elongation was linearly related to soil temperature and Ψ_W(soil), with Ψ_W(soil) dominant at temperatures above 17 °C. Under mild water stress root growth is commonly either maintained or increased (Sharp and Davies, 1979).

No direct relationship exists between total root length and water uptake (transpiration), this being particularly true for tomato (Tan and Fulton, 1985). Taylor and Klepper (1971) found that for cotton, water uptake per unit root length decreased exponentially with soil water potential. Efficiency of a root system is dictated by the supply/demand relationship (Richards and Rowe, 1977b). Eavis and Taylor (1979) found an inverse relationship between root length and uptake per unit length of root.

According to Schulze (1986b), a change in root water status will lead to:

i/ Change in active ion uptake.
ii/ Change in ion concentration in the xylem.
iii/ Change in growth rate.

Along with reduced water uptake there is a reduction in ion uptake under water stress conditions. Phosphate absorption is believed to be directly related to cell metabolism (Dove, 1966). With water stress, root starch levels and total nonstructural carbohydrate (TNC) increase with small changes in soluble sugar concentration. The

contribution of sucrose to TNC is enhanced due to increased synthesis and decreased utilization such that root Ψ_S rises (Hall *et al.*, 1988).

Under non-stress conditions, with only 5% of the root system unsuberised, less than 25% of total uptake occurs from the unsuberised region (Caldwell, 1976). With woody perennials over 99% of the root system may be suberised (Scott Russell, 1982). Unsuberised root tips, however, may be very important for water uptake in drying soil where fluxes are low (Caldwell, 1976) even though their numbers will decrease. This is because the remaining root system will have increased levels of suberisation (accounting for the increased resistance under water stress (Syvertsen, 1985a)).

Water stress will result in an increase in soil temperature. Effects of temperature on root physiology have been investigated by a number of workers under various conditions. However it should be noted that Abbas Al-Ani and Hay (1983) conclude that temperature effects can only be determined if roots are kept in the same environment throughout their growth. Increasing root temperature will increase shoot growth but only over a limited range (e.g. 10 to 25 °C) (Davis and Lingle, 1961). At high temperatures shoot growth is inhibited (Graves *et al.*, 1989). A positive correlation exists between root temperature and the shoot to root (S/R) ratio (Sattelmacher *et al.*, 1990; Szaniawski, 1983). Root number and length decrease as root temperatures rise above a certain level (Sattelmacher *et al.*, 1990). This may result in an increase in leaf area per gram root dry weight (Graves *et al.*, 1989). A decrease in cell division is the first symptom of excessive temperatures (Sattelmacher *et al.*, 1990). In contrast to the shoot system, root maintenance respiration increases with temperature (Sattelmacher *et al.*, 1990).

Root tips are thought to be the site for sensing small changes in Ψ_W(soil) (Davies *et al.*, 1987a, b). They appear to be hydraulically isolated from the rest of the root system (meristems in general possess a high transport resistance (Nonami and Boyer, 1987; Patrick, 1988; Sharp and Davies, 1979)) and so root tip Ψ_W can change without a change in bulk root Ψ_W (Davies *et al.*, 1987a, b). Extraction of water from immediately around the roots will create a Ψ_W gradient in the adjacent soil (Tinklin and Weatherley, 1968). Hence bulk soil Ψ_W will not be representative of that near the root (Sharp and Davies, 1979). Water will be replenished according to the hydraulic conductivity of the soil (Tinklin and Weatherley, 1968). Tinklin and Weatherley (1968) believe that a great deal of plant water stress is due to Ψ_W gradients developing in the perirhizal zone even when bulk Ψ_W(soil) is high. Such is the case with afternoon water stress where plants apparently have adequate soil moisture available

(Huck *et al.*, 1983). The perirhizal resistance is not believed to be located in the soil but rather at the soil-root interface (Weatherley, 1979). For this reason Scott Russell (1982) believes that 'soil-root interface resistance' is a better term that 'rhizosphere resistance'.

Reid (1985) doubts whether soil hydraulic resistance *per se* limits uptake, since r_{root} is about one hundred thousand times greater than r_{soil} at field capacity. Weatherley (1979) measured a perirhizal $d\Psi_W$ of 8 bar (twice that through the plant). The zone was estimated to be 0.1 cm thick giving a gradient of 80 bar cm^{-1}.

It has been widely accepted over the years that roots and root hairs make uniform and continuous contact with the soil. This assumption has now been shown to be incorrect due to root shrinkage (Scott Russell, 1982). Under high rates of transpirational flux, contraction of roots may occur (Huck *et al.*, 1970). This will result in a vapour gap between root and soil (Lösch, 1984; Sharp and Davies, 1979), greatly increasing soil-root resistance (Weatherley, 1976). Faiz in 1973 (as referenced by Scott Russell (1982)) estimated that the increase in interface resistance was two times greater than total plant resistance. Herkelrath *et al.* (1977) showed that water uptake could be modelled more successfully if root contact was assumed to decrease with soil water content.

Such conclusions contrast with the views of Tinker (1976) who believed that local drying around the root system was unlikely. Uptake rates over the whole root system suggest small Ψ_W gradients but as Tinker (1976) rightly points out, local gradients may be much higher (especially in unsuberized portions and root tips). Also the hydraulic conductivity may be quite different around the root compared with the bulk soil (Huck, 1984). Note that root shrinkage is a consequence of water stress and not a cause of it (Scott Russell, 1982).

The extent to which root hairs increase soil contact under field conditions is also being questioned. Other functions for the root hairs have been put forward including (Scott Russell, 1982):

i/ Anchorage.
ii/ Secretion.
iii/ Source of substrates for rhizosphere.

4.4.2 *WATER STRESS AND ROOT RESTRICTION*

Root restriction of any form leads to a restricted root zone volume (RRZV). The agent causing root restriction may be simply an impenetrable barrier (e.g. compacted soil, wood, concrete, polythene etc.) or a change in root zone composition. The latter includes a change in water potential and mineral content.

A decrease in soil moisture restricts root growth but the exact nature of this restriction has not been clearly analysed. Restriction may in fact be due more to increased soil mechanical impedance than decreased Ψ_W(soil) (Greacen and Oh, 1972; Hoogenboom *et al.*, 1987). Hence soil moisture deficit may simply be a special case of physical restriction. Trickle irrigation is the ideal system to consider when investigating RRZV due to a change in Ψ_W(soil) (Carmi, 1986). A small soil volume may be kept moist, allowing extensive root development within that volume, while the surrounding soil is dry and of low penetrability. Plants growing in such a root environment have analogues with those in containers. Proebsting *et al.* (1989) found that effective rooting volume could be controlled by restricted irrigation practices (specifically in this case regulated deficit irrigation (RDI), see section 1.4) as long as roots were physiologically inactive in the dry regions. It was assumed that inactivity occurred below the permanent wilting percentage.

The effects of RRZV have been studied by a number of workers. Attempts have been made to separate the physiological effects of RRZV from those of water stress. Some appear to have been reasonably successful (e.g. Krizek *et al.*, 1985) but many have not (e.g. Hameed *et al.*, 1987; Tschaplinski and Blake, 1985). The difficulty is that as root volume decreases, so does total plant available water and the ability of the root system to cope with changes in evaporative demand (reduced total uptake capacity). As a consequence, plants in small containers invariably suffer water stress thus confounding the two stress agents. Hameed *et al.* (1987) proposed that the principal effect of RRZV was to induce a water stress in the shoot. For plants in small containers, water stress can limit growth long before the root system becomes physically restricted to any extent. Thus the water content of a container has been found to correlate strongly with plant growth parameters (Karlovich and Fonteno, 1986). From a practical point of view, restricted rooting volume leads to a smaller volume of plant available water. Hence, irrigation must be applied more frequently if water stress is to be minimized.

The effects of water stress expounded thus far, involve disruption to a myriad of biochemical pathways, activation of catabolic and senescent processes and general disruption to growth coordination. The work of Krizek *et al* (1985) revealed the following effects from soil water stress:

i/ Decreased Ψ_W(leaf).
ii/ Decreased Ψ_S(leaf).
iii/ Decreased g_s.
iv/ Decreased Pn.
v/ Decreased S/R ratio.
vi/ Increased leaf carbohydrate levels.
vii/ Decreased leaf nitrogen and phosphorus levels.

In contrast the effect of RRZV *per se* appeared to be characterized by a balanced overall decrease in growth with little or no increase in catabolic or senescent processes (Krizek *et al.*, 1985). Increases in leaf starch level have been measured (Carmi and Heuer, 1981). Changes such as decreased chlorophyll levels, increase in leaf area to root dry weight ratio and increase in correlative bud inhibition as observed by Tschaplinski and Blake (1985) can be attributed to water stress. Proebsting *et al.* (1989) have found, using restricted watering, that decreased rooting volume does not affect Ψ_W(leaf) as much as water stress and has no effect on Ψ_S(leaf).

A number of workers have found no change in assimilate partitioning with RRZV (Cooper, 1972; Hall and Turner, 1986; Krizek *et al.*, 1985; LaRoche, 1980; Richards and Rowe, 1977a), while others have found some preferential translocation to fruit (Carmi, 1986; Carmi and Shalhevet, 1983; Cooper, 1972). Ruff *et al.* (1987) observed a decrease in the S/R ratio while Tschaplinski and Blake (1985) observed an increase. This however was attributed to water stress and even with the work of Ruff *et al* (1987) confounding cannot be ruled out. Al-Sahaf (1984) also measured an increase in the S/R ratio with an increased proportion of DM allocated to stems and less to leaves.

With a decrease in root volume there is a concomitant reduction in extension growth (Carmi, 1986; Carmi and Heuer, 1981; Carmi and Shalhevet, 1983; Ruff *et al.*, 1987) and leaf growth (Mutsaers, 1983b) resulting in smaller plants with fewer leaves (Carmi and Shalhevet, 1983; Richards and Rowe, 1977a). Trees under RRZV are similar to those on dwarfing root stocks (Richards, 1986). Growth reductions may be due to deceased root hair and lateral root initiation (Ruff *et al.*, 1987). Hameed *et al.* (1987) found that roots under a RRZV were thicker with increased dry matter per unit length (7.8×10^{-6} kg m^{-1} c.f. $6.1 \times 10^{-6} \times$ kg m^{-1}). Resistivity (1 / conductivity per unit length) was 25% greater for RRZV plants (Hameed *et al.*, 1987). In a slight contrast to the above, Hanson *et al.* (1987) found that shoot growth was reduced as the length of containers of uniform diameter increased. It appears that tap root growth was restricted leading to lateral proliferation. This would have significantly affected the hormonal balance. More work is needed to confirm these results.

Mutsaers (1983b) explained decreased leaf development under root restriction in terms of nutrient supply limitation. However this is not supported by

Carmi and Heuer (1981). They found that increasing the media nutrient status lead to increased leaf growth of controls but not RRZV plants, despite increases in leaf mineral content.

In contrast to water stress, RRZV does not appear to affect unit shoot rate (USR) (Ruff *et al.*, 1987). Richards and Rowe (1977a) found that RRZV decreased root apex number by 33% and root length by 59%. Various other parameters could be related to these two indicating their dependency either on root surface area (proportional to root length) or meristematic potential. Leaf number, lateral length and top weight were reduced by 27, 28 and 34% (c.f. 33% for root number) while total water uptake, nitrate uptake and leaf area were reduced by 49, 55 and 51% (c.f. 59% for root length). Again, more work is needed in this area to substantiate these findings and test their generality.

Under RRZV the chlorophyll A and B content may increase along with leaf thickness. Roots tend to be more highly branched and water plus mineral uptake activity per unit root length is enhanced (Al-Sahaf, 1984). Al-Sahaf (1984) puts forward two possible factors to explain this enhancement in tomato:

i/ Reduced diffusive resistance.
ii/ Increased cation exchange capacity of the root surface.

Tan and Fulton (1985) suggest that total root surface area or high absorptive capacity may be key parameters.

The differences between water stress and RRZV are likely due to the hormonal changes involved. Hormonal changes under water stress will be reviewed shortly. Work associated with RRZV has centred primarily on CK and GA, both of which appear to decrease (Ruff *et al.*, 1987). Carmi and Heuer (1981) found that GA_3 overcame the reduction in stem growth completely and leaf growth partially, while BA alone greatly increased leaf growth. When applied together, GA_3 and BA removed most of the changes caused by RRZV. Lack of complete growth restoration may have resulted from involvement of other GAs or specific amino acids (Carmi and Heuer, 1981). Richards and Rowe (1977a) also found that BAP overcame reduced leaf growth although the leaves contained lower nutrient levels. BAP reduced root number and length in unrestricted plants but not in those physically restricted. This means that in unrestricted plants, BAP will decrease the uptake of Ca^{2+} but not K^+ (Richards, 1978) because Ca^{2+} is taken up only at the root tips whereas K^+ is taken up by all roots whether suberised or not (Richards, 1978) (explaining a higher K^+/Ca^{2+} ratio measured under irrigation (Marangoni and Rossi Pisa, 1985)).

Of direct significance to the effects of water content on soil resistance is the work on mechanical impedance. Soil impedance (strength) is a function of basic soil properties (texture, structure and bulk density) as well as Ψ_W(soil) (and hence volumetric water content). For a given soil sample, soil impedance will be a constant which is independent of root growth.

Goss (1977) demonstrated that roots were unable to reduce their diameter to enter a small pore. In fact, mechanical impedance characteristically leads to an increase in diameter (Atwell, 1989; Scott Russell, 1982). This radial swelling many help to overcome high soil strength (Atwell, 1989). Mechanical impedance leads to reduced extension growth with a change in cell size and number at the root tip (shorter cells) (Goss and Scott Russell, 1980). The cortex becomes thicker while the stele is unaffected (Atwell, 1989). Nodal roots are induced and lateral root development is enhanced (Goss and Scott Russell, 1980) although overall root dry weight is reduced (Masle and Passioura, 1987). Root hair development is commonly stimulated over much of the root surface (Scott Russell, 1982) while the zone of cell expansion moves closer to the root tip (Atwell and Newsome, 1990). Even with all of these anatomical changes it is observed that impedance is only detrimental once water and/or nutrients become limiting (Scott Russell, 1982). In the shoot system lateral shoot meristems are stimulated by mechanical impedance (Goss and Scott Russell, 1980) but leaf area and shoot dry weight are negatively correlated with soil strength (Masle and Passioura, 1987).

Roots can exert large axial pressures in the range of 9 to 13 bar (Goss, 1977; Scott Russell, 1982) with maximum root pressure correlated with the Ψ_S of the tissue (Veen, 1982). However resistances much smaller than this growth pressure (e.g. 0.2 bar) will induce growth and development changes (Scott Russell, 1982; Veen, 1982) thus implicating yet again the role of plant hormones. Little work has been done in this area. Lachno *et al.* (1982) found that IAA increased 3.5 times in impeded roots (with no change in ABA) and used this to explain the morphological changes observed. Masle and Passioura (1987) observed a decrease in stomatal conductance with increasing soil strength and suggested that shoot responses were due to root hormones.

4.5 **ROOT/SHOOT INTERACTION**

An understanding of growth co-ordination is central to whole plant physiology. In fact any analysis of either the root or shoot system is not complete

without taking account of the interactions between them (Scott Russell, 1982). The term correlation is often used in this area, meaning the influence of one part of the plant over the growth and development of another (Black and Edelman, 1970). Growth coordination must exist between (Wareing, 1970):

i/ Shoot and root.
ii/ Extension and radial growth.
iii/ Vegetative and reproductive growth.

Division of physiological function within a plant into root and shoot has caused two major problems (Luckwill, 1960):

i/ Basipetal/acropetal transport.
ii/ Growth coordination.

The first problem was resolved by the evolution of vascular tissue, the second by making each part dependent on the other (Luckwill, 1960).

4.5.1 *ASSIMILATE DISTRIBUTION*

A plant must possess a root system which is large enough to meet the demand for minerals and water while having a shoot system capable of meeting photoassimilate demand. Photosynthesis is needed to maintain mineral absorption while mineral absorption is required to maintain photosynthesis (Marek and Sailerová, 1984). This is the basis of the functional (nutritional) eqilibrium between shoot and root which was first proposed by Brouwer in 1963 (Lambers, 1983). The size of the absorbing root system, xylem and leaf area are all in balance through a physiological feedback mechanism (Kaufmann and Fiscus, 1985). Along with water and minerals, roots also provide hormones and certain amino acids, with the shoot providing B vitamins (thiamin and biotin) (Wareing, 1970). Roots have generally lost their ability to produce thiamin but are the important site for nitrate reduction (Luckwill, 1960).

Roots re-export a large proportion of the carbon products they import (Khan and Sagar, 1969b). Assimilate partitioning between root and shoot is controlled by (Hunt and Nicholls, 1986):

i/ Absolute amounts of above and below ground stress.
ii/ The stress ratio (K_E = shoot stress / root stress).
iii/ Growth potential of the species.

Gersani *et al.* (1980a) document two hypotheses for internal S/R control which have been put forward by several workers:

i/ Correlative mechanisms control development - metabolites move in response to sink effects.
ii/ Correlative mechanisms control the distribution of metabolites - this in turn controls growth.

Gersani *et al.* (1980a) favour hypethesis (i).

Shoot-root imbalance will lead to either stress development or inefficient growth. Borchert (1975) suggests that cessation of shoot growth under favourable conditions may be due to internal water stress resulting from a superoptimal S/R ratio. Optimum shoot-root allocation depends on both present and future environmental conditions. Continuous growth will only occur if the root and shoot subsystems are in functional equilibrium (Borchert, 1976). A feedback response between shoot and root growth can cause endogenous rhythms even under constant environmental conditions as can external factors on stomata (Borchert, 1975, 1976). This is especially true under water stress conditions (Tschaplinski and Blake, 1985).

Processes which occur between carbon assimilation and growth include (Huber *et al.*, 1985):

i/ Partitioning between nontransport (e.g. starch) and transport (e.g. sucrose) forms.
ii/ Compartmentation of transportable assimilate among various pools.
iii/ Phloem loading.
iv/ Long distance transport.
v/ Phloem unloading and uptake/utilization by sinks.

All of these processes influence, to varying degrees, the distribution of assimilate between plant parts. Growth and development is determined by both environmental factors and genetic information which largely defines the distribution of assimilate (Mutsaers, 1983a). For annual species vegatative growth is supported by current root-assimilated ions. With the change to reproductive growth, root assimilation decreases

and shoot remobilization dominates (Patrick, 1987). Assimilate partitioning may be divided into two components (Fischer and Turner, 1978):

i/ Constitutive partitioning - not affected by environmental conditions.
ii/ Facultative partitioning - dependent on environmental conditions.

Environmental factors operate on assimilate distribution through altering source and sink strength (sources are organs supplying carbohydrate and sinks organs using carbohydrate). If sink strength is reduced, overall demand for assimilate falls unless other sinks take over. Lower demand leads to a reduction in source strength, that is, net photosynthesis, predominantly it would appear through changing hormone levels and not end product inhibition (i.e. buildup of carbohydrate in the leaves) (Patrick, 1987). However it has been found that changing the strength of one source can cause immediate changes in the strength of another. The speed of response indicates a physical signal (c.f. hormones) and Thorpe *et al.* (1983) proposed that this signal was a turgor (Ψ_P) change. With Ψ_P and Ψ_S nearly equal in seive tubes (Lang, 1974), information on solute concentration can be transmitted as a change in pressure potential (Thorpe *et al.*, 1983).

Two simple models for assimilate partitioning have been proposed (Patrick, 1987):

i/ Sink model;

Assimilate use rate	-->	Assimilate concentration in sink cytosol	-- |
Translocation rate	<--	Concentration gradient	<---

ii/ Supply/sink model;

Assimilate use and translocation integrated by independent factors (eg. water supply, hormones).

Partitioning to meristems is most readily regulated by components of the source-path system whereas for expansion/storage sinks regulation may be confined to operation of key sink processes (Patrick, 1987). There may be a significant hydraulic conductivity

limitation to meristems (Patrick, 1987). Stress factors such as water deficits and high/low temperatures in the root zone reduce root sink strength. This can cause a lowering of the photosynthetic rate due to a change in the Rubisco activation state (Janes *et al.*, 1988).

The root and shoot systems compete with one another for assimilates, but for optimum growth it is important that this competition does not lead to inhibition (Gersani *et al.* 1980b). Short term competition can be accounted for by control of assimilate transport (Gersani *et al.*, 1980a). Shoot demand for assimilates may be transmitted to the roots by mineral ion concentration in the phloem. These ions would exert allosteric regulation on the unloading step (Patrick, 1987). Both ABA and cytokinins may also be involved in this process as they affect ion secretion into the xylem. At the source end, auxins stimulate phloem loading and may regulate phloem K^+ uptake (Patrick, 1987).

Various simple relationships exist between aspects of the root and shoot system. For example, Richards and Rowe (1977b) found linear relationships between dry weight increase *versus* water use and final LA *versus* water uptake rate. Root length (or perhaps more precisely root surface area) and LA are related through involvement in water and nutrient uptake while root number and leaf number are related through differentiation processes in the apical meristems (Chung *et al.*, 1982). A linear relationship exists between root and leaf number (also leaf area and root number) (Richards, 1986). LaRoche (1980), working with *Aquilegia canadensis*, found a significant correlation between root dry weight and plant height.

Water stress leads to a decrease in the S/R ratio (Fischer and Turner, 1978; Gales, 1979; Hall *et al.*, 1988; Steinberg *et al.*, 1990; Wilcox-Lee, 1987) due to a greater reduction in shoot growth than root growth. This is the same as the effect of shading (Avery *et al.*, 1979). The water stress may be generated by either low Ψ_W(soil) or high VPD (Fischer and Turner, 1978). Mederski and Wilson (1960) observed a linear relationship between the S/R ratio and soil water content. In some cases root growth may even increase under water stress (Huck *et al.*, 1983) leading to increased root length (Huck *et al.*, 1986) but not total mass (Hoogenboom *et al.*, 1987). The increased sensitivity of leaves over roots to low Ψ_W may be due to the ability of roots to maintain a favourable Ψ_W gradient in the elongating tissue which is needed for continued growth (Boyer and Westgate, 1984) as originally proposed by Greacen and Oh (1972). This Ψ_W gradient is related to the presence of solutes which also maintain a favourable cell turgor (Boyer and Westgate, 1984). Schildwacht (1989) found that roots could osmoregulate over a range of water potentials whereas shoots could not.

4.5.2 *ALLOCATION OF RESPIRATORY ENERGY*

Two general approaches to the study of whole plant responses to a water stress can be identified in the literature. The first approach focuses on assimilate partitioning and maximum relative growth rate (as discussed above), while the second centres on allocation of respiratory energy (Taylor, 1989).

Any adaptive response by a plant to water stress must be associated with an energetic cost where respiratory resources are diverted from growth to maintenance (Taylor, 1989). Maintenance respiration is that which counteracts spontaneous entropy increase and maintains physiological functions as opposed to growth respiration which gives rise to new biomass (Taylor, 1989). An equilibrium exists between the maintenance respiration rates of the root and shoot systems as well as the proportion of photosynthates used in respiration (Szaniawski, 1983). Szaniawski (1987) considered stress tolerance in terms of homeostasis, that is, the tendency to keep internal plant parameters within a certain range. The actual homeostatic capacity (AHC) of a plant was said to equal the ratio of maintenance respiration to total respiration. However this hypothesis appears rather simplistic as it does not include homeorhesis (the tendency to maintain growth patterns under stress) or species specific adaptations (Taylor, 1989). Taylor (1989) believes that stress tolerant plants will allocate a greater proportion of respiratory energy to growth, i.e.:

$$\text{Stress tolerance (T)} = Rg / Pg \qquad 4.2$$

Where: Rg = growth respiration; Pg = gross photosynthesis.

At first this appears to conflict with a generally held view that high stress tolerance is associated with a low maximum relative growth rate (RGR_{max}) as hypothesizes most strongly by Grime (see Grime(1979)) in his C-S-R model (see also Hunt and Nicholls, 1986; Shipley and Keddy, 1988). However this is not the case as long as slow growing plants have low rates of photosynthesis. The correlation between slow growth and stress tolerance may be due to the need to minimize maintenance respiration relative to photosynthetic rate (Taylor, 1989). From the model of Hunt and Nicholls (1986), low RGR_{max} promotes large changes in the allometric k value (see section 4.5.5) under stress which helps to maintain the plants RGR.

4.5.3 *THE SHOOT TO ROOT RATIO*

The simplest root-shoot parameter to calculate and one which is quoted extensively is the Top/Root (T/R) (or Shoot/Root (S/R)) ratio (R/S ratio is also used eg. Atkinson *et al* (1976)). Some authors take S/R ratio as vegetative shoot tissue dry weight over root dry weight and T/R ratio as shoot vegetative plus reproductive tissue dry weight over root dry weight (eg. Richards *et al*., 1979) while others use T/R when calculating the former. This continues to lead to confusion. The distinction of Richards *et al*. (1979) is useful and will be followed in this thesis (note that in the experimental work which follows Sh/R will be used to avoid confusion with the stem to root ratio, Sm/R).

The S/R and T/R ratios are not ontogenically constant (Brouwer, 1983; Hunt and Nicholls, 1986; Richards, 1986) but generally increase with age (Richards, 1986) and show considerable genetic (Brouwer, 1983; Wareing, 1970) and environmental (Brouwer, 1983; Hunt and Nicholls, 1986) variability. They are a reflection not only of assimilate partitioning but also the rate of death and turnover of materials (Fischer and Turner, 1978). Richards *et al*., (1979) demonstrated a linear increase with time for the S/R ratio but an exponential increase for the T/R ratio. This increase can be explained by the fact that roots face progressively deteriorating conditions while shoots do not (Borchert, 1976). Shoot growth will also decrease with time due to increased complexity of the shoot system along with limitations imposed by root growth and depleted soil water reserves (Borchert, 1976).

Overall, the S/R ratio is reasonably stable over a given set of environmental conditions (Wareing, 1979). This is demonstrated by the fact that a set of plants with very different S/R ratios will tend to a common ratio under the same conditions (Barlow, 1960; Brouwer, 1983; Brown and Scott, 1984; Rogers and Booth, 1960; Sanders and Brown, 1976). If conditions change the S/R ratio will tend to a new value which is characteristic of the new set of conditions (Brouwer, 1983).

Four basic models for shoot-root control have been developed, or used, to interpret experimental results (Wilson, 1988):

i/ Allometric relationship.
ii/ Functional equilibrium.
iii/ Thornley carbon/nitrogen model.

iv/ Hormonal model.

The first two have only an empirical basis, but they are extremely useful for gaining an initial insight into the complex area of shoot-root interactions. The allometric relationship succinctly identifies overall changes between the root and shoot as discussed in section 4.5.5 and shown in the experimental work to follow. Although Wilson (1988) points out that most evidence is compatible with the Thornley model, this is based around carbon and nitrogen flux which is just one of the three major physico-chemical links within the plant, the others involving water and hormones (see section 5.7). McIntyre (1987) has developed an hypothesis for various physiological interactions based on water flux, while at the same time there is a bounty of support, in various forms, for controls involving hormone flux (see section 5). Since information is seldom, if ever, redundant in nature, it would seem necessary to incorporate these major fluxes of information into a unified model. This is done in section 5.7.

4.5.4 *THE FUNCTIONAL EQUILIBRIUM*

Luckwill (1960) suggested that plants maintain a constant ratio not of shoot and root weight *per se* but nitrogen uptake to carbohydrate synthesis. Not long afterwards, in 1963, Brouwer proposed the term functional equilibrium for the relationship between uptake of nutrients by the root and synthesis of new carbon material by the shoot (Lambers, 1983). Thornley (1972) stated that total shoot activity and total root activity are proportional under steady state growth. Within this framework comes the requirement, in a terrestrial environment, to balance water uptake and water loss. Therefore water supply will determine the S/R ratio under optimum nutrient conditions (Brouwer, 1983). The S/R ratio may change so as to keep Ψ_W within certain limits (Fischer and Turner, 1978). Brouwer (1983) suggests that environmental changes will not allow the functional equilibrium to exist for long periods of time so that non-equilibrium situations are normal.

To investigate the equilibrium there is a requirement for assessing root system efficiency (c.f. Pn rate for shoots) as first pointed out by Hudson (1960) and this has been achieved by measuring ion absorption. Humphries (1960) measured the K^+ content to estimate relative mineral uptake rates. Under high nutrition K^+ is closely related to gain in dry matter but is less so under poor nutrition. There are usually very similar patterns for cumulative water extraction and K^+ uptake and dry

matter production (Brown and Scott, 1984). Brouwer (1983) noted three problem areas in the investigation of the functional shoot-root relationship:

i/ Results tend to be based on dry matter rather than exposed surface area.
ii/ It is hard to distinguish between structural and nonstructural reserves.
iii/ Differences may occur in the readiness of plants to respond to environmental changes.

The extent to which these factors have influenced findings and results is hard to assess. Point (i) is certainly valid although exposed surface area is a difficult variable to measure.

A functional (or steady state) equilibrium is considered to be the most orderly, efficient and economic state for an open system (Szaniawski, 1987). Hunt (1975) expressed the functional relationship as:

$$W_r / W_s = a + b \times (1 / (SAR_m / USR)) \qquad 4.3$$

Where: W_r = root mass; W_s = shoot mass; W_r/W_s = Mass ratio; SAR_m = Specific absorption rate for element m; USR = Unit shoot rate; SAR_m/USR = Activity ratio; a, b = Constants (0.051 and 45.7 respectively).

In other words, root mass times rate (absorption) is proportional to leaf mass times rate (Pn). This was also used by Richards *et al.* (1979). However Thornley (1975) showed that the same relationship could be expressed very simply as:

$$dM = f_m \times dW \qquad 4.4$$

Where: dM = Change is weight of element m; dW = Change in plant weight; f_m = Fraction of plant weight which is element m.

The functional equilibrium proposed by Brouwer in 1963 was based on nutritional supply limitations (Lambers, 1983). Brouwer (1983) still maintains that nutritional control provides the best explanation for growth changes after plant environment disruption. However Lambers (1983) provides clear evidence that the root system is not supply limited under stress. This is based on the activity of the alternative respiratory pathway which is only engaged when excess carbohydrates are

available and yet still occurs under stress. No firm hypothesis is put forward to redress this conflict except that a regulatory factor is proposed to travel in proportion to the sucrose concentration. Cytokinins have been shown to regulate root nitrate supply but there is no evidence for control of overall dry matter partitioning (Lambers, 1983). The nitrate-cytokinin relationship is as follows (Lambers, 1983);

```
        < N to root from shoot          -->  < root CK production      ---
                                                                        |
--      < N incorporation into shoot    <--  < CK to shoot             <---
|
->      > N to root from shoot
```

4.5.5 *THE ALLOMETRIC RELATIONSHIP*

It was noted previously that the S/R ratio is not ideal for studying root-shoot interactions because it changes with age (size of the plant) (Gales, 1979; Richards, 1986). Thus size variation is confounded and the ratio tells one nothing about the rate of change of organ growth (Troughton, 1956). A more stable and indeed fundamental way of expressing growth and development patterns of organs from any organism is to use allometry (Gales, 1979). Allometric relationships have been known in biology since last century but it was not until 1932 that Huxley demonstrated wide biological significance (Causton and Venus, 1981; Reiss, 1989). They have now been found at the biochemical level as well (Causton and Venus, 1981). According to Szaniawski (1987) the allometric relationship "simply reflects the basic biological fact that any multicellular organism whether plant or animal can survive only if it maintains a balance between its various organs".

The most simple form of the allometric relationship is a linear trend where the following formula applies (Troughton, 1955):

$$Y = b \times X^k \qquad 4.5$$

Where: X = weight of organ; Y = weight of rest of organism; b, k = constants.

Neither the organ nor the organism needs to be growing logarithmically for this relationship to exist (Troughton, 1955). The allometric formula was first used by Snell in 1892 but not until Pearsall in 1927 was it applied to plant root and shoot dry weights

(Troughton, 1956). This development was reiterated by Wareing (1950) and then the work developed further by Troughton (1955, 1956, 1960, 1977). Causton and Venus (1981) give a thorough mathematical treatment of both linear and curvilinear allometry based around three basic theorems. The following point should be noted from these theorems with regard to the shoot-root relationship. If there is a linear allometric relationship between any two plant parts (e.g. shoot and root) then the relationship between any part and the whole will not be linear (Causton and Venus, 1981).

The critical parameter in the allometric relationship is 'k', the exponential growth exponent which Huxley in 1932 termed the "constant differential growth-ratio" (Troughton, 1956) and Hunt and Nicholls (1986) termed the "partitioning ratio (K_P)". Troughton (1955) concluded that "A study of the values of 'k' enabled the effect of treatments on the relative growth of the root and shoot to be far more clearly defined than did a study of the proportions of the plant" (i.e. the S/R ratio). The constants 'k' and 'b' may be found by re-expressing the allometric formula as:

$$\log(Y) = \log(b) + k \times \log(X) \qquad 4.6$$

Hence a plot of log(Y) verses log(X) gives a linear relationship with slope of 'k' and y-intercept of log(b). Since 'k' is proportional to (log(Y) / log(X)) i.e. log(shoot) / log(root) it is an expression of the ratio of relative growth rates of the shoot and root i.e. 'k' is proportional to RGR(shoot) / RGR(root). Under natural conditions it is root growth which is restricted (due to mechanical resistance, water deficits etc.). Hence from the allometric relationship it may be stated that the RGR of the root limits the potential for vegetative growth of above ground parts (Chalmers, 1987). If $k = 1$ the S/R ratio is constant, if $k > 1$ the S/R ratio declines with age and if $k < 1$ the S/R ratio increases with age (Richards, 1986).

Causton and Venus (1981) describe the hypothesis put forward by Nelders in 1963, that if 'k' is not equal to one, growth is demand limited, while if 'k' equals one, growth is supply limited. They note that as 'k' commonly does not equal one, supply limitation may be infrequent. The general range from for 'k' is between 0.3 and 3 (Hunt and Nicholls, 1986). It should be noted here that the value of b is difficult to interpret, being the value for the organ when the rest of the organism is unity (Troughton, 1956). The allometric constant changes with environmental conditions for a given plant (Chalmers, 1987). However it is interesting to note that although flowering and fruiting changes k, as first documented by Pearsall in 1927 (Troughton, 1955), the initial value of 'k' continues throughout fruiting if the weight of

reproductive parts is subtracted (Chalmers and Van den Ende, 1975; Richards, 1981). The allometric relationship can be related to the C/N ratio (as shoots are providing carbon and roots nitrogen) such that as 'k' decreases the C/N ratio increases (Troughton, 1960).

Linear allometric relationships will exist in the vegetative phase as long as environmental conditions are constant (Scott Russell, 1982). There are very few documented cases of curvilinear allometric relationships, the best example being from Currah and Barnes (1979). They found that individual carrot plants followed a curved path. However for any one harvest (i.e. for plants of the same age) linear relationships were found. This means that there is discontinuity between harvests for the whole population. Other examples of discontinuity as well as segmented linear allometry are given by Causton and Venus (1981).

Hunt and Nicholls (1986) conclude that "any growth limiting condition or resource will also induce a change in the resource partitioning of the plant. This will result in proportionally increased allocation of linear size, number or mass in favour of that part of the plant which draws most upon the growth limiting part of the environment". Hence there are well known examples such as nutrient deficiency leading to increased 'rootiness' and shade leading to increased 'shootiness' (Hunt and Nicholls, 1986). Water stress however is more complicated because of direct effects on both plant subsystems and a host of interactive responses.

The slope of the allometric relationship (k) has been observed to decrease in response to increasing water stress (Troughton, 1960), nutrient stress (Hunt and Nicholls, 1986) and light intensity (Hunt and Burnett, 1973; Hunt and Nicholls, 1986; Troughton, 1960).

5 WATER STRESS AND PLANT HORMONES

5.1 CYTOKININS

Roots are the main source of cytokinins (CK) (Carmi and Heuer, 1981), with production in the subjacent tissue of root meristems (Torrey, 1976), although isolated leaves can also produce measurable quantities (Salama and Wareing, 1979). They can be formed independently of tRNA degradation by addition of an Δ^2-isopentenyl group to AMP (Letham *et al.*, 1982). When CK are applied to the root system the S/R ratio decreases (Richards, 1980; Wittwer and Dedolph, 1963) and when applied to shoots the ratio increases (McDavid *et al.*, 1973; Richards, 1980). Thus CK divert assimilate towards points of high concentration, suggesting an important role in assimilate distribution and root-shoot relations (Torrey, 1976).

Along with growth control, CK are involved in the regulation of photosynthesis and senescence (Carmi and Heuer, 1981; Livne and Vaadia, 1972). Removal of root tissue enhances leaf senescence and decreases the photosynthetic rate while application of CK can overcome these changes (McDavid *et al.*, 1973; Itai and Benzioni, 1976). However CK applied to the whole plant can actually enhance senescence (Itai and Benzioni, 1976) due to their detrimental effect on the plant-water balance (Mizrahi and Richmond, 1972). Cytokinins reduce root permeability to water (Vaadia, 1976) and stimulate stomatal opening. The ability of CK to increase the affinity of cells for potassium ions (K^+) (and decrease the affinity for sodium ions (Na^+)) may be important here (Itai and Benzioni, 1976). The net result can be a large decrease in leaf water potential (turgor).

Cytokinins are needed to maintain cell wall extensibility (Davies *et al.*, 1987b) which may explain the correlation between cytokinin levels and shoot growth. Cytokinins increase a plants resistance to stresses in general by increasing membrane stability through stimulation of anabolic processes (Gamburg, 1982).

Blackman and Davies (1985b) have placed central importance on root supplied CK. This is based on the hypothesis that reduced synthesis or transport of a promoter is a more sensitive indicator of root perturbations than increased synthesis of an inhibitor. This hypothesis was first proposed by Itai and Vaadia (1965).

Changes in mineral nutrition, particularly nitrate, cause changes in endogonous cytokinin levels (Bruinsma, 1984; Kuiper D. *et al.*, 1989; Lambers, 1983;

Salama and Wareing, 1979) (see section 4.5.4). Phosphorus has a similar effect but not potassium (Salama and Wareing, 1979). Kuiper D. *et al.* (1989) hypothesize that nitrate is the effecter of cytokinin concentration changes and it may be in fact that enhanced growth following improved nutrition is due to increased cytokinin levels rather than the minerals *per se*. Changes in cytokinin levels under water stress may be modified in part through the interaction with mineral status. Nitrogen and phosphorus levels decrease under water stress (Davies *et al.*, 1987a) detrimentally affecting cytokinin production (Turner, 1986b).

Water stress reduces the amount of CK exported from the roots (Hubick *et al.*, 1986b; Itai and Vaadia, 1965; Rosa da Costa *et al.*, 1987; Torrey, 1976) although the level of synthesis may not change, indicating that the response is due to decreased transport (Rosa da Costa *et al.*, 1987). This changes the amount of CK in the shoot by as much as 50% (Hubick *et al.*, 1986b). Cytokinin levels can be related to the growth rate of the root system (Schulze, 1986b) where, along with reduced export, qualitative changes are observed. For example, Hubick *et al.* (1986b) found a large peak correlating with zeatin glucoside which was not present in unstressed plants. This bound cytokinin may be a function of cytokinin/ABA interaction. Reduced cytokinin activity is associated with enhanced metabolism (Itai and Benzioni, 1976).

5.2 **ABSCISIC ACID**

Abscisic acid is considered to be a general stress hormone (Schulze, 1986b) with its level increasing due to a wide range of external factors, and it is categorized as a growth inhibitor. However it occurs at high concentrations in the growing regions of both root and shoot, thus implying substantial compartmentalisation (Hartung *et al.*, 1982). Mature leaves are the prominent site of synthesis (Bruinsma, 1984) although as noted in the previous section, root produced ABA could be very important in root-shoot communication (Davies *et al.*, 1987a). Synthesis of ABA may be confined to plasmids which would explain the differential production capacity between roots and leaves, although supply of the mevalonate precursor may also be rate limiting in the root system (Baker and Lachno, 1989).

There are three aspects to the control of tissue ABA levels (Hubick and Reid, 1988):

i/ Cellular pH.

ii/ Rate of synthesis, catabolism and conjugation.
iii/ Rate and direction of transport.

While ABAH can diffuse through membranes, ABA cannot and so distribution is according to pH gradients with up to 80% in chloroplasts which have a high pH (Hartung *et al.*, 1982).

The effects of ABA at the cellular level are wide-ranging. It stimulates sucrose uptake in root tissue (Brenner *et al.*, 1982) while inhibiting the flux of K^+ into guard cells (Itai and Benzioni, 1976; Mansfield, 1976). Its site of action on guard cells is the apoplastic side of the plasmalemma (Schulze, 1986b). Abscisic acid reduces the capacity of the cell wall to respond to acidity (Davies *et al.*, 1987a) and also the efflux of H^+ (Marrè, 1982). However its effect on increased root membrane permeability leads to an initial release of H^+ and stimulation of root growth (Evans and Mulkey, 1982). There are four responses of a root to ABA (Fiscus, 1982):

i/ Fast release of solutes.
ii/ Rapid increase in volume flux through root.
iii/ Slow buildup of ion transport activity.
iv/ Decrease in hydraulic conductivity (under high concentrations only).

It appears that under nonstress conditions ABA does not have a major role in shoot growth regulation as long as the potential for growth is high (Powell, 1982). However under water stress, ABA changes are now considered to be very important. Bunce (1990) concludes that "understanding the control of ABA synthesis and movement during dehydration and its mechanism of action is becoming increasingly important to a better understanding of responses to water stress". The glucosyl ester of ABA, which unlike phaesic acid, does not decline after water stress is removed, may be used as a cumulative indicator of water stress history (Lösch, 1984). It should be noted however that although exogenous ABA can induce the water stress changes to stomatal aperture it cannot induce many of the biochemical changes which occur under water stress including accumulation of sugar, disaggregation of polysomes and increase in certain mRNAs. This implies that other mechanisms are involved (Creelman *et al.*, 1990).

Water stress increases the capacity of tissue to synthesize ABA (Creelman *et al.*, 1990; Itai and Benzioni, 1976; Zhang and Davies, 1987; Zeevaart and Boyer, 1982), especially as turgor loss approaches (Walton, 1980). At the same time the rate

of degradation may be reduced (Itai and Benzioni, 1976) although others have reported a stimulation as turgor falls (Hartung *et al.*, 1982). The answer to this conflict may lie in the fact that ABA metabolism is more rapid in the dark than light, possibly due to increased ethylene production at that time (Zeevaart and Boyer, 1982). Under prolonged stress, the levels of conjugated ABA (B-D-glucopyranosyl abscisate) also increase (Zeevaart and Boyer, 1982) although there may be no effect on ABA transport rates (Hubick and Reid, 1988). Biosynthesis, conjugation and metabolism of ABA take place preferentially in extraplastidic compartments (Hartung *et al.*, 1982).

Stimulation of synthesis and redistribution of compartmentalized ABA are in response to changes in Ψ_P such that total ABA levels in apple leaves correspond to Ψ_P changes and not Ψ_W (Davies and Lakso, 1978). Turner (1986b) identified that ABA concentrations increased linearly with turgor. Water stress, from either a lack or excess, causes chloroplast pH to drop (Cowan *et al.*, 1982; Smith and Ager, 1988) releasing ABA into the cytoplasm and from there the apoplast. In flooding treatments, Smith and Ager (1988) believe the sequence of events is as follows:

i/ Decreased assimilation rate.
ii/ Decreased 3PGA in chloroplast at expense of orthophosphate.
iii/ Decreased chloroplast pH.
iv/ ABA release from chloroplast.
v/ Stomatal closure.

When roots become water stressed their ABA concentrations can increase dramatically (though not always (Larsson *et al.*, 1989)). With only a 4 bar stress, Walton *et al.* (1976) observed a ten fold increase after only one hour, with this increase due to *de novo* synthesis. There was also an increase in the ABA metabolite DPA (dihydrophaseic acid) (Walton *et al.*, 1976). Many other workers have now observed root ABA increases (Baker and Lachno, 1989; Neales *et al.*, 1989; Zhang and Davies, 1989a, b), sometimes of up to 32 fold (Hubick *et al.*, 1986b) but generally at least an order of magnitude or more (Davies *et al.*, 1987b). Baker and Lachno (1989) found that a stress of -2 bar was needed before ABA concentrations increased. This may be compared with the situation in leaves where a Ψ_W threshold for ABA accumulation is generally assumed (Rudich and Luchinsky, 1986) which may be close to, or at, the turgor loss point ($\Psi_P = 0$) (Rudich and Luchinsky, 1986; Raschke, 1982). Blake and Ferrell (1977) found rapid ABA increases at a Ψ_W(leaf) of -10 to -12 bar.

In the root system it has been observed that root ABA levels can change without a change in bulk water potential or even turgor. However it is known that

ABA is synthesized in root tips (Zhang and Davies, 1987) and these may be hydraulically isolated from the rest of the root system (Davies *et al.*, 1987a). Therefore, Ψ_P changes in the root tips resulting from changes in Ψ_W(soil) could modify ABA synthesis and as such, amounts available for export to the leaves (Zhang and Davies, 1989a, b). Export could then occur through small transpiration fluxes or by dehydration of turgid roots loaded with ABA (Zhang and Davies, 1987). Zhang and Davies (1989b) found that root tip Ψ_W depended upon soil Ψ_W and root size, with finer roots loosing turgor more rapidly. They proposed that fine roots in the surface soil layers could supply ABA to the leaves thereby causing a reduction in g_s and leaf growth. In turn, more assimilate would be available for root growth in wetter regions.

The initial response to water stress in leaf tissue may involve changes in ABA distribution which, as noted previously, are related to pH gradients. Water stress results in a lowering of chloroplast stroma pH allowing ABA release into the cytoplasm and apoplast (site of action on guard cells) (Davies *et al.*, 1987a). The problem of high ABA levels under nonstress conditions and a lack of detectable increase in leaf levels with water stress in some cases (although stomata close) has already been discussed. Compartmentalisation and root ABA moving directly to the site of action have been used to explain these results (Davies *et al.*, 1987b). There will undoubtably be interactions between ABA levels and those of other hormones, especially CK. Reduced cytokinin level increases the sensitivity of stomata to ABA (Blackman and Davies, 1985b). Interactions with mineral status are also proposed. Decreased levels of N and P under water stress would increase the sensitivity of leaf processes to a constant level of ABA (Davies *et al.*, 1987a) by reducing cytokinin production in the root (Turner, 1986b). The effect of ABA on stomatal closure is increased under N deficiency (Davies *et al.*, 1987a). ABA may affect nutrient uptake via an increase in the membrane permeability of roots which, even under nonstress conditions, are somewhat leaky (Gardner, 1965).

Accumulation of ABA in the leaves is generally far higher than that required for stomatal closure. The excess may have a role to play in ameliorating the deleterious effects of water stress within the protoplasm (Creelman *et al.*, 1990). Abscisic acid may also function to inhibit shoot growth relative to root growth (Creelman *et al.*, 1990) which, combined with promotion of water flux in and out of the root system (i.e. increases root hydraulic conductivity) (Torrey, 1976) and stomatal closure, would lead to recovery of plant water status (Mizrahi and Richmond, 1972).

5.3 GIBBERELLINS

Giberellins appear to be produced both in the shoot and root (McDavid *et al.*, 1973) although evidence for root production is not conclusive (Graebe, 1982) with synthesis only observed in isolated roots (Bruinsma, 1984). They have a major role in stem growth and internode elongation (Carmi and Heuer, 1981) and hence increase the S/R ratio (Currah and Thomas, 1979). It is known however that GAs are converted to different forms in the root tips. The best known example is that of GA_{19} which is produced in the shoot, translocated to the root, converted to GA_1 and then re-exported to the shoot (Torrey, 1976). GA_{19} is inactive in shoot tissue whereas GA_1 is highly active (Bruinsma, 1984). Giberellins cause changes in Ψ_S gradients and cell wall extensibility. They stimulate cell elongation and synthesis of wall material but do not promote proton efflux as auxin does. Stimulation of elongation may be due to enhancement of Ca^{2+} uptake into the cytoplasm, as applied Ca^{2+} is strongly inhibitive (Jones 1982).

Generally GA levels decrease with water stress (Torrey, 1976) although the effect may sometimes be minimal (Hubick *et al.*, 1986b). Levels in the shoot also decrease under water logging (Livne and Vaadia, 1972). Wareing and Phillips (1981) state that gibberellins have no obvious role in water stress phenomena. However considering the complex interactions between all plant hormones it would seem inevitable that gibberellins are involved in certain aspects of water stress physiology.

5.4 AUXINS AND POLYAMINES

Auxins are produced predominantly in shoot meristems and transported basipetally in polar fashion (Phillips, 1971). As with ABA the protonated form (IAAH) has a relatively high diffusive permeability and so distribution is pH dependent (Rubery and Astle, 1982). Movement also appears to occur through two other carriers, an H^+/IAA^- symport and an electrogenic uniport (Rubery and Astle, 1982). Within the roots auxin is transported acropetally towards the root tips, predominantly in vascular tissue (Torrey, 1976), with increased rates at higher pH.

The most dramatic effect of auxin is to stimulate proton excretion from cells. This efflux is mediated by a vanadate sensitive, and therefore probably plasma-membrane located, ATPase. Auxin does not activate the ATPase directly but may

modify the concentration of an ATPase effecter. No specific counter-ion is needed but there is a requirement for continued protein synthesis (Cleland, 1982). Proton excretion is associated with the acid growth hypothesis as has already been discussed (Evans and Mulkey, 1982) where the H^+ gradient is utilized for (Marrè, 1982):

i/ Cation uptake (electrogenic uniport).
ii/ Anion and amino acid uptake (electrogenic and electroneutral symport with H^+).
iii/ Sugar uptake (electrogenic symport).
iv/ Efflux of Na^+ and Ca^{2+} (electroneutral antiport with H^+).

Auxin also enhances cell division, especially DNA replication (Gamburg, 1982), while auxin stimulation of both cell division and enlargement is commonly associated with increased respiration (Gamburg, 1982).

Synthetic auxin analogues tend to have opposite effects to those of IAA. This is due to analogues binding at the site of action but not effecting a response (Cox *et al.*, 1985).

Polyamines have similar effects to auxins but are not as active in cell elongation (Guern *et al.*, 1982). Their antistress effects on membrane survival are mediated by (Bagni *et al.*, 1982):

i/ Stabilization of negatively charged plasmalemma.
ii/ Involvement in nucleic acid biosynthesis and degradation (stabilizes polynucleotides).

Auxin has a central role in plant morphology through apical dominance. This is believed to be a function of shoot apex produced IAA and root produced cytokinin (Wareing and Phillips, 1981). Control exerted by shoot apex IAA on shoot cells in general may be greater that previously thought if a frequency dependent IAA signal is operating as suggested by Zajaczkowski *et al.*, 1983).

Wareing and Phillips (1981) conclude that auxins, as with gibberellins, have no obvious roles in water stress physiology. Again however it may be hypothesized that significant roles will be found in the future. It has been known for some time in fact that the level of IAA oxidase increases under water stress (Livne and Vaadia, 1972; Todd, 1972) resulting in increased degradation of IAA. Also, as noted above, polyamines can stabilize cell membranes and polynucleotides (Altman *et al.*, 1982) which will increase water stress tolerance. Far more work is needed on polyamines in

all areas of plant physiology to clarify their significance and interactions with the main hormone groups.

5.5 **ETHYLENE**

Ethylene is a unique plant hormone because it is gaseous. Its synthesis is stimulated by auxin and hence specific responses to auxin and ethylene are often poorly defined. Ethylene causes a change in the direction of cell enlargement, with decreased elongation and increased lateral expansion as ethylene concentration increases (Osborne, 1982). Cells differ in their response to both auxin and ethylene. Osborne (1982) has identified three types:

i/ Cells enlarge by auxin and not ethylene (majority of shoot cells).
ii/ Cells enlarge by ethylene and not auxin (abscission zone).
iii/ Cells enlarge by ethylene and auxin (epinastic zone).

Ethylene generally inhibits root elongation as does high auxin concentration (Torrey, 1976) with the inhibition being reversible (Torrey, 1976). Low concentrations, however, can actually stimulate root extension in some plants such as rice (Jackson, 1982). Ethylene production is inhibited by light, this being attributed to low C_i (Woodrow and Grodzinski, 1984). Ethylene may act as a link between growth and development phenomena and aspects of basic metabolism (Woodrow and Grodzinski, 1984). The response of stomata to ethylene appears complex with both increased and decreased opening possible (De Lorenzi and Giulivo, 1985).

Although some workers have found no response of ethylene levels to water stress (Hubick *et al.*, 1986b), ethylene synthesis has been shown to increase. However this increase will be offset by increased ABA and decreased auxin plus cytokinin production (Davies *et al.*, 1987a). In some cases ethylene production may increase as much as thirty times and then decrease even though water stress continues (Lösch, 1984). Wilting enhances synthesis of ACC (1-aminocyclopropane-1-carboxylic acid), the precursor of ethylene. Application of BA or IAA to plants before water stress increases ethylene synthesis whereas ABA inhibits synthesis of both ethylene and ACC (Wang and Seffans, 1985). A residual metabolite from the ethylene pathway (N-malonyl-aminocyclopropane-1-carboxylic acid) can be used as an indicator of stress history (Lösch, 1984).

Osmotic agents increase the conversion of ACC to ethylene, this being dependent on the intensity of stress (i.e. concentration of osmoticum) (Chrominski *et al.*, 1988). Under water stress stomata may become sensitive to ethylene and close as concentrations increase (Davies *et al.*, 1987b). However Masia *et al.*, (1985), using an ethylene releaser (CEPA), found that stomata lost their responsiveness to environmental conditions under high ethylene concentrations.

5.6 **ROOT/SHOOT INTERACTION REVISITED**

5.6.1 *ROOT-SHOOT COMMUNICATION*

Roots are necessary for continued stem growth (Peterson and Fletcher, 1973) and it has been shown that only a small amount of root tissue need be removed to reduce shoot growth whereas a large reduction is needed to change root growth rate (Humphries, 1960). Two possibilities are suggested (Davies *et al.* 1987a):

i/ Roots remove a growth inhibitor produced by the shoot.
ii/ Roots produce a growth promoter needed by the shoot.

Went in 1938 was the first to propose that a factor necessary for shoot growth originated in the root (Skene, 1975). With the advancement in plant hormone research, a feedback control model was developed (Richards, 1986). In this model shoot apex produced auxin stimulated root growth and root cytokinin production. In turn, root cytokinin promoted shoot growth and shoot auxin production (Richards, 1986). This hypothesis was supported by results showing the preferential action of cytokinins on shoot tissue and auxins on root tissue (Gersani *et al.*, 1980a). It is also known that auxin transport is polar and basipetal while cytokinins move preferentially in acropetal fashion (Gersani *et al.*, 1980b).

To elucidate the chemical link between root and shoot, which is independent of hydraulic conductivity (Schulze, 1986b), analysis has been carried out on xylem (and phloem) exudates. Substances with kinetin-like activity were the first to be identified in root exudate (Itai and Vaadia, 1965). This is appropriate as CK appear the best candidates for a root produced chemical messenger (Bruinsma, 1984). Cytokinins have now been found in the xylem sap from a large variety of plants (see Skene (1975)).

After the discovery of cytokinin-like activity, GA-like substances were found along with growth inhibitors (Jones and Lacey, 1968). Bruinsma (1984) identified 3 cytokinins, 4 giberellins and 4 inhibitors in the root exudate of maize.

Changes in the level of a chemical messenger appear to be related to root cell volume (therefore turgor) changes. This being the case, stronger signals will be generated by root systems with low hydraulic resistance and highly elastic cells (Turner, 1986b).

The role of substances detected in vascular tissue exudate has been investigated by studying both the effect of external factors on natural levels (e.g Itai *et al.*, 1973) and by applying exogenous chemical to various plant parts. Early experiments by Wittwer and Dedolph (1963) revealed that cytokinin in the nutrient solution reduced shoot dry weight, plant height and the S/R ratio (which approached unity). Thus kinetin was found to have the opposite effect to giberellin on these parameters as well as root elongation. The most interesting experiments in recent years have involved splitting the root system and treating the different portions with combinations of factors. Examples of such experiments are listed below:

(a) (Meyer and Gingrich, 1964); Water stress to any portion of the root system led to a decrease in the utilization of carbohydrate before translocation from the shoot was affected.

(b) (Bar-Yosef and Lambert, 1979); Six root sectors used. Some plants grown with roots divided into each sector and some with roots in a single sector. The treatments involved different levels of nitrogen, Ψ_P and O_2 and root growth was followed using the equation:

$$dL/dt = GRC \times L \qquad 5.1$$

(L = root length; GRC = specific root growth rate). For plants in a single sector GRC decreased with Ψ_P while for split root plants GRC was a minimum at a Ψ_P of 1.6 bar (approximating Ψ_W(soil)). It was suggested that with decreasing Ψ_S the plant initiated a greater number of thinner roots so resulting in a lower GRC.

(c) (Erickson and Kirkham, 1979); Roots divided between combinations of nutrient solution and soil. Plants in nutrient solution only had the highest Ψ_W(leaf) and g_s and lost two and a half times more water than those in soil only. Plants in nutrient solution : soil combination lost seven times more water from the nutrient solution side. Highest growth occurred if part of the root system was at zero Ψ_M (i.e. in nutrient solution).

(d) (Tan *et al.*, 1981); Tomato plant roots in quadrants. With 50% of the root system stressed there was no reduction in E, Pn, LA or g_s. The S/R ratio decreased as the amount of stress increased.

(e) (Tan and Buttery, 1982b); Peach roots in four compartments. With 50% of the root stressed there was only a small decrease in E, Pn and g_s, no change in the S/R ratio and increased root growth in the wet quadrants.

(f) (Cohen *et al.*, 1985); Citrus trees under irrigation with only 40% of the root volume wetted showed an increase in uptake from the wet zone but not sufficient to compensate for a 60% reduction in the dry zone.

(g) (Blackman and Davies, 1985b); With part of the root system dry, g_s decreased while Ψ_W(leaf), Ψ_P and leaf ABA content remained unaffected. If cytokinin was applied to the leaves stomata opened.

(h) (Green and Smith, 1988); With half the kiwifruit vine root system not watered, stomata closed.

(i) (Proebsting *et al.*, 1989); Peach tree seedlings with root systems divided into four pots. If water was applied to only some of the pots (reduced effective soil volume), Ψ_W(leaf) and g_s were reduced but not Ψ_S(leaf). However, Ψ_W(leaf) and g_s were unaffected if irrigation was less than full replacement (deficit irrigation).

Results from these experiments have tended to be somewhat conflicting and confusing due to the different plant material, experimental systems, rates of stress induction and stress periods involved. In the longer term plants will adjust to the modified root environment with increased root growth and water uptake from the wetted zones (Cohen *et al.*, 1985; Erickson and Kirkham, 1979; Proebsting *et al.*, 1989). However, within this time frame some very interesting intra-plant interactions can occur which will be addressed in the following three sections. Key results are those of Blackman and Davies (1985b) who observed a change in g_s without a significant change in Ψ_W(leaf) or even leaf ABA content.

ii/ Nutrient solution : Soil.

iii/ Soil : Soil.

Plants in nutrient solution only had the highest Ψ_W(leaf) and g_s and lost two and a half times more water than those in soil only. Plants in nutrient solution : soil combination lost seven times more water from the nutrient solution side. Highest growth occurred if part of the root system was at zero Ψ_M (i.e. in nutrient solution).

(d) (Tan *et al.*, 1981); Tomato plant roots in quadrants. With 50% of the root system stressed there was no reduction in E, Pn, LA or g_s. The S/R ratio decreased as the amount of stress increased.

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(g) (Blackman and Davies, 1985b); With part of the root system dry, g_s decreased while Ψ_W(leaf), Ψ_P and leaf ABA content remained unaffected. If cytokinin was applied to the leaves stomata opened.

(h) (Green and Smith, 1988); With half the kiwifruit vine root system not watered, stomata close.

5.6.2 *THE HORMONE MESSENGER*

It is now recognized in plant physiology that most, if not all, hormone regulated systems involve the interaction between two or more plant growth substances. Root-shoot communication provides a prime example, as reviewed by Sharp and Davies (1989). Although ABA and CK have received the most attention, all five major classes of plant hormone have been implicated in some aspects of the communication system. The work of Blackman and Davies (1985b) suggests that it is a decrease in cytokinin supply from the roots which modifies shoot growth and internal physiology. They state that a decrease in synthesis or translocation of a promoter provides a more sensitive indicator of root perturbations than increase in synthesis of an inhibitor. Cytokinin could act as an integrated indicator of the turgor pressure in all root tips and proportionally control leaf gas exchange (Davies *et al.*, 1987a). However, as Davies *et al.* (1987b) point out, cytokinin levels change by only a few percent following root environment changes whereas ABA levels change by an order of magnitude or more. This suggests that ABA has the central role rather than cytokinin (Zhang and Davies, 1989b).

The evidence for ABA as a root generated signal is now very strong. Critical facts include (Neales *et al.*, 1989):

i/ ABA production by roots.
ii/ ABA transportation in the xylem.
iii/ Appearance of root produced ABA in the leaves.

Zhang and Davies (1989a, 1990) found that xylem ABA varied with soil water content while there was little or no change in leaf ABA levels. This contrasts with Loveys (1984) who concluded that diurnal changes in leaf ABA could be accounted for by xylem ABA levels. Regardless of this conflict, Neales *et al.* (1989) and Zhang and Davies (1990) have shown that xylem ABA concentrations can fully explain changes in stomatal conductance. Zhang and Davies (1990) clearly identify problems in the conclusions drawn by Munns and King (1988) that something else, apart from ABA, was causing stomatal closure in water stressed plants. However there are still some interesting anomalies in the results of Munns and King (1988) which need further clarification. These include the level of stomatal closure elicited by

nonstressed plant sap and the increase in stomatal resistance when ABA was removed from the sap.

Perhaps the most plausible hypothesis for root-shoot communication is that ABA and cytokinin levels interact to give an integrated chemical signal. As cytokinin levels fall the sensitivity of leaf functions to ABA increases (Blackman and Davies, 1985b) while reduction in cytokinin levels may be enhanced by ABA stimulated glucoside formation (Hubick *et al.*, 1986b). Giberellin, ethylene and auxin may join this interaction complex at varying points but at present, knowledge is insufficient to elaborate further.

5.6.3 *THE PLANT - AN OSCILLATORY SYSTEM?*

Since the discovery of plant hormones, chemistry has provided, almost totally, the basis for understanding plant physiological responses. In other words only chemical signals have been considered. The serious flaw in this situation was eloquently discussed by Due (1989). The basic argument is that a signal is characterized by frequency as well as specific carrier properties. Frequency has been ignored in plant physiology despite the fact that there are a myriad of oscillating variables both in and around living organisms (Due, 1989). With frequency carrying information it is possible for simple parameters such as turgor to cause specific responses which have till now been only in the realm of plant hormones. Due (1989) also pointed out that there is commonly only a small response at zero frequency for oscillatory systems. Hence experiments involving either presence or absence of hormone (zero frequency) may give a false picture of plant sensitivity. Due (1989) suggests that plants be treated as a noisy communication channel and not a set of biochemical reactions.

Zajaczkowski *et al.* (1983) provide one of the few good examples of frequency consideration. They state that for harmonious plant development "a higher degree of supracellular morphogenetic information than that obtained by mere hormone gradients seems to be required". A model was proposed based on a wave pattern of polar auxin transport. They noted that as diffusion decreased exponentially with distance, oscillatory systems may be particularly important in large organisms. Environmental factors would exert their influence through interference of the oscillatory system.

It is clear that far more work needs to be done in the area of plant oscillatory systems. This may well be a major thrust of future research and could hold the key to many current controversies.

5.6.4 *WHAT IS THE STRESS SENSING ORGAN?*

One of the most critical yet controversial areas in plant water relations research at present is the question of which part of the plant generally senses the water stress. Kramer (1988) sparked considerable debate and discussion by criticizing experiments where the root system was stressed while the shoot system was not (e.g. Gollan *et al.*, 1986; Passioura and Munns, 1984). It was stated that this was the reverse of the natural situation and that shoot water stress usually developed before significant root water stress occurred. Kramer (1988) believed it was unlikely, under field conditions, that roots were the primary sensors of water stress or that root biochemical signals were as important as direct hydraulic effects of shoot water stress. Schulze *et al.* (1988) were in general agreement with Kramer (1988). They noted that primary sensing could involve both hydraulic (turgor, conductivity, elasticity) and metabolic (reaction rate, membrane transport, translocation) factors.

The criticism of Kramer (1988) needs to be kept in context. He was attacking the extrapolation of results directly to the natural situation which is clearly invalid. However there is still room to use such techniques to investigate physiological processes (e.g. Kuang *et al.* (1990)) so long as caution prevails.

Passioura (1988) suggested that Kramer (1988) was mistaken when postulating that leaves usually experience a water stress first. The argument was put forward that surface soil can dry to low potentials such that shoot effects may frequently be overridden by root signals. Other workers have been in general accord with this hypothesis (Baker and Lachno, 1989; Neales *et al.*, 1989; Zhang and Davies, 1989a, b, 1990). Zhang and Davies (1989a, 1990) suggested that modification of root water relations may be the first change in a drying soil. Leaf expansion and stomatal conductance would then respond directly to soil conditions. Neales *et al.* (1989) reiterated the possibility that shallow roots may dehydrate independently of deeper ones and thus be a source of chemical signals. Zhang and Davies (1989b) made the profound observation that fine root tips in surface soil layers can have a *lower* Ψ_W than the leaves. This arises through evaporation from the soil surface leading to a situation where fine roots can lose water to the surrounding soil. At the same time,

leaf Ψ_W can be maintained by water uptake from deeper roots situated in moist soil layers.

Boyer (1989) believed that Kramer (1988) was correct in suggesting that leaves have a lower Ψ_W than roots and should sense a water stress first (but note of course the results of Zhang and Davies (1989b)). However Boyer (1989) also blended in the root sensor idea stating that roots can communicate decreasing Ψ_W(soil) through, firstly decreasing Ψ_W(xylem) (and consequently increased xylem ABA) and secondly decreasing solute translocation to the shoot. Hence communication both ways was suggested making location of the water stress sensory organ difficult (if not impossible) to explicitly identify (Boyer, 1989).

Baker and Lachno (1989) make the very sensible suggestion that roots may be involved in the 'fine control' of the plant water balance via root ABA effects on stomatal conductance and root hydraulic conductivity. This leaves the way open for leaves to invoke 'coarse adjustments' and initial water stress responses. It is now not a question of which plant part senses water stress but rather how do leaf and root signals vary under different conditions and interact with each other. It must be remembered that water stress development is a process which occurs over a period of time with events in a specific sequence. Water stress sensing is the initial event. There is no doubt that a relatively wet shoot environment - relatively dry root environment (see section 1) can develop naturally. However it will only do so following a period of evapotranspiration. Hence the shoot will tend to be exposed to a high VPD and very likely initiate water stress responses. This is in contrast to experimental conditions where the root and shoot environments are independent. A dry root environment can be created while keeping the shoot system moist.

The other critical factor is that of root zone heterogeneity. From the results of Zhang and Davies (1989b) it does appear that the suggestion of Passioura (1988) is correct. Evaporative loss from the soil surface, in combination with plant uptake, can result in a low Ψ_W(soil) in the upper soil layer, while shoots are supplied by roots in moist soil deeper in the profile.

In concluding this discussion, it is clear that researchers today need to base their conclusions around the *dominant* water stress sensing organ for a *given* set of environmental conditions at a *given* point in time. Any generalizations beyond this strict specification will only lead to ambiguity and misunderstanding.

5.7 **THE PLANT MODEL**

Much has already been established on the hormonal nature of water stress sensing signals, with far more still to be learned regarding hormonal interactions. Consideration of the frequency component of a signal (see section 5) may yet lead to a drastic reevaluation of currently held views. That aside, it is very important in any field of research to regularly bring together known facts in a concise way to highlight areas where information is lacking and where attention should be focused. The best way to do this is in some form of model.

Schulze (1986a) states that "few attempts have been made to understand whole-plant performance as an integrated process of different fluxes, especially carbon, water and nutrients". This is certainly true and would seem to be a very useful goal in the realm of whole-plant physiology.

A schematic presentation of the plant model under consideration is given in Fig. 5.1. The following is a full description of all the component interactions, ordered according to the arrow numbers. For physical links, a simple short description is given, while for metabolic links there is a brief discussion on current knowledge. Abbreviations follow appendix 18.1, with additional symbols explained as they are used. Note that an 'X', used as either a subscript or in double brackets, stands for any plant part (i.e. leaf, stem or root). Young and old leaf components are often shown together as 'YO' in subscripts etc.. The model is inclined towards plant water relations and as such special emphasis is placed on root tips and older leaves in the hormonal balance of the whole plant.

5.7.1 *CARBON FLUX COMPONENTS*

(a) Physical Links:

(C1) $C_a \longleftrightarrow C_i$

Diffusion of CO_2 between bulk air and the substomatal cavity. Rate of movement represents either Pn or Rd in light or darkness respectively. Both boundary layer and stomatal resistances are involved.

(C2) $C_i \longrightarrow A$

Diffusion of CO_2 between the substomatal cavity and the site of carboxylation. Resistances include r_w, r_m and $(Pk)^{-1}$. The relationship between C_i and A is at first linear (slope $\approx$ k) followed by a region of decreasing slope (Ru-1,5-BP regeneration limitation) leading to a constant small slope thereafter.

