EFFECTS OF ROOT EXCISION AND ROOT COOLING ON PRIMARY LEAF EXPANSION IN *PHASEOLUS VULGARIS* L.

by

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I declare that this thesis has been composed by myself and that the work it describes is mine.

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ABBREVIATIONS

A	area
ABA	abscisic acid
^{o}C	degrees Centigrade
CAWL	capacity for acid-induced wall loosening
cm	centimetre
dV/dt	rate of irreversible increase in cell volume
DW, Dwt	dry weight
EEx	elastic extensibility
FW/Fwt	fresh weight
g	gramme
GLC	gas-liquid chromatography
h	hour
HPLC	high-performance liquid chromatography
K	potassium
kg	kilogramme
l	length
$\log_e$	natural logarithm
Lp	hydraulic conductivity
LPW	lamina piece weight
m	metre
mg	milligramme
mm	millimetre
mOsmol	milliosmole
MPa	megapascal
Mwt	molecular weight

N	nitrogen
NAR	net assimilation rate
nm	nanometre
P	turgor pressure; turgor potential
P	phosphorus
P_B	balance pressure
PEG	polyethylene glycol
PEx	plastic extensibility
P/V	pressure/volume
Q	total weight of water lost
RC	root cooling treatment
RE	root excision treatment
RGR	relative growth rate
RR	root excision treatment (regeneration of roots permitted)
RRME	relative rate of midrib extension
R/S	root/shoot
RWC	relative water content
s	seconds
t	time
T	transpiration rate
TEx	total extensibility
TW	turgid weight
V	volume
w	width
WEx	wall extensibility
WLF	wall loosening factor
Y	wall yield threshold; wall yield stress

ABSTRACT

Seven-day old seedlings of French bean (Phaseolus vulgaris L.) were grown on nutrient solution and subjected to root treatments known to affect leaf growth. The first, root excision, involved the removal of all lateral root tissue. The second, root cooling, was achieved by maintaining plants at a root temperature of 10°C, the control temperature being around 19°C.

Both treatments reduced root system size relative to that of control plants and also profoundly affected leaf growth. The rate of primary leaf expansion was significantly reduced within 7 - 9 hours of the beginning of treatment and final areas were less than 40% of those of control plants. Interestingly, when root excision was performed once only and new lateral roots allowed to develop, this reduction in leaf growth was rapidly reversed, even when the amount of lateral tissue produced was low. These findings suggest that a complete and metabolically-active root system is necessary for normal leaf growth, and that the root apices may play a major role in this control.

Leaf cell number was little affected by either treatment, the greatest effect being on leaf cell enlargement. However the hypothesis that root treatments reduced the growth of leaf cells by reducing water uptake and leaf cell turgor could not be proven. Although in the long term root excison did affect leaf water status, over the first 40 hours of treatment when considerable reductions in leaf growth were detected, leaf water potential and leaf turgor were unaffected. Similarly in root-cooled plants, although the rate of leaf enlargement was consistently lower than in control plants, no associated effect on leaf water status could be found. Significantly the content of abscisic acid (ABA) in the primary

leaves increased substantially in response to root cooling, being significantly higher than the control value within one day of treatment and reaching ten times its normal concentration after five days.

Having failed to establish a correlation between the early effects of roct treatments on leaf cell enlargement and changes in leaf turgor, attempts were made to assess the effects of treatments on the biophysical parameters governing cell wall yielding. A technique for measuring wall yield stress was developed but found to be unsuitable for use with the very small leaves being studied. However, the construction of an Instron-type tensiometer allowed the effects of root treatments on leaf tissue elastic and plastic extensibilities to be monitored. Although interpretation of these data was complicated by doubts about the effects of differences in leaf internal structure, both treatments were shown to reduce measured plasticity. In the case of root excision, this reduction persisted throughout the experiment but for root cooling, reversal of the effect took place after several days. Nevertheless the correlation between decreases in leaf extension rate and plastic extensibility indicated that in addition to their effects on leaf water status, plant roots may also control leaf growth by affecting the yielding properties of leaf cell walls.

1. INTRODUCTION

1.1 INTRODUCTION

The relationship between the root and the shoot is a complex homeostatic one in which root:shoot ratio is kept constant, or altered by changes in the relative growth rates of the two sub-systems. Under constant conditions, a functional equilibrium appears to exist, such that the size and activity of the root and shoot are maintained at levels most appropriate to external conditions (Richards, 1980). When a change in the environment takes place, the rate of growth of one sub-system relative to the other changes and the functional balance is restored (Troughton, 1980). Conversely, if environment is kept constant but the root:shoot ratio altered, for instance by removal of part of either sub-system, differential growth rates cause the rapid re-establishment of the original ratio (Brouwer and Kleinendorst, 1965).

The mechanism by which a functional equilibrium is maintained between the root and shoot must be highly complex. One model proposes that the basis of control is the maintenance of a constant carbon:nitrogen ratio (Troughton, 1980). Thus, if nitrogen supply increases, shoot growth is promoted so that carbon assimilation can rise. Conversely, if assimilate supply increases, root growth is enhanced, leading to increased nitrogen uptake. This model is based on the "competition hypothesis" in which it is postulated that the growth of any organ or sub-system is related to its ability to compete for minerals and assimilate (Troughton, 1980). However, several observations suggest that a more complex system of control, possibly involving specific chemical factors, might operate (McDavid, Sagar and Marshall, 1973; Gollnow and Letham, 1978).

One interesting aspect of the problem of root - shoot interactions is the

role of roots in the regulation of the growth and development of leaves. Although this problem has received considerable attention in the past, and although evidence has been obtained to show that roots do indeed influence leaf growth, the mechanisms by which such control might operate remain poorly understood (Gollnow and Letham, 1978). In most published work, the role of roots in the regulation of leaf growth has been investigated by applying root treatments known to affect leaf growth and recording which aspects of root function are altered by treatment. (For review, see Gollnow and Letham, 1978.) Such an approach may identify