(C3) $A \longrightarrow [C(s)/C(m)]_{YOLf}$

Transfer of carbohydrate from current assimilate to assimilate in the leaf pool. Within the leaf pool, interconversion occurs between storage (s) forms (e.g. starch) and mobile (m) forms (e.g. sucrose).

(C4) $[C(s)/C(m)]_X \longleftrightarrow \otimes_{C,X}$

Movement of carbohydrate to and from the site of phloem loading and unloading.

(C5) $\otimes_{C,YOLf} \longleftrightarrow \otimes_{C,Sm}$

$\otimes_{C,Sm} \longleftrightarrow \otimes_{C,Rt}$

Translocation of assimilate between leaf, stem and root portions.

(C6) $[C(s)/C(m)]_X \longleftrightarrow \langle\langle X \rangle\rangle$

Incorporation of carbon into new cellular material and breakdown of cellular material (other than storage forms of carbohydrate), releasing carbon back into the assimilate pool. In leaf tissue only, assimilate can be incorporated into new material without transport in the vascular system.

(C7) $[C(s)/C(m)]_X \longrightarrow R_X$

Loss of carbon from the assimilate pool via metabolic respiration (maintenance and growth) and photorespiration.

(C8) $R_{YOLf} \longrightarrow C_i$

Release of CO_2 within the leaf, so adding to the internal concentration.

(b) Metabolic Links:

(C1) $C_i \bullet\bullet\bullet\bullet> g_s$

If C_i falls, g_s will increase, *ceterus parabus*, due to the maintenance of a high chloroplast pH and thus membrane integrity following equalization of malate synthesis with H^+/K^+ exchange (Eamus and Wilson, 1984).

(C2) $[C(s)/C(m)]_{YOLf} \bullet\bullet\bullet\bullet> A$

Interaction between photosynthetic rate and the amount of photoassimilate in the leaf. A decrease in the rate of export, following lower sink demand (Tromp and Penders, 1986), may reduce photosynthesis due to end product inhibition (Azcon-Bieto, 1986; Ho, 1979). This inhibition could arise through a lack of

metabolically available phosphate when soluble sugars accumulate (Azcon-Bieto, 1986; Mächler and Nösberger, 1984). Accumulation of starch can physically damage the thylakoid membranes and increase CO_2 diffusive resistance (Azcon-Bieto, 1986).

(C3) $[C(s)/C(m)]_X$ ••••> $[C(s)/C(m)]_X$

The effect of substances in the assimilate pool on the interconversion of carbohydrate forms, e.g. starch synthesis can be affected through the inhibition of phosphoglucoisomerase by 3-phosphoglycerate (Sharkey and Vassey, 1989)

(C4) <<X>> ••••> R_X

The effect of tissue mass and growth rate on the rate of respiration. The growth component is proportional to growth rate while the maintenance component is proportional to total dry matter content (Fischer and Turner, 1978), with the links principally via the ATP/ADP ratio (Amthor, 1989).

5.7.2 *NUTRIENT FLUX COMPONENTS*

(a) Physical Links:

(M1) $[M]_{soil}$ <————> $[M]_{Rt}$

Uptake of mineral ions from the soil solution and exchanger, with some replenishment via root death and decay.

(M2) $[M]_X$ <————> $\otimes_{M,X}$

Movement between mineral ion pools and sites of transport tissue (both xylem and phloem) loading and unloading.

(M3) $\otimes_{M,Rt}$ <————> $\otimes_{M,Sm}$

$\otimes_{M,Sm}$ <————> $\otimes_{M,YOLf}$

Transport of mineral ions through the plant in xylem and phloem.

(M4) $[M]_X$ <————> <<X>>

Incorporation of mineral ions into new plant tissue and remobilization of ions from older tissue.

(b) Metabolic Links:

(M1) $[M]_X$ ••••> $[C(s)/C(m)]_X$

The effect of mineral ion concentrations on assimilate partitioning, in terms of the interconversion of carbohydrate forms. Inorganic phosphate (P_i) has a central role in the leaf, integrating carbon fixation, sucrose synthesis and starch synthesis (Patrick, 1988). This involves direct limitation of P_i utilising reactions and also via the ATP/ADP ratio (Azcon-Bieto, 1986). Mineral deficiency will affect the activity of key enzymes including SPS (Patrick, 1988).

(M2) $[M]_{YOLf}$ ••••> A

The effect of mineral ion concentration on the photosynthetic apparatus (enzyme levels and activity, pigment levels, membrane integrity etc.), with key elements including P, N, Mg and Fe. Changes in N supply affect Rubisco content and activity as well as chlorophyll levels (Boyer, 1976a), while low inorganic phosphate limits photosynthesis via a decrease in the ATP/ADP ratio affecting Ru-1,5-BP regeneration (Azcon-Bieto, 1986).

(M3) $[M]_{YOLf}$ ••••> g_s

The effect of mineral ions on stomatal operation. General nutrient deficiency will inhibit proper stomatal functioning, while potassium levels are critical due to the role of this ion in guard cell Ψ_S changes (Lösch, 1984).

(M4) $[M]_X$ ••••> $[H]_X$

The effect of mineral ion concentration on hormone synthesis, conjugation and metabolism, e.g. cytokinin levels decrease under nitrogen and phosphorus deficiency (Bruinsma, 1984; Turner, 1986b).

5.7.3 *WATER FLUX COMPONENTS*

(a) Physical Links:

(W1) $e_{soil} \longleftrightarrow e_{Rt}$

$e_{soil} \longleftrightarrow e_{Rttip}$

Rate of water uptake or in special cases water loss. This occurs when the soil develops a lower Ψ_W, e.g. after excessive fertilizer application or evaporation from surface soil layers.

(W2) $e_{Rt} \longleftrightarrow e_{Sm}$

$e_{Sm} \longleftrightarrow e_{YOLf}$

Movement of water through the xylem in a transpiration stream or under root pressure.

(W3) $e_{air} \longleftrightarrow \Psi_W(air)$

$e_X \longleftrightarrow \Psi_W(X)$

$e_{Rttip} \longleftrightarrow \Psi_W(rttip)$

$e_{soil} \longleftrightarrow \Psi_W(soil)$

Physical relationships between water content (in terms of vapour pressure) and water potential. For the leaf and soil these are commonly called the moisture release curve and soil water retentivity curve respectively, and both exhibit hysteresis.

(W4) $\Psi_W(soil) \longleftrightarrow \Psi_W(root)$

$\Psi_W(soil) \longleftrightarrow \Psi_W(rttip)$

$\Psi_W(root) \longleftrightarrow \Psi_W(stem)$

$\Psi_W(stem) \longleftrightarrow \Psi_W(YOleaf)$

$\Psi_W(YOleaf) \longleftrightarrow \Psi_W(air)$

Water potential gradients through the SPAC.

(W5) $e_{Rt} \longleftrightarrow e_{Rttip}$

$\Psi_W(root) \longrightarrow \Psi_W(rttip)$

The hydraulic link between bulk root system and root tip. This is thought to be poor due to undeveloped vascular connections and hence water potentials may vary markedly in the small volume of root tip tissue as $\Psi_W(soil)$ changes.

(W6) $e_{YOLf} \longleftrightarrow e_{air}$

Diffusion of water vapour between the apoplast of the substomatal cavity and bulk air, with resistances including r_a and r_s. Movement of water into the leaf can occur in a water stressed plant under humid conditions.

(b) Metabolic Links:

(W1) $\Delta\Psi_W(X), \Psi_P(X) \bullet\bullet\bullet\bullet> <<X>>$

The effect of Ψ_P on plant part growth. Cell expansion occurs above a threshold Ψ_P which is capable of breaking cell wall bonds (Greacen and Oh, 1972; Hsiao, 1973; Taylor and Davies, 1985). Cell

expansion also depends on the Ψ_W gradient between the xylem and expanding cells ($\Delta\Psi_W$), which may decrease without a decrease in the Ψ_P of the expanding tissue (Nonami and Boyer, 1989).

(W2) Ψ_W(YOleaf), Ψ_P(YOleaf) ••••> A

Direct effects of Ψ_W(leaf) on photosynthesis. These are now generally considered to be unimportant compared to changes in g_s (Sharkey and Seemann, 1989) although they may become significant under severe water stress. The correlation between Ψ_W and Pn appears to relate to cell volume (and hence predominantly Ψ_P) (Jones, 1973c; Kaiser, 1982), with changes to the thylakoid membrane (e.g. conformation and arrangement of proteins, thickness) being inhibitory (Ben *et al.*, 1988; Boyer and Yonis, 1984; Havaux *et al.*, 1986a, b) (see also section 3).

(W3) e_{YOLf} ••••> A

Independent effects of water content on photosynthesis. These include the concentration of cytoplasmic and chloroplastic constituents, which occurs as cell volume decreases, leading to significant effects on enzyme activity (Kaiser, 1984).

(W4) Ψ_P(YOleaf) ••••> g_s

Effect of leaf turgor on stomata. A decrease in Ψ_P(leaf) will lead to some degree of stomatal closure although, as g_s depends on the Ψ_P of both guard cells and subsidiary cells (Cowan, 1972; Davies and Lakso, 1978; Laisk *et al.*, 1980), the relationship is quite complex, and stomatal aperture is far more stable than Ψ_P(leaf) (Sinclair and Ludlow, 1985).

(W5) vpd_{air} ••••> g_s

The effect of atmospheric vpd on stomata. Guard cells can respond directly to changes in vpd via peristomatal transpiration (Lösch, 1984; Schulze, 1986b) and this involves the hydroactive modification of guard cell ion flux (Schulze, 1986b).

(W6) $\Psi_P(X)$ ••••> $\otimes_{C,X}$

The effect of pressure potential on phloem translocation, and hence the rate of loading and unloading of assimilate. Volume flux of assimilate may be represented by the hydraulic conductivity of the phloem times the Ψ_P gradient between source and sink (Patrick, 1988).

(W7) Ψ_P(root) ••••> $[H]_{Rt}$

Ψ_P(rttip) ••••> $[H]_{Rttip}$

Ψ_P(YOleaf) ••••> $[H]_{YOLf}$

Pressure potential effects on hormone concentrations. A decrease in Ψ_P results in increased ABA synthesis (Davies and Lakso, 1978; Turner, 1986b; Walton, 1980). The major sites of synthesis are

mature leaves (Bruinsma, 1984), but some root production also occurs (especially in the root tips) (Zhang and Davies, 1987). Root tips may be hydraulically isolated and so have a critical role in sensing a soil water deficit (Davies *et al.*, 1987a), while old leaves may protect young leaves from damage by wilting rapidly and discharging ABA into the xylem stream. This wilting results from increased petiole resistance and reduced stomatal sensitivity (Atkinson *et al.*, 1989; Zhang and Davies, 1989a). Roots are the major site of cytokinin synthesis (Carmi and Heuer, 1981; Torrey, 1976). Root export decreases as tissue water potential falls (Hubick *et al.*, 1986b; Itai and Vaadia, 1965; Rosa da Costa *et al.*, 1987; Torrey, 1976) although rate of synthesis may not change (Rosa da Costa *et al.*, 1987).

5.7.4 *PLANT HORMONE COMPONENTS*

(a) Physical Links:

(H1) $\otimes_{H,YOLf} \longleftrightarrow \otimes_{H,Sm}$

$\otimes_{H,Sm} \longleftrightarrow \otimes_{H,Rt}$

Transport of hormones between plant parts. Movement is predominantly basipetal for auxin and acropetal for ABA and CK, while for gibberellin, transport depends on the particular gibberellin involved.

(H2) $\otimes_{H,X} \longleftrightarrow [H]_X$

Loading and unloading of hormone in both the xylem and phloem as well as compartmentalization and conjugation processes in the plant tissue, prior to, and subsequent to transport.

(H3) $[H]_{Rttip} \longrightarrow [H]_{Rt}$

The root tip is believed to be the major site of root ABA production

(b) Metabolic Links:

In the classical definition of a hormone, which comes from animal physiology, the substance is synthesized in one location but has its specific affect in another (Bidwell, 1979). The so-called 'plant hormones' do not fit this definition very well because of apparent nonspecific effects and action close to the site of synthesis. Although the general term 'plant growth regulator' may be used to cover the whole spectrum of natural and synthetic substances (Bidwell, 1979), 'hormone' is still the preferred term of this author for endogenous substances, recognizing the plant and animal differences.

Plant hormone action can occur without transport in the vascular tissue, e.g. ABA may be released from mesophyll cells and diffuse through the apoplast to a guard cell plasmalemma (Raschke, 1982; Zhang and Davies, 1987). Hence in these metabolic links two routes of movement must be considered; (a) transport for a certain distance in the vascular tissue, both within and between organs; (b) production and release close to the site of action, both within cells and tissues. In theory, hormone entering an organ must contribute to the organ pool such that all hormone metabolic links will originate from the conceptualized plant part hormone pool ($[[H]_X]$). In practice however there are problems with this representation, e.g. with high concentrations of ABA in the leaf, a small amount of root produced ABA may not have a *measurable* effect on total leaf level (Zhang and Davies, 1989a) yet significantly reduce stomatal aperture (Zhang and Davies, 1990). This again highlights the importance of understanding hormone compartmentalization, rather than considering total tissue levels.

(H1) $[H]_{YOLf}$ ••••> g_s

Hormonal effects on stomata. Stomatal conductance decreases as ABA levels, specifically in the apoplastic compartment, increase (Davies *et al.*, 1987b). Leaf ABA is produced within the cytoplasm of mesophyll cells and sequestered predominantly in mesophyll chloroplasts (Zhang and Davies, 1987), while movement to guard cells is via the apoplastic compartment (Raschke, 1982; Zhang and Davies, 1987). Root produced ABA has direct access to sites of action outside the guard cell plasmalemma (Atkinson *et al.*, 1989; Davies *et al.*, 1987b) and hence stomata can be affected without measurable changes in $[ABA]_{Lf}$ (Zhang and Davies, 1990). Calcium ions may be involved as secondary messengers in the ABA response (De Silva *et al.*, 1985).

Stomata close as the level of cytokinin decreases (Blackman and Davies, 1985a). With little cytokinin synthesized in the shoot, $[CK]_{Lf}$ is very dependent on root production (Carmi and Heuer, 1981).

(H2) $[H]_{YOLf}$ ••••> A

The effect of plant hormones, produced in the mesophyll cells themselves or elsewhere in the plant, on photosynthetic rate. This link is still largely unresolved, and although ABA can inhibit photosynthesis (Cornic and Miginiac, 1983), current thinking is that direct effects are unimportant compared with stomatal closure (Downton *et al.*, 1988a, b; Loveys, 1984; Sharkey and Seemann, 1989; Terashima *et al.*, 1988).

(H3) $[H]_X$ ••••> $\otimes_{C,X}$

The effect of hormones on phloem loading and unloading of assimilate. Included within this link is the phenomenon of auxin directed transport, which may involve auxin stimulated loading of carbohydrate

and/or potassium ions (Patrick, 1987). Abscisic acid has been shown to inhibit assimilate transport to shoot apices, while both cytokinin and gibberellin stimulate transport (Patrick, 1987).

(H4) $[H]_X$ ••••> $[M]_X$

The effect of hormones on the level of mineral ions in the tissue pool. Root produced cytokinin increases the rate of remobilization of minerals from older leaves (Patrick, 1987).

(H5) $[H]_X$ ••••> $\otimes_{M,X}$

The effect of hormones on mineral ion exchange in the vascular tissue. Shoot produced ABA inhibits the secretion of root minerals into the xylem.

(H6) $[H]_X$ ••••> <<X>>

The effect of hormones on plant part growth. This encompasses the processes of cell division (where auxins and cytokinins are important) (Wareing and Phillips, 1981) and cell expansion (where auxins are important) (Taylor and Davies, 1985). Lateral growth depends on the degree of apical dominance which is related to the auxin/cytokinin ratio (Wareing and Phillips, 1981). In annuals and biennials, stem growth relates to GA levels which rise rapidly before flowering (Wareing and Phillips, 1981).

(H7) $[H]_X$ ••••> $[C(s)/C(m)]_X$

The effect of hormones on the interconversion of carbohydrate forms in the assimilate pools, predominantly between sucrose and starch. Control of this conversion is a critical factor in plant physiology. The key enzyme for sucrose is SPS (Huber *et al.*, 1984; Sharkey and Seeman, 1989; Vassey and Sharkey, 1989), while for plants in which sorbitol is translocated (e.g. apple), sorbitol dehydrogenase is also important (Loescher *et al.*, 1982).

(H8) $[H]_{Rt}$ ••••> Ψ_W(root)

Hormonal effects on root water potential. Root hydraulic conductivity is reduced by cytokinin and increased by ABA. If ABA levels rise and cytokinin levels fall, as can occur under water stress, Ψ_W(root) will increase.

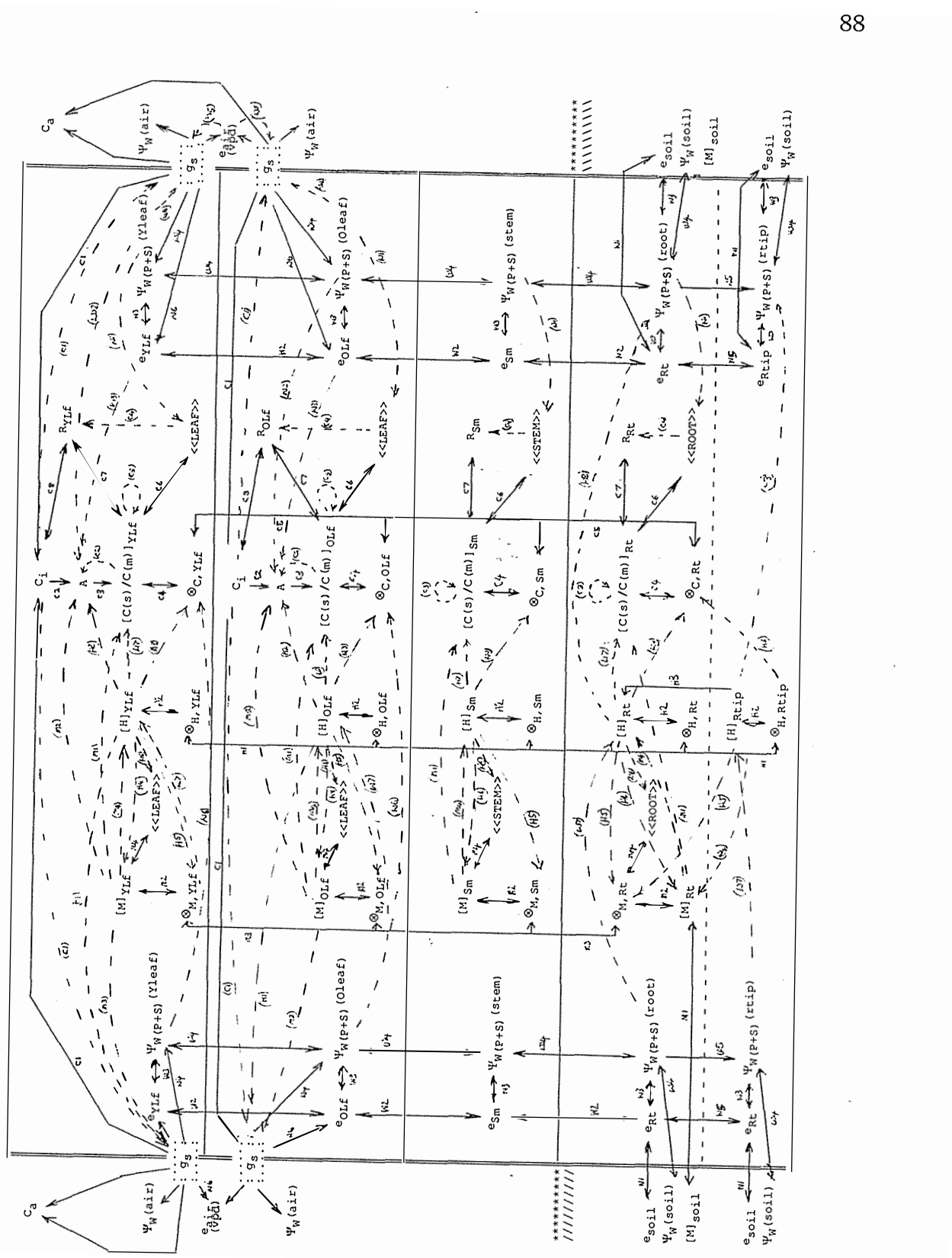

Fig. 5.1 Whole plant model based on the flux of water, minerals, carbon and hormones.

EXPERIMENTAL WORK

WATER STRESS ON VEGETATIVE GROWTH

6 GENERAL MATERIALS AND METHODS

6.1 GENERAL PROCEDURES

6.1.1 *OPERATION OF THE AEROPONIC TANKS : EXPERIMENTS 1 - 3*

The aeroponic tank system described in appendix 18.4 was operated using full strength Coopers nutrient solution (appendix 18.7) at pH 6.5. In experiments 1 and 2, this solution was stored in a 20 l plastic bucket housed within a 100 l polythene container containing 30 l of water. This water formed a water jacket around the nutrient solution, keeping all treatments at the same temperature (± 0.5 °C). In experiment 3 the nutrient solution was located in the 100 l container itself, and with the larger volume it was found that a water jacket was not needed. Nutrient solution temperatures in both cases fluctuated in the range of 15 to 19 °C (from max./min. thermometer located in the storage tank).

The volume of nutrient solution was maintained throughout the experiment by twice daily (approximately 9 am and 4 pm) additions of tap water. This volume was used as an estimate of plant water use (after adjustment for water loss from the tanks with no plants present — estimated to be 0.46 ± 0.06 l/day). Each day (9 am) the conductivity and pH of the nutrient solution was measured (pH; Fisher Accunmet pH meter, model 220, Fisher Scientific, America: Conductivity; Beckman Solubridge, Beckman Instruments inc., America). Stock nutrient solution was added if the conductivity fell below 2.0 mS (Cooper, 1975) (full strength nutrient solution conductivity ≈ 2.5 mS). The pH of the nutrient solution was always found to rise, because tap water in the glasshouse was at pH 8.2, and so nitric acid (2 molar) was added when the pH was above 7.3 (Cooper, 1975)

The ten microjets in each aeroponic tank were checked daily and those not functioning adequately were replaced. Storage tank solutions were replaced every seven days as recommended by Krizek (1985) for polyethylene glycol solutions.

6.1.2 *PLANT RELATED OPERATIONS : EXPERIMENTS 1 AND 2*

Seeds of the tomato (*Lycopersicon esculentum* Mill. cv. Virosa F1) were placed on moist filter paper (Whatman #1) in petri dishes for pre-germination. After 4

days in darkness at 20 °C those seed with emerged radicles of 2 to 3 mm length were transferred individually to 4 cm square plastic pots. Pots contained a 60:40 pumice:sand mixture with incorporated seedling fertiliser mix (appendix 18.6). Pots were distributed over a glasshouse bench and irrigated automatically three times a day via absorbent matting. The glasshouse environmental controller was set to heat at 14 °C and vent at 22 °C.

At the four-leaf stage (approximately 4 - 6 weeks) the seedlings were removed from the pots and all media washed from the root system. To minimise root damage the root system was immersed in a large volume of water and gently oscillated until no more media came loose. Seedlings were then transferred to the aeroponic tanks (appendix 18.4) within the same glasshouse (above temperature regime maintained) with 40 seedlings per tank in a 10 by 4 arrangement. Each seedling was supported by a nylon cord attached to a wooden frame above the aeroponic tank.

After one week, two plants were culled from each aeroponic tank to give a more uniform plant size and then the various treatments commenced. Flower buds were removed every two to three days using forceps in order to keep the plants in a vegetative state. In experiments 1, lateral shoots were also removed to maintain a single stem.

Plants were sprayed for pest and disease control only as required (see appendix 18.9).

6.1.3 *PLANT RELATED OPERATIONS : EXPERIMENTS 3 AND 4*

Refer to section 10.2 and 11.2 respectively.

6.2 **DATA GATHERING PROCEDURES**

6.2.1 *PLANT PART ANALYSIS : EXPERIMENTS 1 - 4*

Plants for plant part analysis were separated into leaf, stem and root portions and the fresh weight determined (to 0.01g). Leaf area was then measured with a leaf area meter (LI-COR LI-3100, Lambda Instruments Corporation, America) and stem length (to 0.5 cm) plus (compound) leaf number recorded. In the case of tomato, leaves at the stem apices of under approximately 1 cm^2 were not included in leaf area

or leaf number measurements (Al-Sahaf, 1984). In experiments 1 and 2, leaves were separated into lamina and petiole portions and weighed. Petioles were passed through the leaf area meter so that an estimate of lamina area could be obtained.

All parts were placed in metal tins and dried at 80 °C for 72 hours in a forced air oven before reweighing (to 0.001 g).

6.2.2 *ROOT LENGTH MEASUREMENT : EXPERIMENTS 1 - 4*

Root length was measured according to the method given in appendix 18.12 using a root length scanner (Comair, Commonwealth Aircraft Corporation Ltd., Australia). Root systems were stored in a cool room at 4°C until needed.

In experiment 3 the length of roots which had growth within the aeroponic tanks (designated 'new' roots), and those present before the experiment began (designated 'old' roots) were measured, according to appendix 18.12, by carefully dividing the two root portions. 'New' roots were easily distinguished because they were a light cream colour as opposed to dark brown. The viability of 'old' roots was estimated after scanning by treating samples with tetrazolium stain and counting the number of stained segments under a disecting microscope.

6.2.3 *ROOT NUMBER : EXPERIMENTS 2 AND 3*

Root number was measured on all samples used for root length scanning (two per plant) in order to calculate a root number for the entire root system (see appendix 18.12). Root number was assessed by counting the number of root branches under a magnification plate (Maggylamp, Newbound and Co. Ltd, Australia) at two times magnification. The root segments were floated in distilled water on a petri dish while counting and the running total recorded on a cumulative counter.

6.2.4 *BUTT CROSS-SECTIONAL AREA : EXPERIMENTS 3 AND 4*

At the start of the experiments stems were marked with white paint, 10 cm above the root system, to give a standard reference point. A value of 10 cm was found to give the best correlation with plant dry weight from a preliminary trial using measurements at 5, 10, 15 and 20 cm (data not shown). Cross-sectional area was

calculated from two diameter measurements taken perpendicular to one another (contact points marked on the white band) using digital calipers (Sylvac sa, Switzerland).

6.2.5 *PLANT HEIGHT : EXPERIMENTS 2 - 4*

Total plant height was measured using a 1 m ruler (to ± 0.5 cm) from a ring painted on the stem at the start of the experiment. For tomato (experiments 1 - 2), the mark was at the point of cotyledon attachment, while for *Pyrus betulaefolia* seedlings (experiments 3 and 4) the mark was 10 cm above the root system (also used for cross-sectional area measurements). In both cases the top of the plant was taken as the apical meristem on the central axis.

6.2.6 *LEAF WATER POTENTIAL : EXPERIMENTS 1 - 4*

Leaf water potential was measured using a pressure chamber (Soilmoisture Equipment Corporation, model 3005, USA) (appendix 18.11). Identical leaves to those used for photosynthesis measurements were selected (see appendix 18.10), with readings made on one leaf per plant from six randomly selected plants per tank.

6.2.7 *LEAF RELATIVE WATER CONTENT : EXPERIMENTS 2 AND 3*

Relative water content (RWC) (formerly water deficit e.g. Ackley (1954)) was measured using the method developed by Barrs and Weatherley (1962). Leaflets were collected into plastic bags (12 -1 pm) and taken to the laboratory, where two 1 cm diameters disks were cut from each leaflet using a cork borer (either side of midrib). Disk pairs were weighed immediately (to 0.001 g), then soaked in distilled water under low light (20 to 50 μmol m^{-2} s^{-1}) for 4 hours. After surface drying under a constant pressure (Turner, 1981), disks were reweighed, and then dry weight determined by drying at 80 °C for 48 hours (see appendix 18.2 for calculation). Preliminary trials revealed that 4 hours soaking gave 98% to 100% full turgidity and that injection errors (Spomer, 1972) over this time were not significant.

6.2.8 *PHOTOSYNTHESIS DATA : EXPERIMENTS 1 - 4*

Measurements of net photosynthetic rate (Pn) (in μmol m^{-2} s^{-1}), stomatal conductance (g_s) (in mol m^{-2} s^{-1}), internal CO_2 concentration (C_i) (in ppm) and transpiration rate (E) (in mol m^{-2} s^{-1}) were made with a portable photosynthesis system (LI-COR LI-6200, Lambda Instruments Corporation, America). On each measurement day 48 readings were made, using two leaves per plant from six randomly selected plants in each aeroponic tank. Leaves were selected according to appendix 18.10.

A detailed description of setting up and calibrating the instrument is given in appendix 18.10, but below are the primary parameter values used:

Atmospheric pressure = 1000 to 1020 mbar
System volume = 1125 cm^3
Leaf area = 5.78 cm^2 (tomato) or 7.60 cm^2 (nashi)
Boundary layer resistance = 0.1775 to 0.1815 s cm^{-1}
Stomatal ratio (STMRAT) = 0.5 (tomato) or 0.0 (nashi)
Maximum desiccant flow rate = 32.0 to 32.4 cm^3 s^{-1}

A stomatal ratio of 0.5 was used for tomato leaves because they have twice as many stomata on the adaxial surface (Rudich and Luchinsky, 1986), while Nashi are hypostomatous and so a stomatal ratio of zero was used.

In the analysis of Pn, g_s, C_i and E, the measured parameters C_a, vpd and PAR were used as concomitant variables (appendix 18.3).

6.2.9 *CO_2 COMPENSATION POINT : EXPERIMENTS 2 AND 3*

The CO_2 compensation point (Γ) was determined by clamping the LI-6200 leaf chamber on a leaf and allowing the CO_2 concentration to drop until the CO_2 exchange rate was low (< 0.01 μmol m^{-2} s^{-1}). Relative humidity was maintained within ± 5% of the starting value by adjusting the flow rate through the desiccant as stomatal conductance changed. Air and leaf temperatures in the chamber rose during a measurement but levels were kept at or below 25 °C by running tap water down the sides of the chamber.

Measurements were either conducted under a bench lamp (400 μmol m^{-2} s^{-1}) (experiment 2) or on a glasshouse bench under natural sunlight (experiment 3). In

both cases the plants were removed from the aeroponic tanks and placed in containers of full strength nutrient solution at least 12 hours before conducting measurements. Two plants per treatment were used, with leaves selected according to (appendix 18.10).

Along with Γ, the initial slope of the Pn *versus* C_i plot (k_{int}) was measured to give an estimate of the carboxylation efficiency (which is theoretically derived from Pn *versus* C_c).

6.2.10 *PRESSURE-VOLUME CURVE ANALYSIS : EXPERIMENTS 1 - 3*

Leaves to be used for pressure-volume curve measurements were collected at 4 pm the previous day and stood overnight with their petioles in a container of distilled water. The container was enclosed in a plastic bag to create a high relative humidity and ensure that full turgidity was reached. The 'bench drying' method was used as outlined in appendix 18.14.

6.2.11 *TISSUE NUTRIENT ANALYSIS : EXPERIMENTS 1 - 4*

After plant part dry weights had been measured, the material was stored in air tight plastic containers until required. The procedure for analysis of calcium, magnesium and potassium is given in appendix 18.16.

6.2.12 *LEAF PIGMENT ANALYSIS : EXPERIMENTS 1 - 3*

In experiment 2 leaf pigments were analysed using the acetone extraction procedure outlined in appendix 18.17, while in experiment 3 extraction was carried out using N,N-dimethylformamide. Two leaflets were used per plant from four randomly selected plants per tank. Leaflets similar to those used for photosynthetic measurements were taken. Two extractions were made on each leaflet and the results averaged.

7 GENERAL INTRODUCTION TO EXPERIMENTS 1 AND 2

7.1 WATER STRESS INDUCING METHODS

A seemingly wide array of methods have been used to induce water stress in plant material. However they can all be placed into four basic categories (Krizek, 1985):

i/ Withholding water.
ii/ Use of 'osmotic' agents.
iii/ Use of semipermeable membranes.
iv/ Regulation of hydraulic conductivity.

A variety of unusual systems can also be found in the literature, based on variables such as plant pot weight (e.g. Hunter and Tonks, 1979) or using ceramic materials (e.g. Karnoski *et al.*, 1984; Perroux, 1979), but these can be grouped in the withholding water category.

Withholding water is the fundamental method of inducing a water stress and has been used most extensively. However it suffers from the important disadvantage that Ψ_W at the root surface cannot be measured precisely (Krizek, 1985). This has led to the use of 'osmotic' agents in whole plant studies, where Ψ_W at the root surface is equal to the water potential of the solution. Note again, however, that solution Ψ_W may have matric as well as osmotic components (e.g. PEG) (Steuter *et al.*, 1981).

An array of substances have been used to generate the decrease in water potential and some examples are given in the table below.

Table 7.1 Water potential modifying substances which have been used in plant water relations studies

Substance	References
Sodium chloride	Slatyer, 1961.
Potassium nitrate	Slatyer, 1961.
Sucrose	Slatyer, 1961.
Mannitol	Greenway and Leahy, 1970; Lawlor, 1970; Parmar and Moore, 1968; Slatyer, 1961; Thill *et al.*, 1979; Trip *et al.*, 1964; Wiggens and Gardner, 1959; Yaniv and Werker, 1983.
Glucose	Wiggens and Gardner, 1959.
PVP	Wiggens and Gardner, 1959.
Ethylene glycol	Greenway and Leahy, 1970.
Polyethylene glycol	Bressan *et al.*, 1981; Carpita *et al.*, 1979; Gergely *et al.*, 1980a Gergely *et al.*, 1980b; Haber *et al.*, 1983; Heyser and Nabors, 1981; Janes, 1966; Janes, 1974; Lang and Thorpe, 1986; Lawlor, 1969; Lawlor, 1970; Mexal *et al.*, 1975; Parmar and Moore, 1968; Shone and Flood, 1983; Shone *et al.*, 1983; Thill *et al.*, 1979; Tingey and Stockwell, 1977; Yaniv and Werker, 1983.

According to Slavik (1974), solutions in direct contact with living cells must have the following properties:

i/ Not be harmful to, or cause undesirable structural changes to, plant cells.
ii/ Not penetrate semipermeable membranes.
iii/ Not be metabolised by the plant or subject to microbial alteration.

No substance currently available fulfills these three criteria completely and hence it is very important to consider all possible effects of the substance used apart from simply changing the water potential. Many of the substances which have been documented in the literature clearly fall well short in at least one of the above three properties. Salts such as NaCl and KCl are readily taken up by plant tissue, while sugars such as glucose and sucrose are rapidly metabolised once inside plant cells. Even large molecules, such as polyethylene glycols, may be taken up by plant roots under certain conditions. Substances such as mannitol have intermediate properties, being absorbed

slowly by plant tissues (Slatyer, 1961; Greenway, 1970) and intact roots (Lawlor, 1970), with a reflection coefficient of 0.8 to 0.9 (Slavik, 1974).

Any uptake of osmotic agent by plants is undesirable in water stress experiments which are focusing solely on changes in plant environment water potential. Problems are compounded when the substance is phytotoxic, as in the case of PEG and this aspect will be discussed in the following section. To overcome uptake problems, systems have been developed in which the plant tissue is separated from the water potential agent by a semipermeable membrane. Useful results have been obtained by this method but in general the systems are limited by the fragility of the membrane and the period over which it retains integrity (Krizek, 1985).

The fourth general method of inducing water stress involves altering the matric potential of the root media by adjusting the height of a water column below the root system and also using materials of differing hydraulic conductivity within this zone (Haan and Barfield, 1971; Snow and Tingey, 1985). Numerous variations on this general system have been reported (e.g. Alvarez and De Datt, 1977). Again such systems can generate useful results but there are limitations due to the physical nature of the setup and the precise control of water level which is required (see Snow and Tingey, 1985).

Hence it can be seen that there is a need to develop simple and yet flexible methods of inducing a controlled water stress which do not suffer from the variety of problems mentioned above. The following two experiments were, in part, conducted to achieve this objective, by looking firstly at the use of an osmotic agent (polyethylene glycol), and secondly intermittent misting, both within an aeroponic system.

7.2 POLYETHYLENE GLYCOL AS A WATER STRESS INDUCING AGENT

A large number of osmotically active substances have been used in plant water relations studies to reduce the water potential of the root environment. Mannitol and polyethylene glycol (PEG) have been used extensively over the past twenty years with PEG proving the most popular. High molecular weight polyethylene glycols (>1000) have several useful properties for water stress studies:

i/ Only very slowly absorbed through intact roots (Janes, 1974; Lawlor, 1970).
ii/ Absorbed with a reflection coefficient of approximately one (Janes, 1974).

ii/ Not easily metabolised by plant tissues (Janes, 1974).

iii/ Not easily broken down by microorganisms and are osmotically stable (Thill *et al.*, 1979).

However a number of workers have encountered problems while using PEG. These are summarised below:

i/ Polyethylene glycol lowers the surface tension and increases the viscosity of a solution (Lawlor, 1970) as well as lowering the oxygen content (Mexal *et al.*, 1975; Tingey and Stockwell, 1977).

ii/ Polyethylene glycol is taken up at increased rates through mechanically or osmotically damaged roots (Lawlor, 1970).

iii/ Polyethylene glycol causes leaf tissue damage which is not related to a change in solution osmotic potential (Lawlor, 1970).

iv/ Polyethylene glycol decreases the permeability of the transpiration pathway (Lawlor, 1970).

v/ Polyethylene glycol decreases phosphorus uptake (Tingey and Stockwell, 1977) and may affect general nutrient availability (Steuter *et al.*, 1981).

The importance of using undamaged root systems when working with PEG has been stressed on several occasions (Lawlor, 1970; Yaniv and Werker, 1983). Yaniv and Werker (1983) found that with a 4.5% PEG solution tomato leaves secreted PEG after 24 hours if the roots were intact but after only 20 minutes if the roots were damaged.

Lawlor (1970) concluded that the phytotoxic effects of PEG were most likely caused by blockage of the transpiration pathway. This was supported by the observation that high molecular weight polyethylene glycols cause rapid leaf damage while those of low molecular weight cause only limited damage over an extended period. Polyethylene glycol was found to move initially to the leaf margins (Lawlor, 1970) as would be expected for a substance moving in the transpiration stream (Canny, 1990). Redistribution then occurred within the lamina causing decreased transpiration pathway permeability (Lawlor, 1970).

The problem of decreased oxygen availability in PEG solutions was investigated by Mexal *et al.* (1975). They found that for PEG solutions of greater than 5%, oxygen concentration was inversely proportional to PEG concentration. Oxygen availability, as estimated by a dimensionless mass transport coefficient (DMT), was reduced to 50% at 3 bar and 2 bar for PEG 4000 and 6000 respectively. The DMT coefficient gives a better indication of oxygen availability to plant roots than relative

oxygen solubility, for example, because viscosity and diffusion coefficients are involved. Mexal *et al.* (1975) concluded that oxygen availability could be limiting root metabolism even at a relatively low PEG concentration.

With these phytotoxic effects in mind it is perhaps surprising that several recent papers have been fully in favour of using PEG. Gergely *et al.* (1980a) grew apple trees in PEG solutions (of up to -7.5 bar) for 14 days with no phytotoxicity problems reported. Lang and Thorpe (1986) were able to repeat experiments using PEG on the same plant over several days and concluded that the direct effect of PEG was not a toxic one. Shone *et al.* (1983) used PEG solutions of up to -8 bar and found no side effects caused by toxicity or oxygen deficiency. A -8 bar solution was considered the maximum for the hydroponic system used, as oxygen availability became limiting at -12 bar (Shone and Flood, 1983).

A full range of PEG molecular weights from 200 to 20000 have been used in water relations studies. Janes (1974) suggested that 1000 - 1540 was the best range while in recent times 4000 - 6000 have been most extensively used (Krizek, 1985). The compromise which must be made is between greater uptake as molecular weight decreases (Janes, 1974; Lawlor, 1970) and more severe xylem blockage as molecular weight increases (Lawlor, 1970).

Extensive use has been made of PEG in plant physiological studies without, it would appear, a clear understanding of the nature of PEG solutions. Steuter *et al.* (1981) have shown that the high molecular weight polyethylene glycols (i.e PEG 1000 and above), at concentrations used in water relations studies, behave like colloids such that the major component of Ψ_W is in fact Ψ_M. They propose that PEG should be referred to as a 'matricum' rather than an osmoticum. Unfortunately this information has not been considered in the number of studies involving PEG since 1981, with workers still referring to Ψ_S changes. The identification of matric rather than osmotic forces in a PEG solution represents a further advantageous characteristic because of the dominant matric component in soils.

7.3 **PRELIMINARY TRIALS WITH PEG**

Very little use has been made of the aeroponic growing system in plant physiological research. As yet, no work has been reported involving the system in combination with osmotic agents. Therefore a preliminary experiment was conducted in order to investigate the suitability of PEG as an osmotic agent for water stress studies in the aeroponic system. A range of polyethylene glycols were tested (PEG

1000, 4000 and 6000) to see if an optimum molecular size could be found with respect to the compromise mentioned above. Details of the experiment will not be given, but the following discussion is relevant to subsequent work.

7.3.1 *POLYETHYLENE GLYCOL IN THE AEROPONIC TANKS*

Problems were encountered in the aeroponic system when using PEG 6000 due to the increased solution viscosity. The viscosity of a 25% solution of PEG 4000 at 20 °C is 12 cS, while for PEG 6000 it is 20 cS (BDH Chemicals). In the preliminary trial a 15% solution of PEG 6000 was used. Microjets installed in the tanks did not distribute the solution as evenly as solutions of lower viscosity, while the droplet size was larger (visual assessment). There was also a far greater tendency for the microjets to block. On average one jet blocked per day in the PEG 6000 tank compared with only 0.25 jets per day for the other three tanks. These factors together meant that a constant watering of all plant root systems in the PEG 6000 tank could not be assumed.

In the absence of PEG, the above situation is analogous to intermittent misting treatments used in later experiments, where the misting was cycled on and off. As discussed in section 9.4.9, plants do not respond to lack of misting for several minutes after the pumps have been switched off, and even after two hours the effect is not excessive. However, with PEG present the situation is very different because the water potential of the adhering solution will fall rapidly as water is lost from the root surface. Thus roots receiving inadequate water will be exposed to water potentials well in excess of that of the nutrient solution (in this case -3 bar).

With 'osmotic' agents in an aeroponic system the assumption will be made, for later discussion, that the water potential at the root interface is constant, that is, it is independent of transpiration rate. This will be the case if solution flow rate over the root surface is far greater than the rate of water uptake. The rate of watering per unit volume of tank will be constant (provided the jets remain operative). If uniform distribution is assumed throughout the tank then:

Watering rate	= pump flow rate	/ tank volume		7.1
($m^3\ m^{-3}\ s^{-1}$)	($m^3\ s^{-1}$)	(m^3)		
	= 6.7×10^{-5}	/ 0.413		
	= $1.622 \times 10^{-4}\ m^3\ m^{-3}\ s^{-1}$			

If the root system is assumed to be a cylinder of diameter 0.15 m and length of 0.5 m (average tomato plants grown for 19 days in the aeroponic tanks) then the maximum volume of water the root system could receive is:

Vol. of water	= root volume	x watering rate	7.2
($m^3 s^{-1}$)	= (m^3)	($m^3 m^{-3} s^{-1}$)	
	= ($\prod$ x (($0.15)^2$ / 4) x 0.5) x 1.622×10^{-5}		
	= 1.43×10^{-6} $m^3 s^{-1}$		

When plants were this size, they were found to use an average of 8.0×10^{-5} m^3 between 9 am and 5 pm which translates to a water use of 2.8×10^{-9} $m^3 s^{-1}$. Hence in this situation there was a 500 fold difference between the rate of watering and rate of uptake, giving strong support to the assumption of a constant water potential around the root system.

Having said this, it is possible that the physiology of the plant could have been significantly affected even if only a small percentage of the root system received inadequate water. Attention has been given in recent years to the possibility that water stress to a portion of the root system could affect leaf physiology (primarily stomatal conductance) without a change in bulk leaf water status. Blackman and Davies (1985b) found such a response using maize when half of the root system was stressed (see section 5). The proposed hypothesis involved interaction between CK and ABA. Under water stress, root CK production is reduced and ABA production increases which could lead to higher leaf ABA and lower leaf CK. Reduced CK levels would increase stomatal sensitivity to ABA (either from the root or leaf) (Blackman and Davies, 1985b). This hypothesis and variations thereof will be discussed in detail later (see in particular section 17) but it is raised here to support the notion that uneven watering of PEG solution in an aeroponic system could lead to significant physiological changes.

It is difficult to draw firm conclusions regarding the question of oxygen limitation in the root zone. An aeroponic tank, by name alone, suggests a well aerated root environment and this is certainly true for straight nutrient solution. Roots are surrounded by a continuously moving, well aerated film of water which is in direct contact with air. However, when PEG is added the situation is more complex. Measurements of the nutrient solution oxygen concentration (O_2 electrode determinations) showed that concentrations did not fall below 5 mg l^{-1} (70% relative solubility) at a water potential of -3 bar. This figure is in agreement with the results of Mexal *et al.* (1975) who concluded that O_2 transport in PEG solutions could be

insufficient to meet the plants respiratory demand. However, it is believed that in an aeroponic system oxygen availability will not constitute a limiting factor due to the continuous flow of a thin film of solution over the roots. With little depletion of O_2 at the root surface, viscosity and diffusion characteristics of the PEG solution are less importance.

7.3.2 *PLANT UPTAKE OF PEG*

In agreement with Janes (1974) no relationship was found between transpiration rate and PEG uptake. This indicates a high reflection coefficient at the root interface (epidermis) for all three PEG molecular weights. Levels of uptake overall were low compared with those quoted by other workers using hydroponic systems. Plants were found to contain 6 mg of PEG 1000 after 7 days at -3 bar (Fig. 7.1), while Janes (1974) found levels as high as 30 mg for the same time and osmotic potential. This difference could reflect the different species used, tomato *versus Capsicum annuum*, or to 'osmotic' shock. Janes (1974) transferred plants straight into -3 and -5 bar solutions which may well have caused root damage. Lawlor (1970) clearly demonstrated that PEG uptake was far greater through damaged than intact roots. According to Krizek (1985), increments in 'osmotic' potential should be less than or equal to -2.5 bar.

Differences in the experimental system may also have been important. Roots grown in an aeroponic system are morphologically distinct from those produced under hydroponics. Aeroponic roots are consistantly thicker, although the dry weight per unit length appears similar. Due to the lack of physical support laterals are short and stubby, reaching only 1 to 1.5 cm in length and it is possible that these roots exhibit a greater resistance to PEG uptake. Janes (1974) has suggested that there are two barriers to PEG uptake within the root, between the epidermis and xylem, which are likely to be the epidermis itself and the endodermis. The existence of a second barrier was also demonstrated in these preliminary experiments using different molecular weights. For a given solution cencentration, it was found that root concentrations were similar while leaf concentrations varied markedly (Fig. 7.1). Thus it would appear that the tomato root epidermis does not discriminate between molecular sizes whereas the inner barrier will only allow the passage of small molecules, below an average molecular weight of 4000. However, the inner barrier may not be as clearly defined as suggested by Janes (1974).

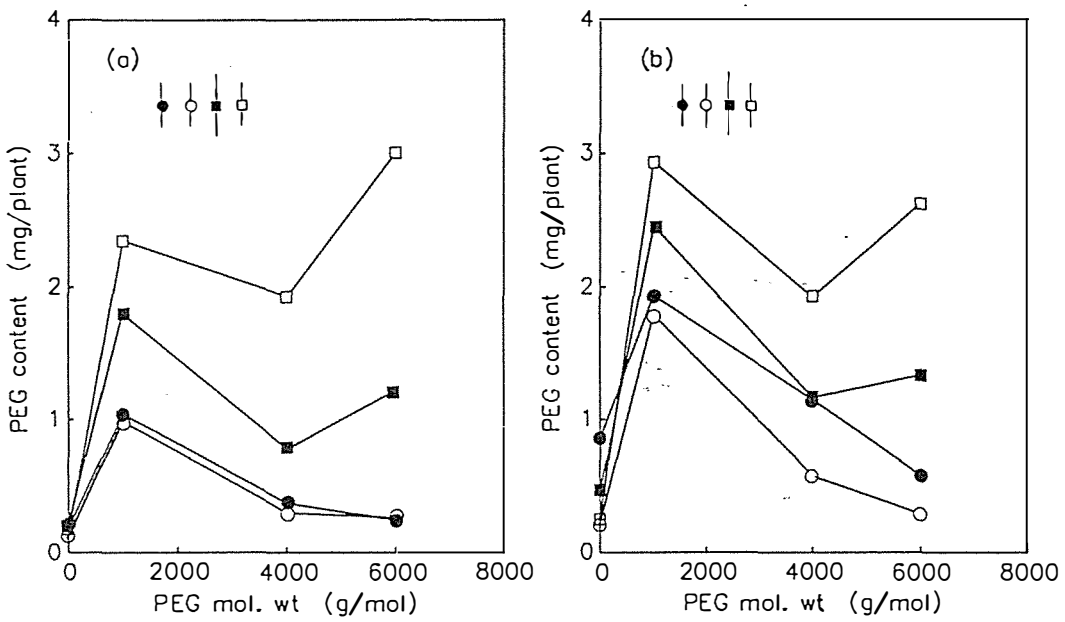

Fig. 7.1. The effect of polyethylene glycol in the nutrient solution of an aeroponic system (at Ψ_W = -3 bar) on the polyethylene glycol content of plant tissue. Each point is the mean of 4 measurements (2 samples per plant from 2 plants). Bars show pooled s.e. for each plant part. (a) After 8 days. (b) After 19 days. ●, young leaves; ○, old leaves; ■, stem; □, root.

Results from Fig. 7.1 show that the stem tissue contained significant amounts of PEG in all three treatments. Higher stem levels in PEG 1000 plants suggested that some discrimination occurred around the endodermal region but that discrimination also occurred within the xylem of the main stem.

From Fig. 7.1 it can be seen that most of the PEG uptake occurred within the first seven days and so it is possible that this was due to the presence of damaged and dying roots. In section 4 it was noted that when plants are transferred from one root environment to another, roots from the old environment tend to become dysfunctional and new ones with different morphological characteristics are produced. This was found to occur when transferring tomato seedlings from solid media to the aeroponic system, as Al-Sahaf (1984) found for transfer to a hydroponic system. The old root system degenerated rapidly in the aeroponic tanks, with new roots produced from the base of the stem. Although the new root system had established before treatments were commenced, uptake may have occurred through partially functional old roots. There is also the possibility that 'osmotic' shock caused damage to the newly developing roots, though this is unlikely as water potentials were decreased at only -1.5 bar per day, compared with the -2.5 bar maximum recommended by Krizek (1985).

8 EXPERIMENT 1 : WATER STRESS STUDIES ON TOMATO USING PEG 4000

8.1 INTRODUCTION

A number of workers have investigated the effect on plant growth of different water potentials in the root environment, generated using PEG. There have been no reports however involving an aeroponic system. Lawlor (1969) used PEG 4000 from -0.4 to -8.0 bar in the nutrient solution of a hydroponic system, and measured a decrease in all aspects of growth with decreasing 'osmotic' potential. Wilting and cessation of leaf growth were found to occur at 'osmotic' potentials of -8.0 bar. Gergely *et al.* (1980a) used PEG 4000 ranging from -0.5 to -7.5 bar on young apple trees. Water use per leaf area and dry weight increase per leaf area were shown to be linearly related to solution 'osmotic' potential. It was found that after an adaptation period of 4 days, PEG induced 'osmotic' stress could be related to a corresponding soil moisture stress. That is, -0.5 bar appeared to equate to 75% of field capacity, while -4.0 bar equated with the calculated wilting point for the soil. Haber *et al.* (1983) investigated the effects of PEG 4000 at -1.5 to -7.5 bar on young peach seedlings and found that shoot growth, root growth and water use were reduced as 'osmotic' potential decreased. Root growth was reduced markedly at -1.5 bar, with little further decrease thereafter. Both Gergely *et al.* (1980b) and Haber *et al.* (1983) found that calcium uptake was reduced as Ψ_S decreased and Haber *et al.* (1983) related this to the decrease in unsuberized root surface.

Shone *et al.* (1983) found no effect on CO_2 uptake or assimilate partitioning when PEG 4000 at -3 bar was applied to the whole root system or PEG 4000 at -8 bar was applied to the upper half of the root system. However with PEG 4000 at -8 bars around the upper roots and -6 bar around the lower roots, CO_2 uptake and the shoot/root ratio were reduced. No effect on respiration was observed at -8 bar.

The above results show a variety of responses to different levels of water potential. These reflect in part the use of different systems and different plant material. In order to provide a basis for critically comparing literature results and the results from further water stress trials to be conducted, it was decided to carry out an experiment using PEG 4000 in the aeroponic system. Solutions with water potentials ranging from -0.5 to -6 bar were used, to cover the mild water stress range, without

involving concentrations which have clearly been shown to produce unwanted side effects (generally above -7 to -8 bar). The aim was to develop an efficient water stressing system in which plant growth could be accurately modelled.

8.2 MATERIALS AND METHODS

For a detailed description of general experimental procedures see section 6.

8.2.1 *EXPERIMENTAL INFORMATION*

Pre-germination of tomato seed commenced on 1 May 1988 and seedlings were transferred to the aeroponic tanks six weeks later. Harvest 0 was carried out after 7 days with one further harvest following 30 days of treatment.

Lateral buds were removed with forceps every 2 - 3 days to maintain a single stem and thus minimize shoot competition.

8.2.2 *TREATMENT INFORMATION*

On day 0, storage tanks were replaced by 10.6 l of full strength Coopers nutrient solution at pH 6.5, with PEG 4000 added according to Table 8.1 (Polyethylene glycol 4000, Laboratory reagent, BDH Limited, Poole, England: average molecular weight = 3300 to 4000). The nutrient solution itself had a water potential of -0.5 bar.

The relationship between PEG 4000 concentration and solution water potential was determined using a thermocouple psychrometer (Wescor HR 33T Dew Point Microvoltmeter, Wescor Inc., USA) (appendix 18.13) (Fig. 8.1). This relationship was in close agreement with those of Gergely *et al.* (1980a), Lawlor (1970) and Steuter *et al.* (1981). A cubic function was fitted to the data in order to calculate solution water potential, which was subsequently checked when the tank solutions were made. The PEG 4000 was incorporated over a period of three days to avoid 'osmotic' shock.

Table 8.1 Treatment specification.

TANK #	Stress level	Amount added (g/tank)			Ψ_S (bar)		
		Day 0	Day 1	Day 2	Day 0	Day 1	Day 2
1	SL2	600	622	0	1.2	3.0	3.0
2	SL3	600	600	659	1.2	2.9	5.9
3	SL0	0	0	0	0.5	0.5	0.5
4	SL1	0	606	0	0.5	1.2	1.2

8.2.3 *DATA GATHERING PROCEDURES*

Plant part analysis was carried out on days 7 and 30. Photosynthetic measurements were made on days 15, 17, 21 and 29 (11 am to 1 pm), while dark respiration measurements were made on day 28 (11 pm) using the same procedure except but with plants in darkness (moonlight only). Leaf water potential measurements were made on days 5 and 17, 21 and 29 (12pm to 1 pm), with total plant height measured on days 3, 9, 15 and 21. Other measurements included leaf pigment analysis (day 34), pressure-volume curve analysis (day 36) and assessment of tissue mineral levels (see section 6.2).

Plants for PEG uptake analysis were taken on day 30 and washed for 15 minutes under running tap water (previously found to be sufficient to remove PEG from the surface of leaf and root tissue). Each plant was separated into leaf, stem and root portions, weighed (to 0.01 g) and then frozen in plastic bags (-20 °C). Plant sap was analysed for PEG according to the method given in appendix 18.15, with two samples of sap collected from each plant part. The sap concentration (in mg/ml) was converted to a dry weight basis using the treatment mean water content of that plant part.

8.2.4 *CALCULATION OF DERIVED VARIABLES*

A full listing of all derived variables is given in appendix 18.2.

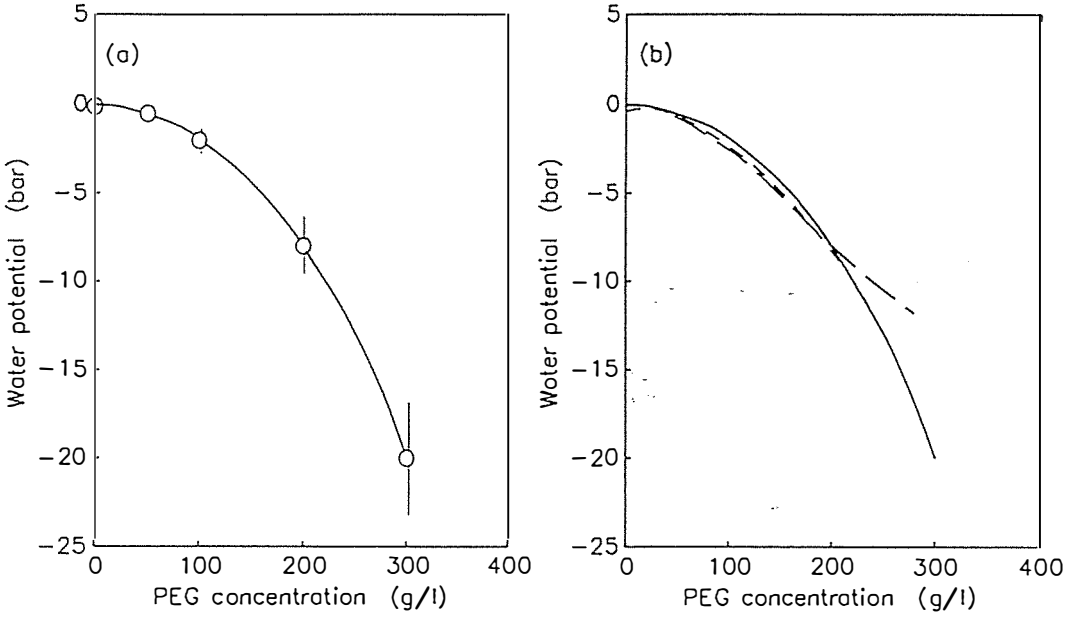

Fig. 8.1. The relationship between polyethylene glycol concentration in an aqueous solution (g/l) and the solution water potential (bar). (a) Water potential measured with a thermocouple psychrometer (n = 6). Bars show ± s.e.; $Y = 0.077 + 0.0028X + 0.00013X^2 + 0.00000027X^3$; $R^2 = 0.9999^{**}$. (b) Comparison of previous relationship (——) with those of Gergely *et al.* (1980) (- - -) and Lawlor (1970) (— —).

8.2.5 *STATISTICAL ANALYSIS*

The experiment consisted of four treatments, with one replicate of each treatment containing 36 subsamples. Hence a simple pooled list design was involved. Description of analysis procedures for all data is given in appendix 18.3.

8.3 **RESULTS**

8.3.1 *PLANT PART ANALYSIS*

Tables 8.2, 8.3 and 8.4 give the treatment means for plant part variables, plant part ratios and plant part water contents respectively. After a treatment period of 30 days, leaf, stem and root dry weights were found to be related in a linear fashion to the solution water potential (Fig. 8.2), as was leaf area (Fig. 8.3). The ratio of plant dry weight to leaf area was similar for all three stress treatments and significantly above the control (Fig. 8.3). Stress treatments 3 and 4 had one less leaf on average than the control and SL1. No differences were found in plant part water contents.

8.3.2 *CANONICAL ANALYSIS ON PLANT PART DATA*

A canonical analysis (see appendix 18.3) was performed using the following plant data;

i/ Leaf dry weight
Stem dry weight
Root dry weight

ii/ Leaf dry weight
Stem dry weight
Root dry weight
Leaf area
Root length

Table 8.2 Effect of different concentrations of PEG 4000 in the nutrient solution of an aeroponic system on leaf number, leaf area, plant height, plant part fresh and dry weights and root length after 30 days (n = 12)

Plant parameter	Solution water potential (bar) -0.5	-1.2	-3.0	-5.9	s.e. mean
leaf number	12.0 a	11.8 a	10.8 b	11.1 b	0.2
leaf area (cm^2)	1406 a	1180 b	971 c	649 d	46
stem lgth (cm)	87 a	81 b	74 c	59 d	2
Fresh weights (g):					
leaf	38.8 a	33.1 b	25.7 c	17.9 d	1.6
lamina	22.0 a	19.0 b	15.2 c	10.3 d	0.8
petiole	16.8 a	14.0 b	10.5 c	6.7 d	0.7
stem	28.1 a	23.1 b	17.9 c	10.8 d	1.0
root	13.4 a	10.4 b	8.4 c	7.4 c	0.6
shoot	66.9 a	56.2 b	43.6 c	28.7 d	1.8
plant	80.3 a	66.6 b	52.0 c	36.1 d	2.0
Dry weights (g):					
leaf	4.24 a	3.73 a	3.08 b	2.19 c	0.21
lamina	3.00 a	2.65 a	2.21 b	1.61 c	0.12
petiole	1.23 a	1.08 a	0.87 b	0.58 c	0.05
stem	2.96 a	2.61 a	2.16 b	1.30 c	0.10
root	0.89 a	0.87 a	0.76 a	0.53 b	0.05
shoot	7.20 a	6.34 b	5.24 c	3.49 d	0.23
plant	8.09 a	7.21 b	6.00 c	4.02 d	0.25

Means with the same letter in each row are not significantly different at the 1% level using the lsd discriminator

Table 8.3 Effect of different concentrations of PEG 4000 in the nutrient solution of an aeroponic system on plant part dry weight ratios after 30 days (n = 12)

Plant parameter	Solution water potential (bar) -0.5	-1.2	-3.0	-5.9	s.e. mean
Lm/Pe	2.46 b	2.47 b	2.54 b	2.76 a	0.04
Lf/Sm	1.43 b	1.42 b	1.42 b	1.67 a	0.01
Lf/Rt	4.79 ns	4.29 ns	4.15 ns	4.36 ns	0.08
Sm/Rt	3.37 a	3.03 ab	2.96 ab	2.64 b	0.07
Sh/Rt	8.15 a	7.32 ab	7.11 b	6.99 b	0.14
SLA (cm^2/g)	469 a	449 ab	443 ab	389 b	20
LAR (cm^2/g)	173 ns	164 ns	162 ns	162 ns	15

Means with the same letter in each row are not significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)

Table 8.4 Effect of different concentrations of PEG 4000 in the nutrient solution of an aeroponic system on the water content (g H_2O / g d. wt) of plant parts after 30 days (n = 12)

Plant parameter	Solution water potential (bar) -0.5	-1.2	-3.0	-5.9	s.e. mean
leaf	8.24 a	7.95 a	7.29 b	7.14 b	0.30
stem	8.53 a	7.91 ab	7.54 b	7.37 b	0.35
root	13.9 ns	11.0 ns	10.2 ns	13.8 ns	1.1

Means with the same letter in each row are not significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)

Results are given in Tables 8.5 and 8.6 for the first two canonical variables (others were not significant). From the between canonical structure (Table 8.5) canonical 1 may be described as a 'shoot discriminator' since it predominantly involves shoot variables, while canonical 2 may be described as a 'root discriminator'. Canonical 1 ranked treatments in order of decreasing nutrient solution Ψ_W, with greater discrimination achieved when leaf area and root length were included (Table 8.6).

Table 8.5 Effect of different concentrations of PEG 4000 in the nutrient solution of an aeroponic system on a canonical analysis - Between canonical structure

Canonical variable	Plant variable LDW	SDW	RDW	LA	RL
(a) Variables used = leaf, stem and root dry weight.					
1	0.99	0.99	-0.53	-	-
2	-0.09	0.01	-0.43	-	-
(b) Variables used = leaf, stem and root dry weight, leaf area and root length.					
1	0.98	0.99	-0.47	0.99	0.55
2	-0.17	-0.10	0.21	-0.06	-0.80

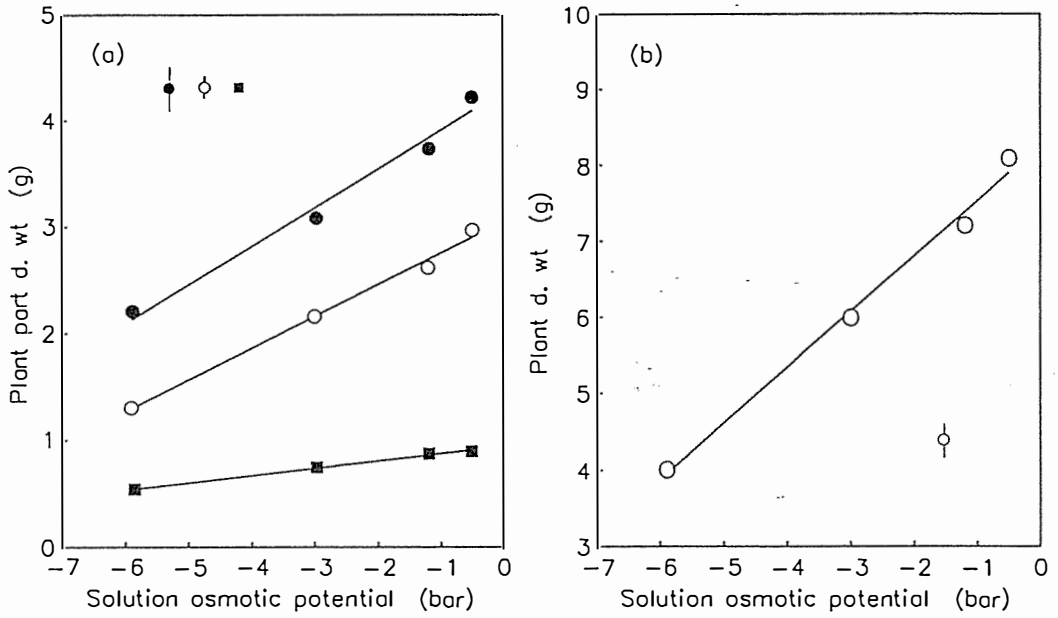

Fig. 8.2. The effect of different nutrient solution water potentials (obtained using PEG 4000) in an aeroponic system on dry weight after 30 days (n = 12). (a) Plant part dry weights. Bars show pooled s.e. for each plant part. ●, leaf; $Y = 4.26 - 0.36X$; $R^2 = 0.98^{**}$:○, stem; $Y = 3.04 - 0.30X$; $R^2 = 0.99^{**}$: ■, root; $Y = 0.94 - 0.07X$; $R^2 = 0.99^{**}$. (b) Plant dry weight (bar shows pooled s.e.); $Y = 8.24 - 0.73X$; $R^2 = 0.99^{**}$.

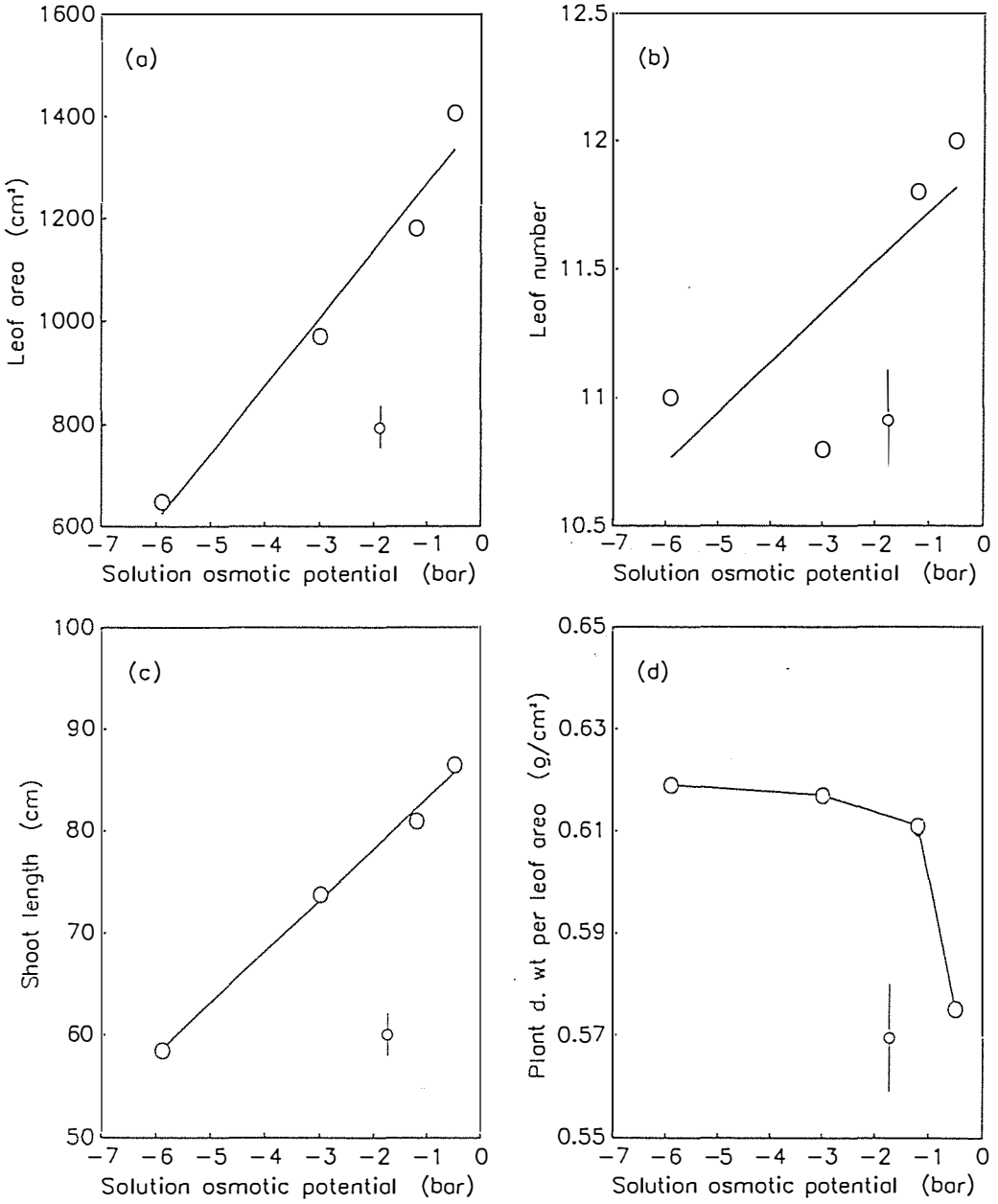

Fig. 8.3. The effect of different nutrient solution water potentials (obtained using PEG 4000) in an aeroponic system on various shoot parameters after 30 days (n = 12). Bars show pooled s.e.. (a) Leaf area; $Y = 1396 - 131X$; $R^2 = 0.97^{*}$. (b) Leaf number; $Y = 11.91 - 0.19X$; $R^2 = 0.63^{ns}$. (c) Shoot length; $Y = 88.12 - 5.00X$; $R^2 = 0.99^{**}$. (d) Dry weight gain over the 30 day period divided by final leaf area.

Table 8.6 Effect of different concentrations of PEG 4000 in the nutrient solution of an aeroponic system on a canonical analysis - Canonical variable class means

Canonical variable	Solution water potential (bar) -0.5	-1.2	-3.0	-5.9	lsd
(a) Variables used = leaf, stem and root dry weight.					
1	3.70 a	0.90 b	-2.08 c	-2.53 c	0.60
2	0.17 a	-0.29 b	-0.12 ab	0.24 a	0.19
(b) Variables used = leaf, stem and root dry weight, leaf area and root length.					
1	5.43 a	0.34 b	-2.30 c	-3.47 d	0.50
2	0.47 a	-1.24 b	0.43 a	0.33 a	0.27

Means with the same letter in each row are not significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)

8.3.3 *PLANT GROWTH ANALYSIS*

Allometric relationships (see section 4.5.5) are shown in Fig. 8.4 and have a significant linear trend. From the equations given in the legend to Fig. 8.4 it can be seen that the slopes (k values) were significantly different between the control and stress levels 2 and 3. A plot of the allometric k value *versus* solution water potential (Fig. 8.5) also shows a linear trend (although the standard errors are large).

Relative growth rate, absolute growth rate, unit leaf rate and unit shoot rate (see appendix 18.2) were calculated using the means from day 0 and day 30 harvests. In Fig. 8.6 it can be seen that all the variables were linearly related to solution osmotic potential, except for root relative growth rate.

8.3.4 *PLANT HEIGHT*

Plant height measurements from day 0 to 21 showed a very strong linear relationship with time (Fig. 8.7) and growth rate, as measured in terms of increase in height per day, was linearly related to the solution water potential (Fig. 8.7).

8.3.5 *ROOT LENGTH*

Root fresh and dry weight are plotted against root length in Fig. 8.8 for the control and SL3. It can be seen that no change in the linear relationship occurred following water stress. This means there was no change in the length per unit weight of root (although there may have been a change in tissue density and mean diameter). The mean root lengths were 100.5 m and 59.8 m for control and SL3 plant respectively. These were significantly different at the 1% level.

8.3.6 *LEAF WATER POTENTIAL*

Leaf water potentials, both at dawn and noon, increased significantly with each addition of PEG to the nutrient solution (Table 8.9), with a high correlation between dawn values and solution water potential. The difference between potentials at noon and dawn was similar for all treatments and averaged 1.8 bar (Fig. 8.9).

8.3.7 *PHOTOSYNTHETIC SYSTEM DATA*

From Table 8.7 it can be seen that the light levels for measurement were low throughout the experiment, except on day 17. Only on this day were significant differences found in net photosynthetic rates, with lower values for SL3 plants. The data for day 17 are plotted in Fig. 8.10 where it can be seen that stomatal conductance, transpiration, and internal CO_2 were linearly related to solution water potential. Thus, 'instantaneous' transpiration measurements and experiment long water use data were highly correlated.

Overall, internal CO_2 was significantly lower in the SL3 plants on each day except day 25. Stomatal conductance and transpiration rate differed on all days, being significantly lower in SL3.

No significant differences were found in dark respiration rates but stomatal conductance values at night were considerably greater for the control plants.

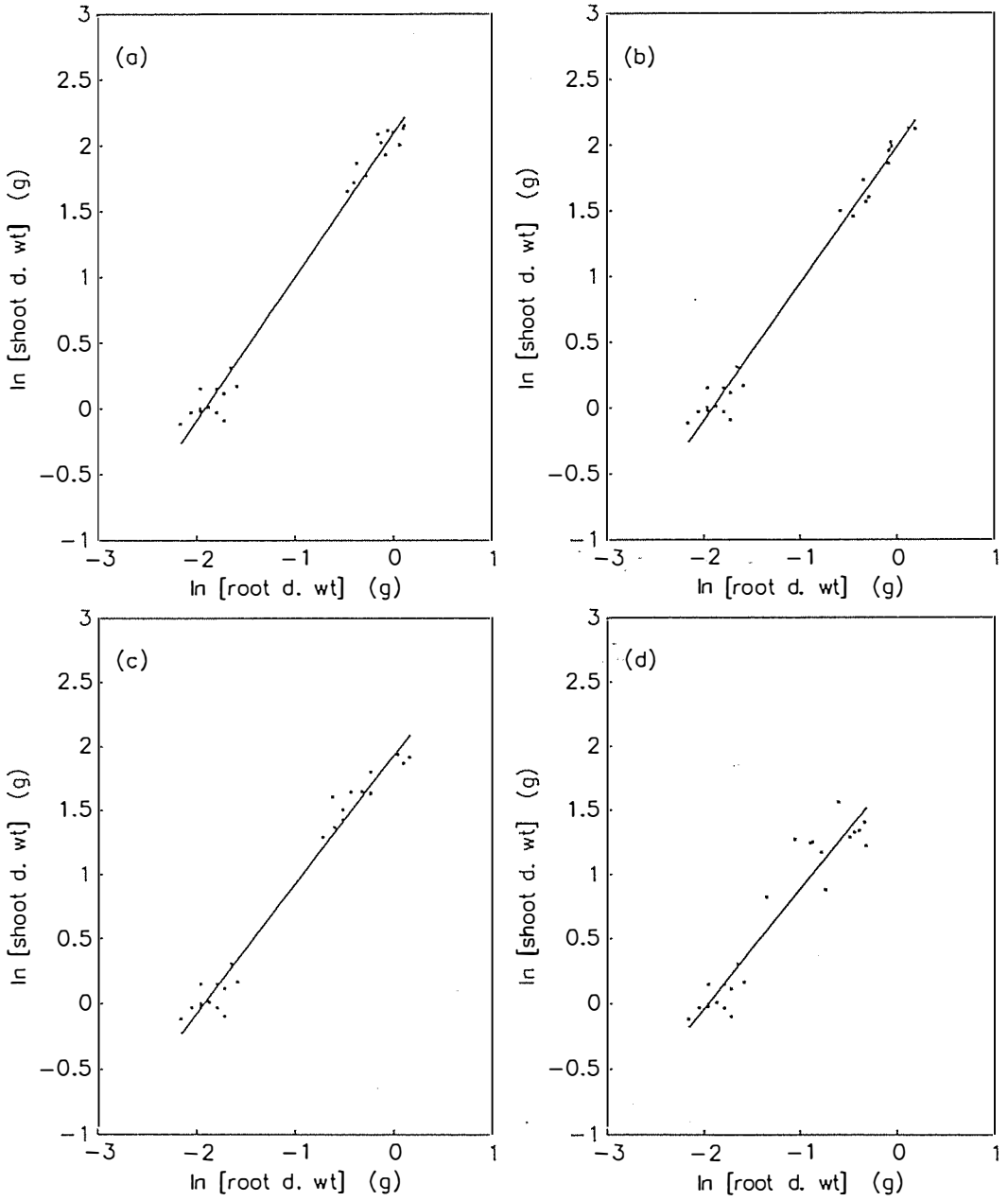

Fig. 8.4. The effect of different nutrient solution water potentials (obtained using PEG 4000) in an aeroponic system on the allometric relationship. (a) 0.5 bar; $Y = 2.09 + 1.09X^{a}$; $R = 0.98^{**}$. (b) 1.2 bar; $Y = 1.98 + 1.04X^{ab}$; $R = 0.98^{**}$. (c) 3.0 bar; $Y = 1.92 + 1.00X^{b}$; $R = 0.97^{**}$. (d) 5.9 bar; $Y = 1.80 + 0.92X^{c}$; $R = 0.90^{**}$. (letters on slope coefficients indicate significant differences at the 5% level using a paired t-test)

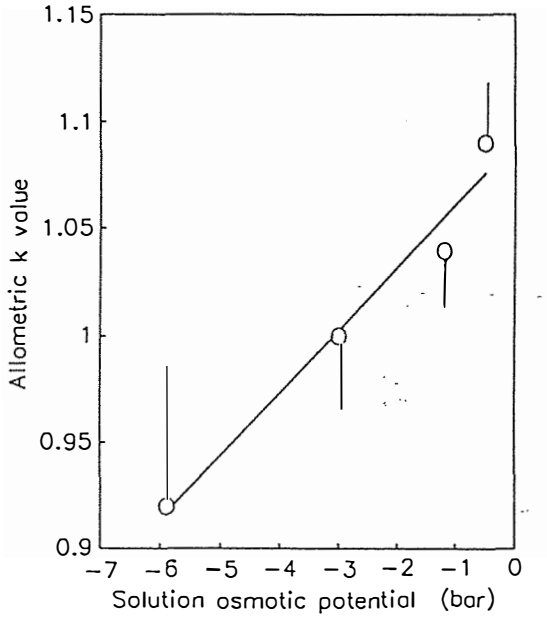

Fig. 8.5. The effect of different nutrient solution water potentials (obtained using PEG 4000) in an aeroponic system on the allometric k value (bars show s.e. of k); $Y = 1.09 - 0.03X$; $R^2 = 0.97^*$.

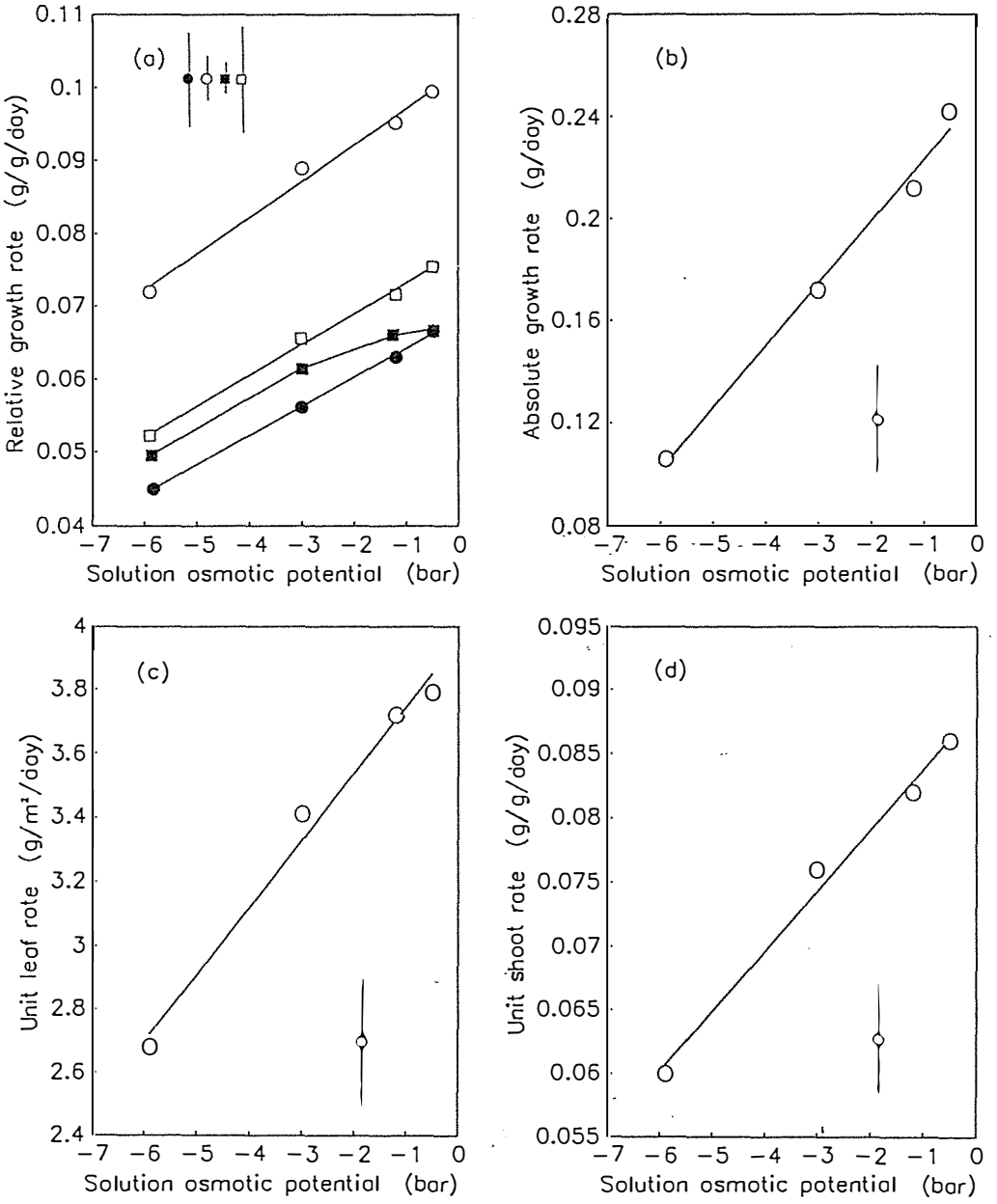

Fig. 8.6. The effect of different nutrient solution water potentials (obtained using PEG 4000) in an aeroponic system on growth analysis parameters (n = 12). Bars show pooled s.e.. (a)●, Leaf RGR; Y = 0.068 + 0.0040X: ○, Stem RGR; Y = 0.102 + 0.0050X: ■, Root RGR: □, Plant RGR; Y = 0.077 + 0.0042X: $R^2 = 0.99^{**}$ for all equations. (b) Absolute GR; Y = 0.25 + 0.0016X; $R^2 = 0.99^{**}$. (c) Unit leaf rate; Y = 3.95 + 0.209X; $R^2 = 0.98^{**}$. (d) Unit shoot rate; Y = 0.089 + 0.0047X; $R^2 = 0.99^{**}$.

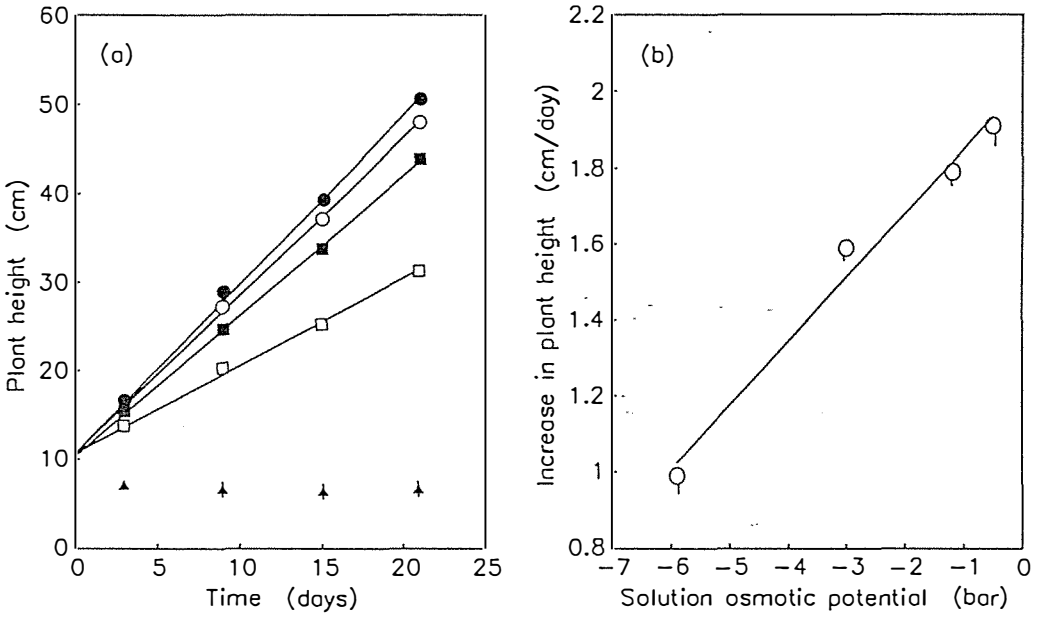

Fig. 8.7. The effect of different nutrient solution water potentials (obtained using PEG 4000) in an aeroponic system on plant height. (a) Plant height *versus* time (n = 24). Bars show pooled s.e. for each measurement time.●, 0.5 bar; Y = 10.61 + 1.91X:○, 1.2 bar; Y = 10.60 + 1.79X:■, 3.0 bar; Y = 10.27 + 1.59X: □, 5.9 bar; Y = 10.59 + 0.99X: $R^2 = 0.996^{**}$ for all equations. (b) Rate of increase in plant height. Bars show s.e. of slope coefficient; Y = 2.01 - 0.17 X; $R^2 = 0.98^{**}$.

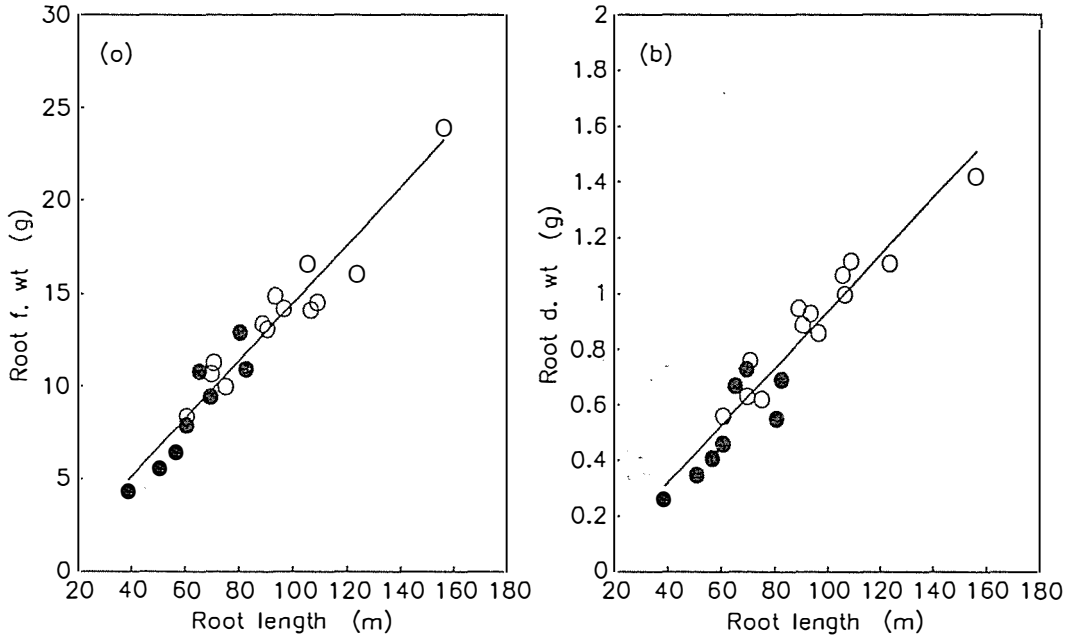

Fig. 8.8. The effect of different nutrient solution water potentials (obtained using PEG 4000) in an aeroponic system on the root weight to length relationship after 30 days. ●, Ψ_W = 0.5 bar; ○, Ψ_W = 5.9 bar. (a) Root fresh weight (all points); Y = -1.13 + 0.16X; $R^2 = 0.93^{**}$. (b) Root dry weight (all points); Y = -0.09 + 0.01X; $R^2 = 0.91^{**}$.

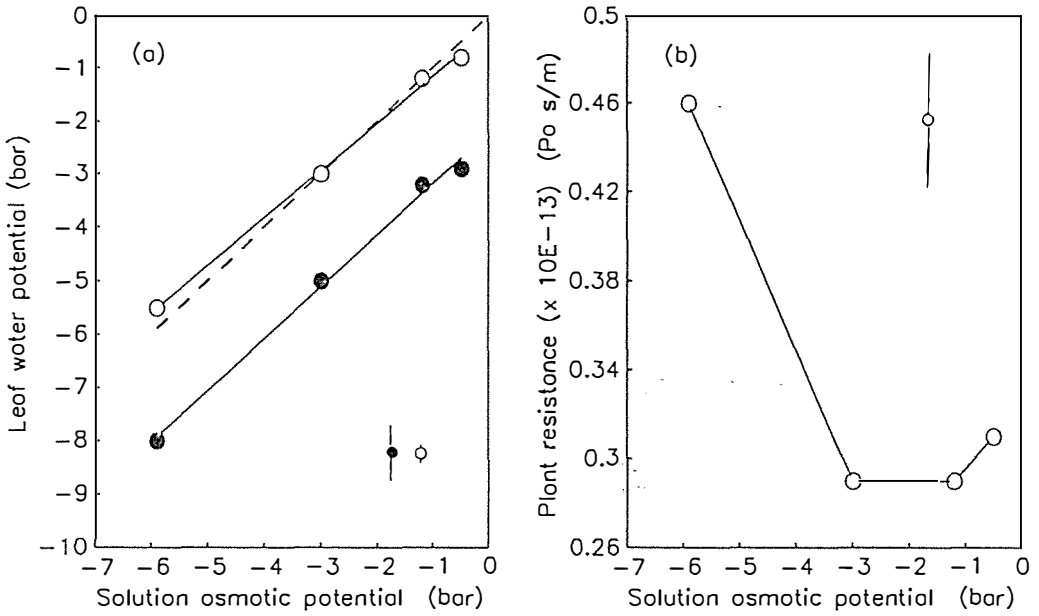

Fig. 8.9. The effect of different nutrient solution water potentials (obtained using PEG 4000) in an aeroponic system on leaf water potential and plant resistance. Bars show pooled s.e.. (a) Leaf water water potential. Each point is the mean of 24 measurements (6 readings per day from 4 days). ●, noon Ψ_W(leaf); Y = 1.87 + 1.02X; R^2 = 0.999**: ○, dawn Ψ_W(leaf); Y = 0.30 + 0.88X; R^2 = 0.997**: ·-·-·, 45° line. (b) Plant resistance ((Ψ_W(leaf) - Ψ_W(solution)) / E) (n = 24).

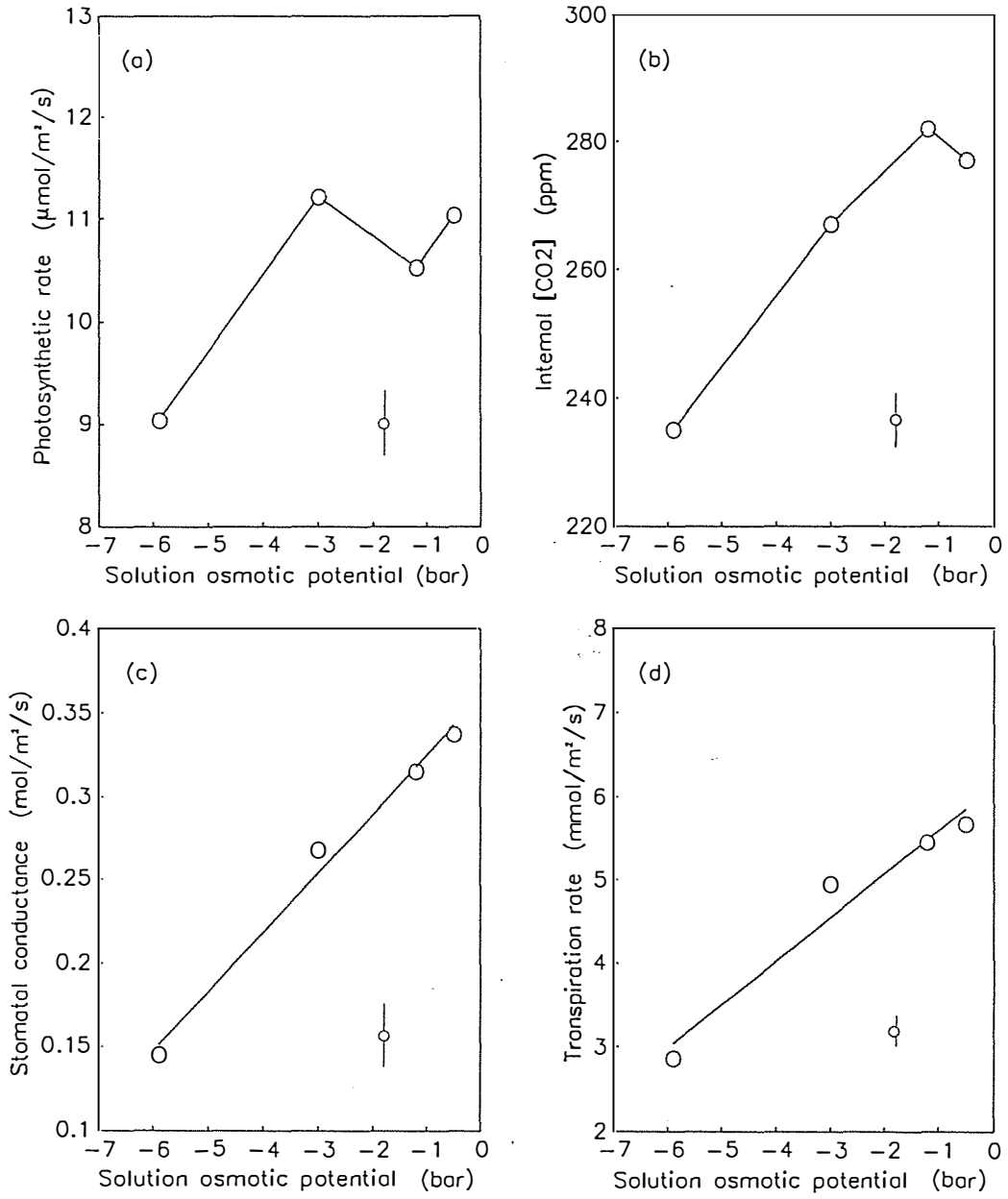

Fig. 8.10. The effect of different nutrient solution water potentials (obtained using PEG 4000) in an aeroponic system on leaf parameters. Each point is the mean of 48 measurements (12 per day from 5 days). Bars show pooled s.e.. (a) Net photosynthesis. (b) Internal CO_2 concentration. (c) Stomatal conductance; $Y = 0.36 + 0.035X$; $R^2 = 0.99^{**}$. (d) Transpiration rate; $Y = 6.11 + 0.52X$; $R^2 = 0.96^{*}$.

Table 8.7 Effect of different concentrations of PEG 4000 in the nutrient solution of an aeroponic system on leaf parameters (n = 12)

Day (PAR)[a]	Soln. Ψ_W (bar)	Pn (μmol $m^{-2} s^{-1}$)	gs (mol $m^{-2} s^{-1}$)	C_i (ppm)	E (mmol $m^{-2} s^{-1}$)
10	-0.5	3.9 ns	0.32 b	334 a	3.6 ab
(221)	-1.2	3.8 ns	0.32 b	333 a	3.6 b
	-3.0	4.5 ns	0.36 a	329 a	3.9 a
	-5.9	3.6 ns	0.17 c	320 b	2.0 c
s.e. mean		0.2	0.01	2	0.1
13	-0.5	3.2 b	0.28 a	333 a	3.1 a
(146)	-1.2	3.3 b	0.30 a	333 a	3.0 a
	-3.0	4.0 a	0.27 a	328 a	2.9 a
	-5.9	3.0 b	0.12 b	308 b	1.4 b
s.e. mean		0.2	0.01	2	0.1
17	-0.5	11.0 a	0.34 a	278 ab	5.7 a
(847)	-1.2	10.5 a	0.31 ab	282 a	5.5 ab
	-3.0	11.2 a	0.27 b	267 b	4.9 b
	-5.9	9.0 b	0.15 c	235 c	2.9 c
s.e. mean		0.3	0.02	4	0.2
25	-0.5	6.5 ns	0.53 a	321 ns	6.9 a
(392)	-1.2	6.9 ns	0.47 a	317 ns	6.4 a
	-3.0	7.7 ns	0.54 a	316 ns	6.5 a
	-5.9	6.5 ns	0.26 b	305 ns	4.4 b
s.e. mean		0.4	0.05	4	0.4
28	-0.5	-4.3 ns	0.043 a	597 ns	0.31 a
(0)[b]	-1.2	-3.1 ns	0.029 b	592 ns	0.19 b
	-3.0	-3.0 ns	0.029 b	590 ns	0.19 b
	-5.9	-3.9 ns	0.030 b	634 ns	0.20 b
s.e. mean		0.3	0.002	26	0.02

Means with the same letter in each column (for a given day) are not significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)

[a] Photosynthetically active radiation in μmol $m^{-2} s^{-1}$

[b] Night readings (photosynthesis value represents respiration rate)

8.3.8 *WATER USE AND PLANT RESISTANCE*

The SL1, SL2 and SL3 plants used 8%, 14% and 43% less water per square metre leaf area per day than the control plants (Table 8.8), giving a linear trend with solution water potential (Fig. 8.11). Water use efficiency was significantly higher for all three stress treatments, with SL3 plants being 27% higher, at 7.23 mg l^{-1} (Fig. 8.11). Plant resistance showed a large increase between SL2 and SL3 (Fig. 8.9).

Table 8.8 Effect of different concentrations of PEG 4000 in the nutrient solution of an aeroponic system on water use as measured in terms of experiment long water use (WU) (n = 30) and instantaneous transpiration rate at noon (E) (n = 12)

Solution Ψ_W (bar)	Water use (WU) (l pt^{-1} d^{-1})	(l m^{-2} d^{-1})	E (l m^{-2} h^{-1})	E/WU[a]
-0.5	0.040 a	0.57 a	0.74 a	14.8 a
-1.2	0.031 b	0.53 ab	0.72 a	12.5 b
-3.0	0.025 c	0.50 b	0.65 b	11.1 b
-5.9	0.013 d	0.38 c	0.42 c	6.8 c
s.e. mean	0.002	0.02	0.03	0.8

Means with the same letter in each column are not significantly different at the 1% level using the lsd discriminator

[a] Both variables in units of l m^{-2} d^{-1}

8.3.9 *PEG UPTAKE ANALYSIS*

The SL1 and SL2 plants contained similar levels of PEG (Table 8.9), these being similar to the levels and distribution found in preliminary experiments (see section 7.3). The SL3 plants had high levels of PEG 4000 in the root system and significantly higher levels in the stem. However, no significant quantities of PEG 4000 were found in the leaves of any treatment plants, so long as they appeared healthy (Fig. 8.12).

The two SL3 plants which exhibited wilting and leaf necrosis had very high leaf concentrations of PEG 4000 (21.1 mg per plant).

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-0.5	0.040 a	0.57 a	0.37 a	15.5 a
-1.2	0.031 b	0.53 ab	0.36 a	16.1 b
-3.0	0.025 c	0.50 b	0.32 b	15.4 b
-5.9	0.013 d	0.38 c	0.19 c	12.0 c
s.e. mean	0.002	0.02	0.03	0.4

Means with the same letter in each column are not significantly different at the 1% level using the lsd discriminator

[a] Both variables in units of l m^{-2} d^{-1}

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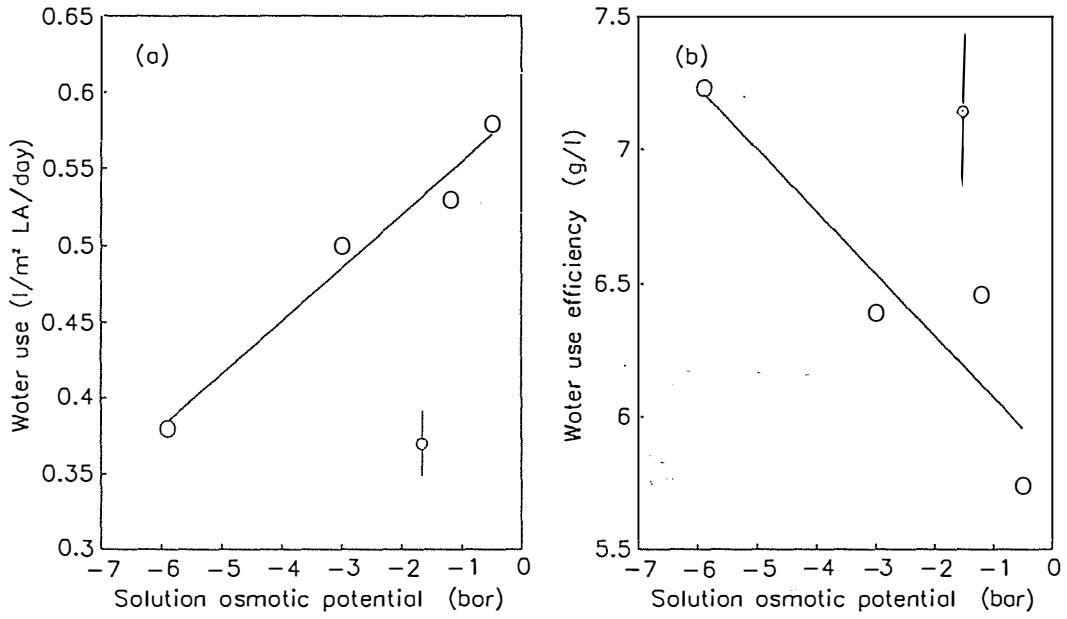

Fig. 8.11. The effect of different nutrient solution water potentials (obtained using PEG 4000) in an aeroponic system on water use and water use efficiency over a 30 day period (n = 30). Bars show pooled s.e.. (a) Water use; Y = 0.59 - 0.03X; $R^2 = 0.97^*$. (b) Water use efficiency; Y = 5.85 + 0.23X; $R^2 = 0.84^{ns}$.

Table 8.9 Effect of different concentrations of PEG 4000 in the nutrient solution of an aeroponic system on the level of PEG within plant tissue after 30 days (n = 8)

Solution Ψ_W (bar)	PEG content mg ml^{-1} sap (mg $part^{-1}$) Leaf	Stem	Root	Plant
-0.50	0.03 ns	0.06 c	0.02 c	
	(1.0)	(1.5)	(0.3)	(2.8)
-1.2	0.06 ns	0.14 c	0.21 c	
	(1.8)	(2.9)	(2.0)	(6.6)
-3.0	0.07 ns	0.12 c	0.45 c	
	(1.6)	(1.9)	(3.4)	(6.9)
-5.9	0.07 ns	0.51 b	3.00 a	
	(1.0)	(4.9)	(20.6)	(26.5)
-5.9	(necrotic)	1.42 ns	0.77 a	1.72 b
	(21.1)	(7.3)	(11.8)	(40.3)
s.e. mean	0.03	0.05	0.10	
	(0.29)	(0.45)	(1.98)	(5.0)

Means with the same letter in each column are not significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)

8.3.10 *CHLOROPHYLL ANALYSIS*

Results from chlorophyll extraction and analysis are given in Fig. 8.13. All pigment concentrations increased as solution water potential decreased, there being a greater increase between -3.0 and -5.9 bar. The chlorophyll a/b ratio fell sharply between -1.2 and -3.0 bar, with no significant decrease thereafter.

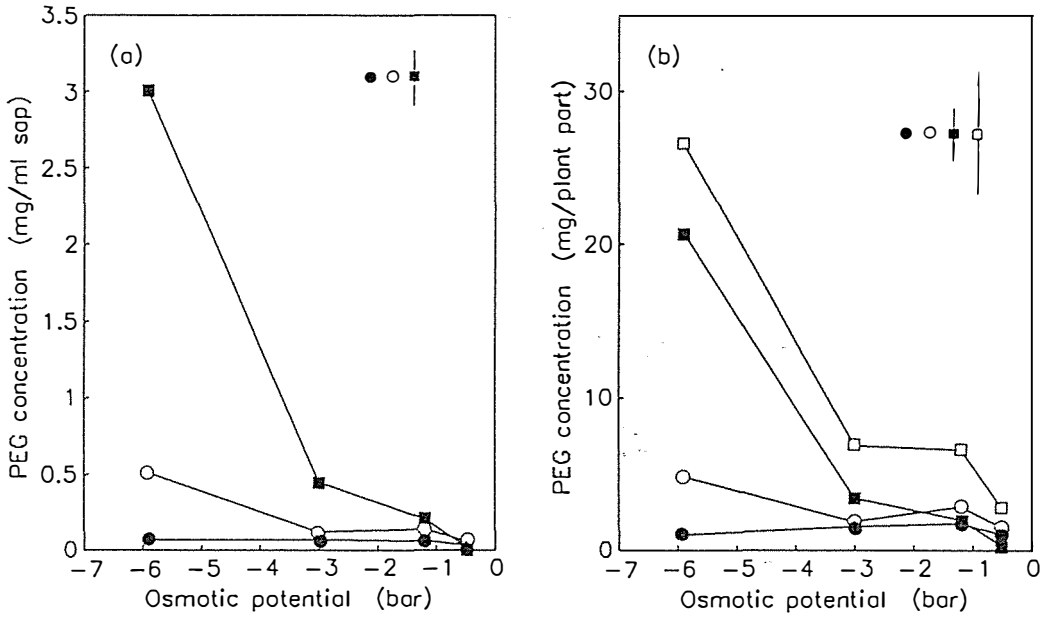

Fig. 8.12. The effect of different nutrient solution water potentials (obtained using PEG 4000) in an aeroponic system on the polyethylene glycol content of plant tissue after 30 days. Each point is the mean of 8 measurements (2 samples per plant from 4 plants). Bars show pooled s.e. for each plant part. (a) mg per ml of sap. (b) mg per plant part. ●, leaf;○, stem;■, root;□, plant.

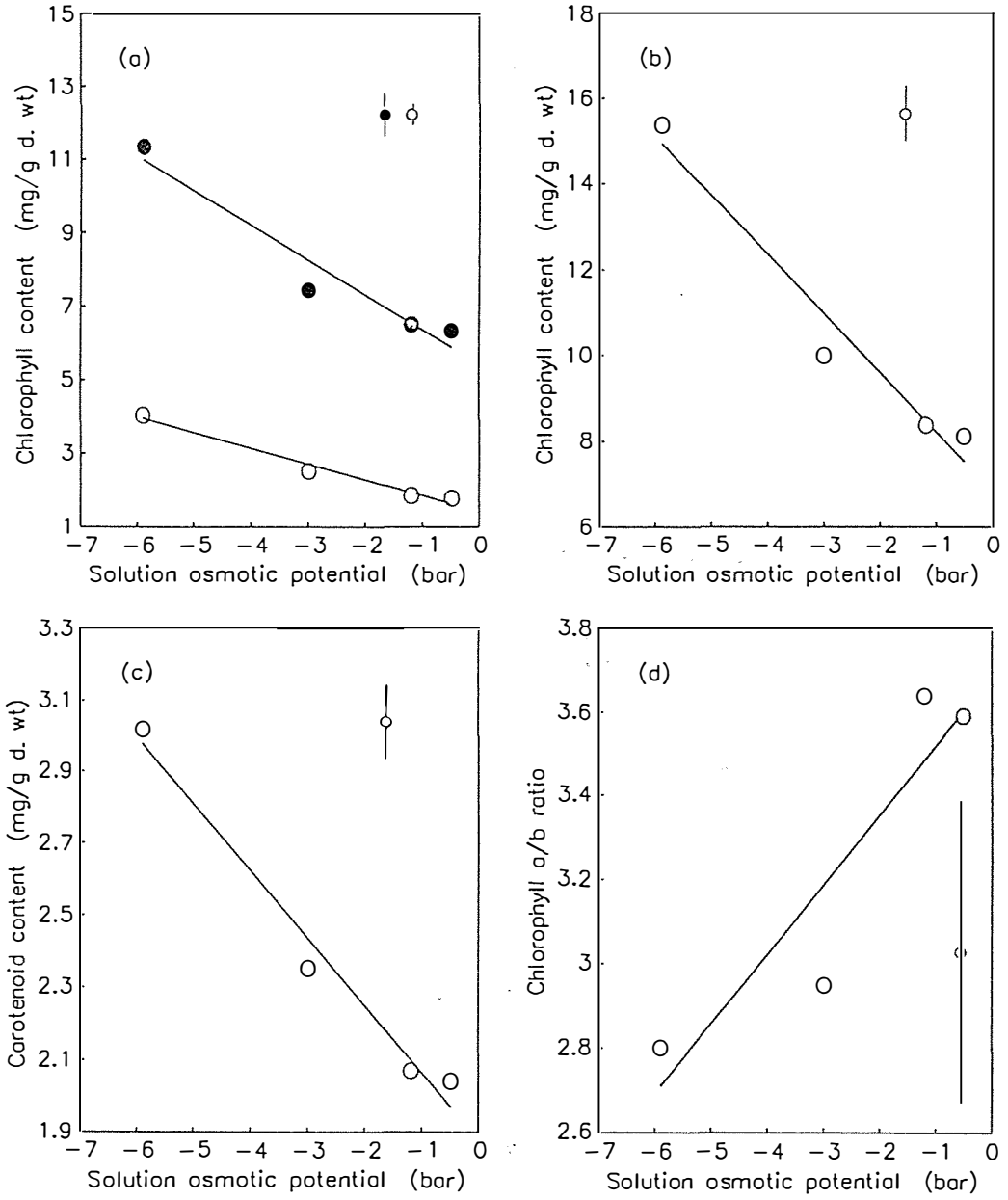

Fig. 8.13. The effect of different nutrient solution water potentials (obtained using PEG 4000) in an aeroponic system on pigment levels after 34 days. Each point is the mean of 8 measurements (2 samples per leaf from 2 leaves per plant off 2 plants). Bars show pooled s.e.. (a) ●, chlorophyll a; Y = 5.42 - 0.94X; $R^2 = 0.94^*$: ○, chlorophyll b; Y = 1.42 - 0.43X; $R^2 = 0.98^{**}$. (b) Total chlorophyll; Y = 6.83 - 1.37X; $R^2 = 0.96^*$. (c) Carotenoid content; Y = 188 - 18.7X; $R^2 = 0.98^{**}$. (d) Chlorophyll a/b ratio; Y = 3.68 - 0.16X; $R^2 = 0.84^*$.

8.4 **DISCUSSION**

8.4.1 *WATER POTENTIALS*

For soil grown plants, Ψ_W(leaf) measured at dawn generally correlates well with Ψ_W(soil) (Garnier and Berger, 1985; Natali *et al.*, 1985a, b, c; Rutter and Sands, 1958) and for plants grown in solutions of different water potential the same sort of correlation would be expected. Such was indeed observed in this experiment. Comparable data from the literature is hard to find because dawn water potential appears to be almost solely a field measurement which is not made under controlled conditions (e.g. a glasshouse). Considering the significance of dawn water potential in understanding the nature of the water stress this situation is unfortunate.

The relationship between Ψ_W(leaf) and solution Ψ_S was linear, with a slope of 0.88 and 1.02 bar bar^{-1} at dawn and noon respectively. Hence, in this experiment, noon Ψ_W(leaf) was closely correlated with root environment Ψ_W. By contrast, Garnier and Berger (1985) observed a poor correlation between the two variables, while Ψ_W(stem) and dΨ_W(stem-leaf) were better predictors of Ψ_W(soil). Deviations of Ψ_W will occur under large fluctuations in the atmospheric environment. During this experiment mild conditions prevailed and consequently the above correlation was observed.

The Ψ_W gradient within the plant was similar for all treatments, being -2.4 bar for the control and -2.1 bar for SL3. The gradient is generally observed to decrease with water stress (Ritchie and Hinckley, 1975) due to stomatal closure reducing the drop in Ψ_W(leaf).

Overall there was a slight decrease in plant resistance for all three stress treatments. Under severe water stress, whole plant resistance increases. The increase is commonly put down to an increase in root resistance (Camacho-B *et al.*, 1974a; Syvertsen, 1985a), this being two to ten times higher than leaf and stem components (Davies, 1986). Reasons for increased root resistance have already been discussed (section 2) and include decreased lateral root growth (Richter, 1976; Teskey and Hinckley, 1981), increased suberization (Syvertsen, 1985a) and a change in uptake pattern from root tips to the absorbing zone (Weatherley, 1979).

In this experiment transpiration was inhibited more than root growth such that water use per unit root length decreased from 4×10^{-4} to 2×10^{-4} l m^{-1} d^{-1}. This would suggest a decrease in root resistance, thus contributing to a decrease in whole

plant resistance, although the reason is unclear. An inverse relationship between root length and uptake per root length is common, such that Ψ_W(leaf) is independent of the leaf area to root length ratio (Eavis and Taylor, 1979).

Plant resistance values calculated in this experiment (0.3 - 0.5 x 10^{13} Pa s m^{-1}) are low compared with those given for tomato (4.7 x 10^{13} Pa s m^{-3} (Hameed *et al.*, 1987)) and woody species (e.g. apple, 10 x 10^{13} Pa s m^{-1} (Landsberg *et al.*, 1976)). The higher values of Hameed *et al.* (1987) may be attributed to the experimental system and measurement technique. With reference to the three plant categories identified by Camacho-B *et al.* (1974a), tomato would fall into category 2, combining strong stomatal regulation with efficient transport. The point to remember is that high resistance is beneficial in a dry environment (Camacho-B *et al.* (1974a). The correlation between hydraulic conductivity and vigour was noted by Syvertsen and Graham (1985) and is worth reiterating here.

8.4.2 *WATER USE*

It can be seen from Table 8.8 that the transpiration rate measured at noon was over fourteen times that estimated from tank water loss for control plants. The tank loss figure represents a 24 hour average. With SL3 plants this difference was halved due to stomatal closure over the midday period. Thus the underlying principle is that transpiration rate fluctuates less with environmental changes as water stress increases.

Under natural conditions, where there is a high evaporative demand relative to supply potential, midday stomatal closure is common. This would appear to be due to large soil-root interface resistances developing, as first suggested by Tinklin and Weatherly (1968). In this experiment the evaporative demand was the same for all treatments but the supply potential was lowered using a water potential modifying agent. This is basically analogous to that of a drier soil as long as there is good soil-root contact. However, as will be discussed later, the development of a vapour gap under water stress, due to root contraction, is now considered to be very important (Herkelrath *et al.*, 1977; Lösch, 1984; Scott Russell, 1982; Sharp and Davies, 1979). Hence it may be concluded that the water stress generated by a water potential modifying agent will be less than that in soil having the same bulk Ψ_W.

Gergely *et al.* (1980a) found a linear relationship between water use per leaf area and solution 'osmotic' potential for young apple seedlings. Such a relationship

was also found in this experiment although the percentage decrease was not as rapid as Gergely *et al.* (1980a) found for apple seedlings. This may reflect the different experimental conditions and plant material.

8.4.3 *SHOOT RESPONSES*

The dry weight per leaf area response in this experiment was quite different from the linear relationship found by Gergely *et al.* (1980a). It increased significantly between -0.5 bar and -1.2 bar and then remained relatively constant. The difference can easily be explained with respect to the different species used. Over an extended growth period dry weight per leaf area would be expected to increase under water stress, as in this experiment, if cell expansion is very sensitive (Hsiao, 1973), thereby limiting leaf development. With the apple seedlings however, relative increase in leaf area over 14 days would not be as great. The dominant factor in contrast would be stomatal limited photosynthesis which would generate a negative relationship as observed (Gergely *et al.*, 1980a).

Total plant height (Fig. 8.7) showed a very strong linear relationship with time for all treatments ($R^2 > 0.99$). This means that after an initial rapid response to the change in solution Ψ_S, activity of the apical meristem was constant under the constant water stress regime. Growth rate in height was linear with solution water potential as were the plant part dry weights after 30 days. Responses can be compared to those of the allometric relationships which were identical in nature but relatively smaller. Height growth rate decreased by 47% at SL3 while the allometric k value decreased 16%. Aspects of plant height increase will be discussed in more detail in experiment 2.

There was a small increase in the lamina to petiole and leaf to stem dry weight ratios at SL3 reflecting an increase in the lamina portion of shoot dry weight (42% to 46%). At the same time, there was a fall in specific leaf area. Tomato leaves under water stress possess smaller epidermal and mesophyll cells (Rudich and Luchinsky, 1986) which may account for this observation.

From the study of Gates (Gates 1955a, b; 1957) on water stress in young tomato plants, the lamina weight ratio was found to decrease while the stem weight ratio increased (Gates, 1955a). Differences may be explained in terms of stress intensity. Gates (1955a) applied a rapid, intense stress (Ψ_W(soil) near the permanent wilting point ($\approx$ -15 bar)) which led to senescent responses not observed in this

experiment. Severe water stress generally leads to leaf aging and abscission. This may be contrasted with nutrient stress which can slow aging and abscission (Schulze, 1986a).

8.4.4 *ROOT RESPONSES*

While root dry weight decreased linearly with solution Ψ_S, it was significantly less sensitive than that of the leaf and stem portions (Fig. 8.2). The slopes were -0.362, -0.295 and -0.068 g bar^{-1} for leaf, stem and root respectively. There was no treatment effect on the linear relationship between root length and root dry weight, that is, root length per unit dry weight was constant. This indicates that there was an overall reduction in root growth without a change in structural characteristics.

Under natural conditions a plant root system may become more extensive as Ψ_W(soil) decreases leading to greater exploration of soil layers (Huck *et al.*, 1983). This will result in an increase in root length (Huck *et al.*, 1986). In the aeroponic system there are no Ψ_W gradients to direct root growth and lateral growth is inhibited by lack of physical support. Hence the root system does not become more extensive as would occur in a completely homogeneous soil.

8.4.5 *SHOOT-ROOT INTERACTION*

Using linear regression for the allometric relationships, 'k' was found to decrease linearly with solution Ψ_S (Fig. 8.5). This is in qualitative agreement with the results of Troughton (1960) who was the first to document decreases in the allometric k value with water stress. The decrease reflects a proportional increase in assimilate partitioning to the root system, this being the part of the plant associated with the growth limiting environmental factor (Hunt and Nicholls, 1986).

8.4.6 *LEAF PHYSIOLOGY*

Stressed plants were observed to be a darker green colour indicating increased pigment concentrations per unit leaf area. A portion of this change can be attributed to the small decrease in specific leaf area. However chlorophyll and

carotenoid contents, on a dry weight basis, increased linearly with solution water potential indicating an absolute increase in pigment levels.

Under water stress chlorophyll content is typically considered to decrease (Gujrathi *et al.*, 1984; Lösch, 1984; Syvertsen, 1985a). This occurs along with a variety of other chloroplast changes such as loss of membrane material, loss of grana thylakoids, increase in starch and accumulation of plastoglobulin (Lösch, 1984). It is clear that light induced chlorophyll formation in etiolated tissue has significant water stress sensitivity (Boyer, 1976; Hsiao, 1973) due to a reduction in the capacity for photochlorophyll formation (Hsiao, 1973) . However green tissue appears to be far less sensitive. Boyer (1976a) provides data showing no significant change in sunflower chlorophyll levels (d. wt. basis) after 3 days of dessication (-20 bar). Boyer (1976a) notes that decreases in chlorophyll level are often associated with water stress induced nutrient deficiency (especially nitrogen) and leaf senescence which are products of long term, severe water deficits. The fact that pigment levels increased in this experiment indicates that the generated stress levels were not sufficient to activate catabolic processes.

Noon leaf water potentials in this experiment averaged -5.0 bar and -8.0 bar for SL2 and SL3 plants respectively. According to Rudich and Luchinsky (1986), stomatal conductance in tomato plants is not affected until leaf water potentials reach -6 to -7 bar. Therefore it may be expected that significant changes in stomatal conductance would only be observed in the highest stress treatment. In fact, stomatal conductance appeared to change at a constant rate with increasing stress. This yet again focuses attention on the threshold response debate which was discussed in section 2. Most threshold like responses can be explained through researchers using resistance rather than conductance (Hall *et al.*, 1976; Tan and Buttery, 1982a). The work of Laisk *et al.* (1980) on aperture distribution provides further strong support for gradual changes.

Photosynthetic rates were significantly lower at SL3 reflecting the fact that stomatal limitation was not significant in the other two stress treatments. Along with the fall in photosynthesis there was a drop in C_i of 15%. This increased to 28% if the adjustment for patchy stomatal closure was applied (see section 3.1.3 and Downton *et al.*, 1988a). Hence stomatal closure can account for the drop in photosynthesis as many workers have recently concluded (Downton *et al.*, 1988a, b; Laisk, 1983; Sharkey and Seemann, 1989). Note that the actual existence of patchiness was not experimentally established in this experiment.

8.4.7 *PERFORMANCE OF PEG 4000*

Phytotoxic symptoms, characteristic of PEG (Lawlor, 1970), were only observed in a few plants at solution Ψ_S of -5.9 bar. These plants were found to have highly significant levels of PEG 4000 in the leaf tissue (21 mg total per plant) while those plants showing no symptoms had nonsignificant levels. The symptoms involved marginal leaf necrosis, chlorosis and wilting.

No significant levels of PEG were found in any plant part for the -1.2 and -3.0 bar treatments. In the -5.9 bar treatment there was a small quantity in the stem (3 to 4 mg per plant stem), with high levels in the root system (20 mg per plant root). Concentrations here, as in the preliminary experiments, were considerably lower than those of Janes (1974) who found 40 to 50 mg per pepper plant root system after 7 days at -5 bar (PEG 4000). Lawlor (1970) obtained leaf PEG contents of under 1 mg per g fresh weight at -2 bar but around 8 mg per g fresh weight at -10 bar (PEG 4000). Results from this experiment equate to about 0.6 mg g^{-1} leaf f. wt. at -5.9 bar for those plants which showed phytotoxic symptoms. These results further support the use of PEG in an aeroponic system to induce water stress. However the -5 to -6 bar limit is a significant disadvantage to the investigation of a full range of water stress levels.

8.4.8 *SUMMARY*

i/ Various measured plant parameters can be related in a linear fashion to solution water potential in an aeroponic system. These include plant part dry weights, leaf area, allometric k value, stomatal conductance, transpiration rate, leaf water potential, height increase over time and pigment concentrations.

ii/ Polyethylene glycol 4000 performs adequately in an aeroponic system up to a solution Ψ_S of -5 to -6 bar. At this point leaf tissue damage occurs due to significant levels of PEG uptake. It is important that plants with damaged root systems are not transferred into PEG solutions.

9 EXPERIMENT 2 : WATER STRESS STUDIES ON TOMATO USING INTERMITTENT MISTING

9.1 INTRODUCTION

With various problems encountered using osmotic agents, especially plant uptake, alternative means of generating a controlled water stress have been sought. These include using a semipermeable membrane to keep the osmotic agent and root system apart (Krizek, 1985). The fundamental method of imposing a water stress, withholding water, usually lacks a high degree of control due to the small root volume in containers generating unwanted variability. The aeroponic system allows for a unique means of withholding water which is potentially far more controllable. All that is required is to turn off the misting pumps periodically (intermittent misting).

Intermittent misting is reported to be used in commercial aeroponic installations to reduce running costs and equipment wear (Massantini, 1985). For example, misting for 5 seconds every 10 minutes during the day and 5 seconds every 30 minutes at night has been found to be sufficient. Hubick *et al.* (1986b) and Hubick and Reid (1988) used a misting cycle of 40 seconds per hour to generate a water stress in sunflower. This decreased leaf water potential from -3.7 bar to -12.3 bar (Hubick *et al.*, 1986b).

Despite the very good results obtained by Hubick and co-workers using their aeroponic system no detailed study has yet been documented involving intermittent misting induced water stress. For this reason the following experiment was undertaken involving withholding misting for three time periods, up to 1.55 hours (off-times), and a continuous misting control. The initial aim was to determine if plants would grow satisfactorily with the misting pumps off for long periods and then if relationships could be found between plant parameters and off-time.

9.2 MATERIALS AND METHODS

For a detailed description of general experimental procedures see section 6.

9.2.1 *EXPERIMENTAL INFORMATION*

Pre-germination of tomato seed commenced on 5 June 1989 and seedlings were transferred to the aeroponic tanks six weeks later. Harvest 0 was carried out after 7 days with a further five harvests carried out as detailed in Table 9.1.

Table 9.1 Harvest information

Harvest #	Day	Plts harvest^{-1}	Plts tank^{-1}
0	0	2	30
1	14	8	22
2	28	8	14
3	49	4	10
4	56	4	6

Every two to three days flower buds were removed to keep the plants in a vegetative state. Plants were sprayed for pest and disease control only as required (see appendix 18.9).

9.2.2 *TREATMENT INFORMATION*

On day 0, storage tanks were replaced with 75 l of full strength Coopers nutrient solution (appendix 18.7) at pH 6.5 and the intermittent misting treatments shown in Table 9.2 were commenced.

Table 9.2 Treatment specification

		Off-Time (hours)			
TANK #	Stress level	Day 0	Day 1	Day 2	On Time (min)
2,5	0	0	0	0	1.0
1,7	1	0.17	0.17	0.17	1.0
4,6	2	0.65	0.65	0.65	1.0
3,8	3	1.55	1.55	1.55	1.0

9.2.3 *DATA GATHERING PROCEDURES*

Plant part analysis was carried out according to section 6.2.1 on the days given in Table 9.1. Photosynthetic measurements were made on days 10, 20, 21, 30 and 40 (12 am to 2 pm) while dark respiration measurements were made on days 25 and 35 (11 pm) using the same procedure but with plants in darkness (moonlight only). Leaf water potential measurements were made on days 5 and 15, 25, 35 and 45 (1 pm to 2 pm). Total plant height was measured on days 4, 8, 21, 35 and 45. Other measurements included leaf pigment analysis (day 34), pressure-volume curve analysis (day 36), assessment of tissue mineral levels using the day 56 harvest, assessment of the CO_2 compensation point (days 21, 35 and 42) and leaf relative water content determination (day 40).

9.2.4 *CALCULATION OF DERIVED VARIABLES*

A full listing of all derived variables is given in appendix 18.2.

9.2.5 *STATISTICAL ANALYSIS*

The experimental design was a randomized complete block (RCB) (2 blocks, 4 treatments) with subsamples. Description of analysis procedures for all data is given in appendix 18.3.

9.3 **RESULTS**

9.3.1 *PLANT PART FRESH AND DRY WEIGHTS*

Treatment means for plant part fresh and dry weights after 14, 28, 49 and 56 days are given in Tables 9.3, 9.4, 9.5 and 9.6 respectively. Plant growth, in terms of total plant dry weight, was approximately exponential over the experimental period as shown in Fig. 9.1. Trends in leaf, stem and root dry weights were all very similar. After only 14 days there were significant differences in all fresh and dry weight

components except for the root. Root dry weight was only significantly different at day 49 when SL1 and SL2 roots were larger than the controls.

Table 9.3 The effect of different misting pump off-times in an aeroponic system on plant part fresh and dry weights after 14 days

Plant parameter	Treatment off-time (hours) 0.00	0.17	0.65	1.55	s.e. mean
Fresh weights (g):					
leaf	35.6 a	28.9 b	23.5 bc	19.6 c	1.8
stem	24.3 a	19.0 b	14.2 c	11.6 d	0.9
laterals	-	-	-	-	-
root	16.0 ns	14.3 ns	13.6 ns	12.4 ns	1.2
shoot	59.9 a	47.9 b	37.5 c	31.2 c	2.6
plant	75.9 a	62.2 b	51.4 c	43.6 c	3.7
Dry weights (g):					
leaf	2.90 a	2.46 ab	2.02 bc	1.80 c	0.17
stem	1.22 a	1.00 b	0.84 bc	0.78 c	0.06
root	0.62 ns	0.62 ns	0.66 ns	0.62 ns	0.05
shoot	4.12 a	3.46 b	2.85 bc	2.58 c	0.22
plant	4.73 a	4.09 ab	3.51 bc	3.20 c	0.27

Means with the same letter in each row are not significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)

Table 9.4 The effect of different misting pump off-times in an aeroponic system on plant part fresh and dry weights after 28 days

Plant parameter	Treatment off-time (hours) 0.00	0.17	0.65	1.55	s.e. mean
Fresh weights (g):					
leaf	84.7 a	64.2 b	52.2 bc	43.0 c	4.2
stem	63.2 a	50.1 b	33.7 c	26.9 c	2.5
laterals	-	-	-	-	-
root	-	-	-	-	-
shoot	147.9 a	114.4 b	85.9 c	69.9 c	6.5
plant	-	-	-	-	-
Dry weights (g):					
leaf	7.10 a	6.01 ab	4.83 bc	4.17 c	0.41
stem	3.90 a	3.43 a	2.38 b	2.16 b	0.19
root	1.41 ns	1.60 ns	1.67 ns	1.63 ns	0.15
shoot	11.01 a	9.44 a	7.21 b	6.34 b	0.60
plant	12.53 a	11.04 a	8.88 b	7.97 b	0.73

Means with the same letter in each row are not significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)

Table 9.5 The effect of different misting pump off-times in an aeroponic system on plant part fresh and dry weights after 49 days

Plant parameter	Treatment off-time (hours) 0.00	0.17	0.65	1.55	s.e. mean
Fresh weights (g):					
leaf	218.3 a	157.9 b	125.8 b	71.5 c	10.7
stem	169.8 a	123.0 b	80.1 c	44.4 d	7.4
laterals	158.3 a	93.0 b	64.5 b	18.9 c	13.3
root	-	-	-	-	-
shoot	388.1 a	280.9 b	205.9 c	115.8 d	17.6
plant	-	-	-	-	-
Dry weights (g):					
leaf	22.6 a	18.7 ab	14.6 b	8.5 c	1.3
stem	15.5 a	13.0 a	8.6 b	5.3 c	0.9
root	4.1 c	5.4 ab	6.2 a	4.7 bc	0.4
shoot	38.1 a	31.7 a	23.2 b	13.8 c	2.1
plant	42.2 a	37.2 ab	29.4 b	18.5 c	2.5

Means with the same letter in each row are not significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)

Table 9.6 The effect of different misting pump off-times in an aeroponic system on plant part fresh and dry weights after 56 days

Plant parameter	Treatment off-time (hours) 0.00	0.17	0.65	1.55	s.e. mean
Fresh weights (g):					
leaf	276.6 a	205.7 b	166.3 b	101.0 c	19.4
stem	203.7 a	167.4 a	114.8 b	57.5 c	17.0
laterals	207.0 a	156.7 ab	103.7 bc	40.3 c	23.7
root	-	-	-	-	-
shoot	480.3 a	373.1 ab	281.0 b	158.5 c	36.5
plant	-	-	-	-	-
Dry weights (g):					
leaf	36.1 a	29.7 ab	22.3 b	12.9 c	2.8
stem	21.9 a	21.3 a	13.5 b	7.7 b	2.1
root	7.0 ns	8.1 ns	10.4 ns	7.5 ns	0.8
shoot	58.0 a	51.0 ab	35.9 bc	20.6 c	4.9
plant	65.0 a	59.1 ab	46.2 b	28.2 c	5.4

Means with the same letter in each row are not significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)

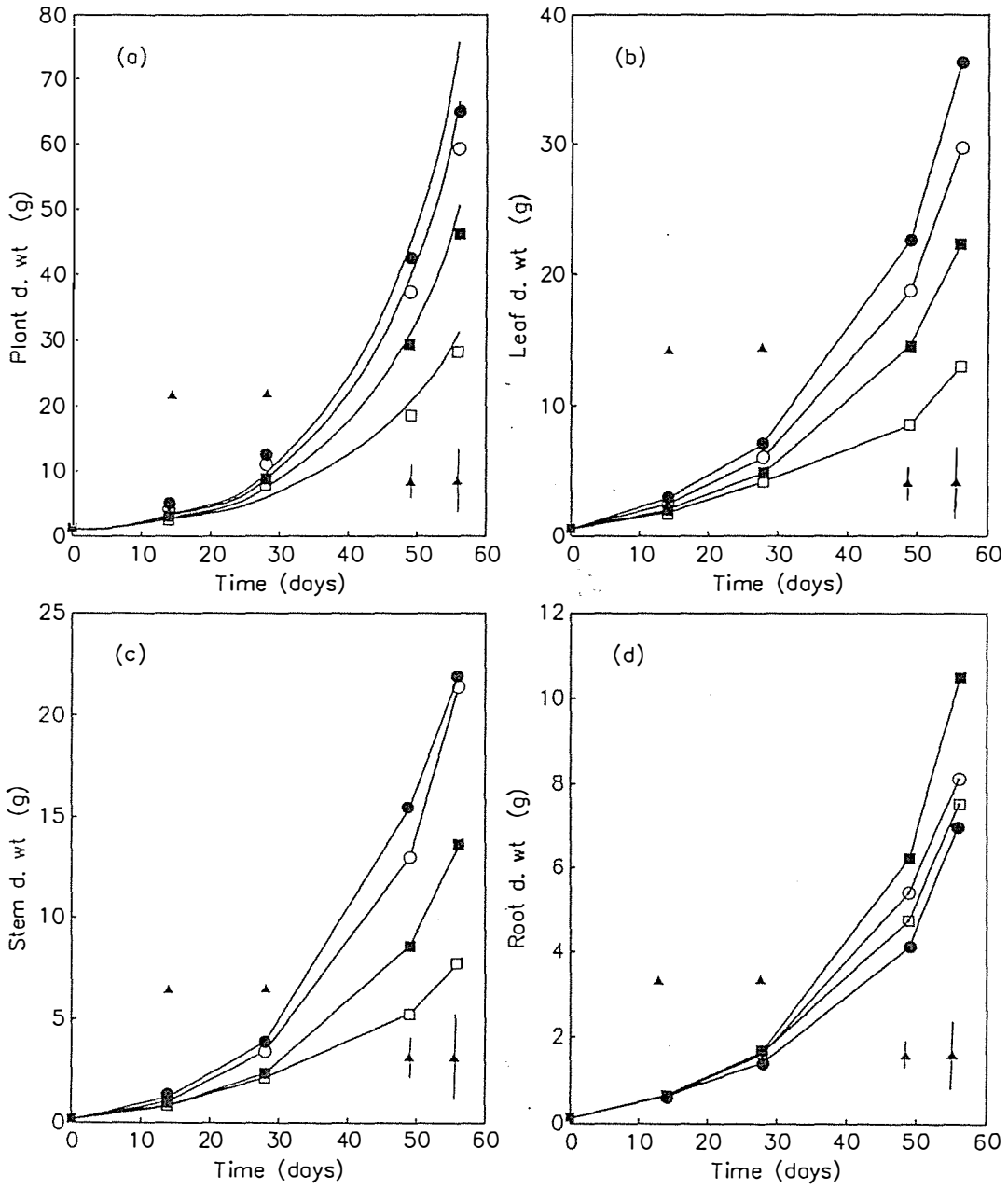

Fig. 9.1. The effect of different misting pump off-times in an aeroponic system on the change in dry weight parameters over time (n = 8). Bars show pooled s.e. for each harvest. (a) Plant dry weight. ●,0.00 hour OT; Y = 1.21 x exp(0.074X); $R^2 = 0.97^{**}$. ○,0.17 hour OT; Y = 1.14 x exp(0.072X); $R^2 = 0.98^{**}$. ■,0.65 hour OT; Y = 1.08 x exp(0.069X); $R^2 = 0.99^{**}$. □,1.55 hour OT; Y = 1.13 x exp(0.059X); $R^2 = 0.97^{**}$. (b) Leaf dry weight. (c) Stem dry weight. (d) Root dry weight.

9.3.2 *PLANT PART DRY WEIGHT RATIOS*

Leaf-root, stem-root and leaf-stem ratios are plotted against the natural logarithm of plant dry weight in Figs 9.2, 9.3 and 9.4. This was done because plant dry weight increased in approximately exponential fashion (Fig. 9.1) and hence ln(PtDW) was a linear function of time. This sort of plot has several advantages over that of dry weight ratio *versus* time (harvest date) (see section 9.4.8) and is discussed fully in section 14. For the control plants, the leaf-root ratio did not change significantly over the experimental period. In contrast, the stem-root ratio increased and the leaf-stem ratio decreased (nonlinearly) with time. Water stress resulted in a progressive decline in both the leaf-root and stem-root ratios over time, such that the stem-root ratio was constant for SL3 plants over the experimental period. Water stress had no significant effect on the rate of decline in leaf-stem ratio, with the trend towards a constant ratio over time. Overall time dependent changes in the leaf-root and stem-root ratios are shown succinctly in Fig. 9.13.

9.3.3 *PLANT PART WATER CONTENTS*

After 14 days of water stress treatment, stem and root water contents were found to decrease progressively with misting pump off-time but leaf water contents differed significantly at SL3 only (Fig. 9.5). Leaf relative water content decreased linearly with misting pump off-time (Fig. 9.5). Note that the difference in the relationship between leaf water content and relative water content reflects in part the different calculation basis, i.e. dry weight and turgid weight respectively.

9.3.4 *LEAF AND ROOT DATA*

Quadratic equations gave the best fit to leaf area data over time (Fig. 9.6) and so these equations were used to adjust water use data for leaf area. Leaf number increased rapidly, when lateral shoot numbers proliferated, in the period between days 28 and 49 (Fig. 9.6). Water stress had a marked effect on lateral shoot development within this period. Average leaf sizes differed significantly only at harvest 2 with the control plants having larger leaves.

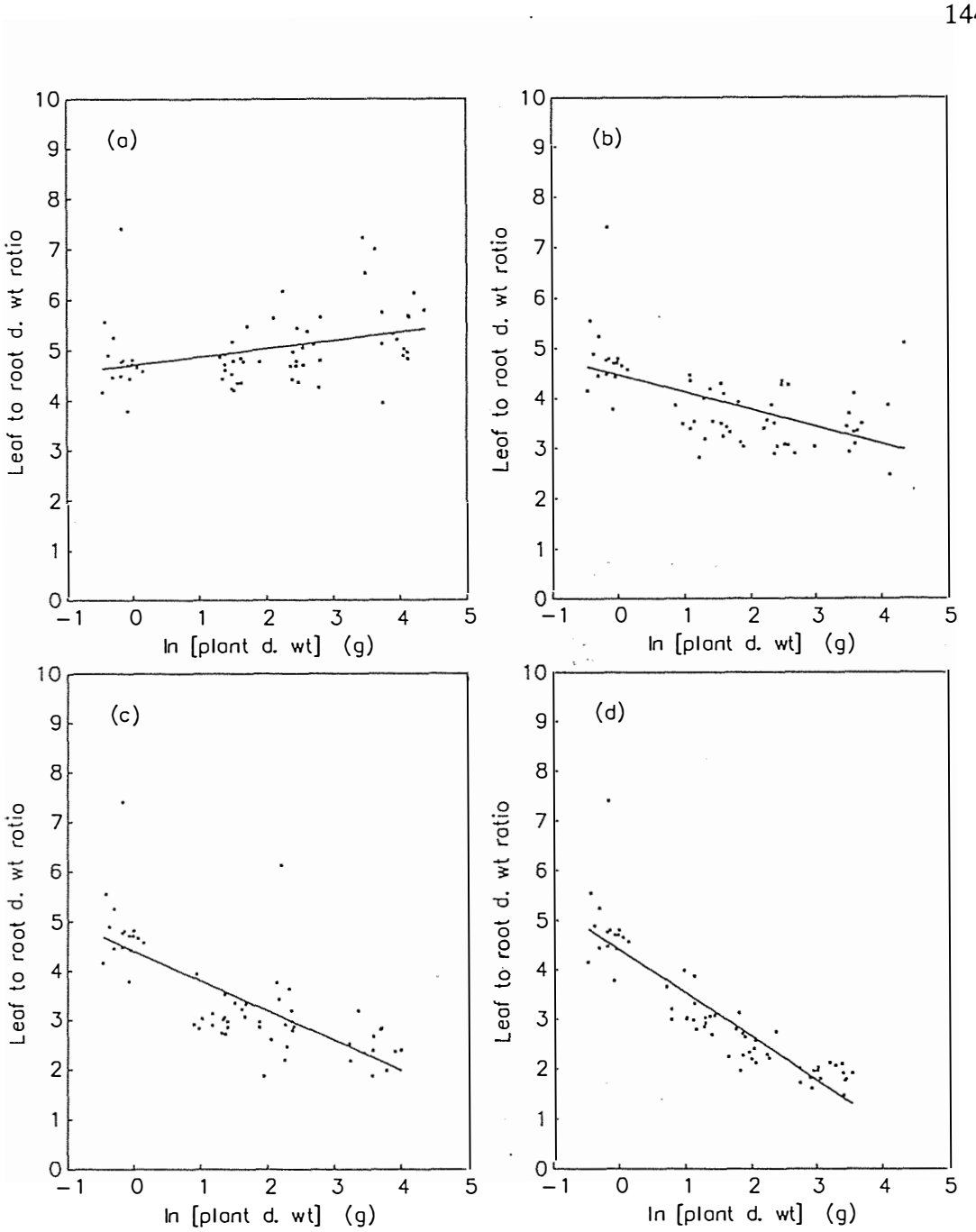

Fig. 9.2. The effect of different misting pump off-times in an aeroponic system on the relationship between the leaf to root dry weight ratio and natural log transformed plant dry weight. (a) 0.00 hour OT; Y = 4.70 + 0.16X; $R^2 = 0.11^{ns}$. (b) 0.17 hour OT; Y = 4.47 - 0.35X; $R^2 = 0.33^{ns}$. (c) 0.65 hour OT; Y = 4.40 - 0.61X; $R^2 = 0.54^{*}$. (d) 1.55 hour OT; Y = 4.41 - 0.88X; $R^2 = 0.80^{**}$.

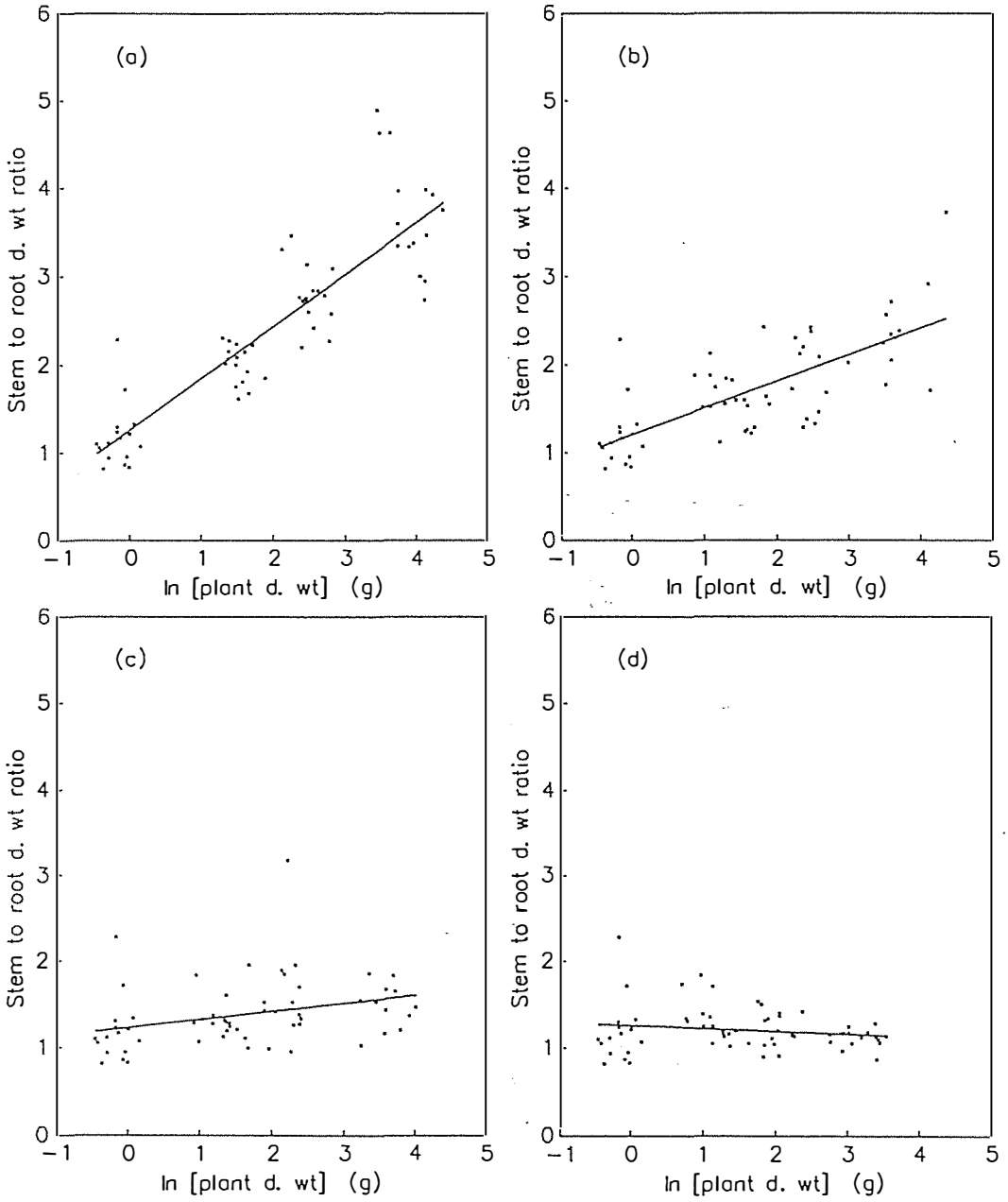

Fig. 9.3. The effect of different misting pump off-times in an aeroponic system on the relationship between the stem to root dry weight ratio and natural log transformed plant dry weight. (a) 0.00 hour OT; $Y = 1.26 + 0.59X$; $R^2 = 0.77^{**}$. (b) 0.17 hour OT; $Y = 1.20 + 0.30X$; $R^2 = 0.52^{*}$. (c) 0.65 hour OT; $Y = 1.23 + 0.09X$; $R^2 = 0.10^{ns}$. (d) 1.55 hour OT; $Y = 1.25 - 0.03X$; $R^2 = 0.02^{ns}$.

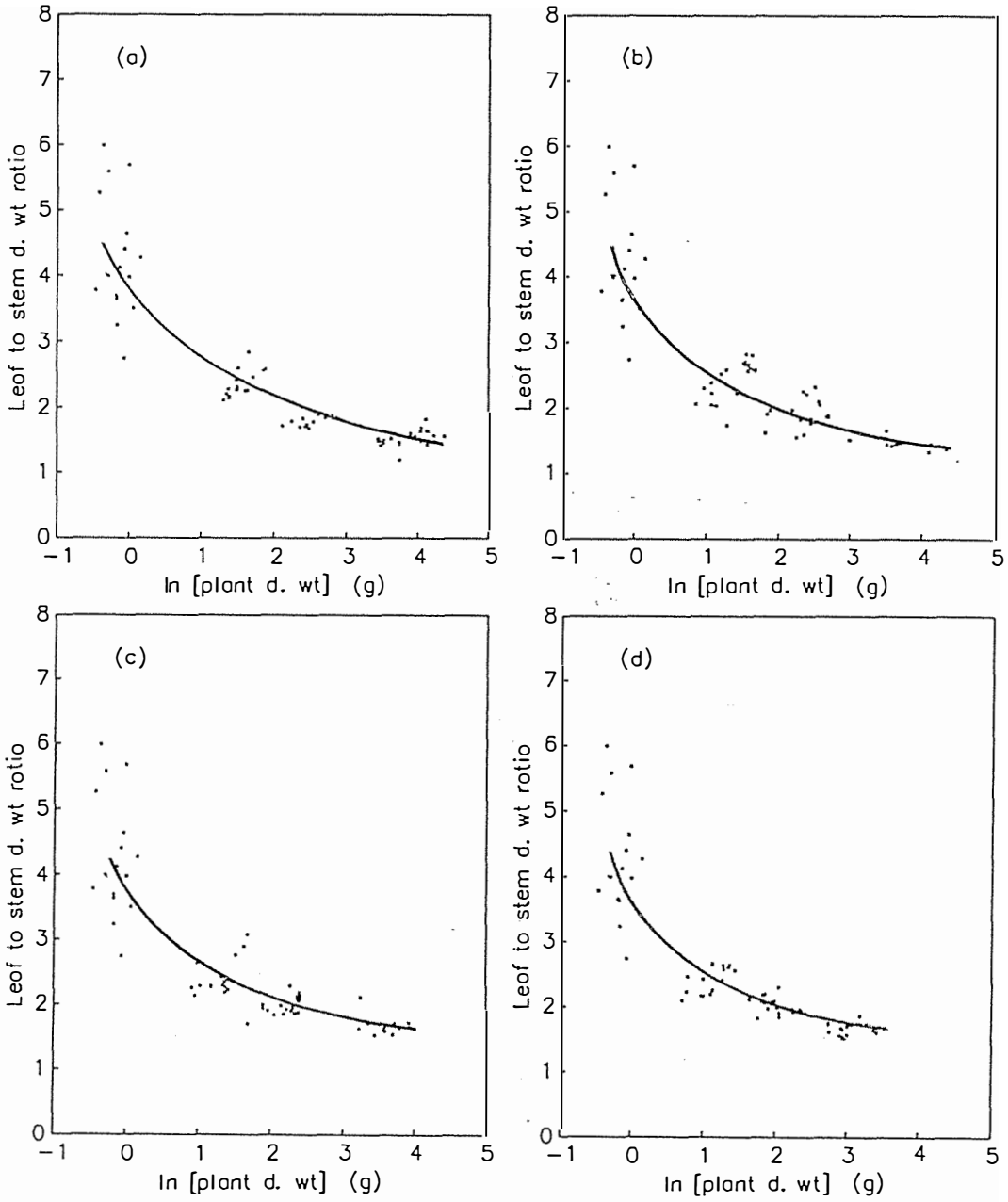

Fig. 9.4. The effect of different misting pump off-times in an aeroponic system on the relationship between the leaf to stem dry weight ratio and natural log transformed plant dry weight (curves fitted by eye). (a) 0.00 hour OT. (b) 0.17 hour OT. (c) 0.65 hour OT. (d) 1.55 hour OT.

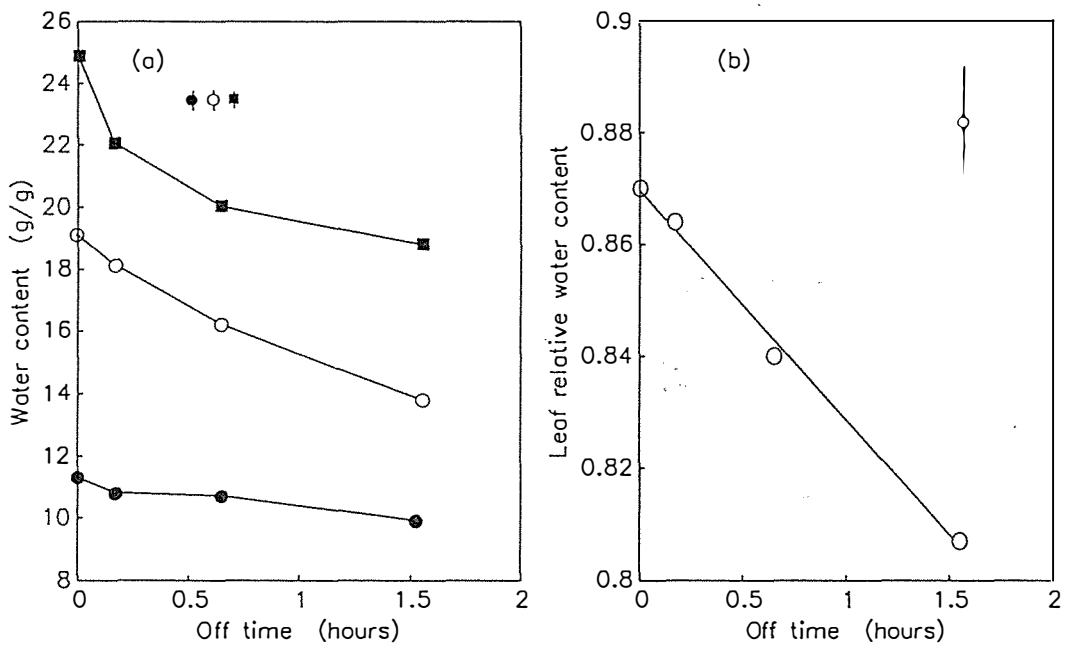

Fig. 9.5. The effect of different misting pump off-times in an aeroponic system on plant tissue water contents. Bars show pooled s.e.. (a) Plant part water contents (n = 8); ●, leaf; ○, stem; ■, root. (b) Leaf relative water content (n = 10); $Y = 0.870 - 0.041X$; $R^2 = 0.995^{**}$.

Increase in root length and root number with time were similar to those of leaf area and plant part dry weights (i.e. tending to exponential) (Fig. 9.7). Only SL3 plants were significantly smaller with respect to root length and number at harvests 2 and 3. Average root length declined steadily with time, after harvest 1, from 22 mm to 14 mm, but there were no significant treatment effects.

The relationships between root length and both root number and root dry weight (Fig. 9.7) showed linear trends. The exception occurred for root length *versus* root dry weight between harvest 2 and 3 when root length per unit dry weight decreases significantly for the three stress treatments. In harvests 3 and 4, root length per unit dry weight and root number per unit dry weight were significantly higher in the control and lower in the SL3 treatment. At no time was there a significant difference between SL1 and SL2 plants.

9.3.5 *LEAF-ROOT RELATIONSHIPS*

Linear trends were found for leaf area *versus* root length and leaf number *versus* root number (Fig. 9.8). Leaf area per unit root length was found to decrease linearly with misting pump off-time (Fig. 9.8). Leaf number per root did not show treatment differences or a uniform trend with off-time (Fig. 9.8).

9.3.6 *CANONICAL ANALYSIS ON PLANT PART DATA*

A canonical analysis was performed on harvests 1 and 3 using the following plant data:

i/ Leaf dry weight.
Stem dry weight.
Root dry weight.

ii/ Leaf dry weight.
Stem dry weight.
Root dry weight.
Leaf area.
Root length.

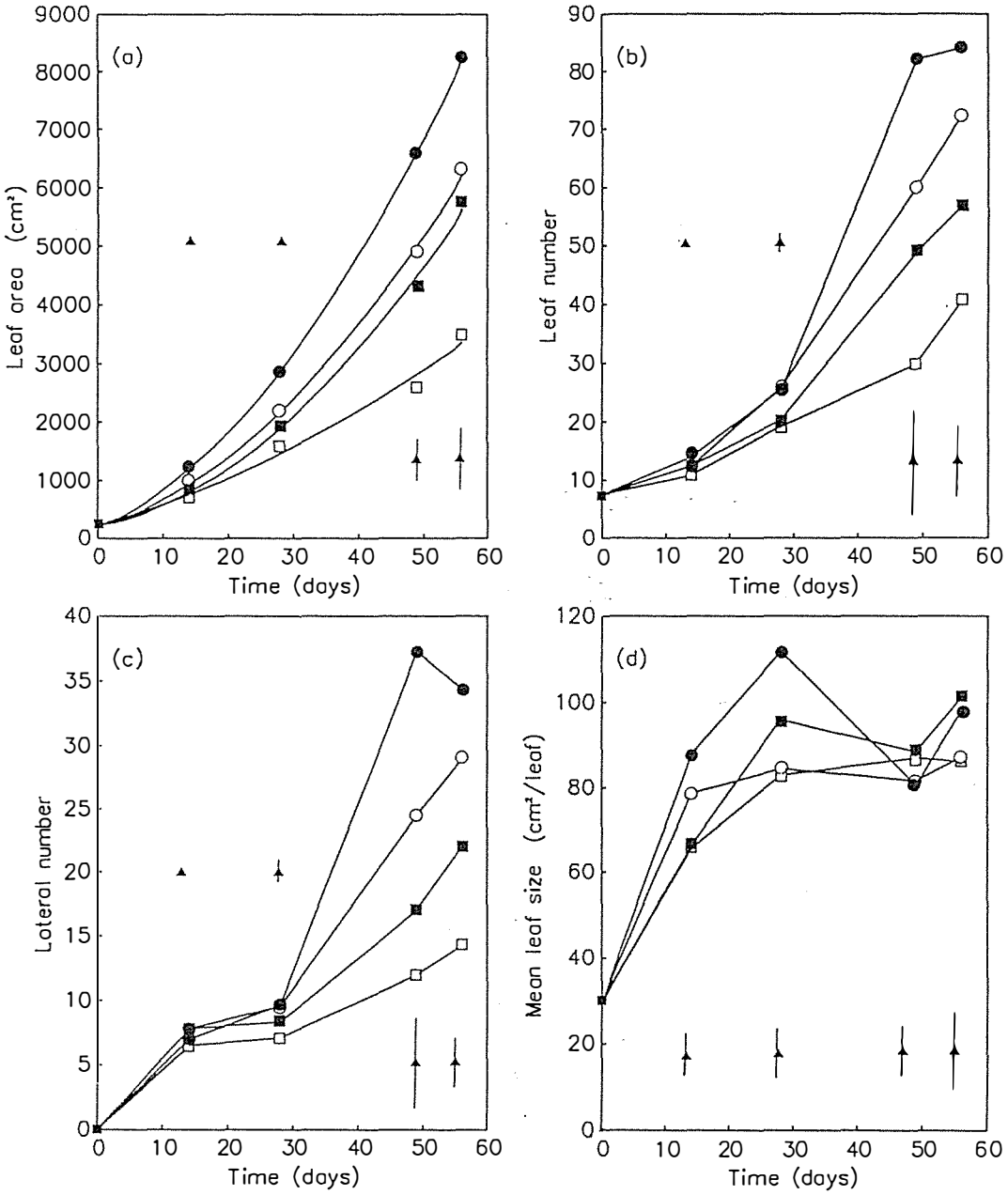

Fig. 9.6. The effect of different misting pump off-times in an aeroponic system on leaf parameters over time (n = 8). Bars show pooled s.e. for each harvest. (a) Leaf area: ●,0.00 hour OT; $Y = 232 + 44.6X + 1.75X^2$. ○,0.17 hour OT; $Y = 244 + 30.1X + 1.37X^2$: ■,0.65 hour OT; $Y = 243 + 21.1X + 1.34X^2$: □,1.55 hour OT; $Y = 222 + 32.0X + 0.43X^2$: $R^2 = 0.998^{**}$ for all equations. (b) Leaf number. (c) Lateral number. (d) Mean leaf size.

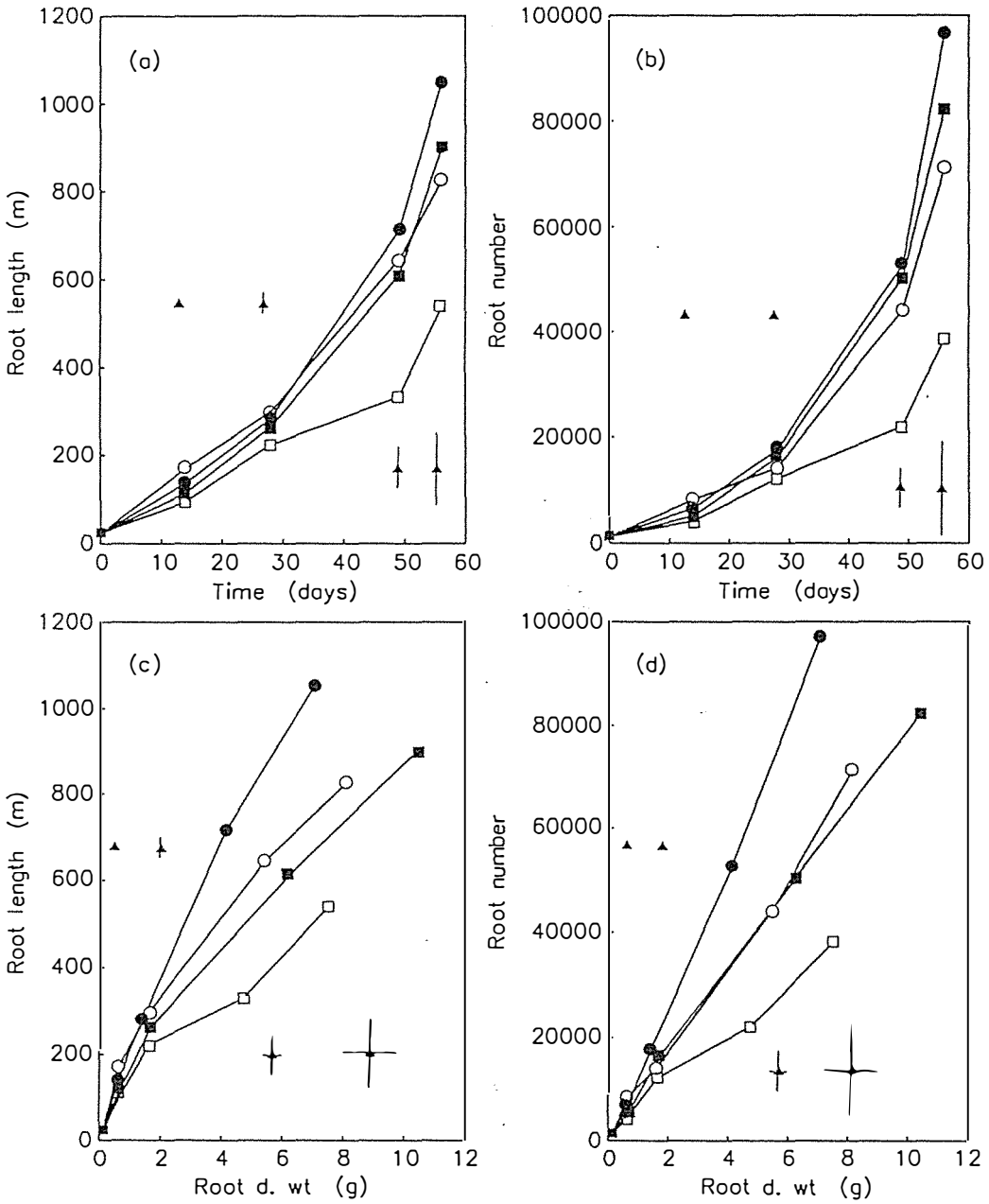

Fig. 9.7. The effect of different misting pump off-times in an aeroponic system on root parameters (n = 8). Bars show pooled s.e. of one (a and b) or two (c and d) variates for each harvest. (a) Root length *versus* time. (b) Root number *versus* time. (c) The relationship between root length and root dry weight. (d) The relationship between root number and root dry weight. ●, 0.00 hour OT; ○, 0.17 hour OT; ■, 0.65 hour OT; □, 1.55 hour OT.

Results are given in Tables 9.7 and 9.8 and shown graphically in Fig. 9.9. Only the first two canonical variables (canonical 1 and 2) were significant at each harvest, with canonical 1 being a 'general' discriminator and ranking treatments in order of decreasing off-time (see appendix 18.3 for more information on canonical analysis).

Considering the analysis involving only plant part dry weights, it can be seen that after 14 days treatments 1 and 2 were significantly different with respect to canonical 1. This canonical variable was highly correlated with leaf and stem dry weight and moderately (negatively) so with root dry weight (see between canonical structure). After 49 days, all treatments were significantly different and canonical 1 was very similar to that from the day 14 analysis, separating out treatments 1 and 2. Canonical 2 was also significant, separated treatments 3 and 4 and being predominantly correlated with root dry weight.

When leaf area and root length were introduced, a greater treatment separation was achieved although trends were basically similar. Leaf area responded identically to leaf dry weight reflecting the high correlation between them and the lack of treatment effects on specific leaf weight. However, root dry weight and root length were oppositely correlated to canonical 1 at both harvest times. This reflects a decrease in the root length to dry weight ratio.

9.3.7 *PLANT GROWTH ANALYSIS*

Average values for relative growth rate, absolute growth rate, unit leaf rate and unit shoot rate are shown in Fig. 9.10. The relative growth rate of stem and leaf decreased linearly with misting pump off-time. Root relative growth rate however was significantly higher for all three stress treatments, showing a maximum at SL2. Absolute growth rate appeared to be linearly related to off-time. Unit leaf rate and unit shoot rate were significantly lower only at SL3.

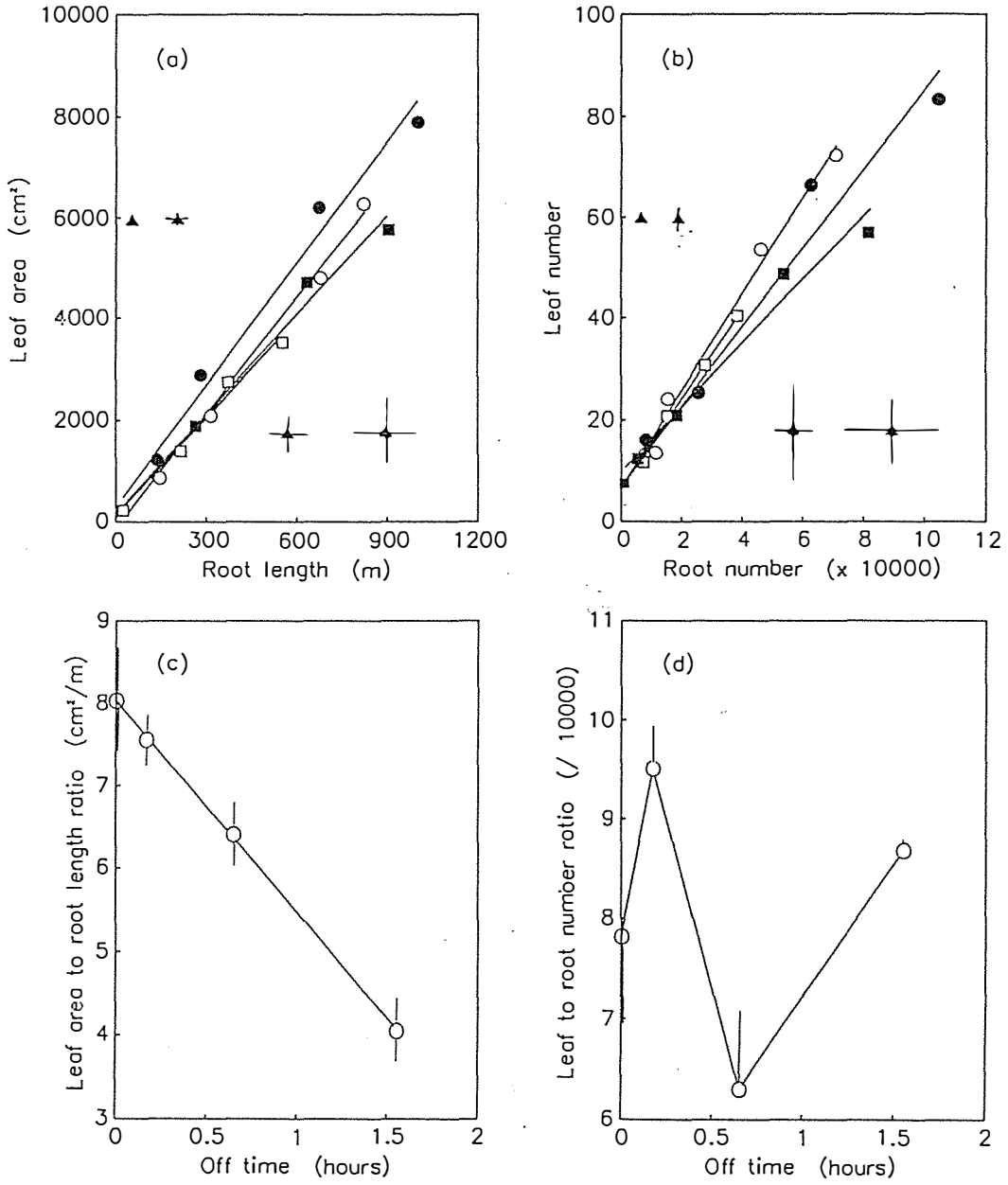

Fig. 9.8. The effect of different misting pump off-times in an aeroponic system on leaf-root relationships. For (a) and (b), bars show pooled s.e. of both variates at each harvest. For (c) and (d), bars show pooled s.e. of slope coefficient. (a) The relationship between leaf area and root length (n = 8): ●,0.00 hour OT; Y = 275 + 8.03X; $R^2 = 0.99^{**}$:○,0.17 hour OT; Y = -132 + 7.56X; $R^2 = 0.99^{**}$: ■,0.65 hour OT; Y = 159 + 6.53X; $R^2 = 0.99^{**}$:□,1.55 hour OT; Y = 121 + 6.41X; $R^2 = 0.99^{**}$. (b) The relationship between leaf number and root number (n = 8): ●,0.00 hour OT; Y = 6.93 + 0.00078X; $R^2 = 0.97^{**}$: ○,0.17 hour OT; Y = 6.67 + 0.00095X; $R^2 = 0.99^{**}$: ■,0.65 hour OT; Y = 9.82 + 0.00063X; $R^2 = 0.95^{*}$: □,1.55 hour OT; Y = 6.67 + 0.00087X; $R^2 = 0.99^{**}$. (c) The leaf area to root length ratio; Y = 8.03 - 2.56X; $R^2 = 0.999^{**}$. (d) The leaf number to root number ratio.

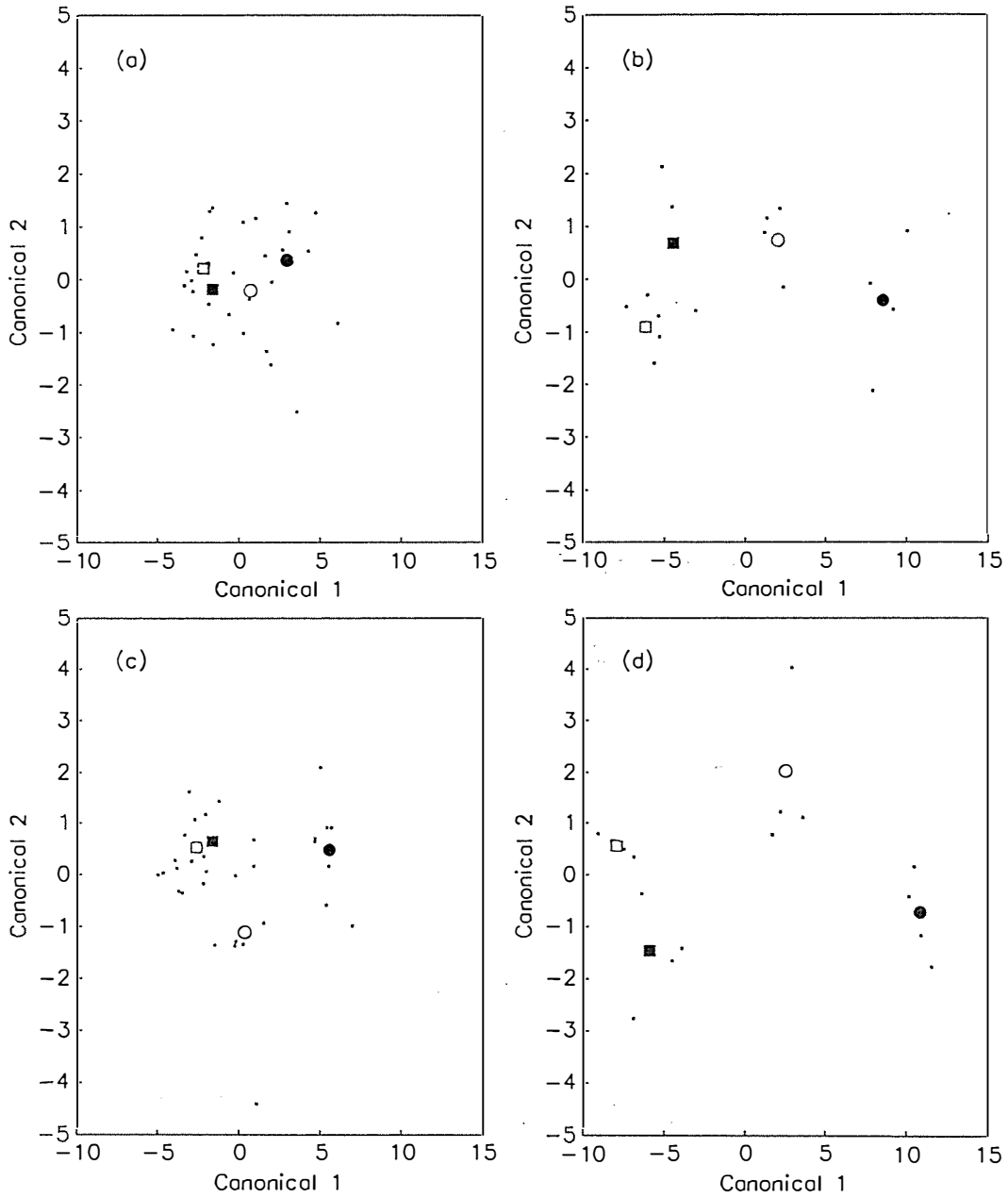

Fig. 9.9. Results from a canonical analysis for plants grown in an aeroponic system with different misting pump off-times. Symbols indicate class means on canonical variables; ●, 0.00 hour OT; ○, 0.17 hour OT; ■, 0.65 hour OT; □, 1.55 hour OT. For (a) (14 days) and (b) (49 days), variables = leaf, stem and root dry weight. For (c) (14 days) and (d) (49 days), variables = leaf, stem and root dry weight, leaf area and root length.

Table 9.7 Effect of different misting pump off-times in an aeroponic system on a canonical analysis - Between canonical structure

Harvest (days)	Canonical variable	Plant variable LDW	SDW	RDW	LA	RL
(a) Variables used = leaf, stem and root dry weight.						
14	1	0.99	0.99	-0.53	-	-
	2	-0.09	0.01	-0.43	-	-
49	1	0.93	0.95	-0.61	-	-
	2	0.35	0.30	0.77	-	-
(b) Variables used = leaf, stem and root dry weight, leaf area and root length.						
14	1	0.98	0.99	-0.47	0.99	0.55
	2	-0.17	-0.10	0.21	-0.06	-0.80
49	1	0.95	0.97	-0.56	0.95	0.79
	2	-0.09	0.02	-0.14	-0.24	-0.19

Table 9.8 Effect of different misting pump off-times in an aeroponic system on a canonical analysis - Canonical variable class means

Harvest (days)	Canonical variable	Treatment off-time (hours) 0.00	0.17	0.65	1.55	lsd
(a) Variables used = leaf, stem and root dry weight.						
14	1	3.70 a	0.90 b	-2.08 c	-2.53 c	0.61
	2	0.17 ns	-0.29 ns	-0.12 ns	0.24 ns	0.84
49	1	8.76 a	1.77 b	-4.66 c	-5.87 d	0.22
	2	-0.47 ab	0.80 a	0.65 ab	-0.98 b	0.71
(b) Variables used = leaf, stem and root dry weight, leaf area and root length.						
14	1	5.43 a	0.34 b	-2.30 c	-3.47 c	0.72
	2	0.47 ns	-1.24 ns	0.43 ns	0.33 ns	0.79
49	1	10.76 a	2.58 b	-5.44 c	-7.90 d	0.99
	2	-0.80 bc	1.79 a	-1.55 c	0.57 ab	0.70

Means with the same letter in each row are not significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)

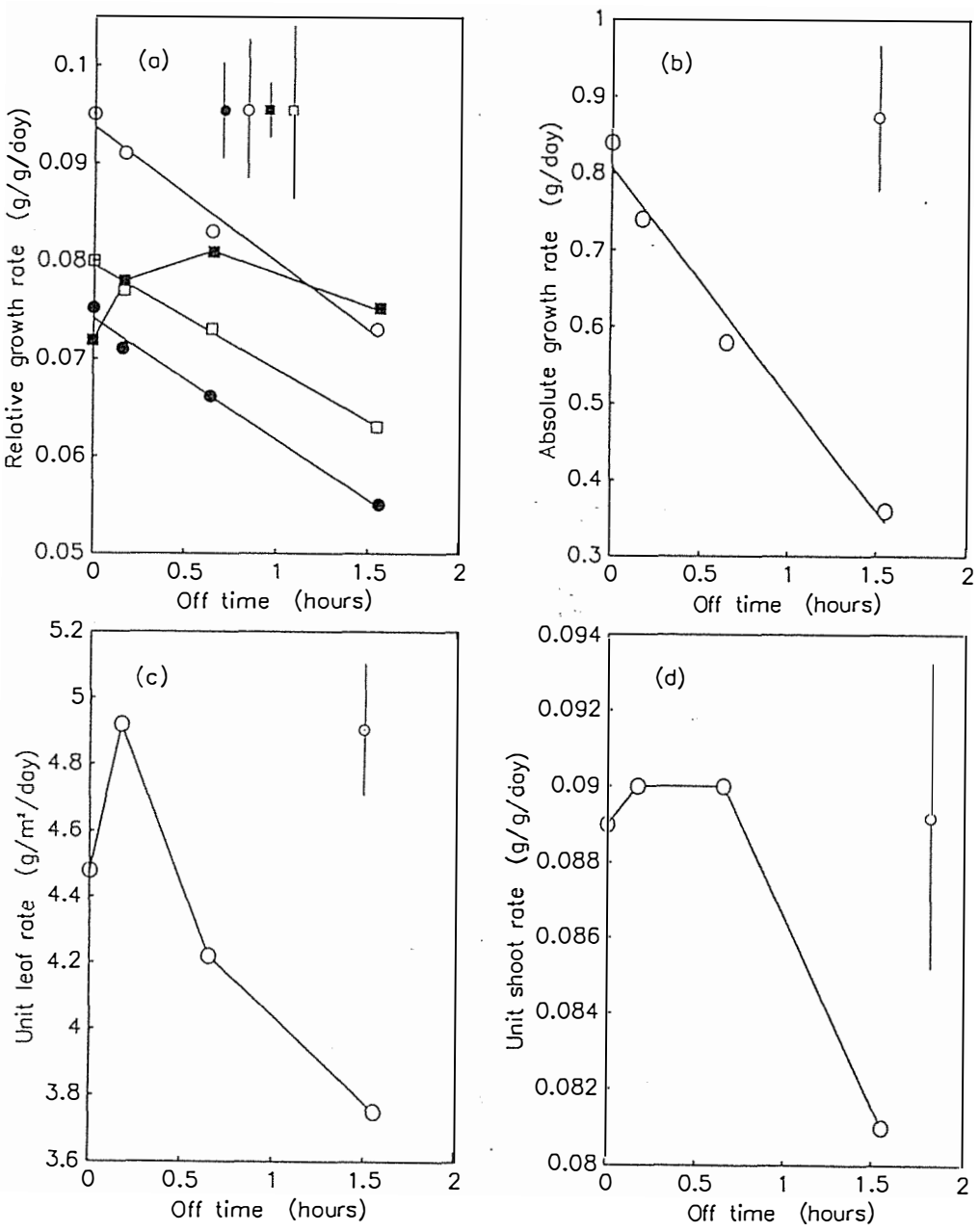

Fig. 9.10. The effect of different misting pump off-times in an aeroponic system on growth analysis parameters (n = 8). Bars show pooled s.e.. (a) ●, leaf RGR; $Y = 0.074 - 0.012X$; $R^2 = 0.99^{**}$:○, stem RGR; $Y = 0.094 - 0.014X$; $R^2 = 0.98^{**}$:■, root RGR:□, plant RGR; $Y = 0.080 - 0.011X$; $R^2 = 0.99^{**}$. (b) Absolute growth rate; $Y = 0.81 - 0.30X$; $R^2 = 0.98^{**}$. (c) Unit leaf rate. (d) Unit shoot rate.

9.3.8 *THE ALLOMETRIC RELATIONSHIP*

Allometric relationships (see section 4.5.5) for shoot versus root dry weights are given in Fig. 9.11 and all appear to be highly linear. Plots of the S/R ratio *versus* natural logarithm transformed plant dry weight are given in Fig. 9.12. For a discussion on these plots see section 9.4.8 and 14). Trends in the S/R ratio *versus* ln(PtDW) plots were linear, with the slopes showing very similar proportional changes to those of the allometric relationship (Fig. 9.13). Also shown on the same plot in Fig. 9.13 is the calculated ratio of RGR(shoot) to RGR(root), which again is in close agreement.

9.3.9 *PLANT HEIGHT*

Mean plant height measurements over the experimental period are shown in Fig. 9.14. They follow a strong linear trend with time (R^2 greater than 0.994 for all treatments). Growth rate, as measured in terms of increase in plant height per day, was linearly related to the misting pump off-time.

9.3.10 *WATER USE*

The SL1, SL2 and SL3 plants used 18%, 36% and 45% less water per m^2 leaf area per day than the control plants from estimates derived from tank water loss (average transpiration rate). Noon transpiration measurements were far more sensitive to off-time, being reduced by 80% at SL3. Consequently the ratio of noon transpiration to average transpiration decreased by over 50% for SL3 plants (Table 9.9).

Water use efficiency was significantly higher for all three stress treatments but peaked at SL2 (WUE = 7.23 mg l^{-1}) (Fig. 9.15). Plant resistance, calculated from Ψ_W(leaf) divided by E, was reduced by over 85% at SL3 (Fig. 9.16).

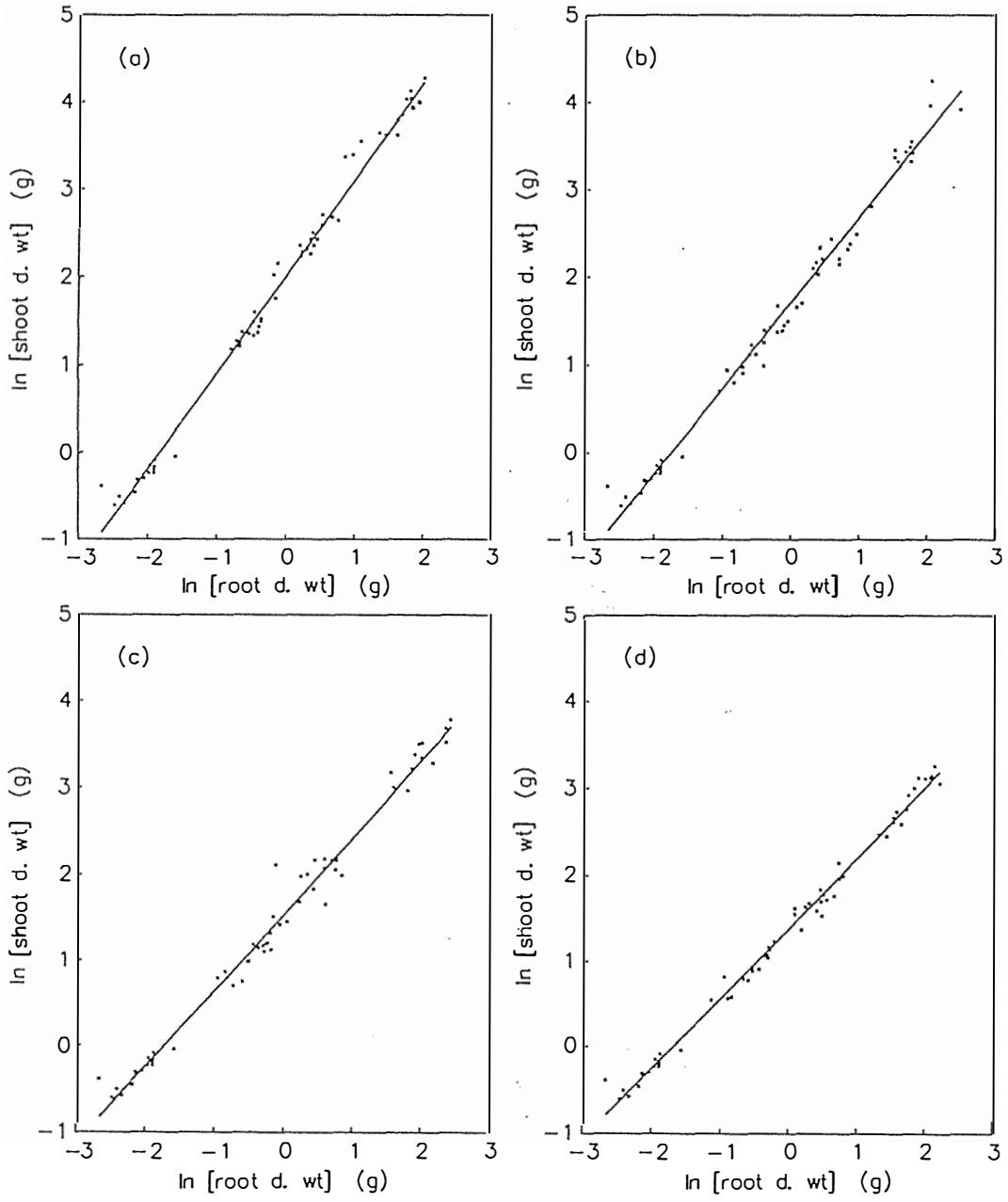

Fig. 9.11. The effect of different misting pump off-times in an aeroponic system on the allometric relationship. (a) 0.00 hour OT; $Y = 2.00 + 1.10X^{a}$; $R^2 = 0.990^{**}$. (b) 0.17 hour OT; $Y = 1.70 + 0.98X^{b}$; $R^2 = 0.986^{**}$. (c) 0.65 hour OT; $Y = 1.53 + 0.89X^{c}$; $R^2 = 0.983^{**}$. (d) 1.55 hour OT; $Y = 1.38 + 0.82X^{d}$; $R^2 = 0.992^{**}$. Letters on slope coefficients indicate significant differences at the 5% level using a paired t-test.

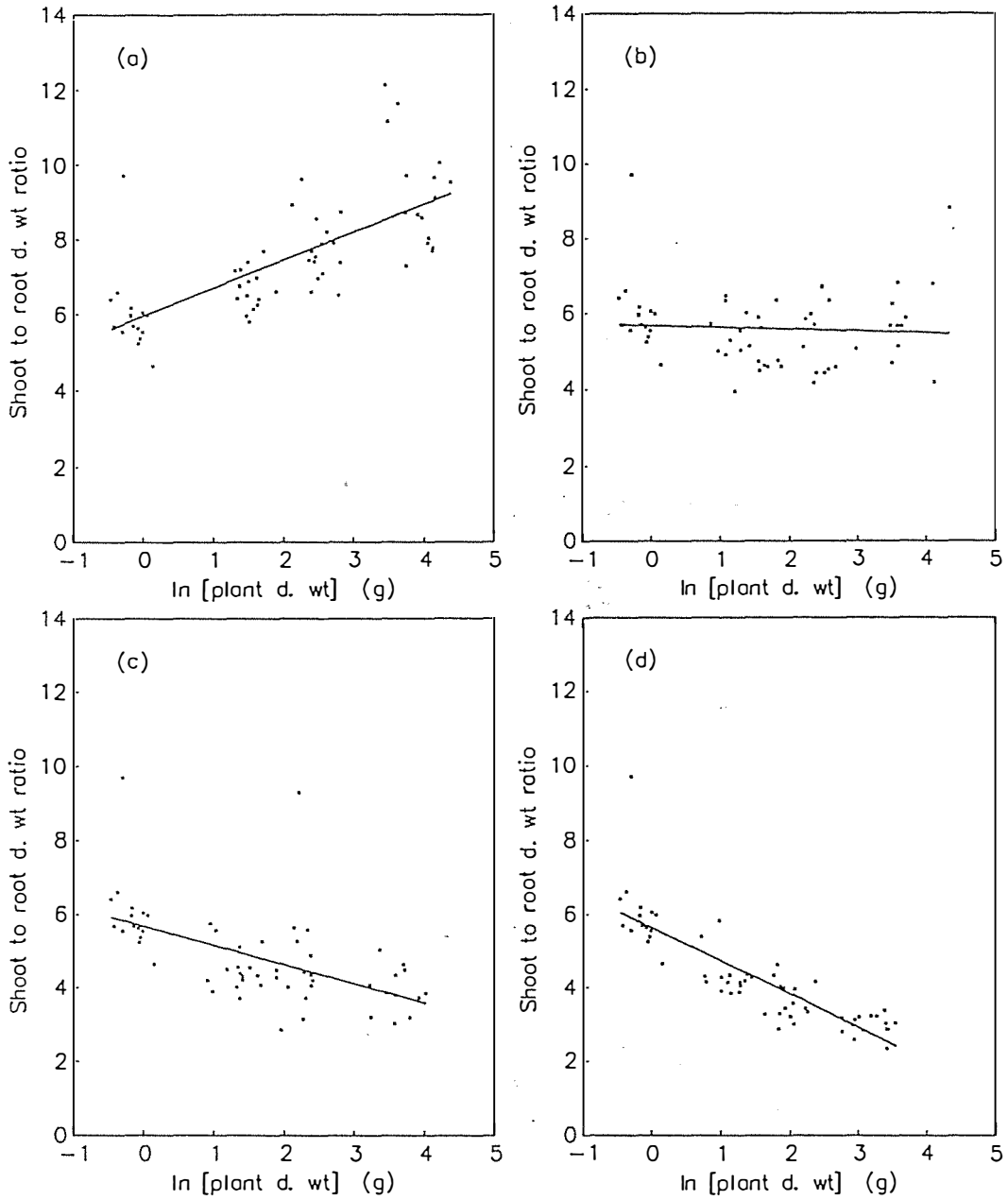

Fig. 9.12. The effect of different misting pump off-times in an aeroponic system on the relationship between the shoot to root dry weight ratio and natural log transformed plant dry weight. (a) 0.00 hour OT; $Y = 5.96 + 0.75X$; $R^2 = 0.50^{*}$. (b) 0.17 hour OT; $Y = 5.68 - 0.05X$; $R^2 = 0.00^{ns}$. (c) 0.65 hour OT; $Y = 5.68 - 0.53X$; $R^2 = 0.32^{ns}$. (d) 1.55 hour OT; $Y = 5.62 - 0.91X$; $R^2 = 0.73^{*}$.

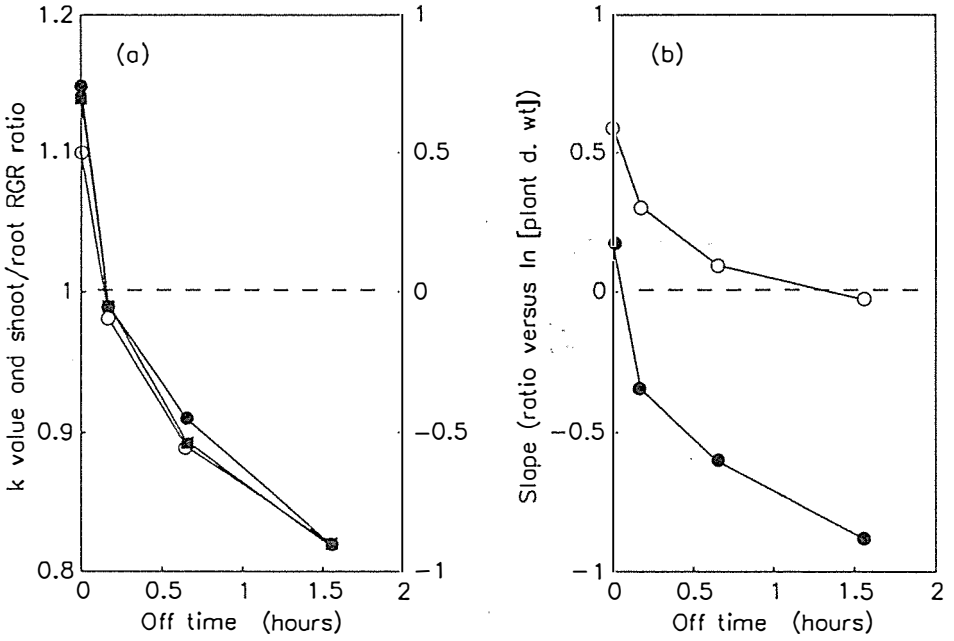

Fig. 9.13. The effect of different misting pump off-times in an aeroponic system on assimilate partitioning parameters. (a)●, the shoot to root relative growth rate ratio:○, the allometric k value: ■, the slope of the shoot/root ratio *versus* natural log transformed plant dry weight relationship. (b) Slope of the plant part ratio *versus* natural log transformed plant dry weight relationship; ●, leaf to root ratio; ○, stem to root ratio.

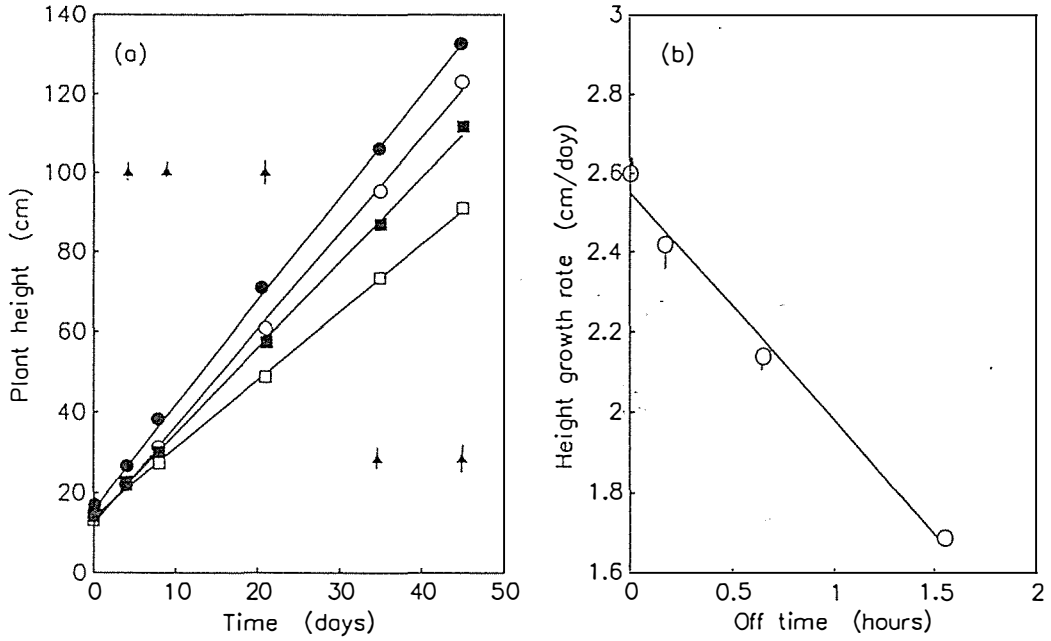

Fig. 9.14. The effect of different misting pump off-times in an aeroponic system on plant height and height growth rate. (a) Plant height *versus* time (n = 24) (Bars show pooled s.e. for each harvest): ●,0.00 hour OT; Y = 15.7 + 2.60X; $R^2 = 0.999^{**}$:○,0.17 hour OT; Y = 12.2 + 2.42X; $R^2 = 0.999^{**}$: ■,0.65 hour OT; Y = 13.1 + 2.14X; $R^2 = 0.999^{**}$: □,1.55 hour OT; Y = 14.1 + 1.69X; $R^2 = 0.999^{**}$. (b) Plant height growth rate. Bars show s.e. of slope coefficients; Y = 2.55 - 0.567X; $R^2 = 0.99^{**}$.

Table 9.9 Effect of different misting pump off-times in an aeroponic system on water use as measured in terms of experiment long water use (WU) (n = 30) and instantaneous transpiration rate at noon (E) (n = 12)

Off-Time (hours)	Water use (WU) ($l\ plt^{-1}\ d^{-1}$)	($l\ m^{-2}\ d^{-1}$)	E ($l\ m^{-2}\ h^{-1}$)	E/WU[a]
0.00	0.31 a	1.04 a	0.47	10.8 a
0.17	0.21 b	0.85 b	0.38	10.7 a
0.65	0.13 c	0.67 c	0.23	8.2 b
1.55	0.09 d	0.58 d	0.10	4.1 c
s.e. mean	0.01	0.04	0.02	0.6

Means with the same letter in each column are not significantly different at the 1% level using the lsd discriminator

[a] Both variables in units of $l\ m^{-2}\ d^{-1}$

9.3.11 *LEAF WATER POTENTIAL*

Mean leaf water potentials at noon were reduced significantly by each increment in stress level (Fig. 9.15). No significant differences were found in the dawn leaf water potential values.

9.3.12 *PHOTOSYNTHETIC SYSTEM DATA - GLASSHOUSE*

Photosynthetic parameters from various measurement days and times are given in Table 9.10. Photosynthetic rate, internal CO_2 concentration, stomatal conductance and transpiration rate were all significantly reduced for each increment in water stress (mean day values) (Fig. 9.17). Adjustment of C_i for patchy stomatal closure (Downton *et al.*, 1988a: see section 3.1.3) led to a more rapid decline (Fig. 9.17).

Two days measurements which were not included in the mean calculations are given for comparison. Day 30 was overcast with PAR levels of only 180 $\mu mol\ m^{-2}\ s^{-1}$. In this case, as in experiment 2 under low PAR, treatment differences in photosynthetic rate were not expressed. Day 21 was hot and sunny and a breakdown in the ventilation system accentuated the conditions. Stomatal conductance values

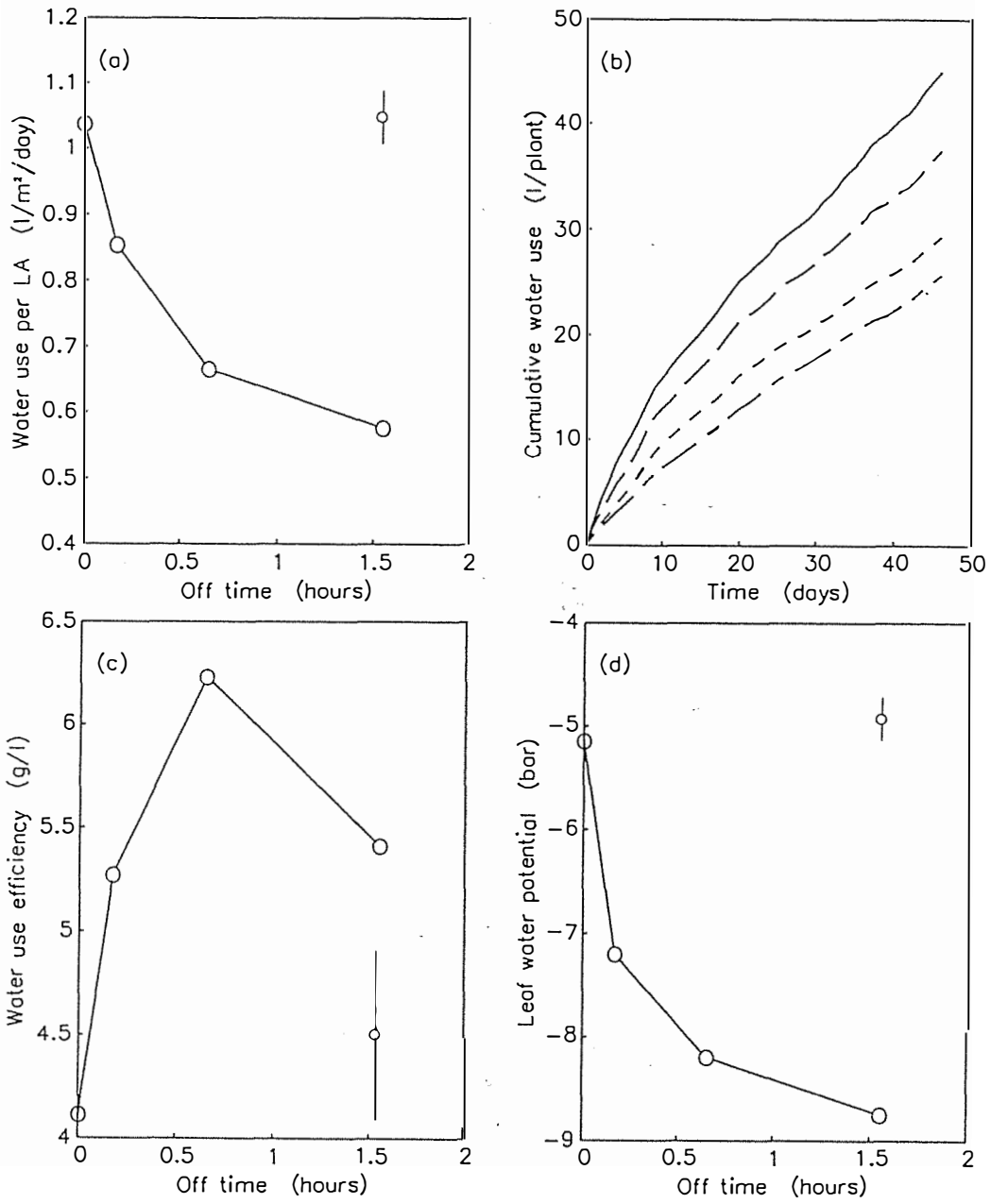

Fig. 9.15. The effect of different misting pump off-times in an aeroponic system on water use parameters and leaf water potential. Bars show pooled s.e.. (a) Water use per unit leaf area (n = 49). (b) Cumulative water use per plant *versus* time; ( ), 0.00 hour OT; ( ), 0.17 hour OT; ( ), 0.65 hour OT; ( ), 1.55 hour OT. (c) Water use efficiency (n = 49). (d) Leaf water potential measured at 12-1 pm. Each point is the mean of 30 measurements (6 readings per day from 5 days).

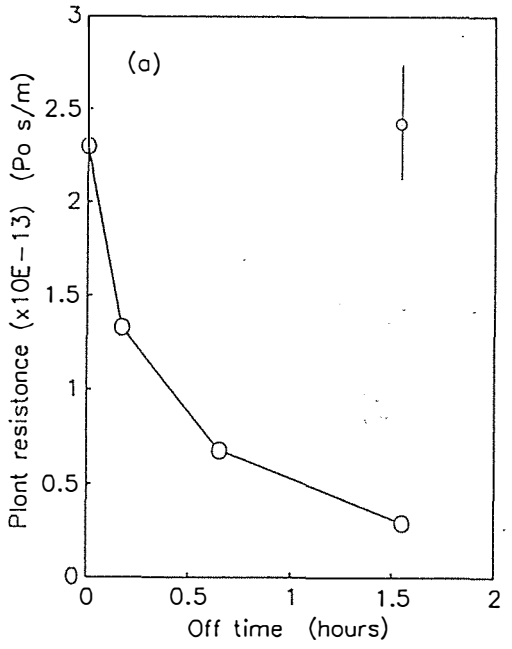

Fig. 9.16. The effect of different misting pump off-times in an aeroponic system on plant resistance ((Ψ_W(leaf) - Ψ_W(solution)) / E) (n = 30). Bar shows pooled s.e..

were very low for all treatments and with moderate photosynthetic rates, internal CO_2 levels were also depressed. Trends with off-time were similar however to those of the mean values.

No significant differences were found in any of the four variables under darkness conditions.

Table 9.10 Effect of different misting pump off-times in an aeroponic system on various leaf parameters

Day[a] (PAR)[b]	Off-Time (hours)	Pn (μmol) (m^{-2} s^{-1})	gs (mol) (m^{-2} s^{-1})	C_i (ppm)	C_i'[d] (ppm)	E (mmol) (m^{-2} s^{-1})
Day	0.00	14.4 a	0.38 a	294 a	[294]	7.3 a
means	0.17	13.4 a	0.31 b	257 b	[242]	5.8 b
(>900)	0.65	9.2 b	0.17 c	232 c	[166]	3.5 c
	1.55	6.8 c	0.05 d	204 d	[123]	1.5 d
s.e. mean		0.7	0.01	5		0.1
Cloud	0.00	2.7 ns	0.46 a	349 ns	[349]	5.3 a
(180)	0.17	3.5 ns	0.37 b	337 ns	[422]	4.5 b
	0.65	3.0 ns	0.31 c	333 ns	[344]	3.7 c
	1.55	3.9 ns	0.19 d	317 ns	[435]	2.9 d
s.e. mean		0.3	0.01	3		0.1
Sun	0.00	9.2 a	0.105 a	198 ns	[198]	2.72 a
(1400)	0.17	8.4 b	0.092 b	193 ns	[180]	2.31 b
	0.65	6.3 c	0.067 c	190 ns	[146]	1.76 c
	1.55	4.7 d	0.045 d	188 ns	[121]	1.25 d
s.e. mean		0.2	0.002	2		0.05
Night	0.00	-1.4 ns	0.28 ns	488 ns	-	2.3 ns
means[c]	0.17	-1.5 ns	0.26 ns	484 ns	-	2.7 ns
(0)	0.65	-1.2 ns	0.26 ns	490 ns	-	2.3 ns
	1.55	-1.4 ns	0.33 ns	486 ns	-	2.6 ns
s.e. mean		0.2	0.02	46		0.2

Means with the same letter in each column are not significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)

[a] Day means from days 10, 20 and 35 (n = 36); Cloud day was day 30 (n = 12); Sun day was day 21 (n = 12); Night means from days 25 and 35 (n = 24)

[b] Units of μmol m^{-2} s^{-1}

[c] Night photosynthetic value represents respiration rate

[d] C_i adjusted according to the equation of Downton *et al.* (1988a) (see equation 3.3 of chapter 3)

9.3.13 *PHOTOSYNTHETIC SYSTEM DATA - LABORATORY*

An example of the laboratory data collected for single leaflets is given in Fig. 9.18 and several features are worthy of noting. Firstly the Pn *versus* C_i curve conforms to the usual trend, with an initial linear portion followed by a flattening off at higher C_i. The point of greatest change in slope occurs at a C_i corresponding to that when the atmospheric CO_2 concentration is at ambient. It was found that linearity was only evident up to a C_i of 102 ± 10 ppm and this point did not differ significantly between treatments. There were however significant differences in the slope of these lines (k_{int}). Water stress increased k_{int} although a difference was not found between SL1 and SL2 (Table 9.11). The CO_2 compensation points (X-intercept of linear portion) also increased slightly with stress level.

The most obvious feature of the C_i *versus* g_s curve (Fig. 9.18) was an abrupt increase in g_s at a C_i of around 200 ppm. Again this corresponds to ambient CO_2 concentrations. Above a C_i of 200 ppm there was little further decrease in g_s.

A feature evident in all Pn *versus* g_s plots was a step like appearance. This occurred due to a step-wise closure of stomatal aperture.

Table 9.11 Effect of different misting pump off-times in an aeroponic system on the CO_2 compensation point and k_{int} (n = 12)

Off-Time (hours)	CO_2 compensation point (ppm)	k_{int} (mol m^{-2} s^{-1})
0.00	47.5 b	0.058 a
0.17	48.0 b	0.067 b
0.65	50.0 a	0.065 b
1.55	50.2 a	0.075 c
s.e. mean	0.8	0.004

Means with the same letter in each column are not significantly different at the 1% level using the lsd discriminator

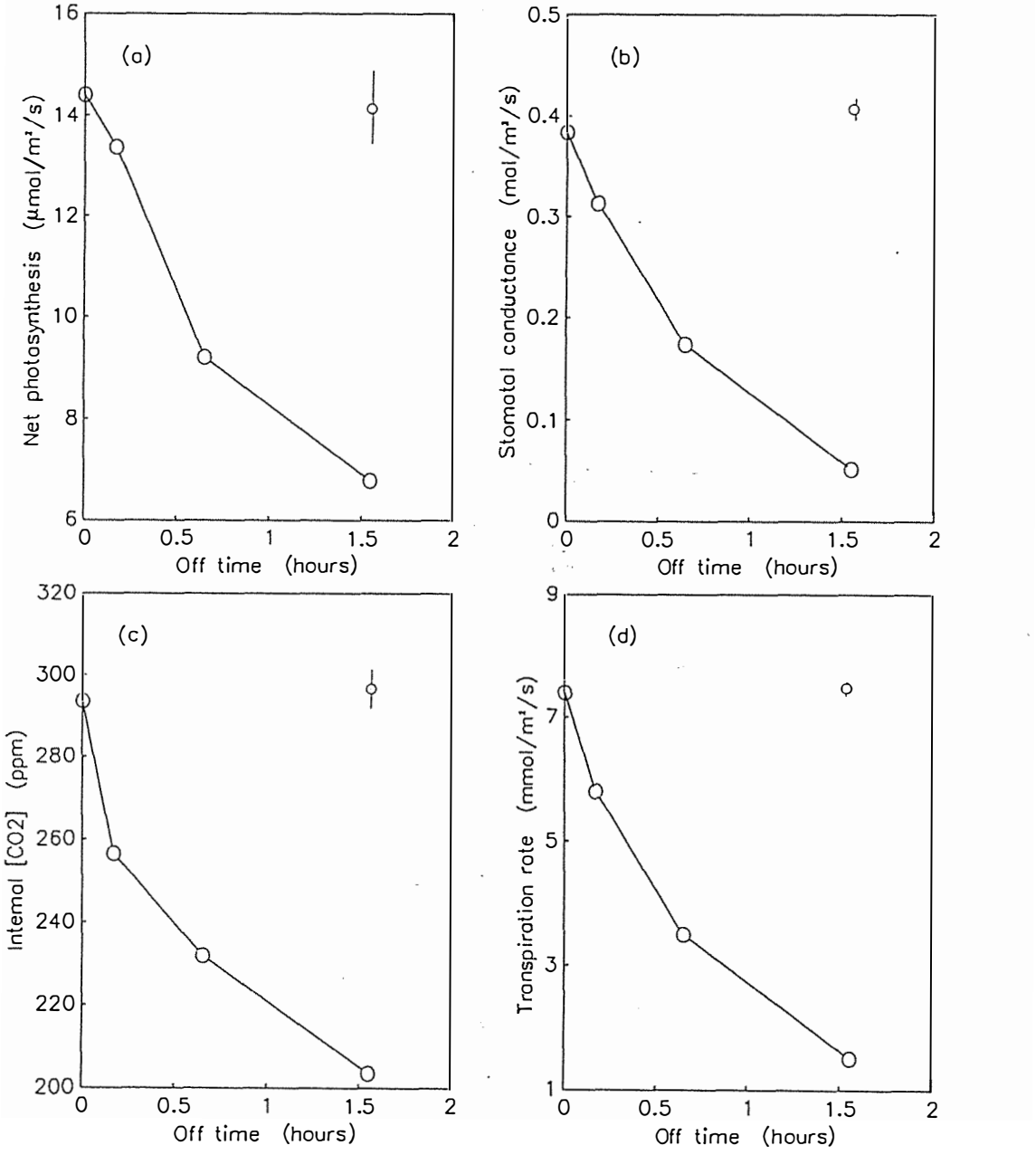

Fig. 9.17. The effect of different misting pump off-times in an aeroponic system on leaf parameters. Each point is the mean of 60 measurements (12 per day from 5 days). Bars show pooled s.e.. (a) Net Photosynthesis. (b) Stomatal conductance. (c) Internal CO_2 concentration. (d) Transpiration rate.

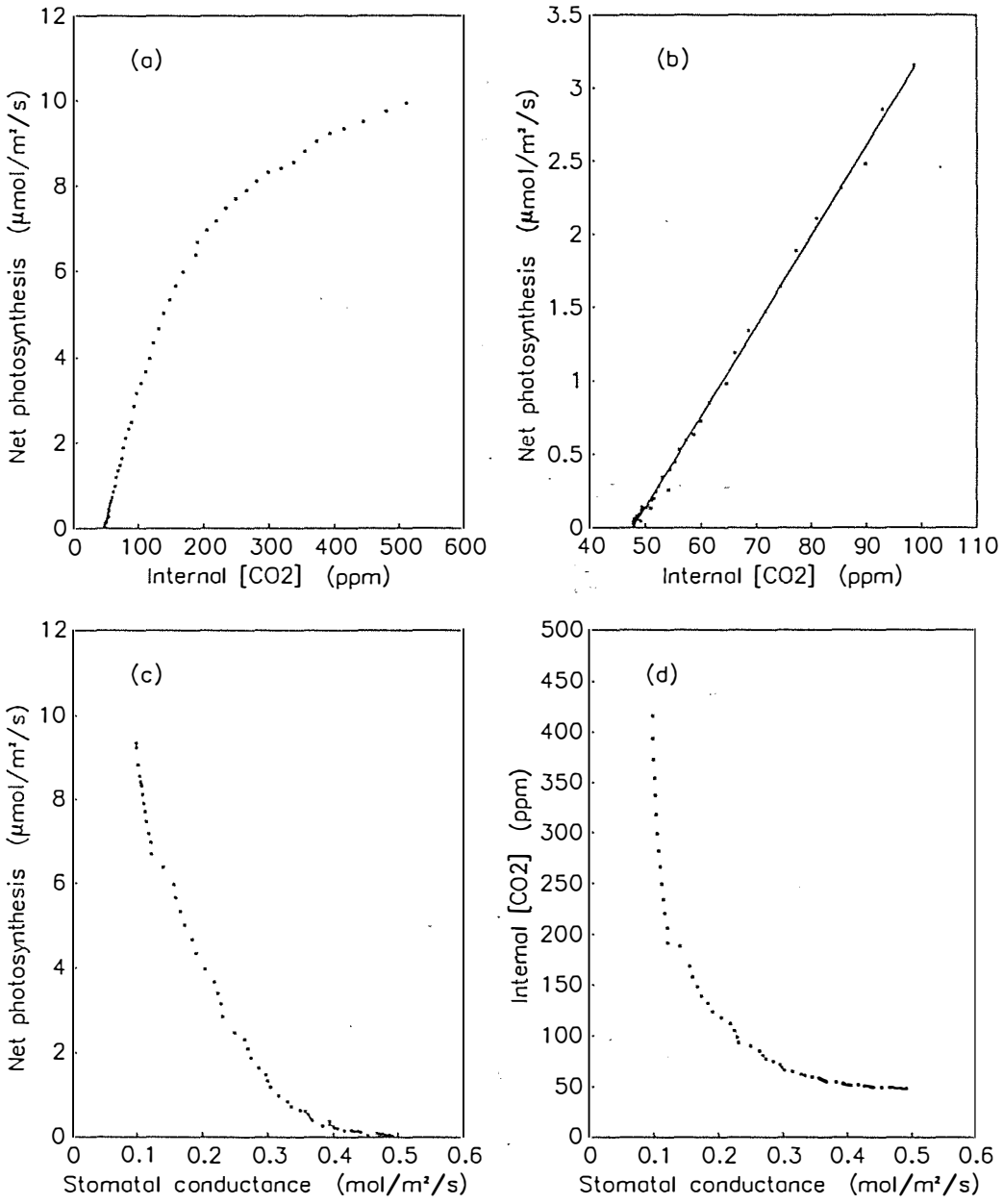

Fig. 9.18. An example of results collected under laboratory conditions for tomato leaves exposes to PAR = 400 µmol $m^{-2}s^{-1}$ within a sealed cuvette. Air CO_2 concentration was initially 800 ppm. For (b); Y = -2.94 + 0.062X; $R^2 = 0.997^{**}$.

9.3.14 *CHLOROPHYLL ANALYSIS*

All pigment concentrations increased with increasing stress level such that linear trends were evident with misting pump off-time (Fig. 9.19). The chlorophyll a/b ratio was higher for all three stress treatments but showed a partial decline at SL3.

9.3.15 *PRESSURE-VOLUME CURVE ANALYSIS*

Pressure-volume curve analysis (appendix 18.14) revealed that a decrease in osmotic potential at full turgidity occurred in both the leaves and roots under a water stress (Table 9.12). For leaf tissue there was a concomitant decrease in the RWC at turgor loss (RWC_o).

Table 9.12 Effect of different misting pump off-times in an aeroponic system on pressure-volume curve parameters for either leaflets or whole root systems determined using the bench drying method (appendix 18.14)[a]

Plant part	Off-Time (hours)	RWC_o	X	Ψ_S(sat) (bar)	a (bar^{-1})
Leaf	0.00	0.85 a	0.65 ns	-6.5 a	13.3 ns
	0.17	0.83 ab	0.72 ns	-8.5 a	12.0 ns
	0.65	0.81 b	0.63 ns	-9.2 b	10.9 ns
	1.55	0.72 c	0.61 ns	-9.5 c	9.0 ns
s.e. mean		0.01	0.04	0.4	2.0
Root	0.00	0.81 ns	0.15 ns	-3.8 a	10.5 ns
	1.55	0.80 ns	0.10 ns	-6.0 b	11.1 ns
s.e. mean		0.05	0.10	0.5	2.0

Means with the same letter in each column are not significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)

[a] For leaves, n = 12; for roots, n = 4.

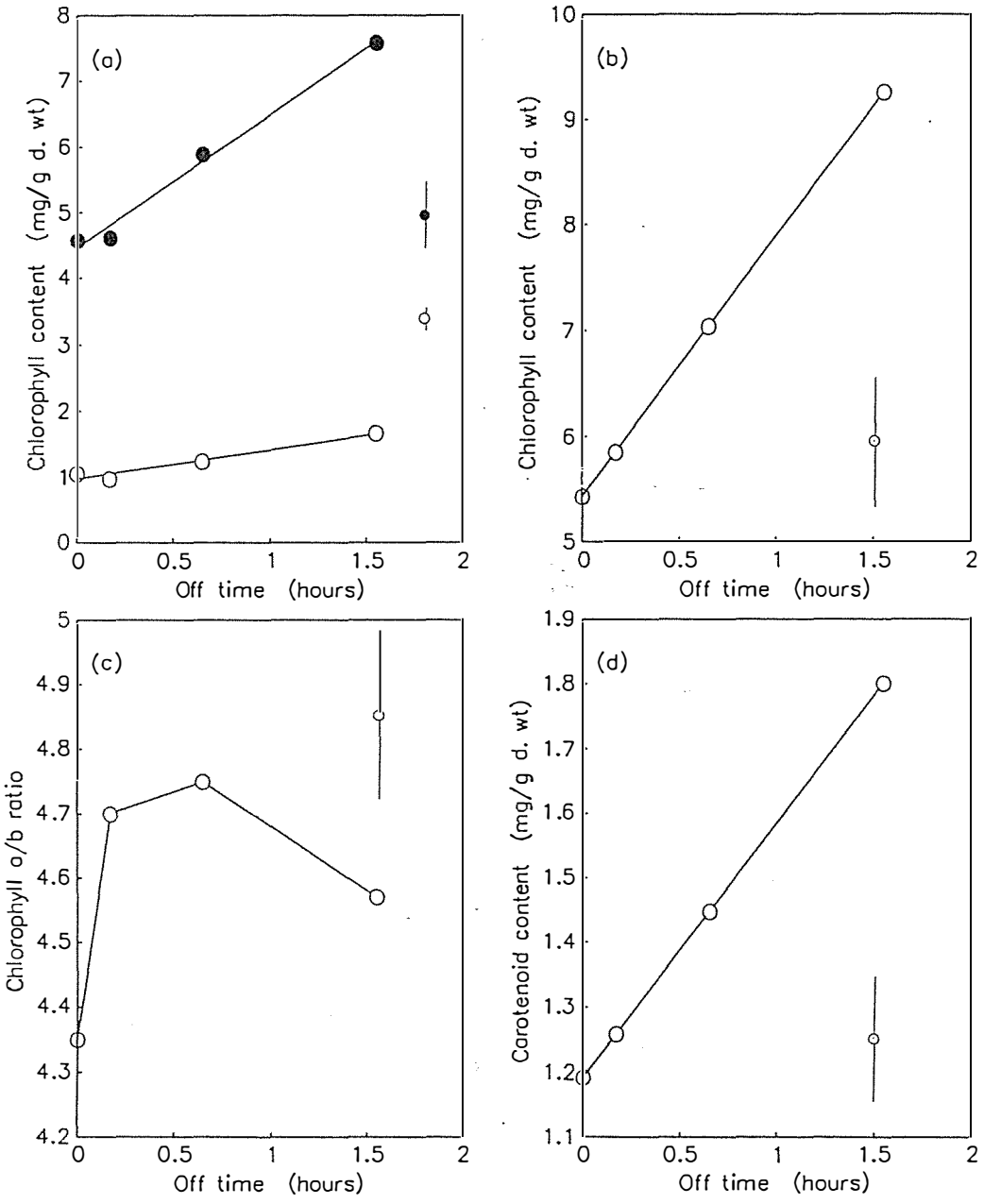

Fig. 9.19. The effect of different misting pump off-times in an aeroponic system on leaf pigment levels after 50 days. Each point is the mean of 16 measurements (2 samples per leaf from 2 leaves per plant off 4 plants). Bars show pooled s.e.. (a) ●, Chlorophyll a; Y = 4.46 - 2.03X; $R^2 = 0.99^{**}$: ○, Chlorophyll b; Y = 0.98 - 0.43X; $R^2 = 0.97^{*}$. (b) Total chlorophyll; Y = 5.43 - 2.47X; $R^2 = 0.99^{**}$. (c) Chlorophyll a/b ratio. (d) Carotenoid content; Y = 1.19 - 0.39X; $R^2 = 0.97^{*}$.

9.3.16 *FOLIAR NUTRIENT ANALYSIS*

No significant differences were found in the leaf levels of potassium, magnesium and calcium between the water stress treatments (Table 9.13). This indicates that nutrient deficiency did not cause any of the observed differences in growth and development. Levels were close to the range considered by Dorofaeff (1981) to be optimal for vegetative growth.

Table 9.13 Effect of different misting pump off-times in an aeroponic system on potassium, magnesium and calcium levels in leaf tissue (mg g^{-1} d. wt) after 49 days (n = 4)

Off-Time (hours)	Potassium	Magnesium	Calcium
0.00	39.0 ns	4.3 ns	29.0 ns
0.13	37.3 ns	4.0 ns	27.5 ns
0.52	34.9 ns	3.5 ns	26.9 ns
2.00	35.0 ns	4.8 ns	25.8 ns
s.e. mean	3.3	1.0	3.1
Ideal[a]	40-50	4-6	15-25
Deficiency[a]	< 30	< 2	< 10

Means with the same letter in each column are not significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)
[a] From Dorofaeff (1981)

9.4 **DISCUSSION**

9.4.1 *THE INTERMITTENT MISTING SYSTEM*

The use of intermittent misting to generate a controlled water stress eliminates the problems which occur with osmotic agents, the principle ones of which are plant uptake and contamination (Krizek, 1985) (see also experiment 1). It is a simple yet unique variation on the withholding water technique which has and will continue to be the fundamental means of water stressing plants (Krizek, 1985). The important difference is in the level of control. When water is withheld from a potted plant, water content and potential changes which occur in the root zone are hard to define. Only bulk parameters are generally used such as change in pot weight. Even

the use of an instrument such as the neutron probe is not ideal due to variability within a limited volume of media.

With intermittent misting in an aeroponic system the level of watering is precisely controlled and can be repeated without uncertainty. The unknown factor however is the exact relationship between misting pump off-time and plant growth parameters, to which this experiment addressed itself.

9.4.2 *PLANT HEIGHT*

The simple measurement of total plant height showed an unexpectedly strong linear relationship with time (R^2 above 99.4% for all treatments) (Fig. 9.14). This is surprising for two reasons. Firstly, the plants were growing approximately exponentially with respect to dry weight increase (Fig. 9.1) and secondly, water stress had a large effect on lateral shoot development. After 56 days, lateral shoot dry weight was 25 g for the control and only 5 g for SL3 plants. The linear relationship indicates that activity of the apical meristem was constant over the experimental period and not affected significantly by lateral development. The same strong linear trends were observed in experiment 1 using PEG although in this case laterals were removed. In both experiments the slopes of the linear trends could be related in a linear fashion to the variable representing the level of stress. That is, solution Ψ_W and off-time for experiments 2 and 3 respectively.

These results suggest that for tomato, total plant height during vegetative growth is a simple yet useful measure of growth response under stress. It has been used as a general growth parameter for tomato by other workers (e.g. Coleman and Greyson (1976)).

Change in plant height with time has been found to be useful in other areas. Bacci *et al.* (1985) observed a sharp change in the slope of the height-time relationship when maize plant growth switched from exponential to linear. This point of growth change is hard to locate using other parameters such as total dry matter. In this experiment such a change was not observed, primarily because plants were maintained in a vegetative state.

9.4.3 *SHOOT GROWTH*

The major contribution to a change in shoot development with water stress came from an increase in apical dominance. This was indicated by the lateral shoot dry weight figures given above. Both lateral shoot number and percentage dry weight in laterals were reduced by more than 50% after 56 days in the SL3 plants. The critical period occurred between days 28 and 49 when lateral shoots proliferated rapidly in the control plants. Steinberg *et al.* (1990) found for peach that decreased dry matter production under water stress was related to the drop in shoot extension growth and leaf production.

Both the initiation and early growth of shoot tissue is said to be consistent with assimilate supply limitation (Patrick, 1988). This can be related to low levels of hormone production by the vegetative meristem and poorly developed vascular stands (Patrick, 1988). With a reduction in productivity under water stress the plant cannot support as many active meristematic regions. Hence reduced lateral growth could be attributed to lack of assimilate supply. However, the 'nutritive theory' of apical dominance is not generally supported (McIntyre, 1987; Wareing and Phillips, 1981), although changing mineral levels will have an effect on hormone levels e.g. cytokinin (Salama and Wareing, 1979).

The loss of a Ψ_W gradient between the xylem and meristem due to lower Ψ_W(xylem) may be critical, as hypothesized by Mcintyre (1987). Nonami and Boyer (1989) clearly showed that loss of this gradient stopped growth without a change in turgor of the growing tissue itself.

With respect to plant hormones, apical dominance is known to be a function of IAA produced by the stem apex and tissue cytokinin content (Wareing and Phillips, 1981), with IAA inhibiting and cytokinin promoting lateral shoot development. Production and transport of root cytokinin (Rosa da Costa *et al.*, 1987; Hubick *et al.*, 1986b; Itai and Vaadia, 1965; Torrey, 1976) decreases under water stress such that shoot cytokinin levels can fall by over 50% (Hubick *et al.*, 1986b). This could lead to a marked increase in the auxin/cytokinin ratio and hence an increase in apical dominance.

Along with those factors mentioned above, the role of ABA may be significant as it stimulates membrane transport of sugars (Patrick, 1988). The level of ABA in the shoot apex will increase under water stress through stimulated synthesis and import from roots and older leaves (Zhang and Davies, 1989a, b). Older leaves can contribute significant amounts of ABA into the xylem (Atkinson *et al.*, 1989; Zhang and Davies, 1989a). This is because stomata become insensitive to ABA with age thus allowing Ψ_W(leaf) to drop and ABA production to increase (Atkinson *et al.*, 1989).

In summarizing the increase in apical dominance under water stress, the following factors may be involved:

i/ Reduction in assimilate supply.
ii/ Loss of Ψ_W gradient between xylem and meristem.
iii/ Increase in auxin/cytokinin ratio.
iv/ Increase in ABA levels.

It is possible, in fact likely, that most, if not all, of these factors interact to generate the dominance effect. This is an interesting area for further research.

9.4.4 *ROOT GROWTH*

Root growth, in terms of root dry weight, actually increased with water stress up to an off-time of 0.65 hours. There was a concomitant decrease in root length and a large reduction in specific root length (54% for SL3 plants after 56 days). Large reductions in specific root length have been reported for other plants. Aina and Fapohunda (1986) found values for maize plants were reduced by over 50% for plants receiving half the water of controls.

In some cases, under soil conditions, water stress can result in an increase in root length (Huck *et al.*, 1983; Huck *et al.*, 1986) but not mass (Hoogenboom *et al.*, 1987). Scott Russel (1982) states that the two most important root factors associated with plant survival under water stress are rapid elongation of the root axis into wetter zones and extensive root branching. Both will lead to an increase in root length. The situation is very different under aeroponic conditions because there is no gradient in water potential away from the root axis and also no physical support for lateral root growth away from the vertical plane. Hence an increase in root length would not be expected and indeed did not occur.

9.4.5 *WATER USE*

The amount of water held by roots in an aeroponic system will be proportional to total root length as long as root diameter and surface charge density remain constant. Root diameters were not measured in this experiment. However

there was no significant difference in average root length and hence basic root structure between treatments although there was a change in root length per unit dry weight. Without further information, constant root diameter and charge density will be assumed.

After 56 days of treatment root length was reduced by 15% to 20% for SL1 and SL2 plants (which were not significantly different) and 50% for SL3 plants. Therefore the SL3 plants had a much reduced volume of free water available at the beginning of each misting cycle. However the important quantity to consider here is that of transpiring area to volume of available water as estimated by the leaf area to root length ratio. After 56 days this had reduced by 50% for SL3 plants. This decrease would be predicted since the available water had to support plants for 1.55 hours. Along with the decrease in leaf area per unit available water, there was a decrease in water loss per unit leaf area due to stomatal closure. For SL3 plants this reduction was 43%. Hence for the SL3 plants it may be estimated that the rate of water use per unit of available water was less than one third that of the controls.

Although water use per leaf area decreased logarithmically with off-time, water use efficiency peaked at an off-time of 0.65 hours and decreased significantly at 1.55 hours. This indicates that in the 1.55 hour treatment stomatal closure had occurred to the point where photosynthesis was severely limited by the rate of CO_2 diffusion. Support for this contention comes from the fact that C_i decreased with each increment in stress (see section 9.4.6 and 3.1.3).

In agreement with experiment 1, mean water use showed a similar trend to that of noon transpiration rate. However noon transpiration rate was more sensitive to off-time reflecting the peak stress conditions around noon with the resulting degree of stomatal closure (stomatal conductance reduced by 87% at SL3). For the control, noon transpiration rate was eleven times that of mean water use while for SL3 plants it was only four times. As in experiment 1, total plant resistance fell significantly with water stress. This reflects a high degree of stomatal control over leaf water potential.

9.4.6 *LEAF PHYSIOLOGY*

Chlorophyll and carotenoid contents were found to increase linearly with off-time, while the chlororphyll a/b ratio peaked at SL2 (Fig. 9.19). Similar increases were also observed in experiment 1. The change in the chlorophyll a/b ratio is interesting because it is one of the few plant parameters which showed a peaked response.

The high correlation usually found between g_s and photosynthesis (Flore *et al.*, 1985; Jarvis, 1976; Munger *et al.*, 1987) was observed in this experiment (r = 0.98). However IRGA C_i followed an identical trend to that of g_s, falling sharply with off-time, which contrasts with many results where C_i appears constant (Raschke and Resemann, 1986; Smith and Ager, 1988; Tenhunen *et al.*, 1984). When C_i was adjusted according to the equation of Downton *et al.* (1988a) (see section 3.1.3), which accounts for patchy stomatal closure, the rate of decline was even greater (Fig. 9.17). The distribution of stomatal apertures was not measured in this experiment, but regardless of whether patchiness occurred or not, it is clear that stomatal closure was the major limitation to photosynthesis as many recent workers have concluded (Downton *et al.*, 1988a, b; Laisk, 1983; Loveys, 1984; Sharkey and Seemann, 1989; Terashima *et al.*, 1988).

9.4.7 *WATER POTENTIALS AND OSMOTIC ADJUSTMENT*

The process of osmotic adjustment was described in detail in section 2. It should be remembered that osmotic adjustment involves the 'active' accumulation of solutes and not hydro-passive changes resulting from a change in cell volume (Turner and Jones, 1980), which is the reason for always quoting osmotic potentials at full turgor (Ψ_S(sat)). Average cell volume relative to fully turgid volume (estimated by leaf RWC) decreases with increasing water stress (e.g. Fig. 9.5) and hence noon Ψ_S values will always deviate further than those of Ψ_S(sat). In this experiment it was found that Ψ_S(sat) decreased by 2.2 bar and 3.0 bar for root and leaf tissue respectively at SL3, giving a relative change of 46% in leaf tissue and 58% in root tissue. These results are in close qualitative agreement with those of Oosterhuis and Wullschleger (1987) who found a larger absolute change in leaves but a larger relative change in roots of cotton (leaf adjustment, 4.1 bar (40%); root adjustment, 1.9 bar (63%)). Similar results were obtained by Turner *et al.* (1987) for lupin species. The greater ability of roots to osmotically adjust has been given prime importance by Boyer and Westgate (1984) who theorized that this allows root growth to continue under water stress, leading to a decrease in the S/R ratio (or perhaps more correctly, a decrease in the allometric k value).

9.4.8 *ASSIMILATE DISTRIBUTION*

From the allometric relationships (Fig. 9.11), three important points arise:

i/ The relationships are linear for all treatments.
ii/ The control has a slope greater that 1 (i.e. 1.10).
iii/ The slope decreased with increasing water stress.

These points will be discussed later in relation to other experiments (section 14.2).

For the control plants, an allometric k value of 1.10 means that the S/R ratio increased with time. A similar result was documented by Richards (1981), who also noted that the the value was maintained during reproductive growth if fruit weight was subtracted from top weight. Tomatoes show the characteristic rapid increase in T/R ratio during fruit growth (e.g. Van der Post, 1968) such that the allometric k value increases significantly (Richards, 1981).

A decrease in slope of the allometric relationship represents a relative increase in assimilate distribution to the root system and the linear response over the entire experimental period (8 weeks) showed that this alteration was constant. There must have been an initial period of rapid change within the first week of treatment followed by maintenance of this pattern thereafter. The important point is that the allometric relationship did not revert back to the slope of the control plants. This would have been the case if plants had fully adjusted to the altered conditions resulting in a change in S/R ratio but not in rate of change in S/R ratio (see section 14.2). In this experiment, where the allometric relationship showed a constant slope there was a change primarily in the latter.

In Fig. 9.2, 9.3, 9.4 and 9.12, plant part dry weight ratios were plotted against natural logarithm transformed plant dry weight. The reason for doing this is that several advantages are gained from using time linearized plant dry weight on the abscissa (see section 14). In particular trends are more clearly identified because the small number of time nodes (harvest dates), in experiments such as this, are removed. It will also be noted from a comparison of Fig. 9.11 and 9.12 that the transformed plant dry weight plots are considerably more sensitive with respect to curvilinear trends and abnormal points. Thus they are very useful companions to the allometric relationship which is well known for being overly robust (Causton and Venus, 1981).

From the slopes of the plant part ratio plots in Fig. 9.2 and 9.3 (Fig. 9.13), it is seen that the greatest contribution to S/R ratio change came from the leaf to root ratio which decreased sharply with time as water stress increased. Such a response

would be expected in a herbaceous plant where leaf growth is the major contributor to total plant growth and is known to be very sensitive to water stress (Hsaio, 1973). Under nonstress conditions (control plants) the leaf to root ratio was basically constant (change over time not significant) which is yet another way of expressing the functional equilibrium between leaf and root. By contrast, the stem to root ratio increased markedly with time (Fig. 9.13), in line with the need for more structural tissue to support the expanding leaf area. Note that the leaf to stem ratio plots (Fig. 9.4) showed a clear nonlinear trend, tending towards a constant ratio over time which was not significantly affected by the water stress treatments. The initially rapid ratio decline can again be attributed to structural stem tissue development.

In the table below a comparison is made between assimilate distribution, assessed in terms of the allometric relationship, and two measures of carbon gain. The first is noon net photosynthetic rate, an instantaneous measurement at a point of rapid photosynthesis and high radiation load in the diurnal cycle. The second measure, absolute growth rate, gives a mean value for carbon gain over the experimental period in terms of dry weight increase per day.

Table 9.14 The relative effect of different misting pump off-times in an aeroponic system on assimilation and partitioning parameters

	Plant parameter (% of control)		
Off-Time (hours)	Allometric k value	Pn ($\mu mol/m^2/s$)	Ab.GR (g/day)
0.00	100	100	100
0.17	89	94	89
0.65	81	69	69
1.55	75	50	45

The two measures of carbon gain agree within the limits of experimental error for all three stress treatments. Thus in this experiment noon carbon exchange rate provided an adequate estimate of relative productivity, but the generality of this relationship, for water stress originating in the root environment, requires further data for conformation. A poor correlation would be expected when comparing between environments in which transient water stress results from large fluctuations in atmospheric evaporative demand. This would lead to greater reductions in the absolute growth rate.

The allometric k value is less sensitive to water stress than the rate of carbon gain. This is to be expected considering the boundaries imposed on dry weight distribution by the functional equilibrium between root and shoot (see section 4).

9.4.9 *OFF-TIME RELATIONSHIPS*

A glance at the relationships between many important plant parameters and misting pump off-time revealed two basic trends, linear and concave curvilinear, the latter being remarkably similar in form and approximated by logarithmic fits. Logarithmic relationships between growth parameters and other types of stress are well known, for example nutrient stress (Hunt and Burnett, 1973). This is not the case however for water stress when measured in terms of root media water potential. Experiment 1 identified linear trends between plant parameters and solution water potential. This means that the relationship between misting pump off-time and a constant nutrient solution water potential, which would generate the same level of plant water stress, is nonlinear. Considering the nature of the aeroponic misting system (see sections 7.3 and 12) this situation is to be expected. The rate of water stress development is rapid at first, as nutrient solution on the root surface concentrates, but decreases over time as the plant adjusts to its new root environment of water saturated air.

The 'log off-time' relationships all have highly significant intercepts. This implies that water stress is not exerted on the plant until a certain 'lag' time has elapsed. This lag time is to be expected as the root system is saturated with water at the start of each stress cycle and the supply of free water must be at least partially removed before a water stress will develop.

In this experiment only three points can be used in the 'log off-time' relationships and hence it is not sensible to place any emphasis on the magnitude of the lag periods, but only on their existence. It does however open the way for experiments in the future (perhaps in controlled environment rooms) involving a greater number of treatments. Lag periods could be estimated with a high degree of accuracy and provide a unique numerical measurement for the onset of water stress. Some insight into this information can be gained from looking at the relative decrease in parameters with off-time, as discussed in section 13.2.

The table below specifies off-time relationships as either linear or logarithmic, depending on which is the stronger trend, although it should be

remembered that only three points could be used. For a general discussion on the relationship between plant parameters and misting pump off-time see section 12.3.

Table 9.15 The relationship between different misting pump off-times in an aeroponic system and various plant parameters

	Relationship with Off-Time	
Variable	Linear	Logarithmic
Leaf water potential		*
Water use		*
Leaf RWC	*	
Height increase	*	
Plant resistance		*
LA per RL	*	
Allometric k value		*
RGR ratio		*
d. wt ratios vs f(PtDW)		*
Absolute growth rate	*	
RGR(leaf)	*	
RGR(stem)	*	
RGR(plant)	*	
Photosynthetic rate		*
Stomatal conductance		*
Internal CO_2		*
Transpiration		*
Chla conc.	*	
Chlb conc.	*	
Caroteniod conc.	*	

9.4.10 *WATER STRESS INTENSITY*

From many of the plant parameter responses in this experiment it may be concluded that, for off-times up to at least 1.55 hours, (mild) water stress increased in a simple (nonlinear) fashion. However certain key parameters indicate that a change occurred in the degree of water stress between off-times of 0.65 and 1.55 hours, i.e. from mild to more severe water stress. The location of this transition would depend on aerial environment conditions and hence controlled environments would be necessary to generate more accurate numerical values.

The stress transition is shown clearly by changes in unit leaf rate and unit shoot rate (Fig. 9.10), both of which were significantly lower only for SL3. Furthermore the root relative growth rate, water use efficiency and chlorophyll a/b

ratio all peaked at SL2 implying that root environment conditions became severe enough to restrict compensatory growth. Carbon gain at SL3 was reduced more than leaf water loss, possibly indicating the increased involvement of nonstomatal factors in the inhibition of photosynthesis. Such a response was not indicated by instantaneous measurements of Pn, gs and C_i (section 9.3.12), that is, C_i did not tend to a constant value under increasing water stress. This is an interesting aspect which needs further investigation and highlights the importance of considering both instantaneous measurements and long-term time averaged figures (as is the case for water use efficiency, see section 2.5).

9.4.11 *CANONICAL ANALYSIS*

In its fullest form canonical analysis involves the correlation between two sets of variables (e.g. plant parameters and climate data). In this thesis a more restricted use is defined, wherein one set of variables is replaced by a single parameter, namely the water stress level (Ψ_W(solution) in experiment 1 and OT in experiments 2 and 3). In this form, and the context of the data, the analysis does not explicitly define new information which is not already contained within the set of univariate analyses. However, its usefulness is derived from the ability to generate a succinct, unbiased, statistical summary of the data from a simultaneous analysis of all the included variables. Such has been clearly demonstrated in this experiment (see section 9.3.6). In brief the canonical analysis involves finding a series of linear functions of the included variables which are maximally correlated with the second set of variables (here the stress level), but which are independent of each other. The most important information from the analysis is contained within the between canonical structure (e.g. Table 9.7) and the canonical variable class means (e.g. Table 9.8). The former gives the correlation between each variable and the linear function, while the latter is the actual value of the linear function.

This experiment gives a very good example of the data summary power of the canonical analysis. With reference to Table 9.7 and the data for day 49 involving LDW, SDW and RDW, it will be seen that canonical variable 1 was highly correlated with LDW and SDW but weakly, negatively correlated with RDW. Hence a decrease in the canonical variable 1 class mean (Table 9.8) mainly reflected a decrease in the size of the shoot. Indeed, it can be seen that canonical 1 ordered treatments in terms of decreasing water stress level (Fig. 9.9b). By contrast, canonical 2 was principally

correlated with RDW and thus separated SL1 and SL2 from the control and SL3, which had smaller root systems (see Table 9.5). It should be noted here that canonical 1 actually accounted for 70% of the variation in the experimental variables whereas canonical 2 only accounted for 21% (data not shown) and thus in Fig. 9.9, distribution in the X-axis is considerably more important. When LA and RL were introduced into the analysis overall treatment separation increased (Fig. 9.9). However canonical 1 contained more root information (RDW and RL) while canonical 2 was not strongly correlated with any of the variables (Table 9.7) (accounted for only 14% of the total variation) and tended to separate treatments somewhat erratically as a consequence (Fig. 9.9d).

9.4.12 *SUMMARY*

i/ Intermittent misting in an aeroponic system can be used to generate a continuous range of mild to moderate water stresses.

ii/ Various plant parameters decrease in a logarithmic fashion with increasing misting pump off-time e.g. Ψ_W(leaf), water use per LA, allometric k value, Pn, g_s.

iii/ There is a transition from mild to more severe water stress between misting pump off-times of 0.65 and 1.55 hours. The general location of this transition will depend on environmental conditions.

10 EXPERIMENT 3 : WATER STRESS STUDIES ON *PYRUS BETULAEFOLIA* USING INTERMITTENT MISTING

10.1 INTRODUCTION

Useful results were obtained using intermittent misting in an aeroponic system to generate a controlled water stress in tomato seedlings (experiment 2). However, these results needed both validating and generalizing by conducting trials on a very different plant type, such as a perennial tree species. A considerable amount of work has been carried out on apple trees and seedlings, but little has been done using nashi (*Pyrus serotina*) and related species. Being a new crop in New Zealand, local knowledge on all aspects of plant growth, development and environmental interactions is limited. Three rootstocks are proving useful in this country over the myriad of climatic/edaphic conditions, namely *P. serotina*, *P. calleriana* and *P. betulaefolia*, with no one species being universally superior. *Pyrus betulaefolia* has several characteristics which make it a promising option under marginal conditions, including greater drought resistance. At present *P. betulaefolia* plants are generally propagated from seed, although with the trend towards clonal propagation throughout the fruit industry this will no doubt change.

It was decided to conduct a water stress experiment identical to experiment 2. This would allow comparisons to be made at the physiological level between a woody perennial and a herbaceous annual, as well as give results of practical significance. Off-times up to 2 hours were selected which were equidistant on a logarithmic scale, reflecting the apparent logarithmic relationship between important plant parameters and off-time found in experiment 2.

10.2 MATERIALS AND METHODS

For a detailed description of general experimental procedures see section 6.

10.2.1 *EXPERIMENTAL INFORMATION*

One year old, single stemmed seedlings of *Pyrus betulaefolia* Bunge were purchased from a nursery in 4 cm pots (containing a peat/sand mixture) in September 1988. They were repotted into polythene bags (PB 8) using a 60:40 pumice:sand blend containing the fertilizer mixture (appendix 18.6) and grown on under automatic misting irrigation (on for half an hour, three times a day) for a period of one year. In October 1989 the two year old plants (single stem, approximately 1 m high) were transferred to aeroponic tanks (6 x 2 plants per tank), with the tops of the tanks sealed with two layers of white polythene. Plants were sprayed for pest and disease control only as required (see appendix 18.9).

10.2.2 *TREATMENT INFORMATION*

After 31 days in the aeroponic tanks, intermittent misting treatments were commenced (see Table 10.1). Four harvests were made in total as outlined in Table 10.2.

Table 10.1 Treatment specification

		Off-Time (min)			
TANK #	Stress Level	Day 0	Day 3	Day 6-84	On Time (min)
1,6	0	0.0	0.0	0.0	1.0
4,7	1	7.8	7.8	7.8	1.0
3,5	2	21.0	27.0	31.2	1.0
2,8	3	60.0	90.0	120.0	1.0

Table 10.2 Harvest information

Harvest #	Day	Plts harvest^{-1}	Plts tank^{-1}
0	0	2	10
1	35	2	8
2	63	2	6
3	84	2	4

10.2.3 *DATA GATHERING PROCEDURES*

Plant part analysis was carried out on the days given in Table 10.2, according to section 6.2.1. Photosynthetic measurements were made once a week, while dark respiration measurements were made on days 45 and 75 at 11 pm (under moonlight only). Leaf water potential measurements were also made once a week at 12pm to 1 pm. Other measurements included butt cross-sectional area (days 0, 7, 19, 48 and 69), pressure-volume curve analysis (days 44 and 74), assessment of tissue mineral levels and assessment of the CO_2 compensation point (see section 6.2).

10.2.4 *CALCULATION OF DERIVED VARIABLES*

A full listing of all derived variables is given in appendix 18.2.

10.2.5 *STATISTICAL ANALYSIS*

The experimental design was a randomized complete block (RCB) (2 blocks, 4 treatments) with subsamples. Description of analysis procedures for all data is given in appendix 18.3.

10.3 **RESULTS**

10.3.1 *PLANT PART FRESH AND DRY WEIGHTS*

Plant part fresh and dry weight data for the three harvests on days 35, 63 and 84 is given in Tables 3, 4 and 5 respectively. Plant growth, as measured in terms of total plant dry weight, exhibited a strong linear trend over the experimental period (Fig. 10.1). However, close examination of the data suggested that growth was inhibited more strongly in SL2 and SL3 plants between 35 and 60 days compared to the other two treatments. This was confirmed by looking at leaf and stem dry weights (Fig. 10.5).

The approximate linearity of plant dry weight, and hence overall growth, is strongly supported by the trends in butt cross-sectional area (section 10.3.8). As such, the linear regressions in Fig. 10.1 are taken as validly representing the data, while at the same time, strong curvilinear trends in component plant parts are acknowledged.

Table 10.3 Plant part fresh and dry weights after 35 days (harvest 1) for plants grown in aeroponic tanks with varying misting pump off-times (n = 8)

Plant parameter	Treatment Off-Time (hours) 0.00	0.13	0.52	2.00	s.e. mean
Fresh weights (g):					
leaf	128.8 ns	126.7 ns	121.7 ns	103.2 ns	6.5
stem	158.4 ns	154.2 ns	153.1 ns	122.4 ns	8.5
shoot	274.9 ns	300.9 ns	287.1 ns	225.6 ns	15.2
Dry weights (g):					
leaf	56.7 ns	55.9 ns	49.7 ns	40.6 ns	2.4
stem	73.5 ns	73.8 ns	68.2 ns	58.6 ns	3.8
root;					
new	6.2 ns	8.8 ns	8.6 ns	7.9 ns	0.4
total	47.4 ns	58.1 ns	54.5 ns	48.4 ns	2.1
shoot	130.2 ns	129.7 ns	117.9 ns	99.2 ns	6.3
plant	177.6 ns	187.8 ns	172.4 ns	147.4 ns	8.5

Means with the same letter in each row are not significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)

Table 10.4 Plant part fresh and dry weights after 63 days (harvest 2) for plants grown in aeroponic tanks with varying misting pump off-times (n = 8)

Plant parameter	Treatment Off-Time (hours) 0.00	0.13	0.52	2.00	s.e. mean
Fresh weights (g):					
leaf	200.6 a	182.2 a	130.9 b	105.9 b	5.5
stem	263.3 a	238.4 a	167.9 b	133.9 b	7.7
shoot	464.0 a	420.6 a	298.9 b	239.8 b	13.2
Dry weights (g):					
leaf	75.2 a	68.8 a	50.4 b	41.6 b	2.0
stem	119.9 a	109.9 a	78.9 b	64.8 b	3.5
root;					
new	17.7 ns	19.5 ns	15.9 ns	16.6 ns	0.9
total	70.7 ns	72.8 ns	63.2 ns	63.0 ns	2.0
shoot	195.0 a	178.7 a	129.3 b	106.4 b	5.6
plant	265.7 a	251.5 a	192.5 b	169.4 b	7.6

Means with the same letter in each row are not significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)

Table 10.5 Plant part fresh and dry weights after 84 days (harvest 3) for plants grown in aeroponic tanks with varying misting pump off-times (n = 4)

Plant parameter	Treatment Off-Time (hours) 0.00	0.13	0.52	2.00	s.e. mean
Fresh weights (g):					
leaf	218.8 a	190.3 b	160.8 c	132.5 d	1.2
stem	320.8 a	265.8 a	202.7 b	149.3 b	9.1
shoot	539.6 a	456.1 b	363.4 c	281.9 d	10.3
Dry weights (g):					
leaf	81.0 a	71.7 b	62.1 c	52.9 d	1.3
stem	145.9 a	122.3 a	95.0 b	72.2 b	4.0
root;					
new	32.2 a	28.6 ab	26.0 ab	22.3 b	1.0
total	90.6 a	83.2 ab	77.0 bc	69.4 c	1.2
shoot	226.8 a	194.0 b	157.2 c	125.1 d	4.4
plant	317.5 a	277.2 b	234.2 c	194.5 d	5.1

Means with the same letter in each row are not significantly different at the 1% level using the lsd discriminator

10.3.2 *PLANT PART DRY WEIGHT RATIOS*

In section 9.4.8 a philosophy was initially presented whereby plant part ratios were plotted against plant dry weight transformed so as to be a linear function of time. This type of plot is discussed in detail in section 14. In this experiment plant dry weight was approximately linear with time (Fig. 10.1) so that leaf-root, stem-root and leaf-stem ratios were plotted against untransformed plant dry weight (Fig. 10.2, 10.3 and 10.4). For control plants, the leaf-root and stem-root ratios increased over the experimental period while the leaf-stem ratio decreased significantly. With increasing water stress the rate of increase in leaf-root and stem-root ratio declined such that for SL3 plants the ratios actually decreased. For the leaf-stem ratio an opposite trend was observed such that the marked decline was eliminated under water stress. Note that the regressions for SL2 and SL3 plants are not significant and therefore may be considered as horizontal lines.

Rates of change in plant part ratios are succinctly depicted in Fig. 10.12 where it can be seen that at an off-time of around one hour the three ratio slopes are

zero i.e., the distribution of dry matter between the three major plant parts did not change with time.

10.3.3 *PLANT PART WATER CONTENTS*

Water contents were reduced significantly by the water stress treatment at all three harvest dates. Note the high consistency in water content data for a woody perennial species.

Table 10.6 Effect of different misting pump off-times in an aeroponic system on plant part water contents (g H_2O g^{-1} d. wt) at harvest 1

Harvest time[a] (days)	Plant Part	Treatment Off-Time (hours) 0.00	0.13	0.52	2.00	s.e. mean
35	Leaf	1.619 a	1.606 a	1.579 ab	1.529 b	0.007
	Stem	1.240 a	1.203 b	1.145 c	1.084 d	0.002
63	Leaf	1.640 a	1.628 a	1.594 b	1.578 b	0.003
	Stem	1.191 a	1.158 b	1.126 c	1.093 d	0.001
84	Leaf	1.710 a	1.651 b	1.585 c	1.528 d	0.006
	Stem	1.197 a	1.162 b	1.126 c	1.090 d	0.001

Means with the same letter in each row are not significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)

[a] n = 8 at 35 and 63 days; n = 4 at 84 days

10.3.4 *LEAF AND ROOT DATA*

Leaf area increase slowed dramatically between harvests two and three (35 and 63 days) as the level of water stress increased, such that in SL2 and SL3 plants no significant increase occurred (Fig. 10.5). However, between harvests three and four (63 and 84 days) control and SL1 plants exhibited and abrupt decrease while SL2 and SL3 plants showed a significant recovery of growth. Significant differences in leaf

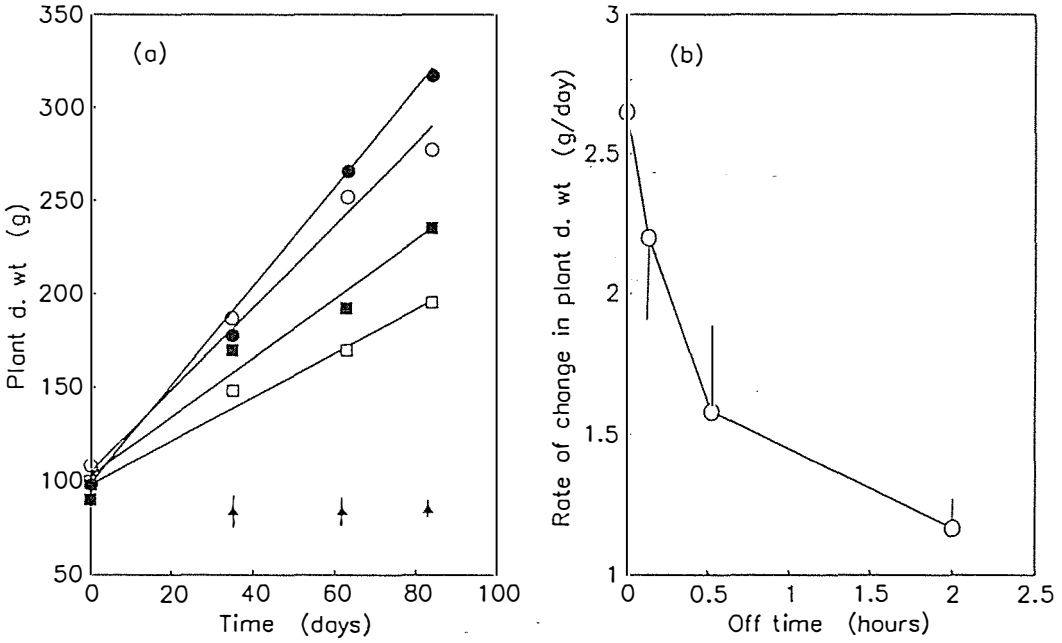

Fig. 10.1. The effect of different misting pump off-times in an aeroponic system on plant dry weight and rate of change in dry weight. (a) Plant dry weight *versus* time (n = 8). Bars show pooled s.e. for each harvest: ●, 0.00 hour OT; Y = 90.3 + 2.71X; $R^2 = 0.99^{**}$:○, 0.13 hour OT; Y = 101.1 + 2.23X; $R^2 = 0.98^{**}$: ■, 0.52 hour OT; Y = 100.7 + 1.59X; $R^2 = 0.96^{**}$: □, 2.00 hour OT; Y = 98.0 + 1.17X; $R^2 = 0.98^{**}$. (b) Rate of change in plant dry weight. Bars show s.e. for slope coefficients.

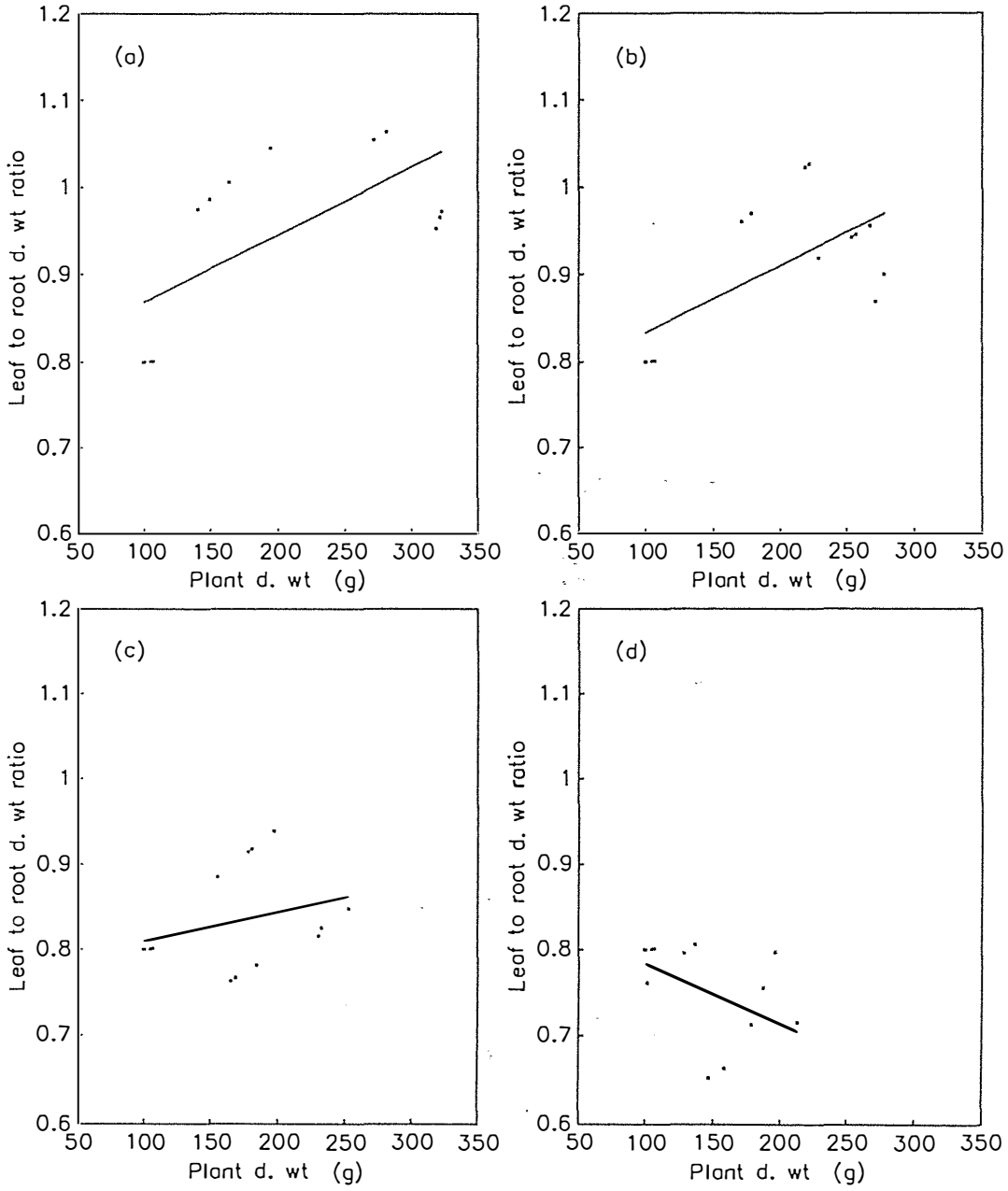

Fig. 10.2. The effect of different misting pump off-times in an aeroponic system on the leaf/root ratio *versus* plant dry weight relationship. (a) 0.00 hour OT; $Y = 0.79 + 0.00078X$; $R^2 = 0.46^*$. (b) 0.13 hour OT; $Y = 0.76 + 0.00077X$; $R^2 = 0.42^*$. (c) 0.52 hour OT; $Y = 0.77 + 0.00035X$; $R^2 = 0.09^{ns}$. (d) 2.00 hour OT; $Y = 0.85 - 0.00068X$; $R^2 = 0.21^{ns}$.

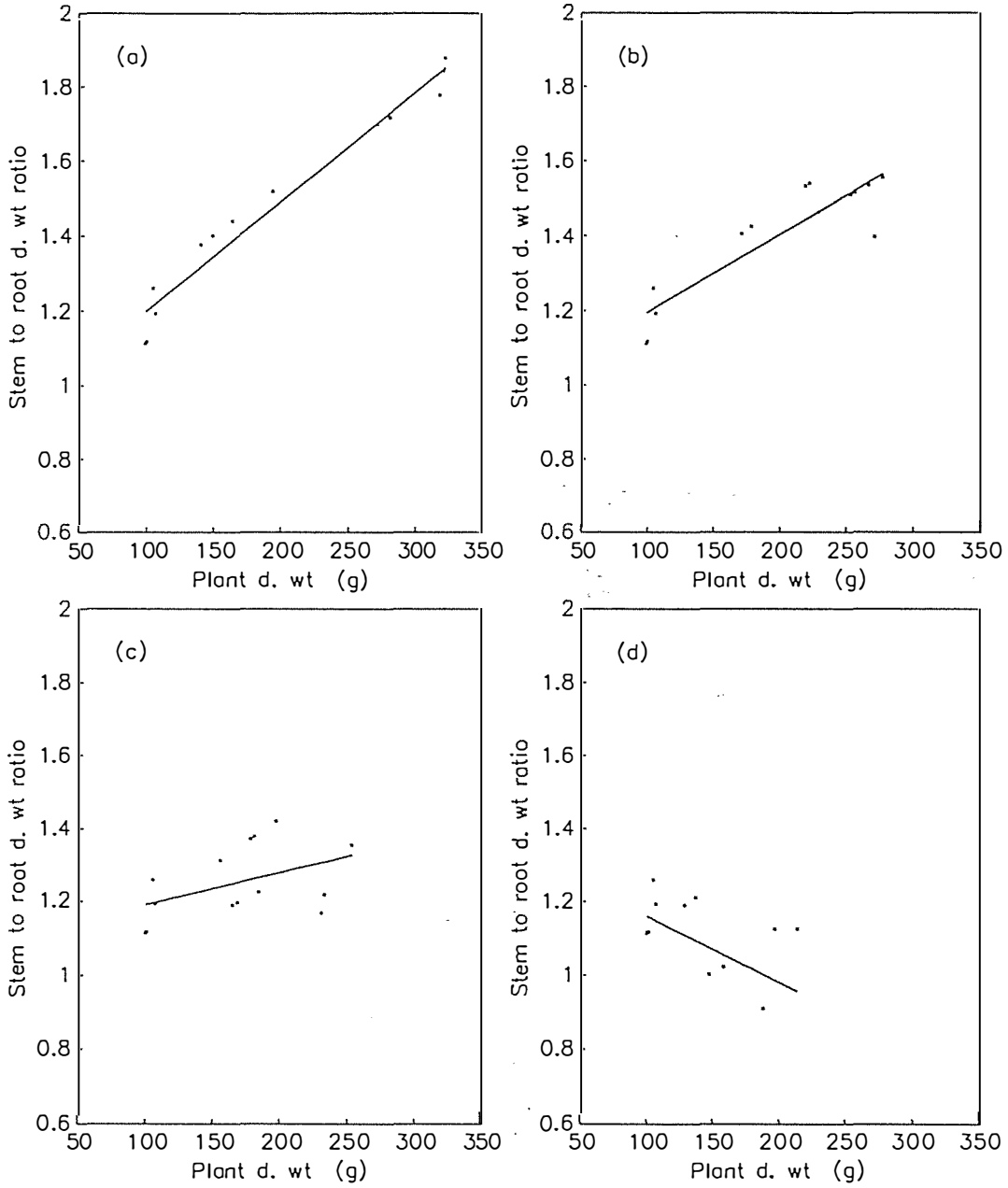

Fig. 10.3. The effect of different misting pump off-times in an aeroponic system on the stem/root ratio *versus* plant dry weight relationship. (a) 0.00 hour OT; $Y = 0.91 + 0.0029X$; $R^2 = 0.96^{**}$. (b) 0.13 hour OT; $Y = 0.99 + 0.0021X$; $R^2 = 0.81^{**}$. (c) 0.52 hour OT; $Y = 1.10 + 0.0009X$; $R^2 = 0.20^{ns}$. (d) 2.00 hour OT; $Y = 1.34 - 0.0018X$; $R^2 = 0.23^{ns}$.

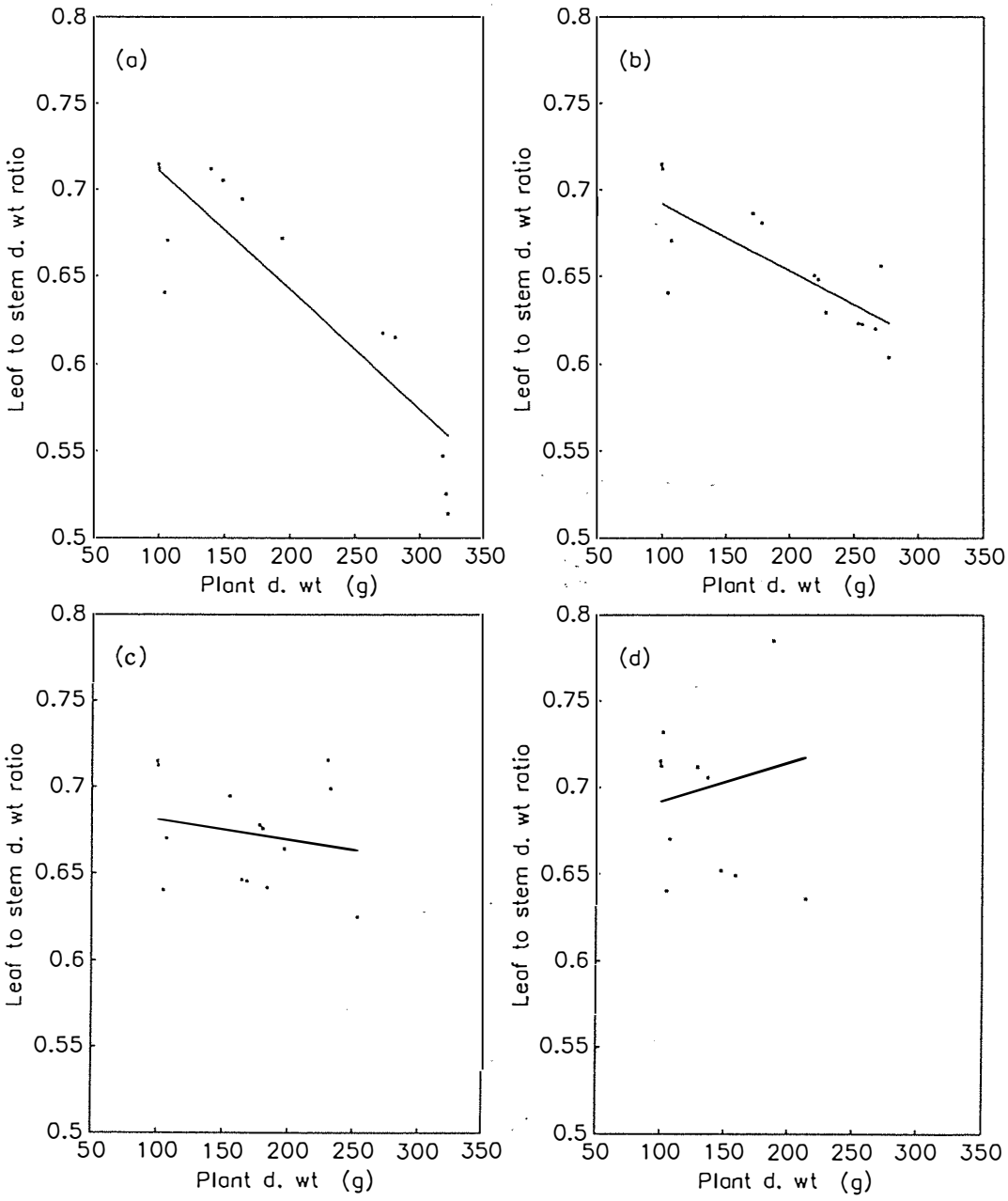

Fig. 10.4. The effect of different misting pump off-times in an aeroponic system on the leaf/stem ratio *versus* plant dry weight relationship. (a) 0.00 hour OT; $Y = 0.78 - 0.00068X$; $R^2 = 0.78^{**}$. (b) 0.13 hour OT; $Y = 0.73 - 0.00039X$; $R^2 = 0.60^{*}$. (c) 0.52 hour OT; $Y = 0.69 - 0.00012X$; $R^2 = 0.04^{ns}$. (d) 2.00 hour OT; $Y = 0.67 + 0.00022X$; $R^2 = 0.02^{ns}$.

area occurred after 63 days and by day 84 all four treatments were different, with a 40% reduction at SL3. Leaf area data were fitted using logarithmic functions as shown in Fig. 10.5 and these functions were then used to adjust water use data.

Total stem length followed a very similar trend to that of leaf area, confirming that meristematic activity had slowed after 35 days, in a way which was dependent on the level of water stress. Shoot length was reduced by 40% at day 84 for SL3 plants.

By contrast to the shoot system, root growth, in terms of dry weight or total root length, showed no convex tendencies over time and overall the root system was not as sensitive as the shoot system to the water stress treatments (Fig. 10.6). Root dry weight was significantly different only at harvest 3 (day 84). With respect to new roots, that is, roots produced in the aeroponic tanks, the difference was only between control and SL3 plants, while old root dry weight was depressed more strongly, indicating a redistribution of stored material into new tissue under water stress. Total root length increased in a linear fashion with time (Fig. 10.6), as did new root length (figure not shown). However there were no significant differences at any harvest date for either new or total root length.

Linear relationships were found for leaf area *versus* root length (Fig. 10.7), with water stress causing the slope of this relationship to decrease (Fig. 10.8) because leaf growth was inhibited more than root growth. The decline with off-time followed the characteristic concave shape.

The root length to dry weight ratio declined with time (Fig. 10.8). For control plants this decline was approximately linear (Fig. 10.8) whereas for the three stress treatments the decrease was rapid in the first 35 days and then less so thereafter. After 84 days the length/weight ratio was almost identical for all four treatments with respect to both old and new roots. Old roots had a consistently lower length/weight ratio and for control plants the difference remained constant at approximately 19 m g^{-1}. The viability of old roots, less than 2 mm in diameter, was estimated to be 50%, 18%, 9% and 4% after 0, 35, 63 and 84 days respectively for the control plants. In the SL3 treatment comparable figures were 40%, 10%, 2% and 1%.

No significant treatment differences were found at any of the harvest dates for either leaf area ratio or specific leaf area.

10.3.5 *CANONICAL ANALYSIS ON PLANT PART DATA*

A canonical analysis was performed on each harvest using the following plant part data:

i/ Leaf dry weight.
Stem dry weight.
Root dry weight.

i/ Leaf dry weight.
Stem dry weight.
Root dry weight.
Leaf area.
Root length.

Only at harvest 3 were the treatments significantly different (significant Wilks' Lambda test) and results from this analysis are given in Tables 10.7 and 10.8. Only the first canonical variable was significant and this ranked treatments in order of decreasing off-time. From the between canonical structure it can be seen that canonical 1 was a general discriminator but was most strongly correlated with stem dry weight. Leaf area and root length were correlated in a similar fashion to leaf and root dry weight. This indicates that treatments did not significantly affect the relationships between these variables, as has already been identified.

Table 10.7 Effect of different misting pump off-times in an aeroponic system on a canonical analysis for data collected after 84 days (n= 4) - Between canonical structure

Canonical variable	Plant variable LDW	SDW	RDW	LA	RL
(a) Variables used = leaf, stem and root dry weight.					
1	0.91	0.99	0.96	-	-
(b) Variables used = leaf, stem and root dry weight, leaf area and root length.					
1	0.92	0.99	0.97	0.95	0.96

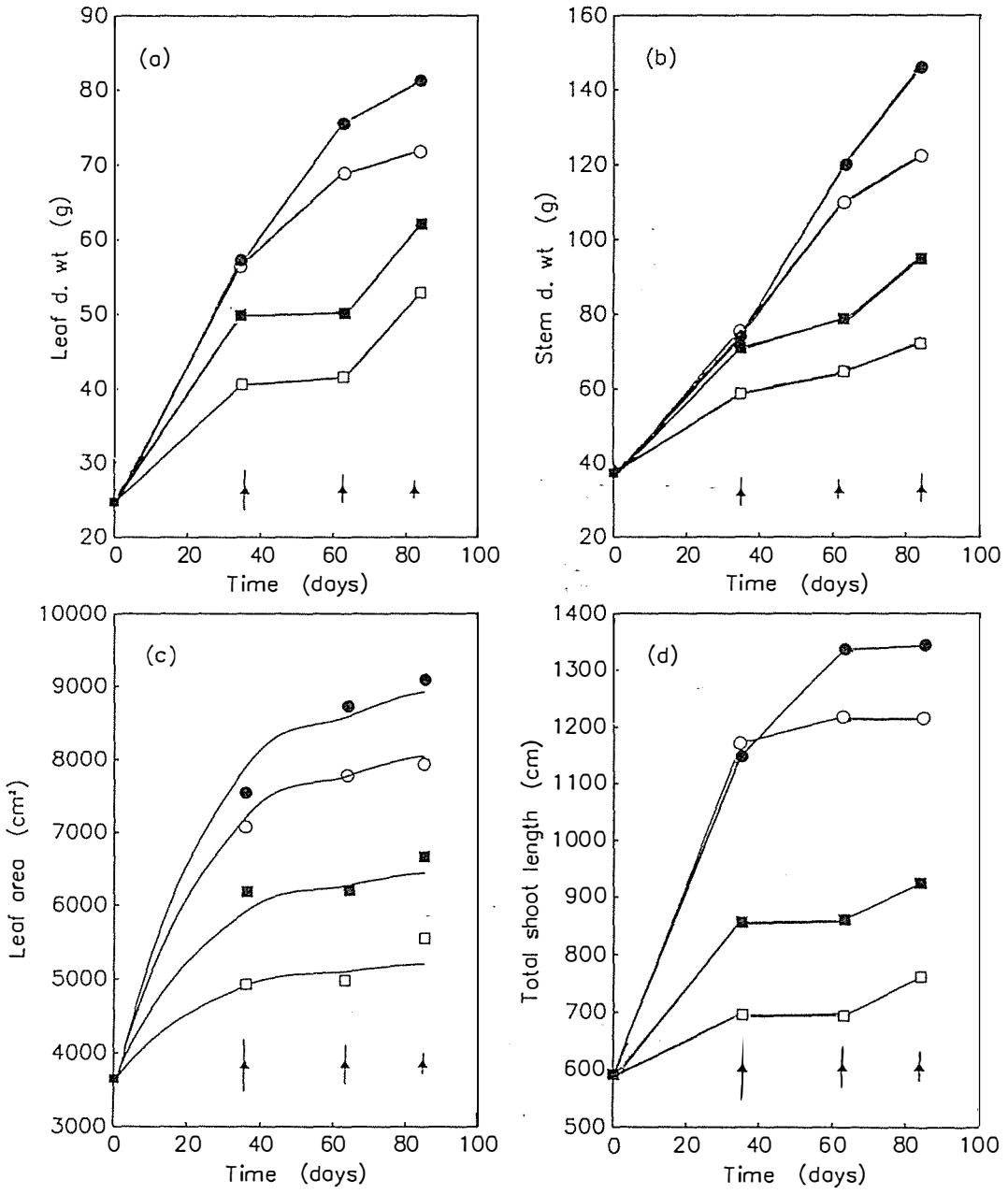

Fig 10.5. The effect of different misting pump off-times in an aeroponic system on shoot parameters over time (n = 8). Bars show pooled s.e. for each harvest. (a) Leaf dry weight: ●, 0.00 hour OT: ○, 0.13 hour OT: ■, 0.52 hour OT: □, 2.00 hour OT. (c) Leaf area: ●, 0.00 hour OT; Y = 3630 + 1191 x ln(X); $R^2 = 0.99^{**}$: ○, 0.13 hour OT; Y = 3686 + 982 x ln(X); $R^2 = 0.99^{**}$: ■, 0.52 hour OT; Y = 3719 + 612 x ln(X); $R^2 = 0.92^{**}$: □, 2.00 hour OT; Y = 3676 + 343 x ln(X); $R^2 = 0.85^{*}$. (d) Total shoot length.

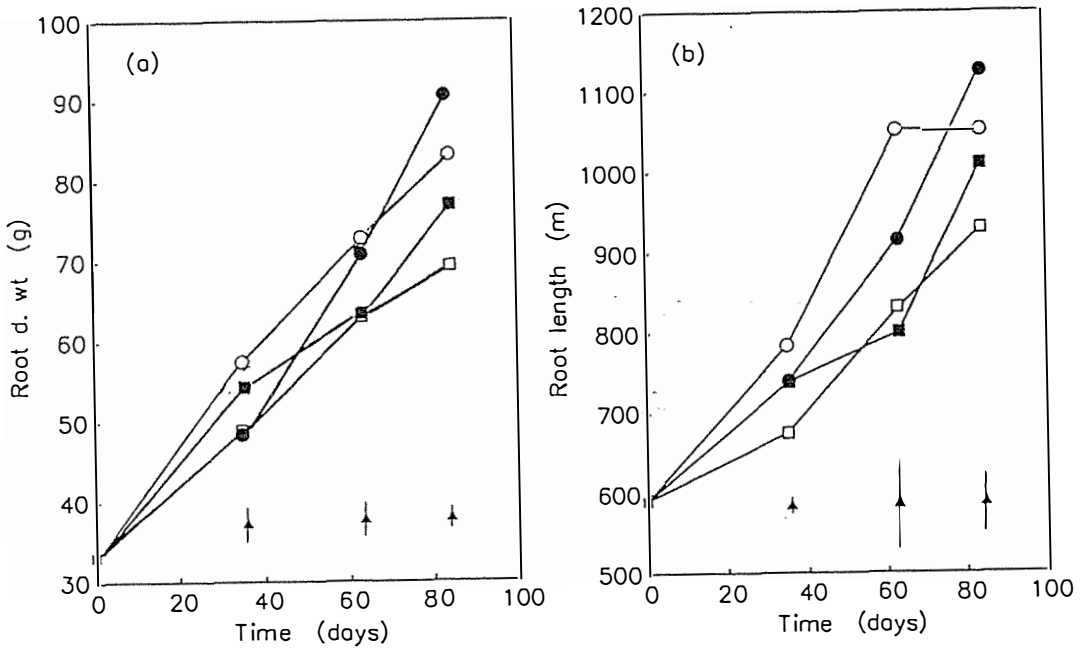

Fig. 10.6. The effect of different misting pump off-times in an aeroponic system on root parameters over time (n = 8). Bars show pooled s.e. for each harvest. (a) Root dry weight:●, 0.00 hour OT: ○, 0.13 hour OT:■, 0.52 hour OT:□, 2.00 hour OT. (b) Root length.

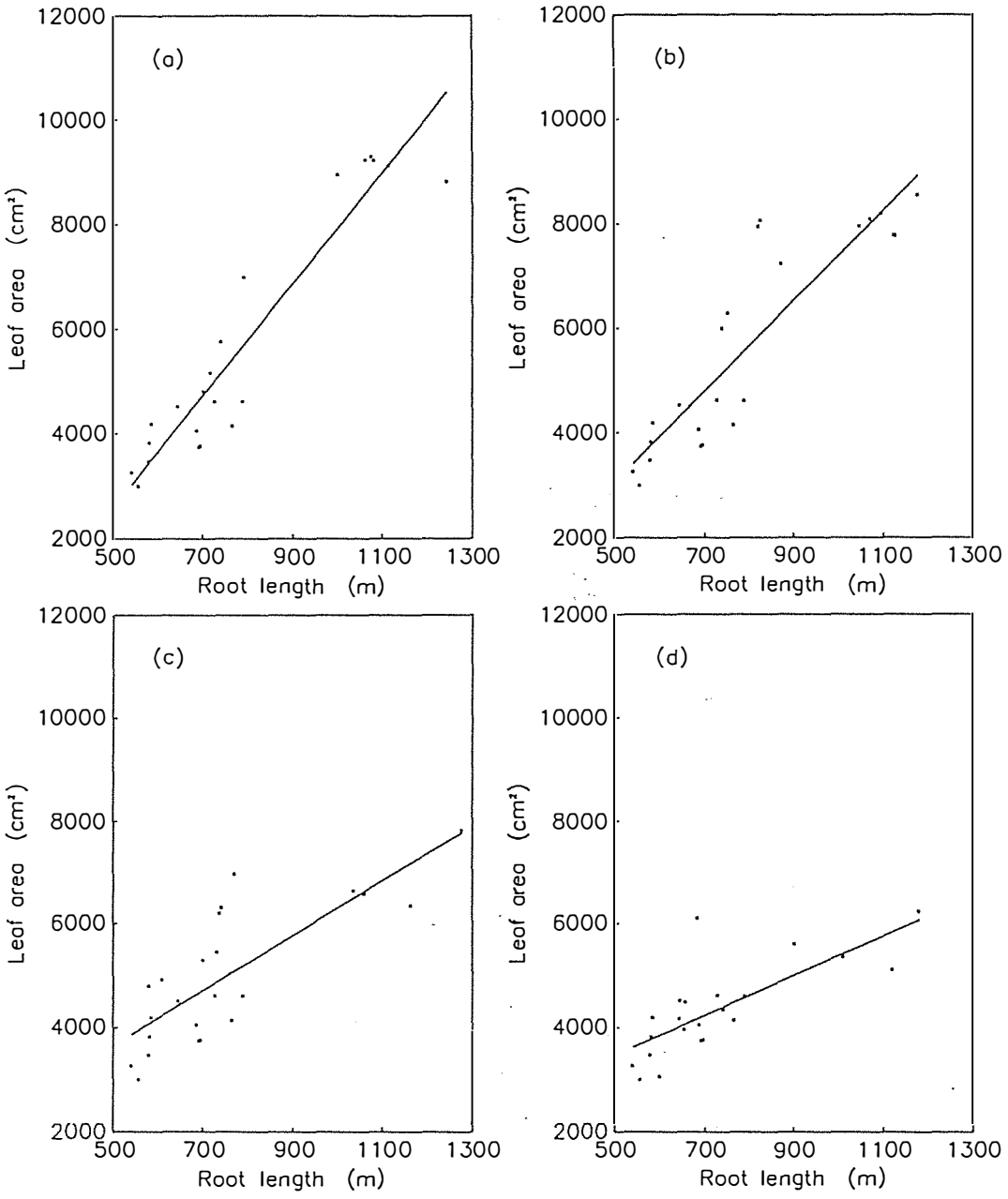

Fig. 10.7. The effect of different misting pump off-times in an aeroponic system on the relationship between leaf area and root length. (a) , 0.00 hour OT; $Y = 314 + 0.084X$; $R^2 = 0.90^{**}$. (b) , 0.13 hour OT; $Y = 288 + 0.091X$; $R^2 = 0.79^{**}$. (c) , 0.52 hour OT; $Y = 163 + 0.118X$; $R^2 = 0.63^{*}$. (d) , 2.00 hour OT; $Y = 88 + 0.148X$; $R^2 = 0.57^{*}$.

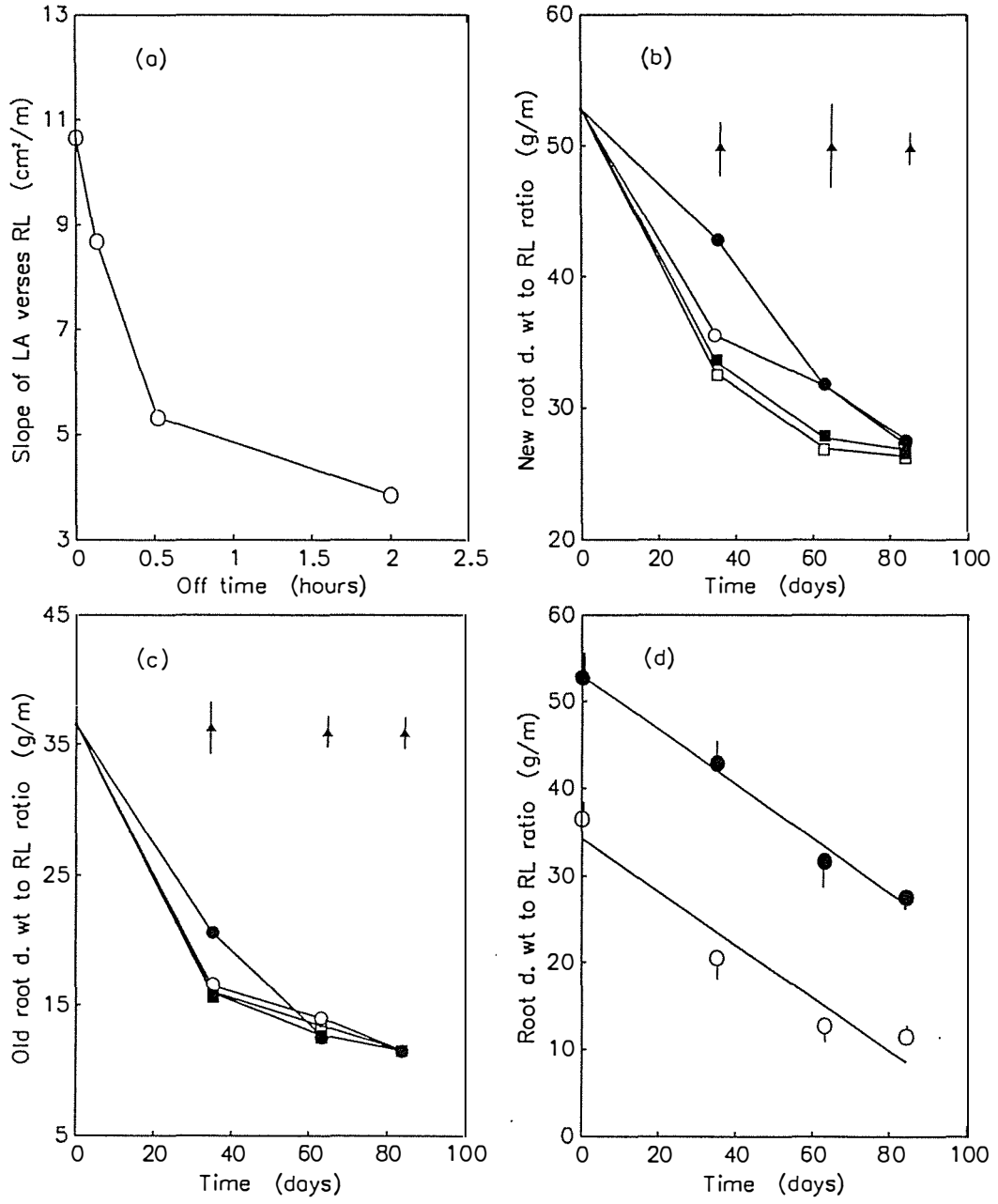

Fig. 10.8. The effect of different misting pump off-times in an aeroponic system on leaf and root relationships. Bars show s.e. of slope coefficients in (a) and pooled s.e. for each harvest in (b), (c) and (d). (a) The slope of leaf area *versus* root length. (b) The root length to dry weight ratio for roots formed within the aeroponic tanks (new roots) *versus* time (n = 8). (c) The root length to dry weight ratio for roots formed before placement in the aeroponic tanks (old roots) *versus* time (n = 8); , 0.00 hour OT; , 0.13 hour OT; , 0.52 hour OT; , 2.00 hour OT. (d) The root dry weight to length ratio for control (0.00 hour OT) plants *versus* time (n = 8): , new roots; $Y = 34.2 - 0.31X$; $R^2 = 0.93^{**}$: , old roots; $Y = 52.9 - 0.31X$; $R^2 = 0.99^{**}$.

Table 10.8 Effect of different misting pump off-times in an aeroponic system on a canonical analysis for data collected after 84 days (n = 4) - Canonical variable class means

Canonical variable	Treatment Off-Time (hours) 0.00	0.17	0.65	1.55	lsd
(a) Variables used = leaf, stem and root dry weight.					
1	2.27 a	0.92 b	-0.96 c	-2.24 d	0.55
(b) Variables used = leaf, stem and root dry weight, leaf area and root length.					
1	3.45 a	1.35 b	-1.25 c	-3.55 d	0.60

Means with the same letter in each column are not significantly different at the 1% level using the lsd discriminator

10.3.6 *PLANT GROWTH ANALYSIS*

Average values for relative growth rate, absolute growth rate, unit leaf rate and unit shoot rate are shown in Fig. 10.9. The RGR of leaf and stem decreased in a logarithmic fashion with off-time. Root RGR also decreased but was not as sensitive, with the reduction at SL3 being 27% compared with 36% and 51% for leaf and stem respectively. Absolute growth rate, unit leaf rate and unit shoot rate also followed similar trends.

10.3.7 *THE ALLOMETRIC RELATIONSHIP*

Allometric relationships for shoot *versus* root dry weight are given in Fig. 10.10 and all appear to be linear although variability increases with stress level. Harvest 0 data are also plotted to show the change in slope. It can be seen that the allometric k value decreased with increasing water stress although there was no significant difference between the control and SL1. The allometric k value is plotted in Fig. 10.12 along with the RGR ratio (shoot/root) and slopes of the S/R ratio *versus* plant dry weight relationships from Fig. 10.11 (for a discussion of these relationships see section 9.4.8 and 14). They are all in general agreement and show the characteristic concave curvilinear trend between SL1 and SL3.

10.3.8 *BUTT CROSS-SECTIONAL AREA*

Mean butt cross-sectional area measurements over the experimental period are shown in Fig. 10.13 and follow a strong linear trend with time, while growth rate, as measured in terms of increase in cross-sectional area per day, was linearly related to the misting pump off-time. Relationships between butt cross-sectional area and leaf area, shoot dry weight, root dry weight and plant dry weight are shown in Fig. 10.14 and follow linear trends.

10.3.9 *WATER USE AND PLANT RESISTANCE*

The SL1, SL2 and SL3 plants used 26%, 33% and 51% less water per metre squared leaf area per day than the control plants thus exhibiting a logarithmically decreasing trend (Fig. 10.15). Water use efficiency was significantly higher for all three stress treatments, showing a rapid increase between off-times of 0.00 and 0.13 hours (Fig. 10.15).

Plants were loosing about five times the amount of water at peak demand (noon transpiration rate, E) compared with average values (WU). However there were no differences in the E/WU ratio indicating that the increased radiation load affected all treatments equally (Table 10.9) .

Plant resistance increased dramatically with off-time (linear fashion) such that there was a five fold increase at SL3 (Fig. 10.16).

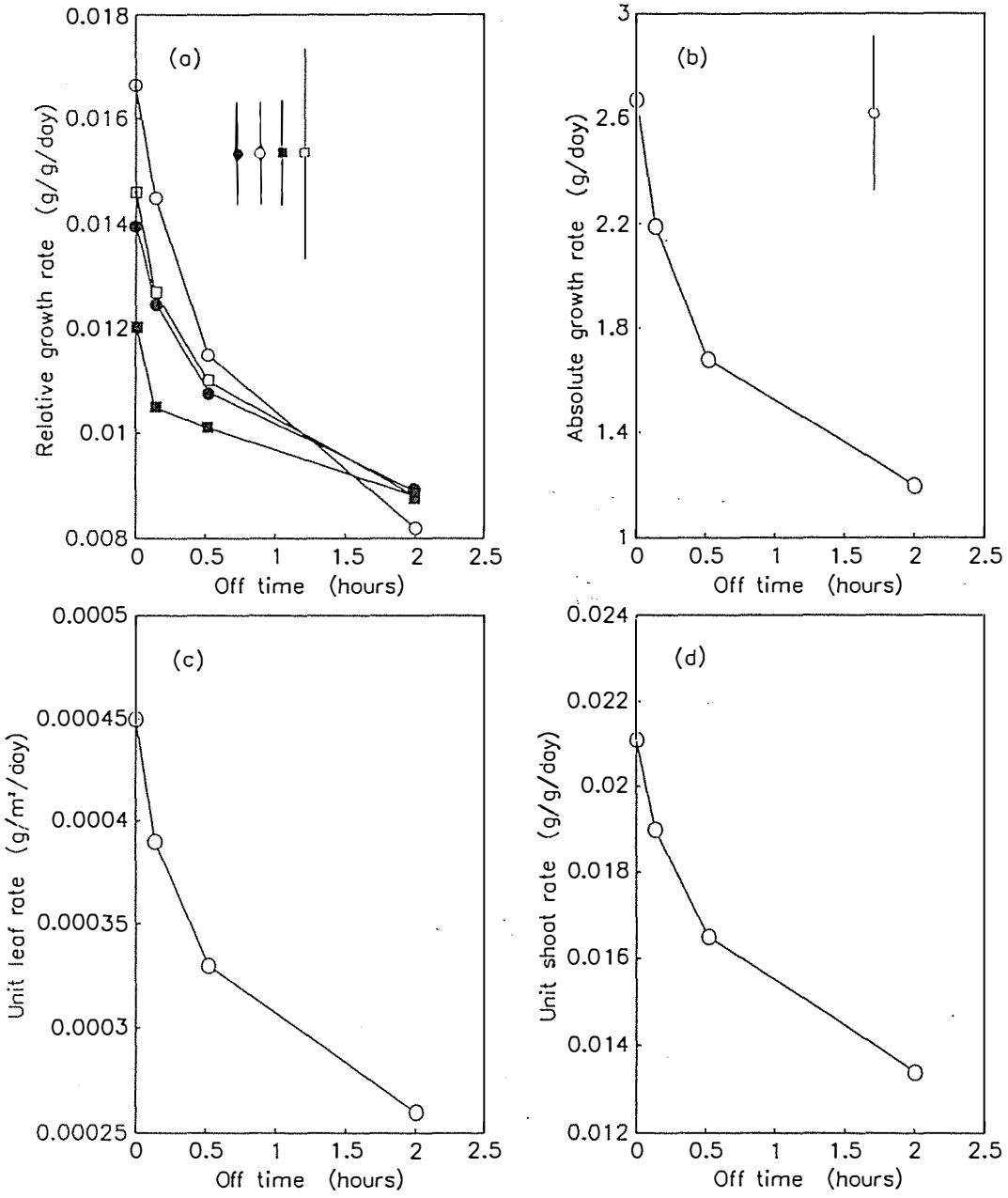

Fig. 10.9. The effect of different misting pump off-times in an aeroponic system on growth analysis parameters (n = 8). Bars show pooled s.e.. (a) Relative growth rate. ●, leaf; ○, stem; ■, root; □, plant. (b) Absolute growth rate. (c) Unit leaf rate. (d) Unit shoot rate.

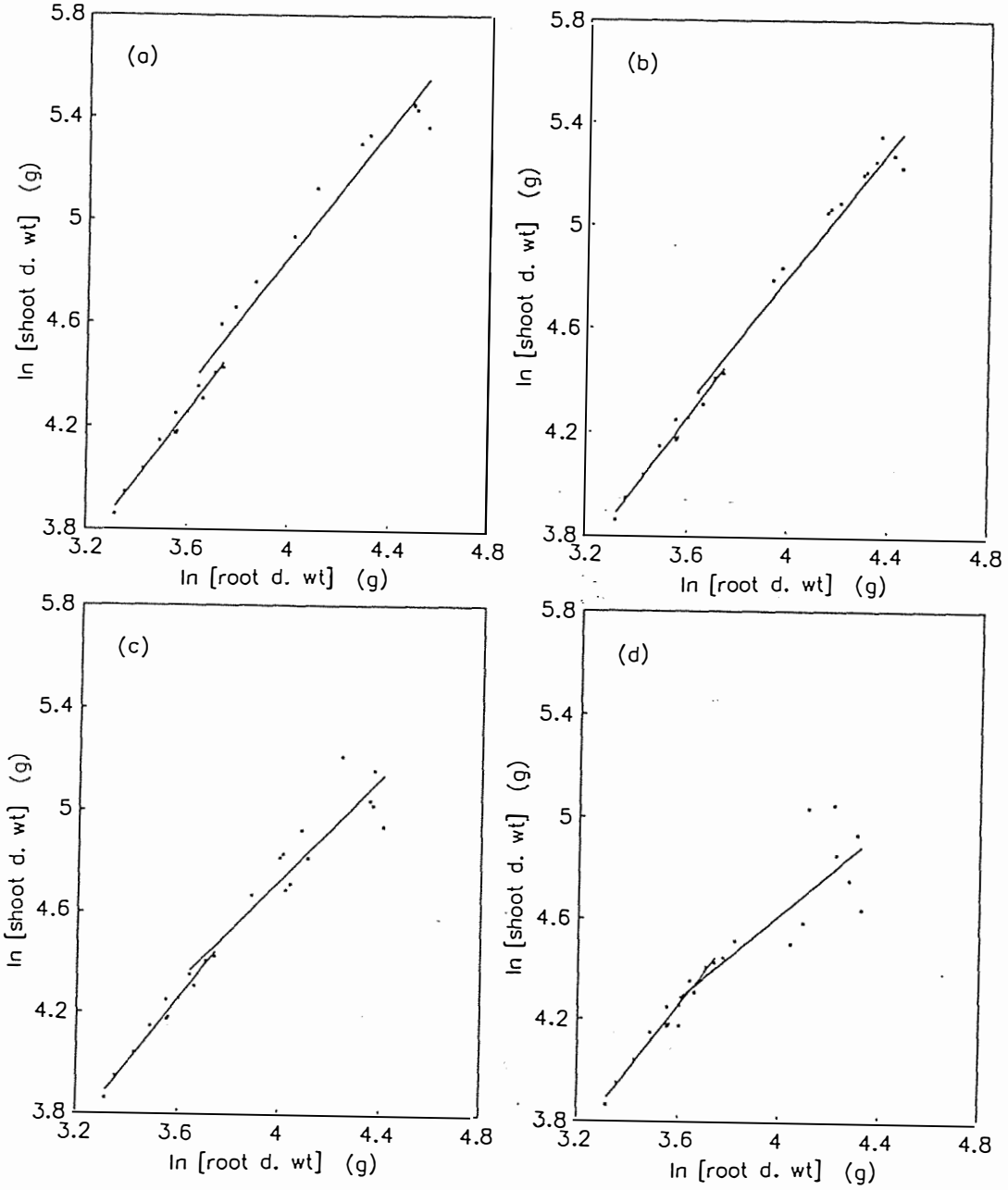

Fig. 10.10. The effect of different misting pump off-times in an aeroponic system on the allometric relationship[a]. (a) 0.00 hour OT; $Y = -0.22 + 1.27X^{a}$; $R^2 = 0.96^{**}$. (b) 0.13 hour OT; $Y = -0.14 + 1.24X^{a}$; $R^2 = 0.96^{**}$. (c) 0.52 hour OT; $Y = 0.67 + 1.01X^{b}$; $R^2 = 0.87^{**}$. (d) 2.00 hour OT; $Y = 1.28 + 0.83X^{c}$; $R^2 = 0.70^{*}$ (letters on slope coefficients indicate significant differences at the 5% level using a paired t-test).

[a] For all treatments the time 0 harvest relationship is; $Y = -0.43 + 1.30X$; $R^2 = 0.97^{**}$.

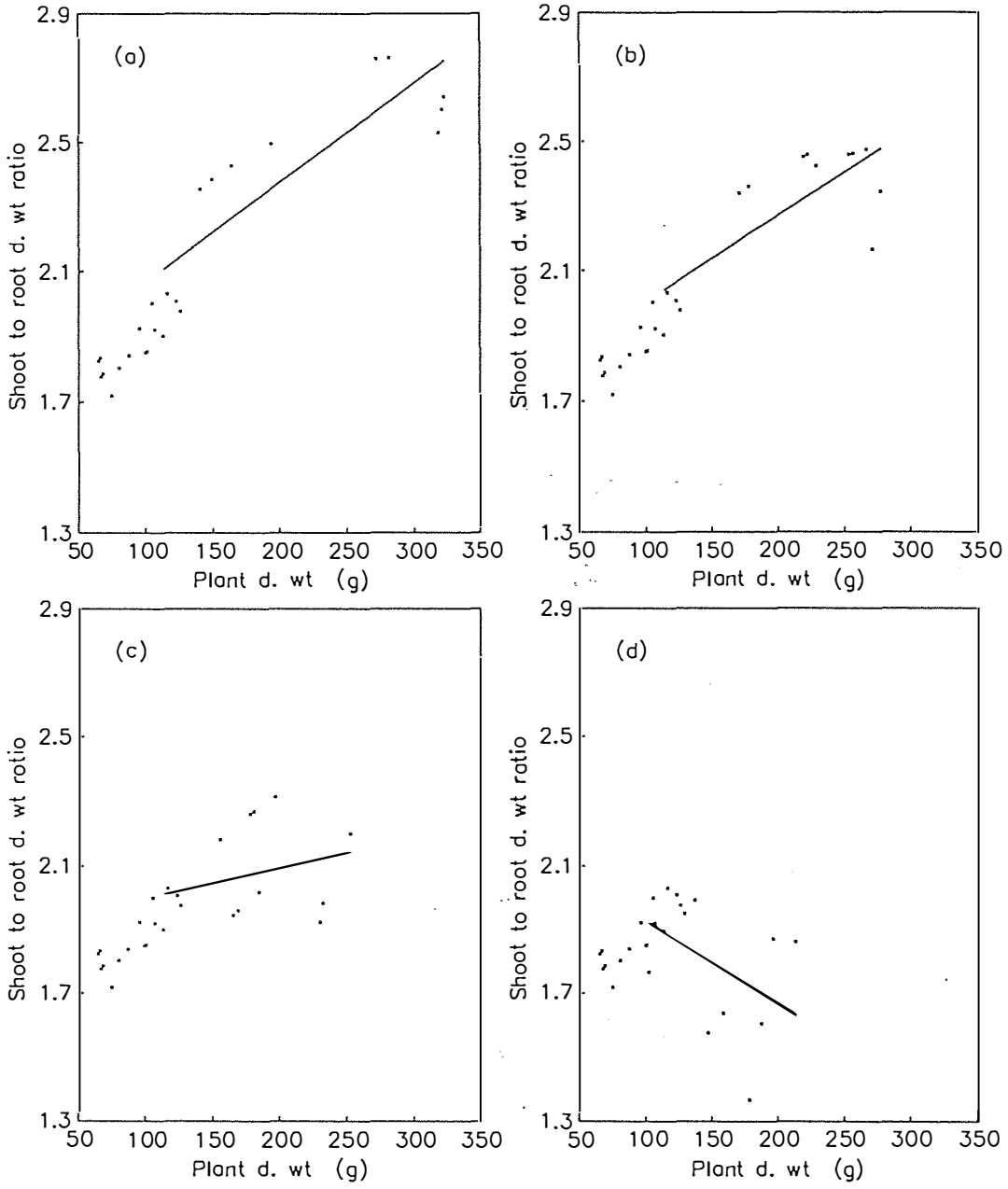

Fig. 10.11. The effect of different misting pump off-times in an aeroponic system on the shoot/root ratio *versus* plant dry weight relationship. (a) 0.00 hour OT; $Y = 1.76 + 0.0031X$; $R^2 = 0.73^{**}$. (b) 0.13 hour OT; $Y = 1.74 + 0.0027X$; $R^2 = 0.62^{*}$. (c) 0.52 hour OT; $Y = 1.91 + 0.0009X$; $R^2 = 0.09^{ns}$. (d) 2.00 hour OT; $Y = 2.19 - 0.0026X$; $R^2 = 0.20^{ns}$.

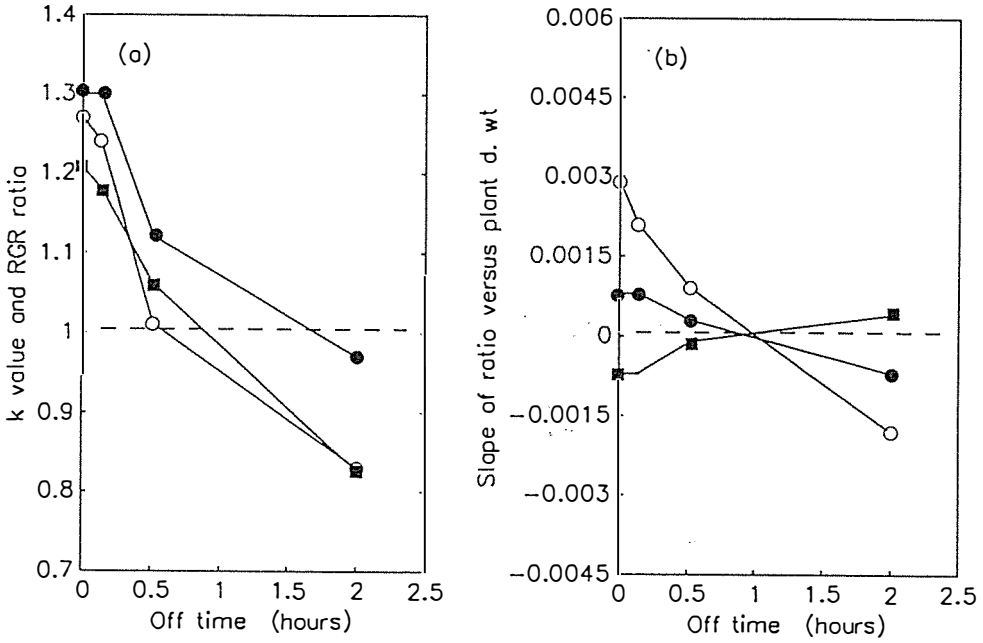

Fig. 10.12. The effect of different misting pump off-times in an aeroponic system on assimilate partitioning parameters (n = 8). (a) ●, shoot to root RGR ratio; ○, allometric k value; ■, slope of shoot/root ratio *versus* plant dry weight. (b) ●, slope of leaf/root ratio *versus* plant dry weight; ○, slope of stem/root ratio *versus* plant dry weight; ■, slope of leaf/stem ratio *versus* plant dry weight.

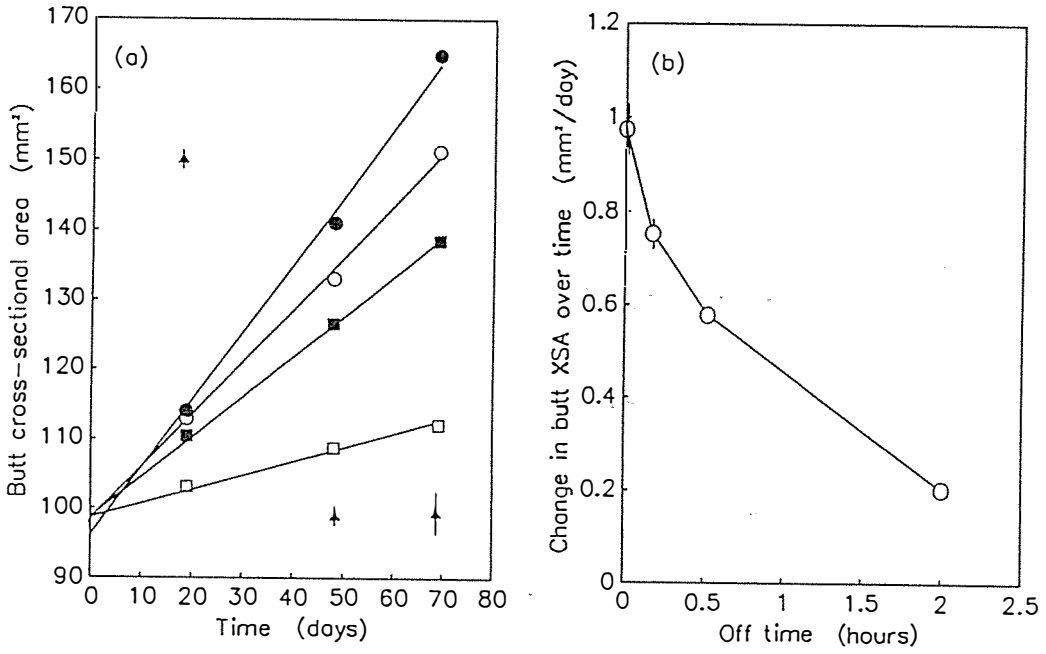

Fig. 10.13. The effect of different misting pump off-times in an aeroponic system on butt cross-sectional area and rate of change of butt cross-sectional area. (a) Butt cross-sectional area (measured 20 cm above the root system) *versus* time (n = 8). Bars show pooled s.e. for each harvest: ●, 0.00 hour OT; Y = 96.1 + 0.97X; $R^2 = 0.995^{**}$: ○, 0.13 hour OT; Y = 98.4 + 0.75X; $R^2 = 0.997^{**}$: ■, 0.52 hour OT; Y = 98.6 + 0.58X; $R^2 = 0.999^{**}$: □, 2.00 hour OT; Y = 98.6 + 0.20X; $R^2 = 0.988^{**}$. (b) Rate of change in butt cross-sectional area. Bars show s.e. of slope coefficients.

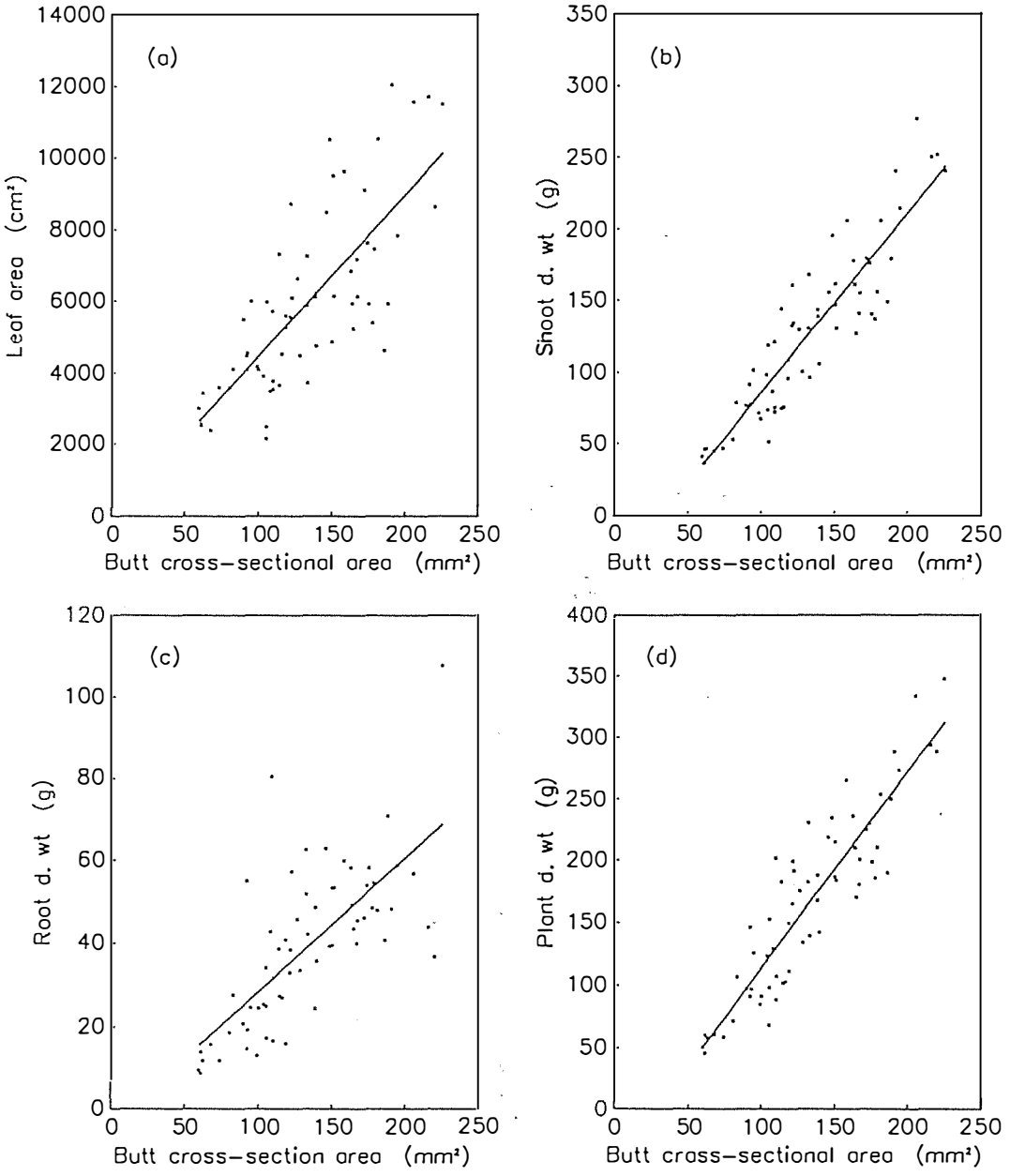

Fig. 10.14. The effect of different misting pump off-times in an aeroponic system on butt cross-sectional area relationships. (a) Leaf area; $Y = -72.6 + 45.2X$; $R^2 = 0.58^{*}$. (b) Shoot dry weight; $Y = -40.3 + 1.26X$; $R^2 = 0.82^{**}$. (c) Root dry weight ; $Y = -3.61 + 0.32X$; $R^2 = 0.50^{*}$. (d) Plant dry weight; $Y = -43.9 + 1.58X$; $R^2 = 0.82^{**}$.

Table 10.9 Effect of different misting pump off-times in the nutrient solution of an aeroponic system on water use as measured in terms of experiment long water use (WU) (n = 30) and instantaneous transpiration rate at noon (E) (n = 12)

Off-Time (hours)	Water use (WU) (l pt d^{-1})	(l m^{-2} d^{-1})	E (l m^{-2} h^{-1})	E/WU[a]
0.00	0.57 a	0.74 a	0.31 a	5.0 ns
0.13	0.39 b	0.55 b	0.26 b	5.6 ns
0.52	0.29 c	0.50 b	0.21 c	5.0 ns
2.00	0.18 d	0.36 c	0.14 d	4.7 ns
s.e. mean	0.03	0.06	0.01	0.2

Means with the same letter in each column are not significantly different at the 1% level using the lsd discriminator

[a] Both variables in units of l m^{-2} d^{-1}

10.3.10 *LEAF WATER POTENTIAL*

Leaf water potentials at noon increased significantly with each increment in the level of water stress and again showed a concave curvilinear trend (Fig. 10.16). No significant differences were found in the dawn leaf water potential values.

10.3.11 *PHOTOSYNTHETIC SYSTEM DATA ANALYSIS*

Photosynthetic rate, stomatal conductance and transpiration rate were all significantly reduced by each increment in water stress, giving rise to the characteristic curvilinear relationship (Fig. 10.17). Internal CO_2 concentration increased with increasing water stress although not significantly between off-times of 0.52 and 2.00 hours. However when C_i was adjusted for patchy stomatal closure (Eq. 3.11) (Downton *et al.*, 1988a: see section 3.1.3), it actually fell sharply with increasing stress. Photosynthetic rate decreased markedly at an off-time of 0.17 hours, corresponding to a similar drop in stomatal conductance, while the CO_2 compensation point was significantly higher above this stress level.

No significant differences were found in the level of dark respiration, which was measured at 1.75 ± 0.2 μmol m^{-2} s^{-1}.

Table 10.9 Effect of different misting pump off-times in the nutrient solution of an aeroponic system on water use as measured in terms of experiment long water use (WU) (n = 30) and instantaneous transpiration rate at noon (E) (n = 12)

Off-Time (hours)	Water use (WU) ($l\ pt\ d^{-1}$)	($l\ m^{-2}\ d^{-1}$)	E ($l\ m^{-2}\ h^{-1}$)	E/WU[a]
0.00	0.57 a	0.74 a	0.16 a	5.2 ns
0.13	0.39 b	0.55 b	0.13 b	5.7 ns
0.52	0.29 c	0.50 b	0.10 c	4.8 ns
2.00	0.18 d	0.36 c	0.07 d	4.7 ns
s.e. mean	0.03	0.06	0.01	0.2

Means with the same letter in each column are not significantly different at the 1% level using the lsd discriminator

[a] Both variables in units of $l\ m^{-2}\ d^{-1}$

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No significant differences were found in the level of dark respiration, which was measured at $1.75 \pm 0.2\ \mu mol\ m^{-2}\ s^{-1}$.

Table 10.10 Effect of different misting pump off-times in an aeroponic system on various leaf parameters

Day[a] (PAR)[b]	Off-Time (hours)	Pn (μmol $m^{-2} s^{-1}$)	gs (mol $m^{-2} s^{-1}$)	C_i (ppm)	C_i'[d] (ppm)	E (mmol $m^{-2} s^{-1}$)
Sun	0.00	5.94 a	0.124 a	253 d	[253]	2.4 a
means	0.13	3.90 b	0.097 b	264 c	[201]	2.0 b
(>1000)	0.52	2.84 c	0.079 c	284 b	[179]	1.6 c
	2.00	1.85 d	0.053 d	291 a	[148]	1.1 d
s.e. mean		0.08	0.008	2		0.1
Night	0.00	-1.5 ns	0.039 a	502 ns	-	0.6 ns
means[c]	0.13	-1.7 ns	0.031 ab	499 ns	-	0.5 ns
(0)	0.52	-1.8 ns	0.029 ab	493 ns	-	0.3 ns
	2.00	-2.0 ns	0.025 b	490 ns	-	0.3 ns
s.e. mean		0.2	0.006	5		0.2

Means with the same letter in each column are not significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)

[a] Sun means pooled over six days, n = 72; night means pooled over 2 days, n = 24

[b] Units of μmol $m^{-2} s^{-1}$

[c] Night photosynthetic value represents respiration rate

[d] C_i adjusted according to the equation of Downton *et al.* (1988a) (see equation 3.3 of chapter 3)

The CO_2 compensation point was found to increase with water stress as shown in the following table.

Table 10.11 Effect of different misting pump off-times in an aeroponic system on the CO_2 compensation point (n = 4)

Off-Time (hours)	CO_2 compensation point (ppm)
0.00	78.1 b
0.13	79.5 b
0.52	82.1 a
2.00	83.0 a
s.e. mean	1.0

Means with the same letter in each column are not significantly different at the 1% level using the lsd discriminator

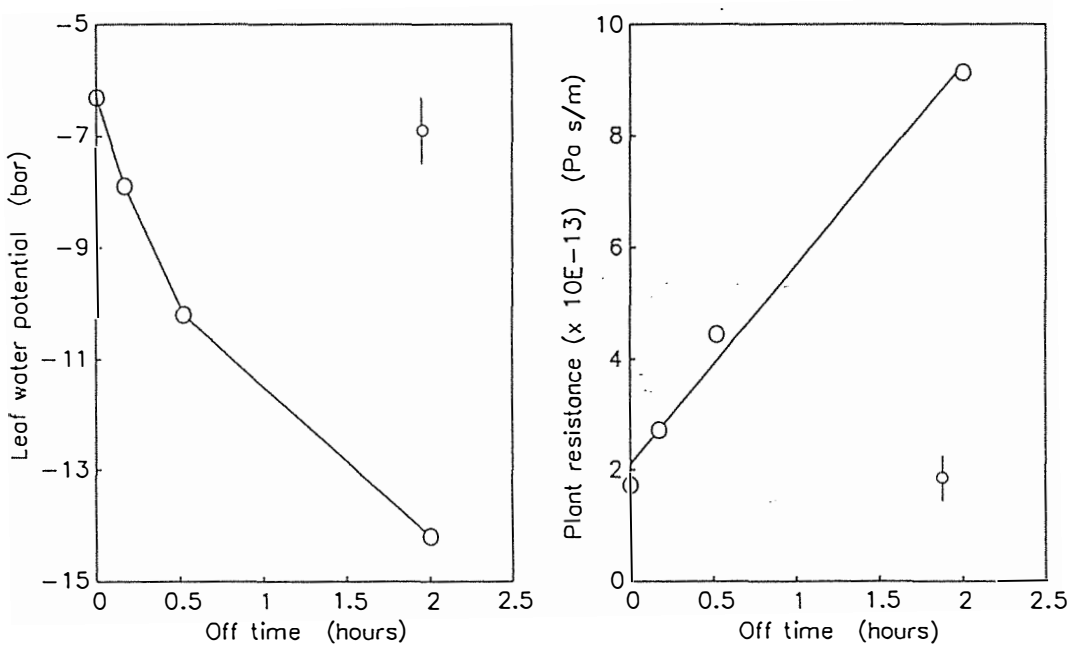

Fig. 10.16. The effect of different misting pump off-times in an aeroponic system on leaf water potential and plant resistance. Bars show pooled s.e.. (a) Leaf water potential. Each point is the mean of 30 measurements (6 readings per day from 5 days). (b) Plant resistance ((Ψ_W(leaf) - Ψ_W(solution)) / E) (n = 30); Y = 2.11 + 3.14X; $R^2 = 0.99^{**}$.

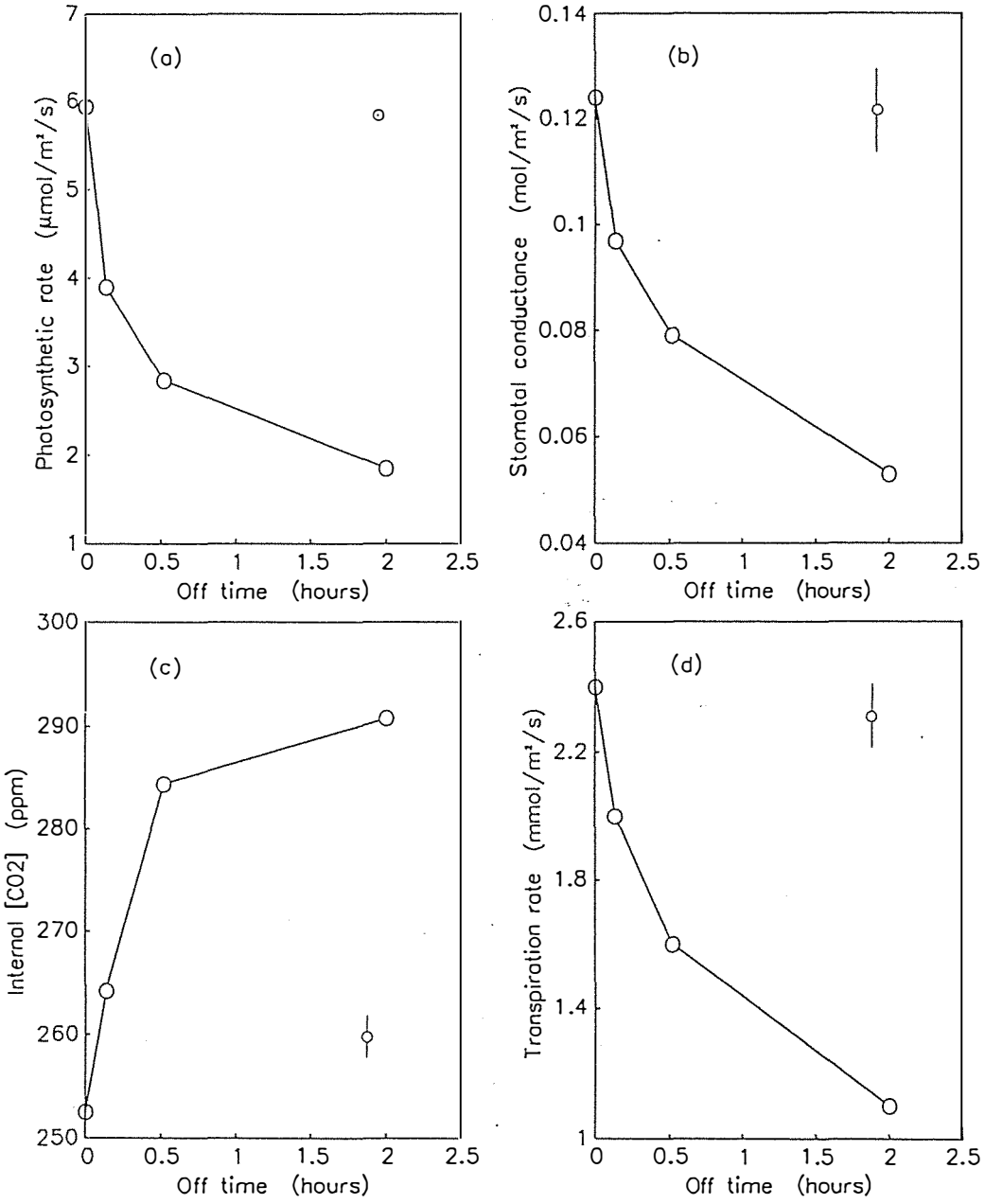

Fig. 10.17. The effect of different misting pump off-times in an aeroponic system on leaf parameters. Each point is the mean of 60 measurements (12 readings per day from 5 days). Bars show pooled s.e.. (a) Photosynthetic rate. (b) Stomatal conductance. (c) Internal CO_2 concentration. (d) Transpiration rate.

10.3.12 *PRESSURE-VOLUME CURVE ANALYSIS*

From analysis of the pressure-volume curve data, obtained using the bench drying method (appendix 18.14), it was found that Ψ_S(sat) decreased with each increment in off-time. Associated with this was a decrease in the relative water content at turgor loss (RWC_O), the symplastic fraction and the exponential constant (a).

Table 10.12 Effect of different misting pump off-times in an aeroponic system on pressure-volume curve parameters for whole leaves determined using the bench drying method given in appendix 18.14

Off-Time (hours)	RWC_o	X	Ψ_S(sat) (bar)	a (bar^{-1})
0.00	0.81 a	0.65 a	-16.2 a	9.3 ns
0.13	0.78 ab	0.61 ab	-16.8 ab	9.1 ns
0.52	0.70 ab	0.55 bc	-18.3 b	8.7 ns
2.00	0.61 b	0.52 c	-20.1 c	7.8 ns
s.e. mean	0.05	0.03	0.9	1.9

Means with the same letter in each column are not significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)

10.3.13 *FOLIAR NUTRIENT ANALYSIS*

No significant differences were found in the foliar levels of potassium, magnesium and calcium between the water stress treatments, indicating that nutrient deficiency did not cause any of the observed differences in growth and development. Levels were generally slightly above those considered by Clark (1987) to be optimal for vegetative growth with the exception of magnesium. Young nashi trees appear to have a specific requirement for magnesium above that of similar fruiting trees although this is not true for mature plants (Clark, 1987).

Table 10.13 Effect of different misting pump off-times in an aeroponic system on potassium, magnesium and calcium levels in leaf tissue (mg g^{-1} d. wt) after 84 days (n = 4)

Off-Time (hours)	Potassium	Magnesium	Calcium
0.00	15.0 ns	2.1 ns	17.9 ns
0.13	14.7 ns	1.8 ns	16.7 ns
0.52	13.6 ns	1.6 ns	16.1 ns
2.00	12.9 ns	1.9 ns	15.4 ns
s.e. mean	2.0	0.4	1.5
Ideal[a]	12.0	2.5	15.0
Deficiency[a]	2.7-4.1	0.9-1.1	6.6

Means with the same letter in each column are not significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)

[a] From Clark (1987) for leaf analysis in mid-season (February) under sand culture

10.4 **DISCUSSION**

10.4.1 *BUTT CROSS-SECTIONAL AREA*

In contrast to the exponential growth exhibited by tomato plants (experiment 2), the *P. betulaefolia* seedlings grew in approximately linear fashion, as measured in terms of both total plant dry weight and butt cross sectional area. The response of these two variables to off-time was highly correlated (r = 0.99).

For tree species, butt size (expressed as either circumference or cross-sectional area) is generally considered to be a good estimator of overall tree size and growth (Pearce, 1952; Westwood and Roberts, 1970). Chalmers and van den Ende (1975) found strong correlations between butt circumference and a variety of tree parameters for peach trees, while Webster and Brown (1980) correlated increase in butt circumference with mean crop load for apple trees.

Results from the experiment described herein are a further demonstration of this close relationship (Fig. 10.14). Plant, shoot and root dry weight, along with leaf area, were all linearly related to butt cross-sectional area, but the level of water stress was a non-significant variable in these regressions. This suggests that moderate environmental stresses may not influence the butt size relationships, at least with respect to vegetative components. Chalmers and van den Ende (1975) note the strong

influence of environment and management on tree productivity and suggest that high correlations between butt size and tree growth will only be obtained under uniform conditions.

The linear increase in butt cross-sectional area with time, and its correlation with stress effects on overall plant growth, can be compared with total plant height measurements for tomato plants (experiment 2). Both parameters were found to increase linearly with time, regardless of the water stress treatments.

10.4.2 *SHOOT GROWTH*

For all four treatments, both total shoot length and leaf area growth slowed down after day 35 of the experiment, the curtailment increasing with the level of stress. However, SL2 and SL3 plants showed a slight recovery in growth between 63 and 84 days while in control and SL1 plants growth continued to decline.

The exhibited growth patterns are characteristic of perennial tree species which show a strong vegetative growth flush early in the season (e.g. peach and pear, see Chalmers *et al.* (1984)). Clearly, however, water stress treatments affected this natural periodicity, causing growth to slow more rapidly early in the season. Plants responded with a relative increase in growth rate later in the season, indicating a certain level of adaptation to their water stress environment.

The importance of the stem component in a perennial tree species has been clearly demonstrated in this experiment. Stem RGR was reduced over 50% by the SL3 treatment while leaf RGR was reduced by only 34%. With a decrease in productivity, assimilate was preferentially partitioned into leaf and root at the expense of stem growth. Plant part ratio changes (Fig. 10.2, 10.3 and 10.4) provide a further insight, with the Sm/Rt ratio showing greatest sensitivity (note that these plots have time linearized plant dry weight on the abscissa as discussed in section 9.4.8 and 14). A rapid increase in the Sm/Rt ratio for control plants exemplifies the importance of the stem as a storage organ through the growing season.

Longer term changes in dry weight distribution within a perennial crop can be compared to within season changes as observed here. These were discussed in detail for peach by Chalmers and van den Ende (1975), while Avery (1970) found that the stem to root ratio in apple decreased with time towards some equilibrium value.

10.4.3 *ROOT GROWTH*

The transfer of plants from a solid medium to aeroponic tanks results in the growth of roots with different characteristics ('tank' roots) and loss of the old root system ('bag' roots). Such a change in root anatomy will occur whenever the root environment is changed significantly (Al-Sahaf, 1984). With tomato seedings, the old root system degenerated rapidly within 7 to 10 days and this was within the pre-treatment period so that only 'tank' roots were considered. With young *P. betulaefolia* trees however, the old root system remained throughout the experiment. Fine roots, those less than approximately two millimetres in diameter, quickly became nonviable (visual assessment and tetrazolium stain) and a large proportion (at least 50%) had died before treatments were begun. They remained attached throughout the experiment however due to the lack of microorganisms in the aeroponic environment. The larger structural roots gave rise to new 'tank' roots and consequently increased in dry weight through the experiment. No structural roots (greater than two millimetres diameter) were formed in the aeroponic tanks during the experiment.

In Fig. 10.8 it can be seen that the 'bag' roots (not including structural) had a significantly lower root length to dry weight ratio than the 'tank' roots throughout the experiment, and for control plants this difference was constant at 19 m g^{-1}. In other words, the 'tank' roots were more succulent, as would be expected in an aqueous environment with no water stress. During the 1988-89 growing season, *P. betulaefolia* trees, in planter bags on sand beds, would certainly have experienced water stress because of the limited root volume (5 l). Considering the high proportion dead 'bag' roots during the experimental period, the difference between 'tank' and 'bag' roots would be even greater with respect to viable roots alone.

The root length to dry weight ratio decreased over time, reflecting the proportional increase in dry weight gain by the roots as the growing season progressed. Water stress resulted in an initial sharp decrease in the ratio due to reduced growth and tissue hardening (increase in dry matter content), with 'bag' and 'tank' roots both exhibiting the same trends. By the end of the experiment however, the ratios were almost identical for all treatments (27 ± 0.5 m g^{-1}) which means that water stress increased the rate at which roots 'matured' without affecting innate characteristics. Clearly, the final ratio reached was a very stable parameter for this species under aeroponic conditions.

10.4.4 *LEAF PARAMETERS*

Both Pn and g_s fell sharply with increasing off-time and were highly correlated (r = 0.99). By contrast, internal CO_2 concentration actually increased from 252 to 290 ppm at SL3, suggesting that the photosynthetic limitation was largely nonstomatal in nature. However the situation is completely different if C_i is adjusted using the equation of Downton *et al.* (1988a) (see section 3.1.3) based on the phenomenon of patchy stomatal closure, with adjusted values being correlated with g_s (r = 0.90) (Table 10.14) indicating that stomatal closure could fully account for the drop in Pn. This provides a good example of the possible dangers of using IRGA derived C_i values to interpret photosynthetic limitations.

10.4.5 *WATER USE*

Water use per unit leaf area decreased rapidly with increasing misting pump off-time, while water use efficiency increased with each stress increment, from 4.7 to 6.7 g d. wt l^{-1} H_2O. Water use per unit root length ('tank' roots only) decreased from 0.71 to 0.30 ml m^{-1} day^{-1} at SL3. Noon transpiration rate was approximately five times that of average water use (measured by tank water loss) for all treatments, which reflects the smaller degree of stomatal control compared with tomatoes in the previous experiment (where g_s was reduced by 85% compared with 50% here). The difference between noon and average water loss figures for the control, in itself, suggests transient water stress over the midday period, although this could not have been very large considering the measured noon Ψ_W(leaf) values (-6.3 bar average).

Total plant resistance increased markedly with water stress (over 500% at SL3). This is in accord with the discussion of Camacho-B *et al.* (1974a) who state that pear species combine strong stomatal control with a low efficiency transport system (i.e. high internal resistance), an adaptation to relatively dry environments.

10.4.6 *WATER POTENTIALS AND OSMOTIC ADJUSTMENT*

Apple trees have been found to osmotically adjust to a greater degree than most other horticultural plants tested (Lakso *et al.*, 1984), and it may be speculated, without supporting information, that Asian pear species would be similar in nature.

Lakso *et al.* (1984) observed a drop in Ψ_S(sat), through one season, of around 20 bar for mature apple leaves (-20 bar (early season) to -40 bar (late season)), results being similar to those of Goode and Higgs (1973). Davies and Lakso (1978) followed the diurnal change in water potential components of apple seedlings. The Ψ_S of irrigated and non-irrigated plants changed by 3 and 6 bar respectively with a change in Ψ_W of 5 and 8 bar, while the diurnal change for field grown trees was found to be somewhat greater (10 bar) (Davies and Lakso, 1979). Note of course that Ψ_S(sat) changes would be significantly less than these values.

In this experiment osmotic adjustment at full turgor was in the order of 4 bar at SL3 (-16.2 to -20.1 bar) corresponding to a change in noon Ψ_W of 7.9 bar. Hence results are comparable to those described above for apple.

10.4.7 *ASSIMILATE DISTRIBUTION*

Linear relationships were found between leaf area and root length (Fig. 10.7), as in experiment 2. Root length and leaf area are linked through their involvement in water and nutrient uptake, while root and leaf number associate through differentiation processes (Chung et al., 1982). Richards (1986) found a linear relationship between root and leaf number as well as root number and leaf area. The slopes for leaf area *versus* root length decreased logarithmically with increasing off-time (Fig. 10.8) showing that leaf development was affected more than root development. This was supported by the decrease in RGR's, 27% and 51% for root and leaf respectively. In contrast to the tomato plants of experiment 2, root relative growth rate decreased with each increment in stress.

Allometric relationships appeared linear although the variation was greater than in experiment 2, which will reflect in part, the use of seed propagated trees rather than clonal material. *Pyrus betulaefolia* shows a high degree of seedling variability (Loreti and Morini, 1977), but this was reduced as much as possible by selecting plants of uniform size, shape and leaf pattern. Differences in stress history may also have contributed to the inter-plant variation. Although the trees were watered three times a day, while growing outside, the limited root volume (5 l) would have accentuated variation in application rate and evaporative demand. Water was applied through mini-sprinklers above the trees such that watering pattern was influenced by wind conditions. Stress history can be very important in determining how a plant responds to subsequent periods of stress (Conroy *et al.*, 1988; Jones and Turner, 1978; Morgan, 1984).

In accordance with previous results and the findings of Troughton (1960), the allometric k value decreased with increasing off-time. The same parameter calculated via RGR(shoot)/RGR(root) and the comparable parameter d(S/R)/d(PtDW) (see section 9.4.8 and 14) showed reasonable agreement with 'k' (Fig. 10.12). Note how the S/R ratio *versus* PtDW plots highlighted the variability in the data, as discussed in section 9.4.8. For a general discussion on changes in the allometric relationship see section 14.2.

10.4.8 *OFF-TIME RELATIONSHIPS*

In this experiment, as in experiment 2 where tomatoes were used, many plant parameters were found to decrease in a logarithmic fashion as misting pump off-time increased. However in contrast to experiment 2 (Table 9.15), no parameters were found to be linearly related (Table 10.14).

Table 10.14 Effect of different misting pump off-times in an aeroponic system on various plant variables

	Relationship with Off-Time	
Variable	Linear	Logarithmic
Leaf water potential		*
Water use		*
Butt XSA increase		*
PLDW increase		*
LA per RL		*
Allometric K value		*
RGR ratio		*
S/R ratio vs PLDW		*
Absolute growth rate		*
RGR(leaf)		*
RGR(stem)		*
RGR(plant)		*
ULR		*
USR		*
Photosynthetic rate		*
Stomatal conductance		*
Transpiration		*

10.4.9 *SUMMARY*

i/ The logarithmic decrease in plant parameters with aeroponic misting pump off-time, as observed using tomatoes (experiment 2), was also found when *P. betulaefolia* seedlings were used. This suggests that the response may be independent of plant type and growth pattern.

ii/ For plants growing linearly, a linear allometric relationship is still observed, which agrees closely with the shoot/root RGR ratio and S/R *versus* plant dry weight ratio.

iii/ Water stress in *P. betulaefolia* is characterized by a significant relative decrease in stem dry weight accumulation and a relative, but not absolute, increase in root partitioning.

11 EXPERIMENT 4 : ROOT RESTRICTION STUDIES ON *PYRUS BETULAEFOLIA*

11.1 INTRODUCTION

Root restriction (or restricted root zone volume (RRZV)) and water stress are two closely associated growth limiting conditions because under natural conditions, physical restriction of the root system will decrease both the current and future water uptake capacity. Scott Russell (1982) has highlighted the importance of continued root axis elongation into wetter zones to minimize water stress, something that cannot occur under root restriction.

Even under what appear to be very controlled experimental conditions, the elucidation of root restriction responses without confounded water stress has proved difficult, and indeed, Hameed *et al.* (1987) were drawn to the conclusion that the principle effect of a RRZV was induced water stress. However Krizek *et al.* (1985) have shown, in one of the most useful experiments, that there are clear differences between the two conditions, with water stress causing wide spread disruption to internal physiology (i.e. decreased Ψ_W(leaf), g_s, Pn, S/R ratio, nitrogen level and phosphorous level) and RRZV causing no such disruption.

Hydroponic systems have proved the most successful in the quest for root restriction responses, due to the ability to precisely control conditions in the root zone. A common system configuration has involved growing plants in aerated containers of nutrient solution with no solid media present (Al-Sahaf, 1984; Cooper, 1972; Krizek *et al.*, 1985; Richards and Rowe, 1977a), thereby allowing direct access to the root system.

The root restriction - water stress area has considerable practical significance, for example, under drip irrigation the root system is restricted by a sharp change in soil water potential (water content). If the root bowl is kept moist while the surrounding soil is dry this situation is directly analogous to the hydroponic system described above. Proebsting *et al.* (1989), using fruiting peach trees, found that root zone volume could be effectively controlled by restricted irrigation practices, or more specifically in this case, RDI. Root restriction treatments did not affect Ψ_W(leaf) as much as water stress treatments, and in fact Ψ_S(leaf) was unaffected.

In view of the confusion surrounding root restriction induced responses, and the practical significance involved, a restriction experiment was conducted using nashi

rootstock seedlings (*Pyrus betulaefolia*) in a hydroponic system. Thus, results could then be compared with water stress studies on the same species carried out in the aeroponic tanks.

11.2 MATERIALS AND METHODS

For a detailed description of general experimental procedures see section 6.

11.2.1 *EXPERIMENTAL INFORMATION*

Seedlings of *Pyrus betulaefolia* Bunge, as described in section 10.2, were transferred from polythene bags (PB 8) containing a 60:40 pumice:sand blend to a hydroponic growing system (appendix 18.5) in December 1988. Three harvests from six blocks (Fig. 11.1) were made as defined in Table 11.1.

Table 11.1 Harvest information

Harvest #	Day	# Plants/block
0	0	2
1	20	4
2	60	8

Plants were sprayed with an insecticide only as required to control insect pests (see appendix 18.9).

11.2.2 *TREATMENT INFORMATION*

The two treatments applied were as follows:

i/ Unrestricted (control) : Root zone volume = 10 l.
ii/ Restricted : Root zone volume = 0.04 l.

Stock nutrient solution (appendix 18.8) was housed within two separate containers and an automatic controller released this solution, along with nitric acid (2 M), to maintain a conductivity of 2.5 mS and a pH of 6.5 respectively. An outflow pipe removed 25% of the hydroponic system solution each week to prevent the buildup of minerals and root exudate. Compressed air was bubbled into each plant container through two microtubes and these were checked weekly for blockages. In the case of restricted plants, one microtube was located in the 10 l bucket and the other in the 40 ml root restriction vessel (see appendix 18.5).

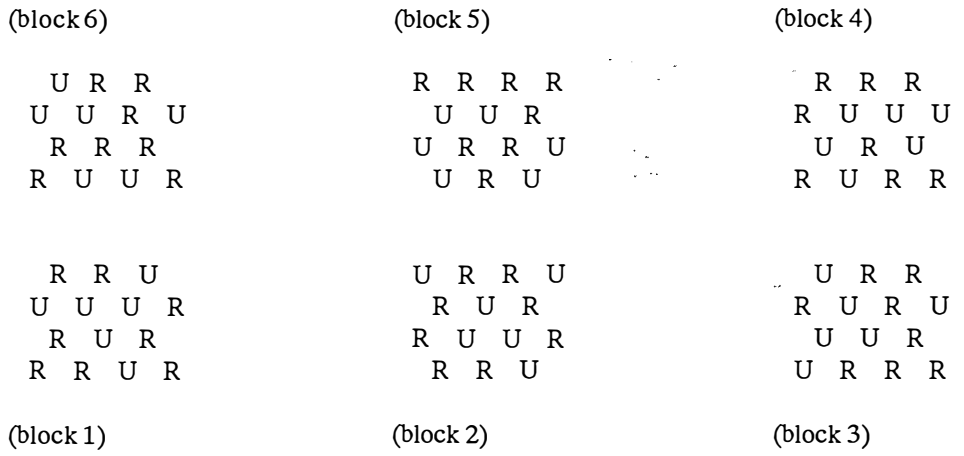

Fig. 11.1. Experimental layout for *P. betulaefolia* seedling trees in the circulating hydroponic system; U = unrestricted; R = restricted.

11.2.3 *DATA GATHERING PROCEDURES*

Plant part analysis was carried out on the days specified in Table 11.1, according to the methods of section 6.2.1. Photosynthetic measurements were made on days 7, 21, 35 and 49 at 12 pm to 2 pm, while leaf water potential measurements were made on days 8, 22, 36 and 50 at 1 pm to 2 pm. Other measurements included plant hydraulic resistance (day 53) and assessment of tissue mineral (day 62) (see section 6.2.11).

In order to measure plant hydraulic resistance, two plants per treatment were removed from the hydroponic system and placed in 20 l buckets of full strength nutrient solution containing four compressed air microtubes. At 9 am the following

Stock nutrient solution (appendix 18.8) was housed within two separate containers and an automatic controller released this solution, along with nitric acid (2 M), to maintain a conductivity of 2.5 mS and a pH of 6.5 respectively. An outflow pipe removed 25% of the hydroponic system solution each week to prevent the buildup of minerals and root exudate. Compressed air was bubbled into each plant container through two microtubes and these were checked weekly for blockages. In the case of restricted plants, one microtube was located in the 10 l bucket and the other in the 40 ml root restriction vessel (see appendix 18.5).

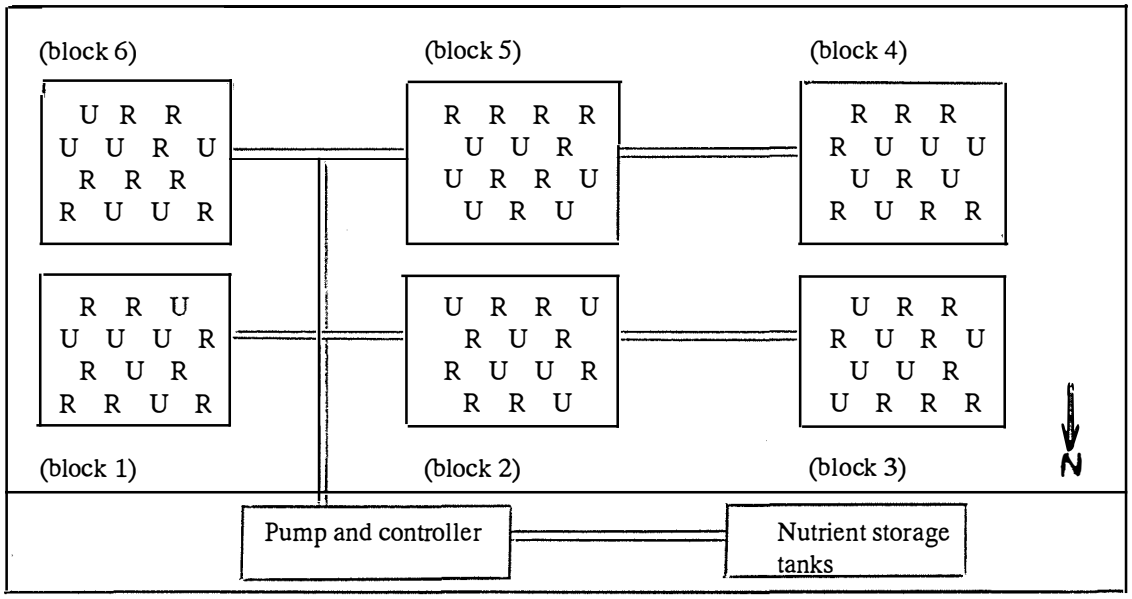

Fig. 11.1. Experimental layout for *P. betulaefolia* seedling trees in the circulating hydroponic system; U = unrestricted plant; R = restricted plant.

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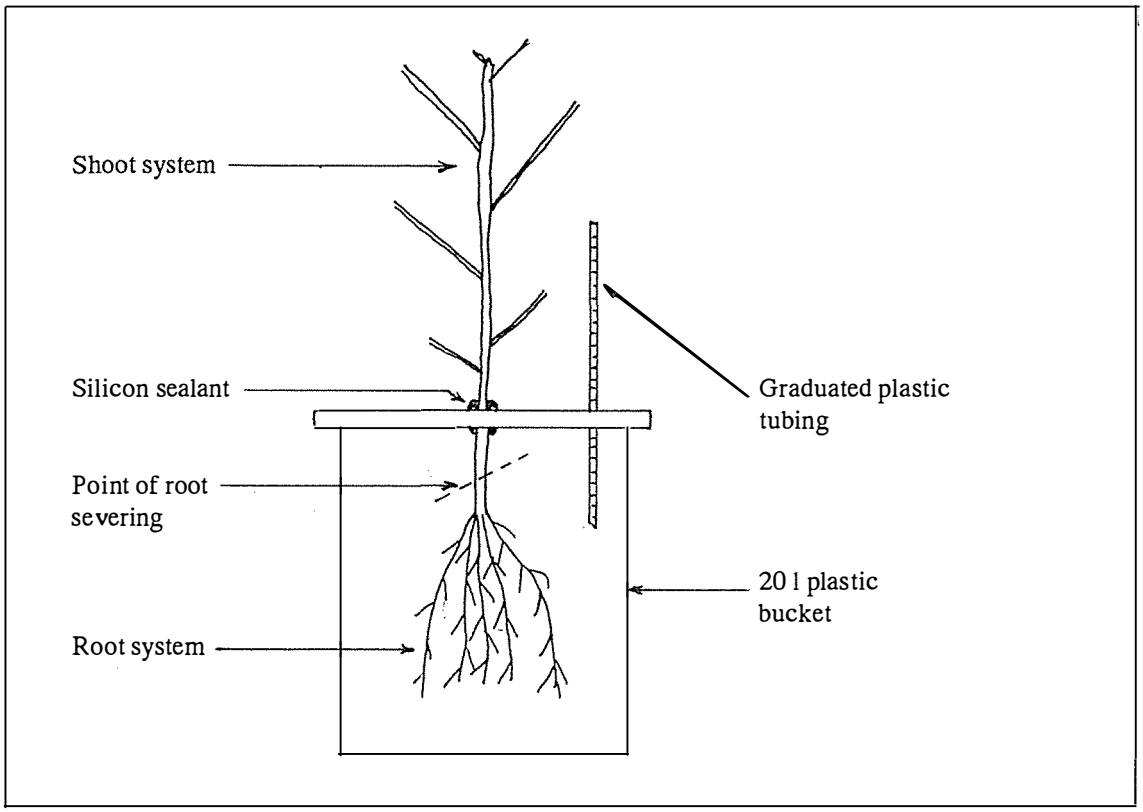

Fig. 11.2. The setup for measuring hydraulic conductivity in *Pyrus betulaefolia* seedlings.

11.3.2 *PLANT PART DRY WEIGHT RATIOS*

After 60 days the restricted plants had a larger stem to root ratio and a smaller leaf to stem ratio than the unrestricted plants (Table 11.3), due to a greater inhibition of root and leaf growth relative to stem growth. Leaf and root growth appeared to be affected equally, with no change in the leaf to root ratio, but there was an overall increase in the shoot to root ratio under root restriction.

Table 11.2 Plant part fresh and dry weights for root restricted and unrestricted plants grown in a hydroponic system for 60 days (n = 8)

Plant parameter	Treatment unrestricted	restricted	s.e. mean
Fresh weights (g):			
leaf	34.80 ns	32.94 ns	1.60
stem	27.50 ns	26.37 ns	1.04
shoot	62.30 ns	59.31 ns	2.30
Dry weights (g):			
leaf	14.07 a	12.43 b	0.40
stem	12.85 ns	12.26 ns	0.40
root	9.10 a	7.73 b	0.33
shoot	26.92 a	24.69 b	0.53
plant	36.02 a	32.42 b	0.65

Means in the same row with different letters are significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)

Table 11.3 Plant part dry weight ratios for root restricted and unrestricted plants grown in a hydroponic system for 60 days (n = 8)

Plant parameter	Treatment unrestricted	restricted	s.e. mean
Lf/Sm	1.09 a	1.01 b	0.02
Lf/Rt	1.55 ns	1.61 ns	0.07
Sm/Rt	1.41 b	1.59 a	0.05
Sh/Rt	2.93 b	3.21 a	0.09

Means in the same row with different letters are significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)

11.3.3 *PLANT PART WATER CONTENTS*

No significant differences were found in leaf or stem water content (Table 11.4).

Table 11.4 Plant part water contents (g H_2O g^{-1} d. wt) for root restricted and unrestricted plants grown in a hydroponic system for 60 days (n = 8)

Plant parameter	Treatment unrestricted	restricted	s.e. mean
Leaf	1.56	1.63	0.05
Stem	1.13	1.14	0.04

No means were significantly different at the 5% level

11.3.4 *LEAF AND ROOT DATA*

Leaf area and root length were both reduced significantly by the restriction treatment, while leaf number increased, resulting in a 27% reduction in mean leaf size (Table 11.5). The reduction in root length was greater than that of leaf area such that the leaf area to root length ratio increased. There was also a significant 13% reduction in root length per unit dry weight.

11.3.5 *CANONICAL ANALYSIS ON PLANT PART DATA*

A canonical analysis was performed on harvest 3 using the following plant data:

i/ Leaf dry weight.
Stem dry weight.
Root dry weight.

ii/ Leaf dry weight.
Stem dry weight.
Root dry weight.
Leaf area.
Root length.

Table 11.5 Leaf area, leaf number and root length for root restricted and unrestricted plants grown in a hydroponic system for 90 days (n = 8)

Plant Parameter	Root Treatment unrestricted	restricted	s.e. mean
leaf area (cm^2)	1923 a	1690 b	80
leaf number	311 b	374 a	10
root length (m)	647 a	478 b	30
RL/RDW ratio ($m\ g^{-1}$)	71 a	62 b	4
LA/RL ratio ($m^{-2}\ m^{-1}$)	2.97 b	3.53 a	0.08
LA per leaf (cm^2)	6.18 a	4.52 b	0.25

Means in the same row with different letters are significantly different at the 1% level using the lsd discriminator

Table 11.6 Canonical analysis on plant part data for root restricted and unrestricted plants grown in a hydroponic system for 90 days (n = 8)

(a) Between canonical structure

Treatment	Plant part Leaf	Stem	Root	LA	RL
Canonical 1	0.99	0.99	0.99	0.99	0.99

(b) Canonical variable class means

Treatment	Canonical 1	s.e. mean
unrestricted	0.80 a	0.10
restricted	-0.69 b	0.09

Means in the same column with different letters are significantly different at the 1% level using the lsd discriminator

The Wilk's Lambda test was only significant for the second analysis (1% level) and in this case only the first canonical variable was significant (1% level). It can be seen from Table 11.6 that the first canonical variable was a general discriminator.

11.3.6 *THE ALLOMETRIC RELATIONSHIP*

Allometric relationships for shoot *versus* root dry weight are given in Fig. 11.3 and appear linear. The slope (k value) increased significantly (5% level) under root restriction.

11.3.7 *LEAF WATER POTENTIAL*

Leaf water potentials at dawn were not significantly affected by the restriction treatment (Table 11.7). However noon leaf water potential decreased under root restriction, reflecting the greater leaf area to root length ratio, and indicating that restricted plants experienced a certain degree of water stress over the midday period.

Table 11.7 Leaf water potentials (bar) for root restricted and unrestricted plants grown in a hydroponic system for 60 days (n = 72 - pooling over three days)

Treatment	Ψ_W(leaf) (bar) Noon	Dawn
unrestricted	-6.3 a	-1.5 ns
restricted	-7.9 b	-1.7 ns
s.e. mean	0.5	0.4

Means in the same row with different letters are significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)

11.3.8 *PHOTOSYNTHETIC SYSTEM DATA ANALYSIS*

Photosynthetic rate, internal CO_2 concentration, stomatal conductance and transpiration rate were not significantly affected by root restriction (Table 11.8) although the unrestricted plants tended to have a higher conductance. This would be expected due to the difference in leaf water potential.

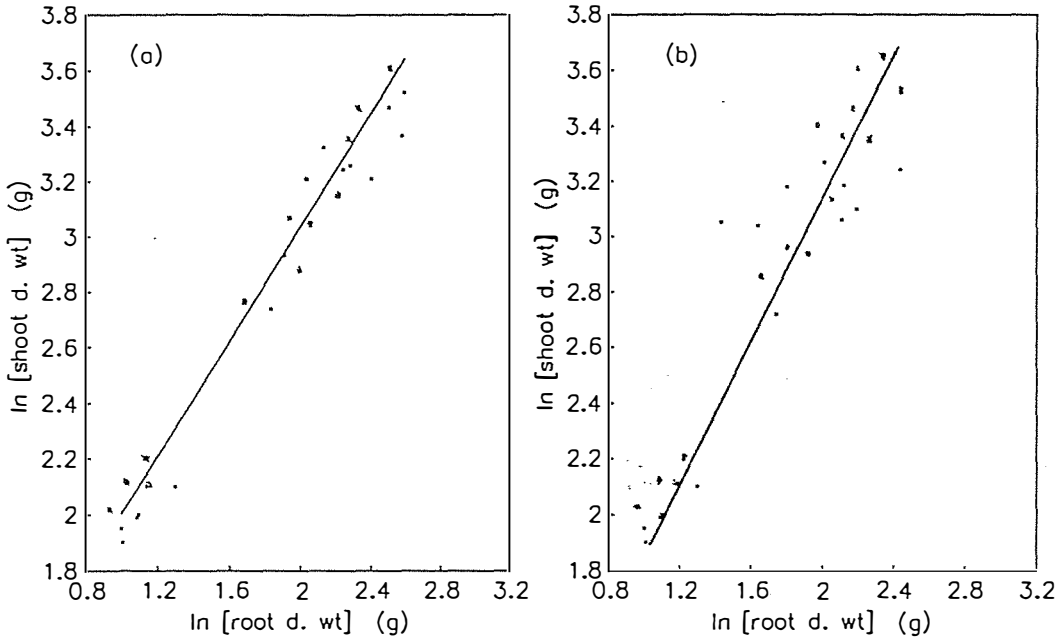

Fig. 11.3. The effect of a restricted root zone on the allometric relationship. (a) Unrestricted; $Y = 0.84 + 1.02X^{b}$; $R^2 = 0.98^{**}$. (b) Restricted; $Y = 0.77 + 1.20X^{a}$; $R^2 = 0.97^{**}$ (letters on slope coefficients indicate significant differences at the 5% level using a paired t-test).

Table 11.8 Leaf parameters for root restricted and unrestricted plants grown in a hydroponic system for 60 days (n = 72 - pooling over three days where PAR > 800 μmol m^{-2} s^{-1})

Treatment	Pn (μmol) (m^{-2} s^{-1})	gs (mol) (m^{-2} s^{-1})	C_i (ppm)	E (μmol) (m^{-2} s^{-1})
unrestricted	4.7[a]	0.13	260	0.0022
restricted	4.3	0.10	270	0.0019
s.e. mean	0.5	0.02	25	0.0002

[a] No significant differences between means in the same column at the 5% level

11.3.9 *HYDRAULIC RESISTANCE*

Root resistance increased by 260% under the restriction treatment, giving rise to a 62% increase in total plant resistance. Stem resistance, which did not increase significantly, was relatively small (Table 11.9).

Table 11.9 Plant part hydraulic resistance (10^{13} Pa s m^{-3}) estimates for root restricted and unrestricted plants grown in a hydroponic system for 60 days (n = 2)

Treatment	Petiole	Stem	Root	Plant
unrestricted	0.2 ns	1.8 ns	0.8 b	2.8 b
restricted	0.2 ns	2.2 ns	2.1 a	4.5 a
s.e. mean	0.1	0.2	0.2	0.4

Means in the same column with different letters are significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)

11.3.10 *LEAF NUTRIENT ANALYSIS*

No significant differences were found in the levels of the three nutrients analysed (Table 11.10). Therefore the restriction treatment did not appear to cause nutrient deficiencies which would have confounded growth responses. Levels were close to those considered ideal by Clark (1987) for mid-season growth.

Table 11.10 Effect of root restriction treatments in a hydroponic system on potassium, magnesium and calcium levels in leaf tissue (mg g^{-1} d. wt) after 60 days (n = 8)

Treatment	Potassium	Magnesium	Calcium
unrestricted	10.0 ns	1.9 ns	14.9 ns
restricted	9.7 ns	1.9 ns	12.7 ns
s.e. mean	2.5	0.5	2.5
Ideal[a]	12.0	2.5	15.0
Deficiency[a]	2.7-4.1	0.9-1.1	6.6

Means with the same letter in each column are not significantly different at the 1% level using the lsd discriminator (ns = not significant at the 5% level)

[a] From Clark (1987) for leaf analysis in mid-season (February) under sand culture

11.4 **DISCUSSION**

11.4.1 *SHOOT AND ROOT RESPONSES*

The decrease in leaf water potential for RRZV indicates that the treatment induced a water stress under significant evaporative demand, as others have found (Hameed *et al.*, 1987; Tschaplinski and Blake, 1985). Hence absolute delineation of root restriction responses is not possible, although useful comparisons may be made with experiments involving water stress under unrestricted conditions.

The main plant responses involved a decrease in the size of root and leaf components, with the root component most strongly inhibited. This led to an increase in the leaf area to root length ratio and hence an increase in the susceptibility to water stress. The size of the stem component was unaffected and, relative to the other components, it received more dry matter. These responses are in agreement with those of Al-Sahaf (1984), who found that the shoot to root ratio of tomato plants increased under root restriction, with a greater proportion of dry matter allocated to the stem and less to the leaves.

Reductions in shoot extension growth (Carmi, 1986; Carmi and Heuer, 1981; Carmi and Shalhevet, 1983; Ruff *et al.*, 1987) and leaf growth (Mutsaers, 1983b) are well known under root restriction. Smaller plants develop with fewer leaves (Carmi and Shalhevet, 1983; Richards and Rowe, 1977a) which, for tree species, are

comparable to those on dwarfing rootstocks (Richards, 1986). In this experiment leaf number actually increased under root restriction, possibly reflecting a greater number of short shoots, although this cannot be confirmed. With the concomitant reduction in leaf area, leaf size was reduced by 27%, which was comparable to the reduction in root length (26%).

Richards and Rowe (1977a) related changes in root length (proportional to root surface area) and root number (proportional to meristematic potential) to the level of change in other parameters and suggested a dependency between them. For example, leaf number, lateral length and shoot weight were reduced to a similar extent as root number, while the change in water uptake, nitrate uptake and leaf area was similar to that of root length. The lack of reduction in leaf number in the experiment described herein, together with the smaller decrease in plant part dry weights compared to root length, suggests that root number was not greatly affected. Indeed, it may even have increased because Al-Sahaf (1984) found that under root restriction, roots tended to be more highly branched with a greater water and mineral uptake capacity per unit length. Increased root number is characteristically found under mechanical impedance (Goss and Scott Russel, 1980).

Increase in whole plant resistance was found to be due to increased root resistance, but overall resistances were lower than those previously quoted for apple (Landsberg *et al.*, 1976), reflecting in part the ontogenic and genetic variation. Hameed *et al.* (1987) observed a seven fold increase in root resistance for tomato plants under physical root restriction, which they used to explain lower leaf Ψ_W and other water stress responses. A similar line of reasoning may be applied to this experiment, although the responses were less marked.

11.4.2 *PHOTOSYNTHESIS AND ASSIMILATE PARTITIONING*

No significant changes in net photosynthetic rate or stomatal conductance were found in line with the results of Krizek *et al.* (1985). This reinforces the idea that root restriction results in a reduction in plant size without significant disruption to internal physiology.

The change in the allometric relationship was opposite to that which occurs under water stress, that is, the allometric k value increased rather than decreased. This is in line with the increase in S/R ratio found after 60 days. Hence root restriction resulted in a relative increase in assimilate partitioning to the shoot (primarily the

stem), as others have observed (Al-Sahaf, 1984; Tschaplinski and Blake, 1985). Clearly this affect will be reduced as confounding of water stress increases, and if water stress becomes dominant, the allometric k value will be observed to decrease. See sections 14 and 16 for further discussion on changes in the allometric relationship under water stress and root restriction.

11.4.3 *HORMONAL INVOLVEMENT*

Growth responses under root restriction may be related to changes in plant hormone levels. Both cytokinin and gibberellin have been observed to decrease under root restriction (Ruff *et al.*, 1987), while application of GA_3 and BA can overcome most of the changes in shoot growth resulting from RRZV (Carmi and Heuer, 1981). Exogenous GA_3 increases stem growth (Carmi and Heuer, 1981), while BA partially restores leaf growth (Carmi and Heuer, 1981; Richards and Rowe, 1977a). Richards and Rowe (1977a) found that BA reduced root number and length in unrestricted but not restricted plants. This reflects the fact that in restricted plants, root growth is already limited by root zone volume. The relative increase in root branching found by Al-Sahaf (1984) may be due to changes in root IAA levels. It has already been shown that mechanical impedance can result in a three and a half fold increase in root IAA levels (Lachno *et al.*, 1982).

Recent work by Thuantavee (1991), using plants with completely unrestricted root systems, has confirmed that gibberellin increases partitioning to the stem (at the expense of the root system). It was further found that continuous application of BA to the root system resulted in a short burst of shoot growth, such that the S/R ratio increased but the allometric k value returned to its original level (see section 14.2). Cytokinin appeared to have no effect in partitioning to the stem.

Results from the experiment in this chapter, and the above literature, strongly suggest that primary changes under RRZV are due to the reduced supply of root produced cytokinin and an imbalance in the cytokinin to gibberellin ratio in the shoot. While a plant can internally adjust to cytokinin levels in moderate excess (by decreasing synthesis and increasing degradation) (Thuantavee, 1991), the imbalance under root restriction will persist, giving rise to a new (higher) allometric k value (e.g. Fig 11.3). However, the increasing S/R ratio increases the chance of water stress, so at some point 'k' will decrease (as shown in experiments 2 and 3). This situation has in the past led to confusion over the physiological responses to RRZV.

More work is needed on plant hormone changes under both mechanical impedance and root restriction because it is an interesting issue both alone and in relation to the whole field of plant water relations. Note that in some cases root pruning has been used to simulate the effects of physical root restriction (e.g. Cooper (1971)). However, when one considers the hormonal changes involved, results from such experiments are hard to interpret. Root pruning removes the apices which are central to root hormonal status and hence the two treatments are by no means comparable.

11.4.4 *SUMMARY*

i/ Physical root restriction led to a relative increase in assimilate partitioning to the shoot, especially the stem portion, with an increase in the leaf area to root length ratio. However, no significant effect on photosynthesis per unit leaf area (Pn) was observed.

ii/ The slope of the allometric relationship was found to increase with increasing root restriction. This is opposite to the response observed under increasing water stress.

GENERAL DISCUSSION

WATER STRESS ON VEGETATIVE GROWTH

12 WATER STRESS INDUCTION

12.1 AEROPONICS AND WATER POTENTIAL AGENTS

The first question to be addressed in the experimental work described herein was 'how do water potential agents perform in the aeroponic system?'. From experiment 1, it was found that dry weight increase was related linearly to solution Ψ_W. Patterns of PEG uptake were similar to those of Janes (1974), who used a simple hydroponic setup, although the level of uptake was considerably lower, suggesting that the use of water potential agents in an aeroponic system could have potential under certain conditions. The unknown physiological effects of PEG which did enter the plants, still represented a major problem for the valid interpretation of water stress results. Thus, alternative methods of generating a controlled water stress are still required, which ideally are both simple in their implementation and yet flexible with respect to the range of water stresses which may be applied. Characterization of the intermittent misting system represents a significant contribution in this area.

12.2 INTERMITTENT MISTING

The results of experiment 2 and 3 were extremely encouraging with respect to the potential of the intermittent misting system. It was found that misting pump off-times of up to at least two hours could be used to generate a controlled 'mild' water stress in very different plant types (woody perennial and herbaceous annual/perennial).

Results showed that a number of key vegetative growth parameters (e.g. Ψ_W(leaf), allometric k value, water use and photosynthetic rate) followed a logarithmic type decrease with misting pump off-time (Tables 9.22 and 10.18). This is an interesting and useful finding considering the different nature of the plant material used and their growth patterns. From these relationships, estimates can be made of the level of plant stress and plant responses over a continuous range of applied water stresses (misting pump off-times). Certain parameters were found to be related linearly to misting pump off-time in the case of tomato and logarithmically in the case of *P. betulaefolia*, e.g. absolute growth rate, relative growth rate, leaf area to root length ratio (Tables 9.22 and 10.18). Such a difference can be explained by considering the respective growth patterns, which were approximately exponential for tomato but tending to linear for *P. betulaefolia*.

Overall, it was found that, for misting pump off-times of up to at least two hours, the level of applied stress and plant responses in the vegetative phase could be predicted. There were no confounding problems of phytotoxicity due to additional chemicals in the nutrient solution, and the system was very simple and flexible in its operation. It could be operated for an indefinite period and easily modified to suit the plant material, unlike systems involving semipermeable membranes or modulated water movement into the root zone. Having established these results it was deemed important to consider the precise nature of the intermittent misting induced water stress, as compared to that generated by water potential agents or under natural conditions.

12.3 INTERMITTENT MISTING INDUCED WATER STRESS

Water can be lost from the surface of roots in an aeroponic tank in three ways, by gravity, evaporation and root uptake. Condensation of water vapour from the atmosphere is the only additive process once the misting pumps are off. This situation is depicted in Fig. 12.1. Measurement of tank atmosphere r.h. revealed that it was in a state of constant saturation, even after the misting had been off for over 1.5 hours and this has two related consequences. Firstly the water potential of the tank atmosphere was close to zero, although it must be remembered that potential is very sensitive to changes in r.h. due to the following relationship:

$$\Psi_W = (R \times T \times \ln(r.h.)) / m_w \qquad 12.1$$

Where: R = Universal gas constant (8.314×10^{-5} bar m^3 mol^{-1} K^{-1}).
T = temperaure (K).
m_w = molecular weight of water (18 g mol^{-1}).

Secondly the evaporative loss from the root surface will be low and not greatly affected by the evaporative demand on the plant. This is because the saturated atmosphere and white tank surface kept temperature fluctuations within the tank to a low level (± 2 °C).

Gravity water loss from the root system will depend on the rate of the other processes and on the characteristics of the root surface. The maximum quantity of water held by a root system will also depend on surface characteristics as well as total root surface area. Root surface area could not be estimated in the aeroponic tank

experiments because root diameters were not measured. However there was no significant differences between treatments in average root length, and hence basic root ball size, although there was a significant change in root length per unit root dry weight indicating some change in root morphology. Without more detailed information on root structure and root surface charge density, it may be assumed that the amount of water held under gravity was proportional to the size of the root system, measured in terms of root length. This assumption was supported by the high correlation found between weight measurements on detached root systems, which had been saturated and allowed to drain, and root length (data not given).

Upon this framework must be laid the process of condensation and plant water uptake from the vapour phase. It is relevant at this point to consider the situation in soil with respect to water uptake under falling Ψ_W(soil). The development of a vapour gap between roots and soil under water stress has been debated for many years (Huck *et al.*, 1970; Lösch, 1984; Scott Russell, 1982; Sharp and Davies, 1979; Weatherley, 1976). It is now definitely known to occur under natural conditions (Scott Russell, 1982), although the extent and importance are yet to be clearly defined. When a vapour gap is present, water must move from the soil particle to root via the vapour phase. Dalton (1989) has recently shown, using hydrogen isotope properties, that there is a vapour component to water uptake. Whether this process increases in importance under water stress is as yet unknown.

Clearly the vapour gap formation situation has direct analogies with the present experimental setup, once free water has been removed from the root surface, and indeed the analogy may be taken even further. For a root system in soil, the root tips may have a significantly different tissue water status than the rest of the root system. This results firstly from their hydraulic isolation (Davies *et al.*, 1987b) and secondly from the following factors:

i/ Continued extension of the root axis.
ii/ The binding ability of root exudates and root cap material.
iii/ Anchorage by root hairs a small distance back from the root tip.

These factors together mean that root tip water status is able to respond in a sensitive way to changes in soil water status. The root tip water stress signal will increase as soil dries through root uptake plus soil surface evaporation and as contact between the root tip and soil particles decreases.

For roots in an aeroponic tank, the situation is seemingly quite different but the end result may be similar. Gravity causes nutrient solution droplets to develop

around the root tips after a misting cycle. Through observation of the root system it was found that the number and size of these water droplets decreased during the stress cycle when misting pumps were off. It is suggested that loss of these droplets through root uptake and evaporation will closely parallel changes in the root environment of aeroponically grown plants. As more root tips loose their water droplets, the root signal will increase, just as it does under natural soil condition.

Comparison should be made between Fig. 12.1 and that of Sharp (1981), from which it was adapted. A misting pump off-time of 0 hours corresponds to moist soil, 0.5 hours to moderately dry soil and 2 hours to dry soil. The situation depicted by Sharp (1981) for a very dry soil did not occur in the experiments described herein but would do so if the r.h. of the tank atmosphere fell significantly below saturation, leading to a net loss of water from the root system.

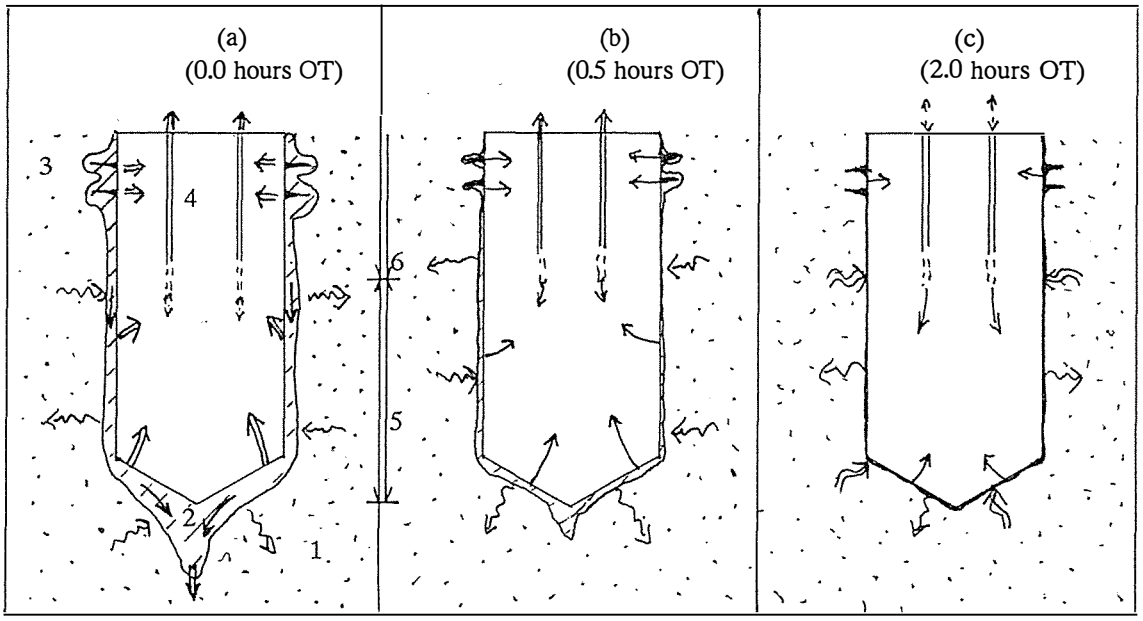

Fig 12.1. A diagram of water flow paths in the root tip, adapted from the diagram of Sharp (1981). Straight arrows, liquid flow; curly arrows, vapour flow. Double shafted arrows indicate relatively greater flux. Broken arrows indicate movement which is dependent on the water potential gradients in the tissue. 1, Saturated air. 2, Nutrient solution (increasing concentration: **a** , **b** , **c**). 3, Root hairs. 4, Xylem. 5, Non-differentiated portion of root tip. 6, Zone of xylem differentiation.

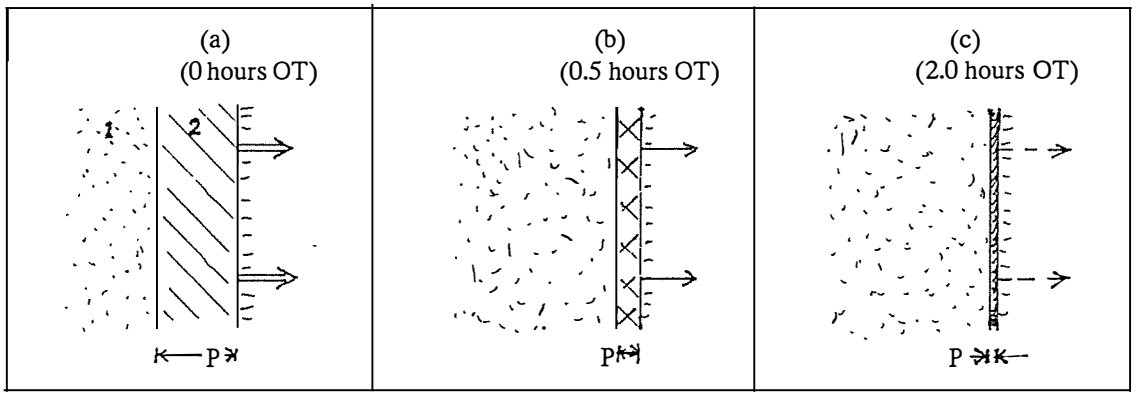

Fig 12.2. A diagram showing proposed water movement at the root surface after different misting pump off-times. The atmosphere is considered to remain saturated regardless of the off-time. See Fig. 12.1 for a description of the arrows and numbered regions. P, Perirhizal zone.

It is now known that both root tips and leaves can sense a water stress and influence whole plant physiology, but their relative importances are still under debate (Boyer, 1989; Kramer, 1988; Passioura, 1988; Schulze *et al.*, 1988) (see section 5.12). Many recent studies on the role of root systems in sensing water stress have been criticized as artificial because part of the root system is stressed while the shoot is kept in a turgid state (by supply from the rest of the root system) (Kramer, 1988; see section 5.4.6). This means that root hormonal signals become, in effect, independent from leaf hormonal signals. This is likely to give a distorted view (Kramer, 1988) although there is certainly information to be gained. Under natural conditions it may be hypothesized that changes in the rate of water uptake, and in turn leaf signals, will be dominant at first due to the large water potential gradient at the leaf surface and continued contact between root tips and soil particles. As the soil dries out further, especially when this is uneven, the root signal will increase in importance. Uneven drying will generally encompass the situation where surface soil layers becoming depleted. This has been investigated through a variety of so—called 'split root' experiments where part of the root system is stressed while the remainder is well watered (see section 5) and such experiments have been able to reveal an important contribution from root produced hormones (Blackman and Davies, 1985b).

Following the above discussion, it is suggested that the intermittent misting system is more closely aligned to the natural situation than many other experimental water stressing systems, including those which only stress the root system and those which create a constant water potential in the root zone (e.g. water potential agents). This is believed to arise due to the proposed similar weighting of root and shoot signals

under the aeroponic conditions. The shoot signals are influenced by natural aerial environment fluctuations while the root signals are believed to be critically related to the free water status of root tips. These points are considered more fully in the following section.

12.4 COMPARISON OF WATER STRESS ENVIRONMENTS

The three environments to be considered here are plants growing in soil, aeroponics + PEG and aeroponics + intermittent misting (Table 12.1). Discussion will centre on an initially saturated system with no further input of water.

In the soil, water is lost through plant root uptake, evaporation from the soil surface and percolation to lower depths. A certain distance from the soil surface or plant roots, Ψ_W(soil) will decrease only slowly, without significant diurnal variation. In the perirhizal zone, Ψ_W(soil) will tend towards bulk soil Ψ_W at night but be lower than soil Ψ_W during the day because of rapid root uptake. Hence there will be a diurnal oscillation, the maxima of which will follow bulk soil Ψ_W. Very similar trends will be followed for water content due to the relationship between content and potential (soil water retentivity curve).

In aeroponic systems the bulk root medium is of course moist air and this was found to remain saturated regardless of whether the misting pumps were off for up to two hours. From equation 12.1 it can be seen that Ψ_W of the air will be only slightly temperature dependent because temperature is in Kelvin. Water content, however, will vary far more widely due to the temperature dependence of saturated vapour pressure.

Within the aeroponic-PEG system water potential and water content in the perirhizal zone will be constant, provided that the rate of misting is far greater that the rate of uptake, as discussed in section 7. In other words, water potential will be independent of evaporative demand, in marked contrast to the natural situation.

The intermittent misting system is at first glance quite different to the above, yet this is not the case. Interesting changes occur however in the perirhizal zone, as indicated by observation and consideration of basic principles. At the start of the misting cycle nutrient solution is present on the root surface and as water is lost this will become more concentrated, thus decreasing Ψ_W. An equilibrium will be reached at some point between root tissue Ψ_W and surrounding solution Ψ_W, after which time the rate of uptake will equal the rate of condensation. Within the perirhizal zone there will be a thin layer of concentrated nutrient solution, surrounded by a layer of saturated

air (see Fig. 12.2). Hence, if air in the tank remains saturated, perirhizal Ψ_W will oscillate with off-time, with a minima corresponding to root tissue Ψ_W. By contrast, perirhizal zone water content will oscillate markedly with off-time depending on the unknown percentage of the zone containing saturated air.

Table 12.1 Comparison of three water stress systems

Region of the root environment	Water Stress System		
	Natural	PEG	IM
Bulk root media Ψ_W	slow change	constant	constant
Bulk root media water content	slow change	constant	change with Ta[a]
Root interface Ψ_W	diurnal change	constant	oscillates with OT
Root interface water content	diurnal change	constant	oscillates with OT and Ta

[a] Ta air temperature

One parameter which identifies a significant difference between the intermittent misting environment and others, is that of dawn leaf water potential. When water potential agents are used, Ψ_W(leaf) tends to equilibrate with solution Ψ_W at the end of the dark period (as in experiment 1) and this is similar to the natural situation where Ψ_W(leaf) correlates with Ψ_W(soil). However under intermittent misting regimes, no significant differences between off-times are seen in dawn leaf Ψ_W (see experiments 3 and 4). This is because root interface Ψ_W is solely dependent on water uptake rates (i.e. the aerial environment) and not on bulk root environment Ψ_W which is constant and high (approximately -0.5 bar). At night, with little or no transpiration, water will be taken up until the Ψ_W(plant) deficit is removed (provided that off-times are not excessive). Such a response is analogous to a moist soil with very low hydraulic conductivity.

13 WATER STRESS ON THE VEGETATIVE GROWTH OF LYCOPERSICON ESCULENTUM AND PYRUS BETULAEFOLIA - A COMPARISON

13.1 PLANT STRATAGIES

Much can be gained in our understanding of plant responses to water and other environmental stresses by comparing and contrasting different plant types. However, in doing this, two separate components must be identified. Along with differences which relate to changes in general plant characteristics, there are a myriad of specific plant adaptations which have evolved to cope with unique environmental conditions. General theories or models on plant functioning tend to ignore this second group, which is to some extent necessary for simplicity, but does limit overall understanding. A good example of this comes from work on partitioning changes under stress. Szaniawski (1987) developed a model based on the partitioning of respiratory energy in which the so called 'homeostatic capacity' of a plant equalled the ratio of maintenance respiration to total respiration. The failure of this model to cope with specific plant adaptations was clearly stated by Taylor (1989). Many other examples could be given, but this serves to illustrate the importance of differentiating between general and specific responses.

An important general model of plant strategies has been developed by Grime (see Grime (1979)). In the C-S-R model three plant strategies are identified, competitive, stress-tolerent, and ruderal. Their responses to stress are given in the following table.

With reference to the plants used in experiments described herein, tomato is an annual/perennial competitive-ruderal while trees such as apple and pear may be tentatively classed as perennial stress-tolerant competitors. Characteristics of these basic classes will be discussed in terms of the observed responses to water stress.

Table 13.1 Responses of the three primary plant strategies from the C-S-R model to environmental stress (Grime, 1979)

Plant strategy	Response to stress
Competitive	Rapid morphogenetic responses which maximize vegetative growth (changes in S/R ratio, leaf area, root surface area)
Stress-tolerant	Morphogenetic responses slow and small in magnitude
Ruderal	Rapid decrease in vegetative growth, resources partitioned into flowering

13.2 RELATIVE RESPONSES OF TOMATO AND *P. BETULAEFOLIA* VEGETATIVE GROWTH PARAMETERS TO WATER STRESS

In the following table, relative parameter values are given for four misting pump off-times. Those from experiment 3 were obtained by interpolation of the appropriate relationships.

13.3 GROWTH AND PARTITIONING

Assimilate partitioning, in terms of the allometric k value, was similarly affected in the two plants except at the first off-time where *P. betulaefolia* was insensitive. Relative growth rate was affected more in *P. betulaefolia* than tomato, due mainly to the strong effect on stem RGR, which was reduced by 50% at the third off-time. Root RGR was one of the most dissimilar parameters measured, increasing significantly for tomato but decreasing at each off-time (though less than leaf and stem RGR) for *P. betulaefolia.* Absolute growth rate reductions were similar.

The fact the only vegetative growth was studied in these experiments meant that the ruderal component of the water stress response was not expressed i.e. rapid

Table 13.2 The relative effect (percent of control) of different misting pump off-times in an aeroponic system on various plant parameters for tomato (T) and *P. betulaefolia* (P)

	Treatment Off-Time (hours)							
	0.00		0.17		0.65		1.55	
Parameter	T	P	T	P	T	P	T	P
'k' value	100	100	89	98	81	79	75	69
RGR(plant)	100	100	96	85	91	74	79	65
RGR(leaf)	100	100	95	89	88	77	73	64
RGR(stem)	100	100	96	87	87	69	77	49
RGR(root)	100	100	108	85	113	83	104	76
Ab. GR	100	100	89	80	69	59	45	50
Pn	100	100	94	63	69	47	50	35
g_s	100	100	79	78	46	62	15	48
$-\Psi_W$(leaf)	100	100	141	127	161	170	172	210
Water use	100	100	83	72	64	59	58	53
WUE	100	100	129	120	151	123	132	136
Resistance	100	100	59	137	26	221	13	500

flowering. There was however good expression of the competitive component, with plant RGR depressions minimized by an increase in root RGR, and relatively small reductions in shoot RGR components. Although the change in allometric k value was similar in *P. betulaefolia*, this was the result of larger decreases in the RGR of all plant parts. Hence the competitive component was considerably weaker in this woody perennial and the threshold stress level needed to change 'k' was correspondingly higher. Lag time estimates (see section 9.4.9) were 2 and 7 minutes for tomato and *P. betulaefolia* respectively.

13.4 **STOMATA, PHOTOSYNTHESIS AND WATER USE**

Following the discussion on plant strategies (section 13.1), tomato was classed as a competitive-ruderal and *P. betulaefolia* was a stress-tolerant competitor. This classification leads to clear predictions about assimilate partitioning but not necessarily about specific changes in g_s, Pn and water use. Generally, water stress should have a greater effect on g_s in tomato and, with lower plant resistances, WUE should be higher. Specific differences will depend on the plants being compared and

hence the comparison is not as useful as that relating to assimilate partitioning. Nevertheless, it is worthwhile in this instance to bring together the two types of experimental material in order to gain a better understanding of their responses to the intermittent misting generated water stress.

Net photosynthesis was more sensitive in *P. betulaefolia*, while stomatal conductance was far more sensitive in tomato, being reduced more than any other parameter except resistance (Table 13.2). Water use per leaf area reductions were similar, with *P. betulaefolia* being slightly more sensitive. Trends in WUE were quite different for the two plants, tomato showing an increase up to the second off-time and *P. betulaefolia* showing a continued increase. This meant that in relative terms they were only significantly different at the second off-time. Plant resistance (Ψ_W(leaf)/E) was the most unusual parameter listed above, with a reduction of 81% and increase of 500% for tomato and *P. betulaefolia* respectively at SL3.

For *P. betulaefolia* smaller reductions in stomatal aperture over the midday period were associated with greater decreases in Ψ_W(leaf). Interestingly however reductions in Pn were greater in *P. betulaefolia* which may be explained by the climatic conditions during the two experiments. The tomatoes were grown in spring where noon PAR levels did not exceed 800 μmol m^{-2} s^{-1} and averaged around 500 μmol m^{-2} s^{-1}. For the *P. betulaefolia* seedlings grown over summer, noon values ranged between 1000 and 1500 μmol m^{-2} s^{-1}. This difference was reflected in the absolute values of stomatal conductance i.e. 0.125 and 0.380 mol m^{-2} s^{-1} for control *P. betulaefolia* and tomato plants respectively. Hence for tomato, the decrease in g_s was large but Pn was not so affected because absolute g_s values were higher and PAR levels lower (light limitation).

The difference in Pn between the two species at the first off-time was surprisingly large and opposite to the difference in the allometric k value (Table 13.2). Again however this may be attributed to the fact that in the tomato trial, photosynthesis was not greatly CO_2 limited by stomatal closure. Note that treatment differences in Pn were not related to changes in SLA, which were small to nonsignificant in both experiments. Charles-Edwards (1979) suggests that photosynthetic rate should be expressed on a unit volume or unit dry weight basis in order to indicate intrinsic activity. This would clearly be important when comparing different plant types.

In experiments 1, 2 and 3, comparison was made between noon water loss and average water loss ratio, on a LA basis. For tomato, this decreased from 14 to 7 and 11 to 4, in experiments 1 and 2 respectively (Control *versus* SL3). However for *P. betulaefolia* it remained constant at around 5. This again reflects in part the high stress load placed upon *P. betulaefolia* under noon summer conditions.

It is interesting to note that the leaf area to root length ratios were very similar for tomato and *P. betulaefolia* (c.f. 8.0 -> 4.0 and 10.5 -> 4.0 for SL0 -> SL3). This is to be expected if, as was hypothesized in section 12, volume of available water is proportional to root length. Under natural conditions there is no simple relationship between root length and available soil water (although there will be a general trend over time) and hence there is no tight proportionality between root length and transpiration (Tan and Buttery, 1982b).

The response of total plant resistance is interesting, and the five fold increase for *P. betulaefolia* no doubt reflects a large increase in root resistance, as has generally been observed (Camacho-B *et al.*, 1974a). Root resistance is usually two to ten times higher than that of the shoot component (Davies, 1986). The significant decrease in resistance with water stress for tomato is unexpected. However storage water was not accounted for in the resistance calculation which is a general source of error and misinterpretation (Lösch, 1984). It will be noted that the water content of tomato is ten times that of *P. betulaefolia* and hence plant capacitance will be considerably greater, leading to an overestimation of flow through the SPAC and underestimation of total plant resistance.

14 THE ALLOMETRIC RELATIONSHIP REVISITED

14.1 THE LINEARITY PROBLEM

Basic theory behind the allometric relationship and the mathematics of simple linear allometry were discussed in section 4. Linear allometry is by far the most common in the botanical field, there being very few clear examples of curvilinear functions (e.g. see Currah and Barnes (1979)). However there is a significant problem regarding the validity of fitting linear regressions to many sets of data. Causton and Venus (1981) point out that if a curvature in the relationship is present it will tend to be slight and thus may easily be missed. Part of the reason for this is the strong linear trend which will always exist between parts of any growing organism regardless of other underlying functions.

One approach to the above problem is to use statistical methods such as a test for linearity and analysis of the linear regression residuals. This however removes the worker from all underlying physiology. A second obvious, though seemingly underutilized, approach is to look at trends over time in the plant part ratios themselves. It will be remembered that a linear allometric relationship means that the S/R ratio is changing at a constant rate with time, except when k=1 where the S/R ratio is itself constant. Hence the plot of S/R verses time will also give a straight line. A problem is encountered however in the visualization of such a plot when sequential harvests have been taken giving only a few time nodes. This is usefully overcome by using plant dry weight transformed so as to be a linear function of time. Such plots are shown for experiments 2 and 3 in Fig. 9.12 and 10.11. In experiment 2, the tomato plants were growing approximately exponentially and so the natural logarithm of plant dry weight was used. In experiment 3, the nashi seedlings exhibited a linear growth trend with respect to both total plant dry weight and butt cross-sectional area such that untransformed plant dry weight was used. Transformed plant dry weight has commonly been used to simplify the trends in growth analysis variables, because growth rate is size dependent (Hurd, 1977). Obviously any size dependent variables could be used for such transformations (Hurd, 1977) but in this case total dry weight is the most useful and efficient.

In Fig. 9.12 it can be seen that the S/R ratios did indeed change linearly with time, as suggested by the close fitting linear allometric trends (Fig. 9.11). It should

also be noted that points which appear to deviate slightly in the allometric plot do so far more noticeably in the S/R ratio plot. In other words, the S/R ratio plot is considerably more sensitive to variation because it uses linear scales, and this is an important attribute.

There is a direct relationship between the slopes of the S/R ratio plots and the allometric k values, such that, as mentioned above, when k = 1 the S/R ratio plot should be horizontal (slope (m) = 0). This relationship is derived mathematically as follows;

Plant d. wt *versus* time: $f(W_p) = a \times t + C_1$ 14.1

Allometric relationship: $\ln(W_s) = k \times \ln(W_r) + C_2$ 14.2

S/R ratio plot : $W_s / W_r = m \times f(W_p) + C_3$ 14.3

Where: W_p = Plant dry weight.
W_s = Shoot dry weight.
W_r = Root dry weight.
t = Time.
a, k, m = Slope constants.
C_1, C_2, C_3 = intercept constants.

From 14.3:

$$\ln(W_s) = \ln(W_r) + \ln(m \times f(W_p) + C_3) \quad 14.4$$

Substituting for W_s using 14.2:

$$k \times \ln(W_r) + C_2 = \ln(W_r) + \ln(m \times f(W_p) + C_3) \quad 14.5$$

Rearranging with respect to m:

$$m = (\exp(C_2) \times W_r^{(k-1)} - C_3) / f(W_p) \quad 14.6$$

From Fig. 9.13, the close correlation between allometric k values and S/R ratio trends is apparent, thus giving further support to the linear allometric functions. Similar trends are shown in Fig. 10.12 (experiment 3) involving a very different plant material and growth pattern. In this case the linear allometric trends are not as strong and differences in the rate of change of S/R ratio are clearly indicated within the S/R ratio plots.

The above results suggest that it would be useful to plot S/R (or a comparable variable) ratio *versus* transformed plant dry weight whenever an allometric relationship is used, to aid the investigator in both interpretation and validation.

Unusual data are shown up far more clearly, as are nonlinear trends and small changes in 'k'.

14.2 THE EFFECT OF CHANGING ENVIRONMENTAL PARAMETERS

Following a change in some plant environment parameter which impinges upon the shoot-root relationship, there will be an associated change in the allometric relationship (value of 'k'). Many examples can be found in the literature as discussed in section 4.5.5. However, one aspect which has not been considered until recently is the potential for two basic response patterns (Fig. 14.1). In the first (Fig. 14.1a), 'k' changes rapidly to reach a new value which is associated with the new environmental conditions. This means that there is a different rate of change in the S/R ratio over time. In the second case (Fig. 14.1b), the initial response is the same as in the first, but then 'k' returns to its original level. In other words, the end result is a change in the value of the S/R ratio but not in the rate of change in the ratio over time.

Two recent examples have appeared in the literature documenting this second response. Thuantavee (1991) applied cytokinin to the roots of tomato plants (in an aeroponic system) by incorporating it into the nutrient solution. The allometric relationships from this experiment came out as a series of approximately straight lines, indicating that 'k' initially increased but then rapidly returned to its original level. Thuantavee (1991) believed that this was due to the root systems adjusting to the higher cytokinin levels by decreasing synthesis and increasing degradation. Hence after a short period of time the shoot system would have been receiving the same level of root cytokinin as before the treatments were begun. In a second example, Hughes *et al.* (1991) applied different temperature combinations to asparagus seedlings and observed allometric relationships very similar to those in Fig. 14.1b.

These examples can be compared to experiments described in this thesis, involving water stress and physical root restriction, which generated allometric relationships of type one (Fig. 14.1a) above (see Fig. 9.11, 10.10 and 11.3).

There are two important points to consider in rationalizing these responses. Firstly, if the plant can internally adjust (either partially of fully) to the changed environmental parameter then 'k' can be expected to return to its original level (e.g. Thuantavee, 1991). If this does not occur then a new 'k' value will persist (experiments 2, 3 and 4). Secondly, however, it should be realized that 'k' values other than one cannot be maintained indefinitely, since the S/R ratio will reach a

limiting value. From a basic understanding of plant physiology it will be recognized that the further 'k' deviates from one the greater will be the tendency for 'k' to return to unity. Hence it may be hypothesized that if any treatment (specific plant environment) could be maintained for a long enough period, all allometric responses would tend towards type two above. Consider, for example, physical root restriction, which has been shown to increase 'k' (Fig. 11.3). Over time the increasing S/R ratio will inevitably lead to greater levels of water stress, thus leading to a decrease in 'k' towards its original value.

From the above discussion it is clear that a thorough understanding of experimental systems and whole plant physiology is required in order to properly interpret apparent changes in the allometric relationship. Misinterpretations can easily be made by the unwary researcher, but two key points from this section should significantly reduce potential problems. Firstly, a plot of the S/R ratio *versus* time linearized plant dry weight will more easily identify curvilinearity and unusual data. Secondly, consideration of the time scale of the experiment will focus attention on the type of allometric response (e.g. as in Fig. 14.1).

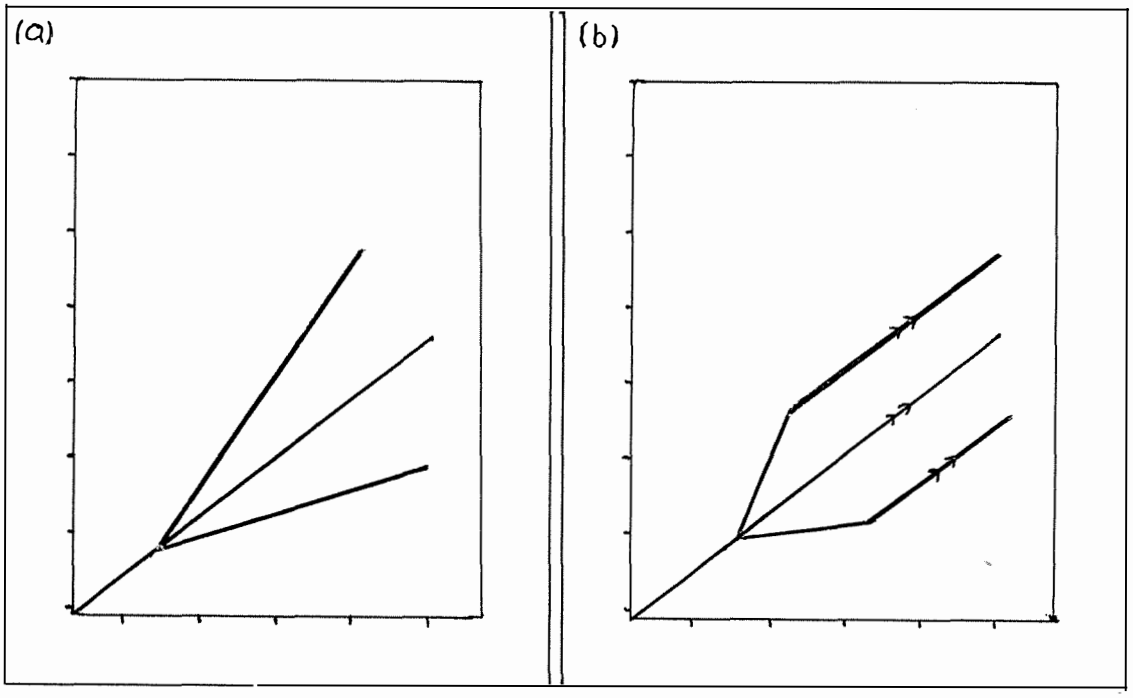

Fig. 14.1 Two idealized responses in the allometric relationship after a change in the root-shoot environment.

15 ASSIMILATE PARTITIONING AND WATER STRESS

15.1 THE SHOOT/ROOT RATIO

In section 4 it was noted that the simplest parameter for investigating assimilate partitioning in plants is the shoot to root ratio (S/R), and its limitations, based on ontogenic (Gales, 1979; Richards, 1986) and genetic (Wareing, 1970) variability have been discussed. It would appear that these limitations have generally been overstated due to the fact that the S/R ratio normally decreases in a simple fashion with plant age (time). A linear change in S/R ratio is implicit the linear allometric relationship, the latter of which has been considered far more useful (Troughton, 1955, 1956, 1960).

The important point in this situation is to think in terms of rate of change in S/R ratio rather than absolute values *per se* (e.g. see section 14). Troughton (1956) stated that the S/R ratio tells nothing about the rate of change of organ growth due to confounding of size variation. This however is not the case if one plots dry weight ratios against plant dry weight transformed so as to be a linear function of time. Good illustrations are presented in Fig. 9.12 and 10.11. Given that changes in the S/R ratio can be simply expressed and that the ratio has significant inherent stability (Brown and Scott, 1984; Wareing, 1979), it is clearly a very useful parameter if handled correctly.

15.2 PARTITIONING, RGR_{max} AND ADAPTATION TO STRESS

For a given plant there will be an optimum S/R ratio (and rate of change of S/R ratio with time) under ideal environmental conditions, in which mineral uptake by the roots and carbon uptake by the leaves are maximized (maximum relative growth rate, RGR_{max}) within the constraints of a functional equilibrium. Any significant change to the environment will change this optimum ratio (e.g. see Fig. 9.11) and reduce overall growth (e.g. see Fig. 9.10). This growth reduction will be minimized by the change in partitioning (e.g. see Fig 9.13). There is however more that one form of partitioning change to consider. Taylor (1989) has indentified two approaches to the analysis of plant stress, which can be found in the literature, and these are based on:

i/ RGR_{max} and dry matter partitioning.
ii/ Allocation of respiratory energy.

With respect to the first approach, Hunt and Nicholls (1986) have considered the situation in terms of three variables:

i/ Partitioning ratio (= allometric k value) (k_P).
ii/ Attainment ratio (RGR/RGR_{max}).
iii/ Stress ratio (k_E).

The stress ratio is composed of two artificial stress indices for the root and shoot environments, R_{SI} and S_{SI} respectively, such that $k_E = R_{SI} / S_{SI}$.

From the mathematical analysis of Hunt and Nicholls (1986) it was shown that a low RGR_{max} promotes a large change in k_P under stress, so minimizing the reduction in attainment. By contrast, if RGR_{max} is high, attainment decreases sharply under stress with little benefit from a change in partitioning. Hunt and Nicholls (1986) related this to plant strategies by suggesting that a high efficiency of attainment for partitioning aids survival in undisturbed habitats whether stress is high or low. In a disturbed habitat where any stress may be severe, change in partitioning is unlikely to be of great benefit compared with rapid ontogeny (Hunt and Nicholls, 1986).

The debate over benefits from a high or low RGR_{max} has been an interesting one. According to Grime and Hunt (1975) a low RGR_{max} is of advantage under unproductive (i.e. high stress) conditions because assimilates will not be exhausted as rapidly and reserves will be accumulated. This may be only partly true however. Szaniawski (1987) modelled plant stress based on the second approach given above (allocation of respiratory energy) and developed the term 'actual homeostatic capacity' (AHC) as the ratio of maintenance respiratory activity to total respiratory activity. This again seems to support the benefit of low RGR_{max} under stress, but Taylor (1989) disagrees, suggesting that it is in fact the ability to divert metabolic energy from growth to maintenance which is important. Hence stress tolerance (T) may be defined as the ratio of growth respiration to gross photosynthesis. This theory can be rationalized with those based on a low RGR_{max} because if photosynthesis is also low there may still be a greater proportion of respiratory energy consumed in growth (Taylor, 1989). Therefore in contrast to the reasons given by Grime and Hunt (1975), a low RGR_{max} may be advantageous if it minimizes maintenance costs relative to photosynthesis (Taylor, 1989).

Experimental results presented herein will now be discussed in the above context.

15.3 PARTITIONING IN TOMATO AND *P. BETULAEFOLIA*

Optimisation of partitioning in any form must relate in part to dry matter partitioning, in terms of the ratio of photosynthetic to nonphotosynthetic tissue. That is, the higher the leaf weight ratio (LWR = leaf weight / plant weight) the higher the potential dry matter productivity, for similar types of plant. When considering different plant types, for example herbaceous and perennial, consideration must be given to the inherent plant structure. Perennial plants have a lower LWR due to the larger amount of structural tissue. From the experiments described herein LWR values obtained for tomato and *P. betulaefolia* were 0.59 and 0.27 g g^{-1} respectively.

Partitioning changes in the two species under water stress were comparable. For misting pump off-times of 0.17, 0.65 and 1.55 hours, the allometric k value decreased by 11%, 19% and 25% respectively in tomato, while for *P. betulaefolia* it decreased by 2%, 22% and 31% respectively. The reductions appeared significantly different only at the first off-time where *P. betulaefolia* plants were little affected.

The relative growth rates for tomato and *P. betulaefolia* control plants were 0.080 g g^{-1} day^{-1} and 0.015 g g^{-1} day^{-1} respectively and these were reduced by 21% and 40% respectively for a misting pump off-time of 1.55 hours. Hence in this simple comparison the suggestions of Taylor (1989) are supported, rather than the low RGR_{max} theory as described previously (section 15.2). With respect to the model of Hunt and Nicholls (1986), relative attainment ratios may be calculated at 0.79 and 0.60 for tomato and *P. betulaefolia* respectively. Note that actual attainment ratios would have been significantly lower than this because in neither case were the control plants in totally ideal conditions. The RGR of tomato control plants (0.080 g g^{-1} day^{-1}) can be compared with the RGR_{max} quoted by Grime and Hunt (1975) of 0.234 g g^{-1} day^{-1}. Light limitations would no doubt have played a major part in this difference. Along with these relative attainment ratios, allometric k values changed by 25% and 31% respectively. Therefore the tomato plants achieved a greater attainment for a smaller change in partitioning compared with *P. betulaefolia.* This is in line with the basic plant strategies discussed in section 13.1, with tomato being a competitive-ruderal and *P. betulaefolia* fitting into the category of a stress tolerant competitor.

Of course tomato and *P. betulaefolia* are so different that a detailed comparison is pointless. However the above discussion is very useful for focusing

attention on the different responses to a similar water stress regime. It is also important to note here the potential usefulness of an aeroponic system for obtaining data where close comparisons are required. The key factor is that the root system is not physically limited in any way, a point taken up in the following section.

15.4 **ROOT AND SHOOT GROWTH - THE LIMITING COMPONENT**

From a study of plant mineral nutrition, Liebig in 1843 developed a so called 'Law of the minimum' which simply stated that the growth of a organism depended on the growth regulating environmental factor which was in shortest supply or greatest excess (Bidwell, 1979). This was clearly a major oversimplification, yet not until Blackman (1905) was the concept refined. In his 'Principle of limiting factors', Blackman (1905) proposed that an increase in any growth regulating environmental factor which was at sub-optimal levels would increase growth, up to the point where the factor was optimal. Although still oversimplified in many ways, this principle has continued to provide a foundation for discussion and debate.

Within a multi-component organism, one part will be limiting the growth of other parts due to the constraints of internal functional relationships between different components. The controlling component will be that which draws most upon the growth limiting environmental factors. Of course, in reality the situation is very complex with so many dynamic factors influencing growth. For a terrestrial plant the limiting factor may change many times over the course of a diurnal cycle. There are also a variety of aspects to plant growth, for example, one must distinguish between vegetative and reproductive growth as their limiting factors many be quite different at any given time.

With respect to vegetative growth, the root system may be considered the limiting plant component for three main reasons (Chalmers, 1988):

i/ Roots must extract water and minerals from the soil which may often be less freely available than light and CO_2 from within the atmosphere. Note however that under an orchard situation, with irrigation and fertilization, light and CO_2 limitation may be critical.

ii/ The soil *per se* presents a physical barrier to growth whereas the atmosphere does not. Inter-plant competition may be considered in this context, both in the root and

shoot environment. However, the generally extensive habit of a root system increases the importance of this factor within the soil (Caldwell, 1987).

iii/ The shoot system is very dependent on the root system for the correct hormone balance (although the reverse is also true). Cytokinins are considered to be the key hormones in this regard (Blackman and Davies, 1985b) (see section 5).

Following on from the above arguments, and in terms of practical plant management, it has been proposed that in order to control and/or modify vegetative shoot growth, it is the root system which should be targeted. This point will be discussed in some detail in section 16. It is important to reiterate here that the initial water stress sensing plant part is still considered to be the shoot system (see section 5.12). Stress develops in the shoot due to a combination of both atmospheric fluctuations and an inadequate water supply from the root system.

In an aeroponic system points (i) and (ii) above do not apply. That is, there is no physical barrier to the root system, and water and nutrients are freely available (in the control treatments at least). This means that the root - shoot relationship will be dependent upon other factors, principally the environmental water status. Which plant part is limiting growth will be dependent on the particular combination of root and shoot environments. For control plants in an aeroponic system (e.g. experiment 2 and 3) the shoot will be the limiting component. In essence this represents a plant growing under ideal soil conditions. As water stress is imposed via the root system, this will become the limiting component. The relative growth rate ratio (allometric k value) may be taken as a general indicator of the growth limiting component, with a lower value representing greater root limitation. However, no exact figures can be used since the ratio is strongly plant type dependent.

16 WATER STRESS AND PHYSICAL ROOT RESTRICTION

16.1 A BASIC COMPARISON

Results from experiments 3 and 4 clearly show the relative differences between water stress and root restriction, as expounded by Krizek *et al.* (1985), and these are summarised in Table 16.1.

Table 16.1 Comparison of water stress and root restriction effects on *P. betulaefolia*[a]

Plant parameter	Water stress	Root restriction
Ψ_W(leaf)	<<	<[b]
g_s	<<	-
Pn	<<	-
'k' value	<<	>
LA/RL	<<	>>

[a] <<, large decrease; <, small decrease; -, no effect; >, small increase; >>, large increase
[b] small decrease due to an unknown level of water stress confounding

16.2 ASSIMILATE PARTITIONING

At the end of section 14 it was stated that plant environment changes may have significant effects on plant size without affecting assimilate distribution. Generally however environmental changes or experimental manipulation will have disproportionate effects on root and shoot. In other words, using the terminology of Hunt and Nicholls (1986), the hypothetical stress ratio (k_E, = shoot stress index / root stress index) is usually nonzero. This leads to increased partitioning in favour of the plant part "which draws most upon the growth limiting part of the environment" (Hunt and Nicholls, 1986).

With respect to experiments described here, two limiting conditions, water stress and physical root restriction have been investigated. Both solely affect the root environment (although soil water deficits under natural conditions can affect air temperature and r.h., particularly within the leaf canopy). Water stress led to a relative increase in partitioning to the roots (decrease in the allometric k value) as observed by

Troughton (1960). This change was more at the expense of stem than leaf tissue. The stress ratio appeared to be linearly related to nutrient solution osmotic potential (experiment 1) and logarithmically to misting pump off-time (experiment 2 and 3).

By contrast, physical root restriction (experiment 4) led to a small relative decrease in partitioning to the root (increase in the allometric k value), as others have observed (Al-Sahaf, 1984; Tschaplinski and Blake, 1985), with a relative increase in partitioning to the stem. This is one of the few clear illustrations of the opposite effects of water stress and root restriction on the allometric relationship of a perennial plant. It is a critical aspect which has significant practical implications and these will now be discussed.

With respect to the statement of Hunt and Nicholls (1986) given above, the limiting factor under root restriction is a plant variable (related to root size) rather than an environmental one *per se*. Hence as the shoot draws upon the root, relative shoot growth will increase, with the extent of the increase determined by uptake and supply (nutrients, hormones etc.) potential of the root system. This will vary significantly between species. In relation to the C-S-R model of Grime (1979) it is the competitive strategy which is critical. Competitive plants, it will be remembered, respond to stress by rapid morphogenetic changes which maximize vegetative growth.

Tomatoes appear to have very flexible root systems, which is in line with their strong competitive component. Al-Sahaf (1984) measured a significant increase in water and mineral uptake capacity per unit length under physical root restriction. Tree species which have a predominantly stress-tolerant strategy would not be expected to have such flexible root systems. Hence partitioning changes should be small, as observed in experiment 4, with greater control of shoot growth for a given degree of root restriction.

16.3 **WATER STRESS AND ROOT RESTRICTION IN PRACTICE**

It was mentioned previously (section 4) that restricted root zone volume can result from a sharp change in any root environment parameter. In the case of physical root restriction it is a change in density, such as may occur under field conditions if a hard pan is present. A sharp change in water content will also present a barrier to root growth and thus create a restricted rooting volume (Chalmers *et al.*, 1983; Proebsting *et al.*, 1989).

In recent times drip irrigation systems have become popular for a variety of horticultural crops, but especially fruit trees. If a drip irrigation system is operated in a

carefully controlled fashion, the root bowl remains moist while surrounding soil stays dry (Levin *et al.*, 1979). Note however that the distribution of water in a given situation will depend on a variety of factors, apart from rate of application, such as, soil type and permeability, rate of percolation and rate of evapotranspiation.

The above situation which can develop under drip irrigation is both physiologically interesting and practically significant. Precise control of water application, in terms of timing, volume and placement, has enabled the vegetative growth control technique of RDI to be developed (see section 1). This technique involves applying a controlled water stress to the trees early in the season when vegetative growth predominates (Chalmers *et al.*, 1981; Chalmers *et al.*, 1983; Chalmers *et al.*, 1986; Mitchell and Chalmers, 1982; Mitchell *et al.*, 1986; Mitchell *et al.*, 1989; Proebsting *et al.*, 1989). The philosophical aim is to provide adequate vegetative growth control while minimizing effects on current and future fruit production.

It is clear from experimental results described herein, and those of others discussed previously, that a root restriction response would be ideal under the RDI system. This is because shoot growth could be contained without inducing a myriad of unwanted metabolic changes e.g. $< \Psi_W$(leaf), $< g_s$, < Pn, etc. as demonstrated in experiment 4 (see Table 16.1). Furthermore, under root restriction the top of the plant is still favoured, in relative terms, with respect to assimilate distribution (the allometric k value increases). This means that there should be relatively more assimilate available for fruit growth, both during the stress period and subsequently under full irrigation. Under water stress, assimilate is partitioned in favour of the root system, a situation which is not conducive to fruit growth and development.

The central question which needs to be answered is the extent to which RDI responses are due to pure water stress or pure root restriction effects. In a recent paper, Proebsting *et al.* (1989) attempted to investigate this question using peach seedlings. It was found that the effects of root restriction and water stress on shoot growth were similar. Note, however, that rooting volume was established using a split root system and not via a moist root bowl. Hence in the restricted treatments a significant amount of the root system was in dry soil. It was assumed that these roots were physiologically inactive (soil below the permanent wilting point), but this assumption may be questioned with respect to hormone production. There was also an unknown degree of water stress confounding in the restricted volume treatments as there was in experiment 4 of this thesis. Perhaps a more critical factor to consider is the fact that fruit growth can actually increase following the RDI stress period (Chalmers *et al.*,

1981; Chalmers *et al.*, 1984; Chalmers *et al.*, 1986; Mitchell and Chalmers, 1982; Mitchell *et al.*, 1986). The ability of the plant to respond in this way may relate, in the first instance, to the extent of the root restriction component.

Under natural conditions there is no doubt that water stress effects will always be present to some extent, for one only needs to consider the experimental difficulty in obtaining unconfounded root restriction (see section 3 and experiment 4). The primary reason is a fluctuating atmospheric environment, because pure root restriction can only be induced successfully in a growth room where the environment is constant. Nevertheless, it is possible that root restriction effects could dominate in the natural environment under certain circumstances. One of the key factors may be inter-plant competition whereby root growth is restricted (Chalmers *et al.*, 1984), in part, by the soil drying effects of other root systems. The restricting effects of inter-plant competition have been graphically illustrated by excavations of whole root systems (Atkinson and Wilson, 1980; Atkinson *et al.*, 1976; Caldwell, 1987; Chalmers *et al.*, 1984; Rogers and Head, 1969). For soil water management systems the implication is that tree spacing will be important, with a relatively close spacing enhancing control over root distribution. Results from Chalmers *et al.* (1981; 1984) have shown that this is indeed the case under RDI.

It is important to remember that any restriction to the root system will increase the potential for water stress. Hence, if the root restriction component is to be maximized under RDI, the root and shoot environments must be kept as constant as possible. Obviously this is extremely difficult to achieve under natural environmental conditions. Climates such as those of central Australia (hot and dry over the growing season) will be better than the wildly fluctuating conditions in New Zealand, provided, of course, that irrigation water is plentiful. At the orchard level normal practices, such as providing good shelter, will also contribute to maximizing the root restriction component.

Modifications in the root environment (soil) are easier to achieve than those in the shoot environment (atmosphere), although the ideal root environment to be aimed for has recently been brought into question (Chalmers, 1988). Regardless of the specific characteristics which are aimed for, a uniform soil profile over the orchard is ideal. If nutrient status, structure, texture or water holding capacity change then so must the irrigation and management system, which is expensive and complicated. Soil nutrient status does not need to be naturally high because a low status in combination with fertigation will aid in root restriction. The same is true for topsoil depth, for while a deep topsoil has always been considered ideal, a relatively shallow profile

would be better for root restriction. This revelation, which goes against long held views (Chalmers, 1988), stems simply from the development of efficient, high precision irrigation systems.

Working against vegetative growth control using soil water status is of course rainfall. In temperate climates it will not be possible to maintain a dry bulk soil profile, overall, without the aid of rain-covers (e.g. polythene sheeting down the tree rows (Jones *et al.* (1983; Powell, 1974)). Nevertheless, evapotranpirational losses can create a significant soil water deficit relatively early in the season, even under a temperate climate (Durand, 1990). This deficit can be used to significantly control vegetative growth and hence overall productivity. Furthermore, the benefits of a controlled irrigation system are many sided, with savings to be made through reduced water and fertilizer use (Assaf *et al.*, 1984). For these reasons it is not correct to disregard the possibility of using orchard soil water management techniques in areas receiving a relatively high rainfall. As with any management system, it is a case of weighing up the potential benefits against other options such as different tree training systems and root pruning. Consumer preference for fruit size, shape, colour etc. will, in the final analysis, bear heavily upon the best production techniques to use.

17 SUMMARY OF RESULTS AND CONCLUSIONS

The use of polyethylene glycol as an osmotic agent to generate a controlled plant water stress was investigated within the bounds of an aeroponic growing system using tomato (*lycopersicon esculentum* Mill. cv. Virosa F1). In this system plant roots were suspended in humid air and watered from below via misting jets. Preliminary experiments showed that for a range of polyethylene glycol molecular weights (PEG 1000, 4000 and 6000), PEG 4000 performed the best in terms of a minimum effect on plant growth. This was believed to reflect the interaction between decreased uptake and increased phytotoxicity as molecular weight increases. Performance of polyethylene glycol in the aeroponic system appeared to be better than in the various hydroponic systems which have been reported, so long as increased nutrient solution viscosity did not affect solution distribution within the tank. In this regard problems were encountered with PEG 6000.

The performance of PEG 4000 as an osmotic agent was very good up to a total nutrient solution Ψ_W of around -6 bar. At this level significant amounts of PEG 4000 were found in the leaves of some plants. These plants exhibited classic PEG phototoxicity symptoms. Using PEG 4000, the relationship between nutrient solution Ψ_W and plant parameters during vegetative growth was investigated. It was found that many important parameters were related linearly to Ψ_W(solution). These included plant part dry weights, leaf area, allometric k value, Ψ_W(leaf), net photosynthetic rate and stomatal conductance.

In order to avoid the problems associated with osmotic agents a different water stressing method was required. Intermittent misting was investigated in which the aeroponic misting pumps were cycled on and off for varying periods. This technique has been used by other workers to stimulate hormonal changes but no work has been reported detailing vegetative growth responses.

Investigation of the aeroponic tank environment revealed that the atmosphere remained almost saturated while the misting pumps were off for periods up to at least two hours. Hence misting pump off-times of this duration could be using without any risk of root damage. Tomato plants were subjected to a range of off-times up to 1.55 hours, with a constant on time of 1 minute (to saturate the root system). It was found that important plant parameters could be related in a negative logarithmic fashion to misting pump off-time (e.g. Ψ_W(leaf), plant part dry weights, allometric k value, net photosynthetic rate and stomatal conductance). Results indicated that intermittent

misting in an aeroponic system could be used to generate a continuous range of mild to moderate water stress in which plant parameter responses could be predicted. Theoretical consideration of the induced water stress suggested that it could be related more closely to the natural situation than that involving osmotic agents.

To test the validity and generality of the above results a very different plant type was tested. *Pyrus betulaefolia* was chosen, this being an important root-stock for the asian pear (nashi) fruit crop (*Pyrus serotina*). For misting pump off-times of up to two hours it was again found that plant parameters could be related to the negative logarithm of the misting pump off-time. This was despite the fact that the tomato seedlings grew approximately exponentially while *P. betulaefolia* plants grew in a more linearly fashion. Hence it was concluded that intermittent misting was an ideal method for generating a controlled water stress in both pure and applied experimental work.

Significant differences in the stress response of *P. betulaefolia* and tomato were discussed in relation to the vegetation type and primary plant strategy, that is, either predominantly competitive (tomato) or stress-tolerant (*P. betulaefolia*). The basic difference was that in tomato there was a smaller decrease in relative growth rate (i.e. greater attainment) for a given change in assimilate partitioning.

In order to investigate the practically important issue of physical root restriction *versus* water stress, an experiment was conducted using *P. betulaefolia* in a circulating hydroponic system. Under root restriction (with minimal water stress) no significant differences were found in several important parameters, including net photosynthetic rate. In contrast to the water stress response, assimilate partitioning to the shoot system increased. The relative increase in partitioning was greatest in the stem component, this being the plant part most severely affected by water stress. The implications of these results were discussed in relation to vegetative growth control measures in fruit crops such as regulated deficit irrigation (RDI).

Following on from a literature review covering the spectrum of water stress effects on vegetative growth and internal physiology, a flow diagram model was constructed involving the interaction between carbon, mineral, water and hormone components. From current knowledge it was concluded that primary water stress sensing occurs via the leaf, with the root system providing a complementary, integrated hormonal signal from the root environment. This allows the plant to 'fine tune' the leaf signals generated from large, rapid changes in the atmosphere. It also means that adjustment can be made for trends in soil conditions which may not be correlated with climate variables on a day to day basis.

APPENDICES AND BIBLIOGRAPHY

WATER STRESS ON VEGETATIVE GROWTH

18 APPENDICES

18.1 APPENDIX 1 : LIST OF ABBREVIATIONS

Table 18.1 General abbreviations (in alphabetical order)

Abbreviation	Full description	Units
A	Cross-sectional area (p28 only)	m^2
A	CO_2 assimilation rate	$\mu mol\ m^{-2}\ s^{-1}$
ABA	Abscisic acid	
ACC	1-aminocyclopropane-1-carboxylic acid	
ADP	Adenosine diphosphate	
AHC	Actual homeostatic capacity	
AMP	Adenosine monophosphate	
ANOVA	Analysis of variance	
ATP	Adenosine triphosphate	
ATPase	Adenosine triphosphatase	
BA	Benzyladenine	
BAP	Benzylaminopurine	
BLK	Block	
c	Mole fraction of CO_2	
c_a	Mole fraction of CO_2 in the atmosphere	
C	Solute concentration (p28 only)	$mol\ m^{-3}$
C	CO_2 concentration	$ppm\ (mg\ kg^{-1})$
C_a	CO_2 concentration in the atmosphere	$ppm\ (mg\ kg^{-1})$
C_c	CO_2 concentration at the site of carboxylation	$ppm\ (mg\ kg^{-1})$
C_i	CO_2 concentration in the substomatal cavity	$ppm\ (mg\ kg^{-1})$
C_i'	C_i adjusted using Eq. 3.3 of chapter 3	$ppm\ (mg\ kg^{-1})$
CK	Cytokinin	
d	Day	
Dc	Diffusivity for CO_2	$mol\ m^{-2}\ s^{-1}$
De	Diffusivity for water vapour	$mol\ m^{-2}\ s^{-1}$
DFT	Deep flow technique	
DMT	Dimensionless mass transport coefficient	
d. wt (= DW)	Dry weight	g
e	Water vapour concentration	$ppm\ (mg\ kg^{-1})$
E	Transpiration rate	$mmol\ m^{-2}\ s^{-1}$
f_m	Element m as fraction of plant d. wt	$g\ g^{-1}$
Fru-2,6-P_2	Fructose-2,6-bisphosphate	
f. wt (= FW)	Fresh weight	g
GA(#)	Gibberellin	
g_l	Leaf conductance	$mol\ m^{-2}\ s^{-1}$
GRC	Specific root growth rate	$m\ m^{-1}\ s^{-1}$
g_s	Stomatal conductance	$mol\ m^{-2}\ s^{-1}$
h	Hour	
IAA	Indolyl acetic acid	
IRGA	Infra-red gas analyzer	
Jv	Volume flux	$m^{-3}\ s^{-1}$
k (= k_P)	Slope of allometric relationship	

Table 18.1 Continued (general abbreviations (in alphabetical order))

Abbreviation	Full description	Units
k_E	Stress ratio (shoot to root)	
k_{int}	Initial slope of the Pn *versus* C_i plot	mol m^{-2} s^{-1}
L	Root length (p73 only)	m
LA	Leaf area	m^2
LAR	Leaf area ratio	m^2 g^{-1}
Lp	Hydraulic conductivity	m^3 m^{-1} s^{-1}
LWR	Leaf weight ratio	g g^{-1}
M	Change in weight of element m	g
min	Minute	
m_w	Molecular weight of water	g mol^{-1}
NADPH	Nicotinamide dinucleotide phosphate-H^+	
NADH	Nicotinamide dinucleotide-H^+	
NFT	Nutrient film technique	
PA	Phaseic acid	
PAR	Photosynthetically active radiation	mol m^{-2} s^{-1}
PEG	Polyethylene glycol	
3PGA	3-phosphoglyceric acid	
PMF	Proton motive force	
Pn	Net photosynthetic rate	µmol m^{-2} s^{-1}
Pg	Gross photosynthetic rate	µmol m^{-2} s^{-1}
$(Pk)^{-1}$	'Carboxylation ' resistance	m^2 s mol^{-1}
Ψ_M	Matric potential	bar (= 0.1 MPa)
Ψ_P	Pressure potential	bar (= 0.1 MPa)
$\Psi_{P(leaf)}$	Pressure potential of leaf	bar (= 0.1 MPa)
$\Psi_{P(th)}$	Threshhold pressure potential	bar (= 0.1 MPa)
Ψ_S	Osmotic potential	bar (= 0.1 MPa)
$\Psi_{S(leaf)}$	Osmotic potential of leaf	bar (= 0.1 MPa)
Ψ_S^p	Osmotic potential at $\Psi_P = 0$	bar (= 0.1 MPa)
$\Psi_{S(sat)}$	Osmotic potential at $\Psi_W = 0$	bar (= 0.1 MPa)
Ψ_W	Water potential	bar (= 0.1 MPa)
$\Psi_{W(leaf)}$	Water potential of leaf	bar (= 0.1 MPa)
$\Psi_{W(plant)}$	Water potential of plant	bar (= 0.1 MPa)
$\Psi_{W(root)}$	Water potential of root	bar (= 0.1 MPa)
$\Psi_{W(soil)}$	Water potential of soil	bar (= 0.1 MPa)
$\Psi_{W(stem)}$	Water potential of stem	bar (= 0.1 MPa)
Ψ_g	Gravitational potential	bar (= 0.1 MPa)
q	Flux per unit plant dry weight	m^3 kg^{-1} s^{-1}
Q	Flux per unit leaf area	m^3 m^{-2} s^{-1}
R	Rate of solute flow (p28 only)	mol s^{-1}
R	Resistance (p 18 only)	Pa s m^{-1}
R	Assimilation rate ratio, stressed to nonstressed	
Rg	Growth respiration	µmol m^{-2} s^{-1}
RDI	Regulated deficit irrigation	
RGR	Relative growth rate	g g^{-1} d^{-1}
RGR_{max}	Maximum relative growth rate	g g^{-1} d^{-1}
r.h.	Relative humidity	
RNA	Ribonucleic acid	
RRZV	Restricted root zone volume	
R/S	Root to shoot ratio	g g^{-1}
Ru-1,5-BP	Ribulose-1,5-bisphosphate	

Table 18.1 Continued (general abbreviations (in alphabetical order))

Abbreviation	Full description	Units
Rubisco	Ribulose-1,5-bisphosphate carboxylase oxygenase	
RWC	Relative water content	
RWC_o	Relative water content at $\Psi_W = 0$	
r_a (= BLR)	Boundary layer resistance to water	m^2 s mol^{-1}
r_a'	Boundary layer resistance to CO_2	m^2 s mol^{-1}
r_s	Stomatal resistance to water	m^2 s mol^{-1}
r_s'	Stomatal resistance to CO_2	m^2 s mol^{-1}
r_i	Leaf internal resistance to water	m^2 s mol^{-1}
r_i'	Leaf internal resistance to CO_2	m^2 s mol^{-1}
r_m'	Mesophyll resistance to CO_2	m^2 s mol^{-1}
$\sum g$	Gross extensibility	s m^{-1}
SAR_m	Specific absorption rate	g g^{-1} d^{-1}
SLA	Specific leaf area	m^2 g^{-1}
SPAC	Soil-plant-air continuum	
SPSase	Sucrose phosphate synthase	
SWR	Shoot weight ratio	g g^{-1}
T	Stress tolerance	
Ta	Temperature of air	°C
Γ	CO_2 compensation point	ppm (mg kg^{-1})
TNC	Total nonstructural carbohydrate	
T/R	Top to root ratio	g g^{-1}
TRT	Treatment	
ULR	Unit leaf rate	g m^{-2} d^{-1}
USR	Unit shoot rate	g g^{-1} d^{-1}
V	Flow velocity of solute (p28 only)	m s^{-1}
vpd	Vapour pressure deficit	mbar (= 10^{-4} MPa)
w	Mole fraction of water	
W	Change in plant weight	g
W_p	Plant weight	g
W_r	Root weight	g
W_s	Shoot weight	g
WC	Water content	g g^{-1}
WU	Water use	l m^{-2} d^{-1} or l pt^{-1} d^{-1}
WUE	Water use efficiency	g kg^{-1}
X	Symplastic fraction of water	g g^{-1}

Table 18.2 Abbreviations specific to this thesis (in alphabetical order)

Abbreviation	Full description	Units
LfFW	Leaf f. wt	g
LfDW (LDW)	Leaf d. wt	g
LfTW	Leaf turgid weight	g
Lf/Sm	Leaf to stem d. wt ratio	
Lf/Rt	Leaf to root d. wt ratio	
LmFW	Lamina f. wt	g
LmDW	Lamina d. wt	g
Lm/Pe	Lamina to petiole d. wt ratio	
Lm/Sm	Lamina to stem d. wt ratio	
Lm/Rt	Lamina to root d. wt ratio	
OT	Off-time	h
PeFW	Petiole f. wt	g
PeDW	Petiole d. wt	g
PPA	Plant part analysis	
PtFW	Plant f. wt	g
PtDW	Plant d. wt	g
PUA	Polyethylene glycol uptake analysis	
rl	Root length of a measured root sample	m
RL	Total root length	m
RtFW	Root f. wt	g
RtDW (RDW)	Root d. wt	g
rtdw	Root d. wt of a length measured sample	g
ShFW	Shoot f. wt	g
ShDW	Shoot d. wt	g
SLx	Stress level x	
SmFW	Stem f. wt	g
SmDW (SDW)	Stem d. wt	g
Sm/Rt	Stem to root d. wt ratio	
S/R (Sh/Rt)	Shoot to root d. wt ratio	
TW	Turgid weight	g
We	Weight of expresses sap	g

18.2 **APPENDIX 2 : LIST OF DERIVED VARIABLES**

Note; see appendix 18.1 for a list of abbreviations.

Table 18.3 List of derived variables used in specific experiments (in alphabetical order)

Abbreviation	Formula	Units
Experiment 1 only:		
LfDW	LmDW + PeDW	g
LfFW	LmFW + PeFW	g
Lamina WC	(LmFW - LmDW) / LmDW	$g\ g^{-1}$
Lm/Pe	LmDW / PeDW	$g\ g^{-1}$
Petiole WC	(PeFW - PeDW) / PeDW	$g\ g^{-1}$
Experiment 2 only:		
RL	$\sum_i(rl_i \times (RtDW / rtdw_i))$	m
RtDW	rtdw1 + rtdw2 + rtdw(unmeasured)	g
Experiment 3 only:		
New/Old RL	As for RL using new/old root	$m\ m^{-1}$
RL	Old RL + New RL	m
Experiment 4 only:		
R(petiole)	$(\Psi_W$(uncov'd lf) - Ψ_W(cov'd lf)) / q	$Pa\ s\ kg\ m^{-3}$
R(plant)	$(\Psi_W$(uncov'd lf) - Ψ_W(soln.)) / q	$Pa\ s\ kg\ m^{-3}$
R(stem)	$(\Psi_W$(cov'd lf) - Ψ_W(soln.)) / q	$Pa\ s\ kg\ m^{-3}$
R(root)	$(\Psi_W$(uncov'd lf) - Ψ_W(uncov'd lf)) / q	$Pa\ s\ kg\ m^{-3}$

Table 18.4 List of derived variables used generally throughout the thesis (in alphabetical order)

Variable	Formula	
AGR	$(PtDW_1 - PtDW_2) / (t_2 - t_1)$	$g\ d^{-1}$
LAR	LA / PtDW	$m^2\ g^{-1}$
Leaf WC	(LfFW - LfDW) / LfDW	$g\ g^{-1}$
Lf/Rt	LfDW / RtDW	$g\ g^{-1}$
Lf/Sm	LfDW / SmDW	$g\ g^{-1}$
LWR	LfDW / PtDW	$g\ g^{-1}$
Resistance (R)	$(\Psi_W$(leaf) - Ψ_W(soln.)) / WU	$Pa\ s\ m^{-1}$
PtDW	LfDW + SmDW + RtDW	g
PtFW	LfDW + SmDW + RtFW	g
RGR	$(\ln(PtDW_1) - \ln(PtDW_2)) / (t_2 - t_1)$	$g\ g^{-1}\ d^{-1}$
Root WC	(RtFW - RtDW) / RtDW	$g\ g^{-1}$
RWC	see Eq. 18.6.	
ShDW	LfDW + SmDW	g
ShFW	LfFW + SmFW	g
SLA	LA / LfDW	$g\ g^{-1}$
Sm/Rt	SmDW / RtDW	$g\ g^{-1}$
Stem WC	(SmFW - SmDW) / SmDW	$g\ g^{-1}$
SWR	ShDW / PtDW	$g\ g^{-1}$
ULR	$AGR * (\ln(LA_1) - \ln(LA_2)) / (LA_2 - LA_1)$	$g\ m^{-2}\ d^{-1}$
USR	$AGR * (\ln(ShDW_1) - \ln(ShDW_2)) / (ShDW_2 - ShDW_1)$	$g\ g^{-1}\ d^{-1}$
WUE	dPtDW / dWU	$g\ g^{-1}$

18.3 APPENDIX 3 : STATISTICAL ANALYSIS

All data was analysed using the SAS software package (SAS Institute Inc., North Carolina, USA) on either a mainframe or networked personal computer. Data were sorted (PROC SORT) before analysis according to the classifying variables (i.e. harvest number, treatment number, block number etc.). Thereafter the following analysis procedures were followed.

18.3.1 *DATA TRANSFORMATION FOR ANOVA*

Data for the variables given in Table 18.5 were transformed so as to meet the requirements of analysis of variance (Bartlett, 1947). Normality was then confirmed using PROC UNIVARIATE.

Table 18.5 Transformations performed on raw experimental data

Variable	Transformation
Leaf number	$(X)^{0.5}$
Lateral number	$(X + 0.5)^{0.5}$
Root number	$(X)^{0.5}$

18.3.2 *HARVEST DATA*

Data collected or derived from the sequential harvests included the following variables; plant part fresh and dry weights, leaf area, leaf number, lateral number, stem length, root number, root length, dry weight ratios, SLA, LAR and water contents. Analysis of variance was performed on these variables using PROC GLM with a model appropriate to the experimental design (see Table 18.6). Treatment means were separated by an F protected lsd test at a significance level for F of 5%. With transformed data, treatment means were back transformed for presentation.

Table 18.6 Analysis of variance models (in SAS format) for harvest data in each experiment

Experiment	ANOVA model
1	Variables = TRT
2,3 and 4	Variables = TRT BLK

18.3.3 *NON-HARVEST DATA*

(A) Leaf water potential, PEG content, chlorophyll content, pressure-volume curve data, mineral content, hydraulic conductivity, CO_2 compensation point, k_{int}.

Blocking not considered - pooled list analysis used.

(B) Plant height.

As per harvest data

(C) Photosynthesis system data (Pn, g_s, C_i and E).

In order to account for changes in PAR, vpd and C_a during the measurement period, an analysis of variance with covariance adjustment was performed on all data sets. The data was first adjusted for the concomitant variables via a multiple regression (PROC REG) in accordance with the following model;

$$Y = b_0 + b_1(PAR) + b_2(vpd) + b_3(C_a) \qquad 18.1$$

Where: Y = Pn, g_s, C_i or E

Analysis of variance was then carried out on predicted values using PROC GLM. Means were separated using an F protected lsd test with a significance level for F of 5%.

18.3.4 *CANONICAL ANALYSIS*

A canonical analysis was performed on plant variables using PROC CANDISC, with discimination based on treatment indices. The Wilks' Lambda F test was used to assess the significance of the whole analysis (1% level). Only those canonical variables which had significant F values at the 5% level were used for discussing treatment separation. The canonical variable s.e. was used to

calculate an lsd (5% level) to separate canonical variable treatment means. Note that canonical analysis does not account for the experimental design but rather treats variables as pooled lists.

Multivariate statistical analysis techniques are now becoming more common in general scientific research. However their potential is still greatly underutilized in many fields, one of which is plant science. In the series of experiments described herein, canonical analysis was used to investigate overall plant response to a water stress. Results have proved extremely useful and demonstrated that the variety of plant responses can be succinctly qualified and quantified in a single analysis. It is hoped that through this and other examples, other workers will be encouraged to use the technique for gaining further insight into their data.

18.3.5 *REGRESSION ANALYSIS*

All regressions were performed using PROC REG. Differences between coefficients of separate regressions were investigated using a t-test involving the appropriate s.e. of the difference.

NB: For all regressions involving a measured variable on the X-axis (e.g. allometric relationships), the simple least-squares method is theoretically incorrect and a maximum likelihood method is required (see Causton and Venus (1981)). However, the difference in parameter estimates is generally small, especially as sample size increases. Considering the inherent experimental variability, it was decided to use a least-squares method routinely for all regressions in this thesis. As such it is acknowledged that for the above mentioned regressions, coefficients and their standard errors are slightly in error due to the analysis method.

18.4 APPENDIX 4 : THE AEROPONIC TANK GROWING SYSTEM

The aeroponic tank growing systems used in experiments 1, 2 and 3 were built by the Agricultural Engineering Department at Massey University. Specifications of the system are described below and a diagram given in Fig. 18.1.

Table 18.7 Specifications of the aeroponic system

Component	Property	specification
Tank;		
	size	width 615 mm; length 1200 mm; height 560 mm
	material	16 gauge galvanized steel
Lid;		
	size	width 700 mm; length 1200mm
	material	12 mm marine plywood
	finish	marine varnish
	cross supports	3 x 10 mm aluminium angle flashing
Storage tank;		
	size	100 l or 20 l
	material	black or white polythene
pump;		
	make	Tsurumi submersible pump
	model	Family 10
	flow rate	80 l min^{-1} (max.)
	head	6 m
	rating	100 W (single phase)
manifold hosing;		
	size	16 mm diameter
	material	black polythene
manifold jets;		
	make	Southerncross microjets
	material	plastic
outlet pipe;		
	size	45 mm diameter
	material	clear polythene
liner;[a]		
	material	vinyl swimming pool lining

[a] The liner was used only in experiment 4.

Slots in the lid were 10 mm wide and 1000 mm long (100 mm from each end). The three aluminium cross supports were located at either end and in the middle of the lid so they fitted neatly inside the tank, and were fixed to the lid using five 8 mm screws. Two layers of white polythene sheeting were laid under the supports. The top layer was wrapped over the edges of the lid and stapled on the top while the bottom layer formed an apron below the lid to prevent water loss. The slots

themselves were further sealed once the plant material was positioned by inserting a strip of white polythene sheeting between the two underside sheets.

For experiment 3, the lid consisted solely of the two polythene sheets which were supported by five nylon cords tied around the tanks. The top polythene sheet was fixed down the outside of the tank using waterproof masking tape, while the inner sheet again formed an apron inside. The two slots cut in the sheets were sealed as described above using an interlocking strip.

Aeroponic tanks were supported on concrete blocks to give a 100 mm fall from back to front (back 600 mm and front 500 mm off the floor), enabling water to flow rapidly into the storage tanks. Aeroponic tanks were positioned along the south side of the glasshouse, so that they sloped north, and were spaced 0.8 m apart with 2 m between the last tank and the glasshouse door.

Plant supporting frames were made of either 15 mm diameter wooden doweling or 10 mm aluminium angle flashing and were 500 mm high.

Arrangement of the aeroponic tanks in the glasshouse is shown in Fig. 18.2. Note that the two suspended heating units next to the tanks had large baffles underneath to direct flow upwards, away from the tanks, so that there was no uneven exposure to warm air. The glasshouse possessed full length side and ridge venting but the vent on the side next to the tanks was turned off to prevent excessive air movement. Sliding end doors were also kept closed at all times for the same reason.

18.5 APPENDIX 5 : THE CIRCULATING HYDROPONIC SYSTEM

The hydroponic system used in experiment 5 was built by Mr B. McKay of the Horticultural Science Department at Massey University (Palmerston North, New Zealand) specifically for root restriction studies. The layout of the system is shown schematically in Fig. 18.3 and was based around that of Willumsen (1983) who described it as the deep flow technique (DFT). Nutrient solution was fed to individual plant containers (6 l plastic buckets) via a common flow line such that there was a continuous vertical flow of solution in the containers from bottom to top.

Root restriction vessels consisted of 40 mm lengths of 40 mm diameter PVC piping sealed at both ends with two layers of polythene film. Nutrient solution fed directly into these vessels, along with one compressed air line. Vessels were supported in a plastic housing held by the polystyrene lid of the plant container. Support for the plants themselves was provided by wire loops embedded in the polystyrene. This setup is shown in Fig. 18.4.

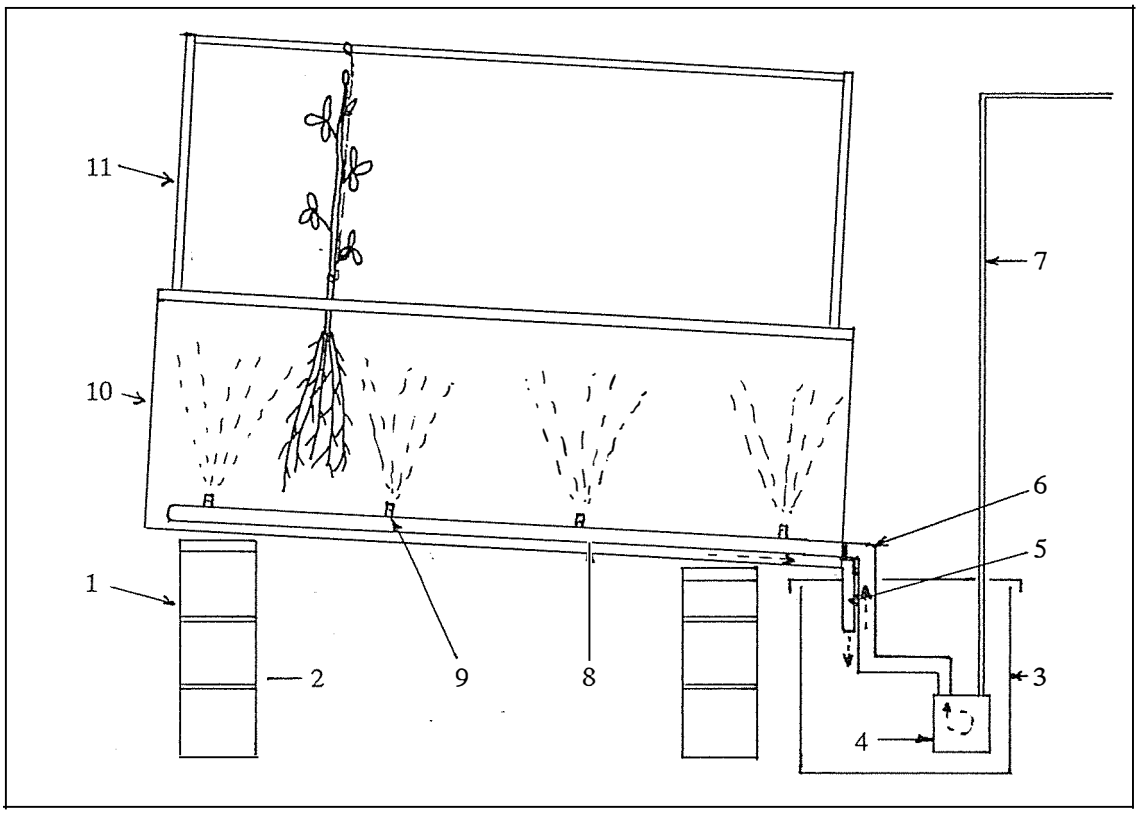

Fig. 18.1. A schematic diagram of the aeroponic system. 1, Aeroponic tank. 2, Concrete block support. 3, Nutrient solution storage container. 4, Submersible pump. 5, Outline pipe. 6, Inlet pipe. 7, Electrical cable. 8, Tank manifold. 9, Microjet. 10, Tank lid. 11, Plant support frame.

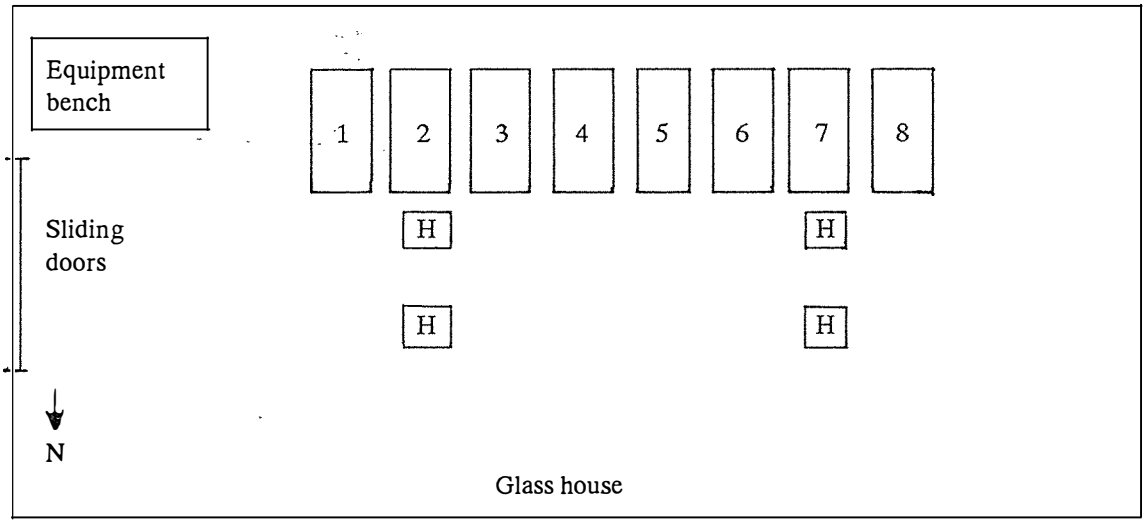

Fig 18.2. Layout of the aeroponic tanks in the glasshouse.

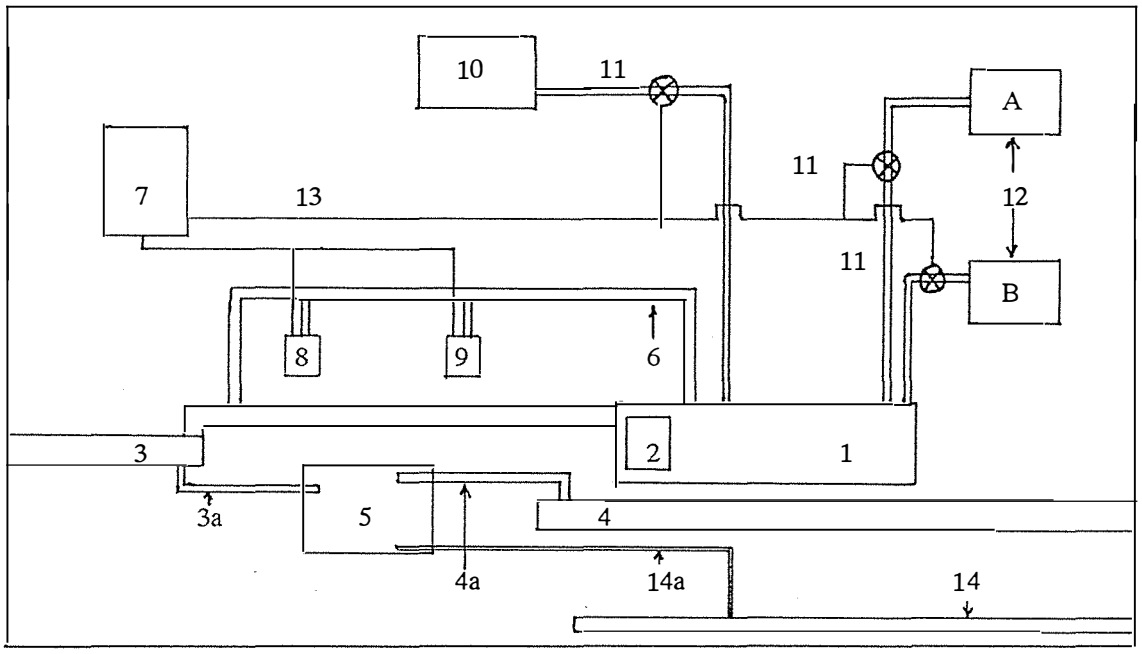

Fig. 18.3. A schematic diagram of the hydroponic system. 1, Nutrient storage tank. 2, Submersible pump. 3, Common supply pipe. 3a, Bucket supply line. 4, Common return pipe. 4a, Bucket return line. 5, Plant bucket. 6, Solution monitoring bypass. 7, Control unit. 8, pH sensor. 9, Conductivity sensor. 10, Acid storage container. 11, Solenoid valve. 12, Nutrient stock solution containers. 13, Electrical wiring. 14, Compressed air piping. 14a, Compressed air microtube.

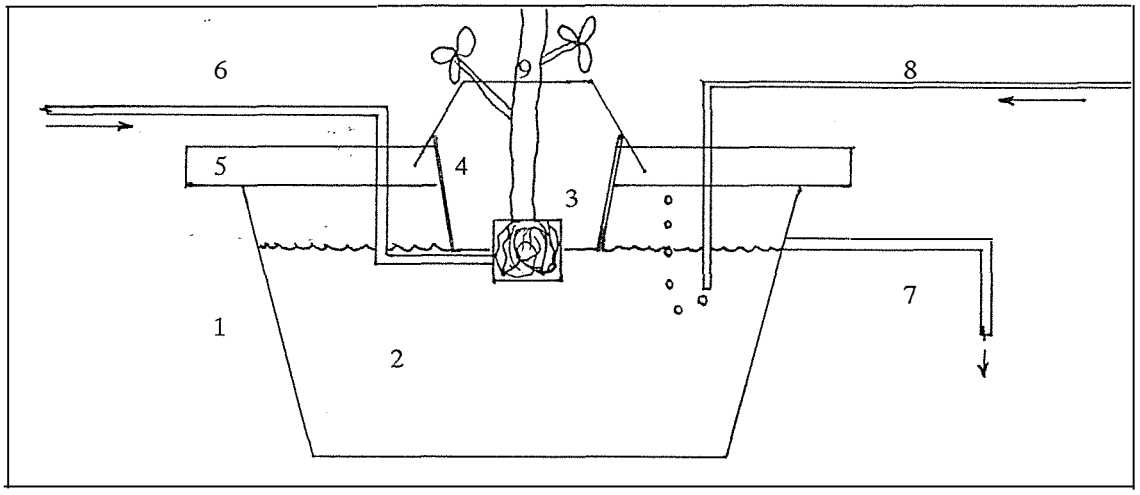

Fig. 18.4. Side view of a plant bucket in the hydroponic system showing the root restriction setup. 1, Plant bucket. 2, Nutrient solution. 3, PVC root restriction vessel with polythene ends. 4, Plastic support. 5, Polystyrene lid. 6, Inlet pipe. 7, Outlet pipe. 8, Compressed air microtubing. 9, Plant support wire.

18.6 APPENDIX 6 : FERTILIZER MIXTURES

Table 18.8 gives the fertilizer mixture used in the growing media for propagating all tomato seedlings. This is the standard seedling fertilizer mixture used by the Vegetable Crops Unit of the Horticultural Science Department at Massey University (Palmerston North, New Zealand). The second column of Table 18.9 gives the fertiliser mixture incorporated into the *P. betulaefolia* seedling growing media. These seedlings were grown in PB 8 planter bags for 12 months with no other fertilizers given. The Nursery Crops Unit of the Horticultural Science Department at Massey University (Palmerston North, New Zealand) uses this mixture for trees and shrubs.

Table 18.8 Seedling fertiliser mixtures

	Quantity (g/100 l of media)	
Fertilizer	Tomato	*P. betulaefolia*
Osmocote (14—6.1—11.6)	225	60
Osmocote (18—2.6—10.0)	-	300
superphosphate	150	-
lime	150	-
dolomite	300	300
Micromax	60	90

18.7 APPENDIX 7 : NUTRIENT SOLUTION FOR AEROPONIC TANKS

The nutrient solution used in the aeroponic system was that described by Cooper (1979) for use with the nutrient film technique (NFT). The first five compounds were kept in separate 2 l Winchester bottles, while the last five (minor elements) were mixed in a single bottle. Stock solutions were made using distilled water and kept in a cool, dark location. A new batch was made for each experiment or as required. The final solution was adjusted to a pH of 6.5 using 2 molar HNO_3.

Table 18.9 Major and minor elements in nutrient soltion

Salt	Final solution			Stock solution	
	g/100		ppm	g/2 l	ml/1l final
potassium phosphate (KH_2PO_4)	26.3	K P	78 62	131.5	4
potassium nitrate (KNO_3)	58.3	K N	254 91	291.5	4
calcium nitrate ($Ca(NO_3)_2.4H_2O$)	100.3	Ca N	168 117	501.5	4
magnesium sulphate ($MgSO_4.7H_2O$)	51.3	Mg S	49 65	256.5	4
sequestrene (NaFe chelate)	7.9	Fe	5.6	158.0	1
manganous sulphate ($MnSO_4.H_20$)	0.610	Mn S	2.2 3.8	12.20	|
boric acid (H_3BO_3)	0.170	B	0.32	3.40	|
copper sulphate ($CuSO_4.5H_2O$)	0.039	Cu S	0.065 0.130	0.78	| - 1
ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$)	0.037	Mo N	0.007 0.001	0.74	|
zinc sulphate ($ZnSO_4.7H_2O$)	0.044	Zn S	0.075 0.037	0.88	|

18.8 APPENDIX 8 : HYDROPONIC SYSTEM NUTRIENT SOLUTION

The nutrient solution used in experiment 4, for a circulating hydroponic system, was a modified Hoagland's nutrient solution which contained chelated iron and chloride ions (in the form of KCl). This solution has been used by the Plant Physiology Division of D.S.I.R. (Palmerston North, New Zealand) in growth cabinets. Stock solutions were made up in two 20 l tanks (tank A and tank B) and diluted by approximately 1 in 200 to give the final solution. The actual dilution process was controlled automatically via a conductivity meter set to maintain a conductivity of 25 mS cm^{-1}.

Table 18.10 Major and minor elements in hydroponic nutrient solution

Salt	final solution		stock solution			
	g/100	ppm	g/l		g/20 l	
TANK A:						
Calcium nitrate ($Ca(NO_3)_2.4H_2O$)	59.04		Ca N	100.20 70.03	118.08	2361.6
Sequestrene NaFe (NaFe Chelate)	2.08		Fe	2.50	4.16	83.2
TANK B:						
Potassium phosphate (KH_2PO_4)	6.81		K P	19.55 15.49	13.61	272.2
Potassium nitrate (KNO_3)	25.28		K N	97.76 35.02	50.56	1011.2
Magnesium sulphate ($MgSO_4.7H_2O$)	24.65		Mg S	24.30 32.00	49.30	986.0
Boric acid (H_3BO_3)	0.143		B	0.250	0.286	5.72
Manginous chloride ($MnCl_2.4H_2O$)	0.091		Mn Cl	0.250 0.320	0.181	3.62
Zinc sulphate ($ZnSO_4.7H_2O$)	0.011 S		Zn 0.012	0.025	0.022	0.44
Copper sulphate ($CuSO_4.5H_2O$)	0.004		Cu S	0.010 0.005	0.008	0.16
Molybdic acid ($Na_2MoO_4.2H_2O$)	0.001		Mo	0.005	0.002	0.04
Potassium chloride (KCl)	0.315		K Cl	1.650 1.500	0.630	12.60

18.9 APPENDIX 9: SUMMARY OF PESTICIDE PROGRAM

Table 18.11 lists all pesticides used in the experiments described herein, while Table 18.12 lists the days on which spraying was carried out for each experiment. Spray was applied according to the manufacturers recommendations using a 5 l backpack sprayer.

Table 18.11 List of pesticides

Trade name	Common name	Application rate	Pests to control
(a) Tomato:			
Attack	Pirimiphos-methyl	1 ml l^{-1}	whitefly caterpillars
Benlate	Benomyl	0.5 g l^{-1}	damping off
(b) *P. betulaefolia*:			
Mavrik (Aquaflow)	Fluvalinate	0.2 ml l^{-1}	mites aphids
Kocide 101	Cupric hydroxide	2.5 g l^{-1}	fireblight

Table 18.12 List of application times

Experiment	Material	Days
(a) Tomato:		
1	Benlate	8 and 15 days after emergence
2	Benlate	10 and 20 days after emergence
3	Benlate Attack	12 and 23 days after emergence 25 and 45 days post-treatment
(b) *P. betulaefolia*:		
4	Kocide 101 Mavrik	Sept. 1988 and Sept. 1989 18, 46 and 72 days post-treatment
5	Kocide 101 Mavrik	Sept. 1988 30 and 50 days post-treatment

18.10 APPENDIX 10 : PROCEDURES FOR PHOTOSYNTHESIS SYSTEM

18.10.1 *INTRODUCTION*

The LI-6200 portable photosynthesis unit has a closed system arrangement and consists of three main components;

i/ Leaf chamber
ii/ Console
iii/ Gas analyzer

The analyzer is a non-dispersive infrared type (Welles, 1986), with the leaf chamber contains four sensors;

i/ Thermocouple (leaf temperature)
ii/ Thermistor (air temperature)
iii/ Vaisala HUMICAP (r.h.)
iv/ LI-COR quantum sensor (PAR)

Further details of the instrument and the calculation procedures were given by Welles (1986) for the previous LI-COR model (LI-6000).

Performance of the LI-6200 was tested by Leuning and Sands (1989) and the significant points to come out of their work are as follows;

i/ The linear data analysis used by the LI-6200 gives similar results for Pn, g_s and C_i compared to rigorous nonlinear analysis as long as chamber r.h. is constant over the measurement period.
ii/ Significant errors occur in the estimates of water vapour flux and these were put down to adsorption / desorption from the chamber walls and leaks in the system. It was suggested that the chamber be stored in similar regime of r.h. and temperature to that encountered during measurement.

18.10.2 *STARTUP PROCEDURE*

NB; The 1 l leaf chamber (with adjustable area blocks) was used for all experiments, and the desiccant flow restrictor was attached to the desiccant tube to allow small flow rates to be accurately controlled.

The startup procedure began by allowing the instrument to warm up for 10 minutes, the flow meter was then zeroed and the condition of the desiccant (dried magnesium perchlorate [$Mg(ClO_4)_2$]) checked visually. If the desiccant was sticking together the tube was emptied, cleaned and refilled, while the effect of less severe clumping was checked by conducting the desiccant test outlined in a following

section. To calibrate the IRGA the steps given below were followed (note that procedures outlined below are taken from the LI-6200 Primer manual (LI-COR Inc., 1989));

(1) Set zero:

i/ Pump on, scrubber off, desiccant on

ii/ All flow through desiccant

iii/ Note flow rate through desiccant (XF value)

iv/ Scrubber on

v/ After 30 seconds set zero CO_2 concentration

vi/ Note flow rate through desiccant

vii/ Scrubber off, pump off

(2) Set span:

i/ Connect span gas to the 'To Pump' socket

ii/ Adjust flow rate of span gas through desiccant to be the same as vi above.

iii/ After 30 seconds set span to span gas concentration

iv/ Disconnect span gas and reconnect hosing

(3) Repeat (1) and (2)

The span gas used throughout contained CO_2 at 425 ± 10 ppm with the remainder N_2. After calibrating the IRGA, boundary layer resistance (in s cm^{-1}) was calculated (see part B below) and values for the parameters BLR (boundary layer resistance), XF (maximum desiccant flow rate) and P (atmospheric pressure) were entered into the instrument. The distance between the area blocks in the leaf chamber was checked and if necessary a new leaf area entered. The photosynthesis system was then ready to take measurements.

The instrument was set to stop measurements after 30 seconds. This gave a CO_2 change of between 0 to 10 ppm. Change in time was selected rather than a change in CO_2 in order to ensure that measurements were completed within a 2 hour period regardless of the prevailing atmospheric conditions.

18.10.3 *BOUNDARY LAYER RESISTANCE MEASUREMENT*

The following procedure was followed;

i/ Pump on, desiccant on, scrubber off, chamber fans on

ii/ All flow through desiccant

iii/ Place wet filter paper (Whatman #1) over measuring area, close chamber

iv/ Adjust desiccant flow to give constant RH

v/ Re-wet filter paper and return to chamber

vi/ After 15 seconds press log (set for a 10 second change in time)

vii/ Record Rs

viii/ Repeat v to vii three more times, average the 4 Rs values, double to give BLR parameter and enter in the instrument

18.10.4 *DESICCANT TEST*

The following procedure was followed;

i/ Pump on, desiccant on, scrubber off, chamber fans on

ii/ Set desiccant flow to 2 $cm^3 s^{-1}$

iii/ Close chamber and after 15 seconds press log (set for a 3% change in RH)

iv/ Check that COND parameter is below 0.01

If the desiccant looked in adequate condition a value for COND of less than 0.01 was always encountered. If a value greater than 0.01 was found the desiccant was replaced and the test repeated.

18.10.5 *MEASUREMENT PROCEDURE*

Before beginning measurements in the glasshouse the flow meter was again zeroed (subsequently re-zeroed every 30 minutes after the pump had been off for 60 seconds) and all major parameters were rechecked to ensure correctness. The following procedure was then followed;

i/ Pump on, desiccant on, scrubber off, chamber fans

on

ii/ Clamp chamber on suitable leaf or leaflet

iii/ Adjust desiccant flow rate to give constant RH

iv/ Clamp chamber on measurement leaf or leaflet

v/ Check that CO_2 is in correct range (i.e. < 380 ppm), RH is relatively constant (if not go back to ii) and the quantum sensor is correctly positioned

vi/ Wait until CO_2 is decreasing steadily (or at least stable) and then press log (set for a 30 second change in time)

vii/ Input treatment number etc. then store reading

viii/ Repeat from iv

Problems were encountered in the glasshouse when trying to keep air CO_2 concentration below say 380 ppm. It was vital to always breath away from the chamber and have a certain degree of air movement.

18.10.6 *PLANT MATERIAL*

In all cases only the youngest fully expanded leaves were used, with selection based on uniform light exposure (generally full sunlight) and lack of obvious tissue damage. For tomato, leaflets adjacent to the apical leaflet were used, with leaves maintained in their natural position after attaching the chamber unless the quantum sensor was under a different light regime. In such cases the chamber was moved slightly to bring the sensor into light similar to that being received by the leaf (visual estimation).

18.11 APPENDIX 11 : MEASUREMENT OF LEAF WATER POTENTIAL

Leaf water potential was measured with a pressure chamber (Soilmoisture Equipment Corporation, model 3005, USA) containing dry nitrogen. The leaf or leaflet to be measured was chosen as for photosynthetic measurements, that is, the youngest fully expanded leaf under uniform light exposure (generally full sunlight) and with no tissue damage. In the case of tomatoes, a leaflet adjacent to the apical leaflet was always used.

The leaf or Leaflet was enclosed in a plastic envelope for 30 seconds before detaching from the plant to minimize water loss (Turner, 1981; Turner and Long, 1980; West and Gaff, 1971). Leaves were detached just above the stem junction and transferred immediately to the pressure chamber,

without recutting the petiole (Turner, 1981). The chamber was lined with wet filter paper and kept away from direct sunlight to maintain a cool, humid atmosphere (Turner, 1981). The amount of petiole protruding from the chamber was kept at 5 mm to reduce extrusion errors (Millar and Hansen, 1975; Turner, 1981).

Pressure was increased at 0.2 to 0.5 bar s^{-1} to within 2 bar of the end point (from preliminary measurements) and then at 0.1 bar s^{-1} thereafter. This is somewhat higher than ideal rates previously quoted (0.03 -0.05 bar s^{-1} (Turner, 1981)) but no loss of accuracy could be detected in preliminary trials, while the measurement time was kept within 50 seconds.

The end point was detected by viewing the cut end of the petiole through a magnifying glass, with illumination via a microscope lamp. In the case of *P. betulaefolia*, the pith region of the petiole turned a darker green colour just prior to the appearance of sap and this meant that the end point could be determined easily and accurately. With tomato, the end point was often hard to detect due to air bubbling from the xylem. In all cases the end point was verified by reducing the pressure one bar, noting disappearance of the sap, and then relocating the sap level. If agreement was not within 0.1 bar the process was repeated and if again unsuccessful the leaf was discarded. Note that the end point occurred at the very first appearance of sap. If pressurization was stopped at this point the sap disappeared and a higher pressure was needed to establish the level. However Campbell and Campbell (1974) found that the initial pressure was most strongly correlated with thermocouple hygrometer readings.

18.12 APPENDIX 12 : MEASUREMENT OF ROOT LENGTH

18.12.1 *GENERAL*

Root length was measured using a root length scanner (Comair, Commonwealth Aircraft Corporation limited, Australia). This instrument uses the intersection method developed by Newman (1966) to give an estimate of total root length in metres. Raw length readings given by the instrument can be corrected for a slight nonlinearity of estimation using a factory calibration given in the instruments user manual;

$$rl(corrected) = -0.2246 + 0.9655 \times rl(raw) + 0.00123 \times rl(raw)^2 \qquad 18.2$$

This correction was applied to all readings before going on to calculate total root system length.

18.12.2 *THE MEASUREMENT PROCEDURE*

All root tissue suitable for measurement (under 2 mm diameter) was cut into approximately 2 cm lengths and distributed evenly over a plastic try with ten 5 cm square grids. The tray had a mesh covered drainage hole to remove water. Two samples were then selected at random from one of more squares (to give a sample length in the range of 20 to 40 m). Each sample was placed on a glass scanning tray in 1 l of tap water and distributed as evenly as possible over the tray using plastic tweezers. Root length was then measured, followed by dry weight determinations on the two samples and the remaining root tissue. Total root length was calculated using each sample and the two values averaged;

$$RL = rl1 \times (RDW / rdw1) \qquad 18.3$$

Where: RL = Total root length

rl1 = Corrected root length for sample 1

rdw1 = d. wt of sample 1

RDW = total root d. wt

(= rdw1 + rdw2 + rdw(unmeasured))

Before each measurement session commenced the scanner calibration was checked using a grid supplied with the instrument and the glass tray was also checked for horizontality using a spirit level.

18.12.3 *PRECISION TEST*

To test the precision of the instrument a large root sample was used (root length estimated over 100 m). At this sample size errors are increased due to root segments falling on top of one another or lying close together and not being differentiated. Sample sizes were generally chosen to have a length in the order of 20 to 40 m which reduced these problems.

The test sample was scanned ten times and before each scan the root segments were removed from the scanning tray, then replaced, giving a completely new distribution. The results were as follows;

Mean : 127.71 m

s.e. mean : 0.83 m

CV : 2.0 %

18.13 APPENDIX 13 : THE THERMOCOUPLE PSYCHROMETER

The instrument used for solution water potential measurements was a thermocouple psychrometer consisting of a Wescor HR 33T Dew Point Microvoltmeter (Wescor Inc., USA) with eight Wescor C-52 Sample Chambers. Deep sample pans were used to hold the nutrient solutions. The instument was calibrated using standard solutions ranging from 0 to 8 bar. Distilled water was used as a blank for all measurements.

18.14 APPENDIX 14 : THE PRESSURE-VOLUME CURVE TECHNIQUE

18.14.1 *EXPRESSED SAP METHOD*

This is the traditional method for obtaining pressure-volume curve data and was first developed by Scholander *et al.* (1964). Accepted theoretically analyses of the pressure-volume curve data are based on Tyree and Hammel (1972), although alternative analyses have been proposed (e.g. Acock, 1975). Pre-weighed, fully turgid plant material is pressurized in a pressure chamber above the balance point so that sap is expressed through the cut end of the petiole or stem. This sap is collected in some way and the weight recorded. After each period of sap collection (say 2 to 10 minutes) the balance point is found. It is then possible to plot balance pressure (commonly the reciprocal) against the weight (or volume) of sap expressed, hence the name 'pressure-volume' curve. The following procedure was adopted based on the methods of several workers (Cutler *et al.*, 1979; Jane and Green, 1983; Sinclair and Venables, 1983; Wenkert *et al.*, 1978).

i/ Plant material left for 15 hours (over night) in distilled water under a humid atmosphere to become fully turgid. With leaves or leaflets only the bottom 2 to 4 mm of the petiole was placed in water.

ii/ Leaf removed from water, petiole blotted dry, 2 mm trimmed from end of petiole, leaf weighed (to 0.001 g), enclosed in plastic bag lined with damp filter paper and then sealed in pressure chamber (Soilmoisture Equipment Corporation, model 3005, USA) with 5 mm of petiole exposed. Pressure chamber operated as described in appendix 18.11.

iii/ Initial balance point found and if greater than 0.2 bar the leaf not used for further measurement. Balance points determined as described in appendix 18.11 except that a pressurization rate of 0.05 bar s^{-}

^{1} was used and the depressurization-repressurization process was repeated until values agreed within 0.1 bar.

iv/ Pre-weighed sap collection capsule placed on exposed end of petiole. The capsule was made from the bulbous end of a disposable plastic pipette which was cut in half and filled with cotton wool. To reuse the capsule, it was simply pulled apart and repacked with cotton wool.

v/ Leaf over-pressurized by 2 to 5 bar for 2 to 10 minutes (Cutler *et al.*, 1979; Wenkert *et al.*, 1978). Both time and over-pressure were increased as more sap was removed.

vi/ Capsule removed and weighed, and then pressure dropped to previous balance point (at 0.1 bar s^{-1}) after which the new balance point was found.

vii/ Steps (iv) to (vi) repeated 15 to 20 times which generally gave at least 8 to 10 reading after the point of turgor loss. In the case of *P. betulaefolia* the procedure usually continued until the over-pressure reached -40 bar, the limit of the pressure chamber used.

viii/ After final balance point obtained, pressure released (at 0.5 bar s^{-1}) and leaf weighed.

ix/ Leaf dried at 80 °C for 48 hours, then dry weight measured (to 0.001 g).

The data analysis procedure is described after the following section.

18.14.2 *BENCH DRYING METHOD*

The above procedure takes over 2 hours for a single leaf. Although this is a major limitation it should be remembered that the original method took 5 to 20 hours (Tyree *et al.*, 1978). Tyree *et al.* (1978) compared the slow Hammel method with the faster Richards method and found that Ψ_S(sat) and Ψ_{SP} were the only two parameters which could be significantly higher using the Richards method. The Richards method was very reliable if the plant material had a high $\sum$ and Ψ_{SP}, a large conducting cross-sectional area and a high conductance (Tyree *et al.*, 1978).

The assumption of potential equilibrium between xylem and leaf cells will always be a point for debate. Certainly for extreme accuracy long equilibration periods must be given (Tyree *et al.*, 1978) but the point at which deviations become practically significant is hard to ascertain. Klepper and Ceccato (1969) compared the pressure chamber with a thermocouple psychrometer and found that the pressure chamber gave lower Ψ_W's below -10 bar, this being attributed to overshooting the end point.

The bench drying method is one means of further overcoming the time constraint. It is possible to obtain data for 8 leaves in around 6 hours compared with only 2 to 3 leaves using the sap expression method. Jones and Higgs (1979) showed that this method agreed closely with results obtained using leaves stressed on the plant, and in fact bench drying was better than the Richards method. Results were in conflict with those of West and Gaff (1971) but the difference could not be explained by Jones and Higgs (1979).

The following bench drying procedure was used in experiments 2, 3 and 4. For whole root systems (experiment 2), modifications to steps (i) and (ii) are given. The method has been used successfully on root systems by Turner *et al.* (1987).

i/ Leaves left for 15 hours (over night) in distilled water under a humid atmosphere to become fully turgid. Only the bottom 2 to 4 mm of the petiole was placed in water.

(Root systems; Whole plants placed 2 l flasks of distilled water and encased in plastic bags overnight.)

ii/ Leaf removed from distilled water, petiole blotted dry and 2 mm trimmed from petiole end. Leaf weighed (to 0.001 g), enclosed in plastic bag lined with damp filter paper and then sealed in pressure chamber (Soilmoisture Equipment Corporation, model 3005, USA) with 5 mm of petiole exposed. Pressure chamber operated as described in appendix 18.11.

(Root systems; Root system severed (2 cm of stem attached) and gently blotted dry. Bark removed from 1.5 cm of the stem and the xylem core pushed through a white rubber pressure chamber sealing ring. Ring clamped into pressure chamber lid and thereafter appendix 18.11 followed.)

iii/ Balance point found (if the initial balance point was greater than 0.2 bar the leaf was rejected). Balance points determined as described in appendix 18.11 except that a pressurization rate of 0.05 bar s^{-1} was used and the depressurization-repressurization process was repeated until values agreed within 0.1 bar.

iv/ Pressure released (at 0.2 to 0.5 bar s^{-1}) and leaf quickly weighed (to 0.001 g)

v/ Leaf placed on bench between a folded paper towel for 1 to 10 minutes (laboratory at 15 to 18 °C and 40% to 50% r.h.), the times being increased as leaf dried.

vi/ Steps (iii) to (v) repeated 15 to 20 times.

vii/ After final balance point obtained, pressure released (at 0.5 bar s^{-1}) and leaf weighed.

viii/ Leaf dried at 80 °C for 48 hours, then dry weight measured (to 0.001 g).

The data analysis procedure is described in the following section.

18.14.3 *ANALYSIS PROCEDURE*

Before analysis of all data sets, leaf weights were converted to relative water contents (RWC) as follows;

i/ Sap expression method:

$$RWC_i = (TW - We_i' - DW) / (TW - DW) \qquad 18.4$$

Where: TW = turgid weight - initial leaf weight

DW = dry weight

We_i' = adjusted total weight of expressed sap at the i_{th} balance point

A small adjustment was made to We_i to account for the loss of water in the chamber (equation 14.2), assuming that loss was constant over the measurement period (Sinclair and Venables, 1983). Losses were in the order of 10% to 15% of total sap weight as found by Sinclair and Venables (1983).

$$We_i' = We_i \times (TW - FW_o) \times (t_i / \sum t) \qquad 18.5$$

Where: $\sum We_i$ = total weight of sap expressed after t_i minutes

$\sum t$ = total time in pressure chamber (minutes)

FW_o = leaf weight after removing from the chamber

ii/ Bench drying method:

$$RWC = (FW - DW) / (TW - DW) \qquad 18.6$$

Where: FW = weight of leaf

The sets of balance pressure and RWC data were analysed using equation 14.4 (Schulte and Hinckley, 1985). Balance pressures were assumed to approximate Ψ_W(leaf) of the attached leaf, in other words it was assumed that Ψ_S(xylem) was close to zero (Ritchie and Hinckley, 1975; Turner and Long, 1980), the plant material was in potential equilibrium and water in the tissue was arranged as it would be in the uncut leaf (Boyer, 1967).

$$\Psi_{Wi} = (\exp(a \times (RWC_i - RWCp)) - 1) + (\Psi_S(sat) / (1- (1 - RWC_i) / X)) \qquad 18.7$$

$$(\Psi_{Wi} = \Psi_{Pi} + \Psi_{Si})$$

Where: RWCp = RWC at turgor loss point ($\Psi_P = 0$)
Ψ_S(sat) = Ψ_S at full turgidity ($\Psi_W \sim 0$)
X = symplastic fraction of total water content
a = constant

Estimates of the four unknowns, RWC_p, Ψ_S(sat), X and a, were obtained using a nonlinear regression procedure (SAS Corporation, USA). Two iterative methods (Gauss-Newton and DUD) were used on each data set as a computational check. Good agreement was always found between the two methods as long as the procedure converged. Convergence failure could generally be corrected by removing the first one or two points in the data set and recalculating RWC's. Problems with initial values have been observed by others, e.g. Jane and Green (1983). The deviations are attributed to excess water taken up by the plant tissue (free water). In the analysis method of Jane and Green (1983) a parameter was added to estimate this extra weight but herein the first one to two points could be removed without significant loss of accuracy as the balance pressure changed by only 0.1 to 0.3 bar. Deviation of the points could clearly be seen by plotting Ψ_{Wi} against RWC_i.

18.15 APPENDIX 15 : ANALYSIS OF PEG IN PLANT SAP

The concentration of PEG in plant sap was determined using a similar method to that given by Hyden in 1956, as described by Janes (1974). The deep frozen plant samples were allowed to thaw and then sap expressed, using a plant tissue crusher (DSIR design, Mt Albert Research Station, NZ), into 25 ml test tubes. After centrifuging (Clements, model GS200, Australia) at 3500 x g for 10 minutes, a 1 ml aliquot was pipetted into a clean centrifuge tube and 0.6 ml of 10% (w/v) $BaCl_2.H_2O$, 0.6 ml of

saturated $Ba(OH)_2$ and 0.6 ml of 5% (w/v) $ZnSO_4.7H_2O$ were added, shaking the tubes between each addition. After standing for at least 5 minutes, tubes were centrifuged at 3500 x g for 5 minutes. The supernatent was poured into a Spectronic 20 cuvette and 3 ml of trichloroacetic acid solution was added (225 g of trichloroacetic acid and 25 g of $BaCl_2.2H_2O$ in 500 ml of water) with mixing achieved by several inversions. After standing for 5 minutes the absorbance was measured at 600 nm on a spectrophotometer (Spectronic 20, Bausch and Lomb, USA). A standard curve relating absorbance to PEG content (mg/ml) (Fig. 18.5) was obtained by making a dilution series of PEG 1000, 4000 and 6000 from 0 to 2 mg/ml. A 1 ml aliquot of these solutions was used in place of the 1 ml of sap supernatent. A blank was prepared with each set of centrifuge tubes, containing 1 ml of deionised water in place of the plant sap. Care was taken to conduct the procedure in a standardised fashion, as stressed by Janes (1974), with only one batch of trichloroacetic acid solution used to avoid changes in concentration. Note that a Spectronic 20 was used in this analysis rather than a more sophisticated spectrophotometer due to the size of the cuvette needed.

18.16 APPENDIX 16 : MINERAL ANALYSIS OF PLANT TISSUE

The method of cation analysis was that used by the Fertiliser and Lime Reasearch Centre at Massey University (Palmerston North, New Zealand). The minerals analysed were potassium (K), calcium (Ca) and magnesium (Mg).

18.16.1 *TISSUE PREPARATION*

Oven dried plant tissue, which had been stored in a dry environment, was ground to a fine powder and the powder placed in 50 ml plastic, screw-top vials.

18.16.2 *TISSUE DIGESTION*

The digesting solution was made by diluting 400 ml of concentrated hydrochloric acid to 5 l with the addition of caesium and strontium at 1000 $\mu g\ ml^{-1}$ (6.336 g and 12.077 g in 5 l respectively). Approximately 0.1 g of tissue powder (weighed to 0.001 g) was placed in a 20 cm boiling tube with 4 ml of the digestion solution. Tubes were heated in a heating block at 250 °C for 4 hours, with a glass funnel in the neck of the boiling tube to aid reflux. The funnel was then removed and the tubes heated

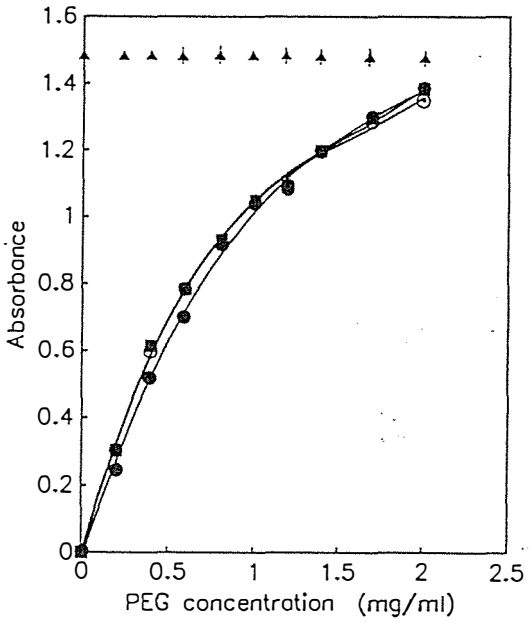

Fig. 18.5. The relationship between polyethylene glycol concentration (mg/ml) and absorbance at 600 nm in an aqueous solution (n = 4). Bars show pooled s.e. for each concentration. ●, PEG 1000; $Y = 1.63X - 0.71X^2 + 0.12X^3$: ○, PEG 4000; $Y = 1.86X - 1.01X^2 - 11.90X^3$: ■, PEG 6000; $Y = 1.85x - 1.01X^2 - 11.32X^3$. $R^2 = 0.999^{**}$ for all equations.

for a further 4 hours at 300 °C. After cooling the residue was redissolved in 5 ml of the digestion solution and then made up to 50 ml in a 50 ml volumetric flask.

18.16.3 *ANALYSIS*

The solutions were analysed on an atomic absorption spectrometer (Instrument Laboratory AA/AE Spectrometer, model 451, USA) using standard solutions of the three elements. These solutions contained the element at 1000 $\mu g\ ml^{-1}$ (along with caesium and strontium at 1000 $\mu g\ ml^{-1}$). Dilution series were made using the standard solutions as follows;

K : 0 to 8.0 $\mu g\ ml^{-1}$: 2.0 $\mu g\ ml^{-1}$ steps
Ca : 0 to 4.0 $\mu g\ ml^{-1}$: 1.0 $\mu g\ ml^{-1}$ steps
Mg : 0 to 2.0 $\mu g\ ml^{-1}$: 0.5 $\mu g\ ml^{-1}$ steps

The sample solutions were diluted 40 times with the caesium/strontium solution before reading and all values subsequently converted to the common units of mg per g dry weight.

18.17 APPENDIX 17 : LEAF PIGMENT ANALYSIS

Two different methods were used for leaf pigment analysis, based on extraction solvents of acetone and N,N-dimethyl formamide respectively. These methods are described below.

18.17.1 *EXTRACTION USING 80% ACETONE*

The following procedure was carried out under low intensity green light at 16 °C. Tomato leaflets were collected in plastic bags and transported immediately to the laboratory, giving a lapse time of less than 30 minutes. Leaves were selected as for photosynthesis measurements (appendix 18.10).

Approximately 1.5 g of lamina tissue (not including midrib) was weighted (to 0.001 g) and then ground in a chilled mortar and pestle with 80% acetone, grinding sand and a pinch of magnesium carbonate ($MgCO_3$). The sample was then filtered through a fritted-glass filter under light suction and the residue washed, until fully decolourised, using 80% acetone. The solution was poured into a 100 ml volumetric flask and 100% acetone added to give an 80% acetone solution. In calculating the volume of acetone required (shown below) a value for the tissue water content (WC) was used which had previously been determined by taking leaf samples the day before and drying them for 24 hours at 80 °C

d. wt of sample = f. wt / (WC + 1) 18.8

vol. 100% acetone = (f. wt - d. wt) x (0.80 / 0.20) 18.9

The volume was then made up to 100 ml with 80% acetone and samples stored in the dark at room temperature for three hours before reading on a dual beam spectrophotometer (Hitachi, model U-2000, Japan) at 470, 646, 663 and 700 nm using an 80% acetone blank. Samples with an absorbance of greater than 0.012 at 700 nm were rejected (Vernon, 1960). Chlorophyll and total carotenoid concentrations were calculated using the equations of Lichtenthaler and Wellburn (1983);

$$Chla = 12.21 \times A_{663} - 2.81 \times A_{646} \quad 18.10$$

$$Chlb = 20.13 \times A_{646} - 5.03 \times A_{663} \quad 18.11$$

$$Carotenoids = (1000 \times A_{470} - 3.27 \times Chla - 104\ Chlb) / 229 \quad 18.12$$

18.17.2 *EXTRACTION USING N,N-DIMETHYL FORMAMIDE*

The following method was carried out under normal laboratory light. Tomato leaflets were collected in plastic bags and transported immediately to the laboratory, giving a lapse time of less than 30 minutes. Leaves were selected as for photosynthesis measurements (appendix 18.10).

Lamina discs were punched from the region midway between midrib and leaf margin using a sharp 0.81 cm^2 cork borer. Two discs were taken from each leaflet (one either side of the midrib), with 4 leaflets per treatment (total of 32 measurements). Two further discs were taken from each leaf and dried at 78 °C for 48 hours before weighting (to 0.001 g) thus allowing pigment concentrations to be converted to a dry weight basis.

Each disc was transferred immediately to a test tube containing 3 ml of N,N-dimethylformamide (DMF) chilled to 4 °C and the tube capped with aluminium foil. After preparing all samples (including two blanks of 3 ml DMF only) the entire rack was encased in foil and stored at 4 °C for 4 days. Absorbance was then read on a dual beam spectrophotometer (Hitachi, model U-2000, Japan) at 700, 663, 646 and 470 nm using the prepared blanks. Pigment contents were calculated as in experiment 1, excluding samples with an absorbance above 0.01 at 700 nm (Vernon (1960) for 80% acetone extraction).

Chlorophyll and total carotenoid concentrations were calculated using the equations of Lichtenthaler and Wellburn (1983) given above. Although these are for 80% acetone, Moran and Porath (1980) showed that performance of the two solvents was very similar.

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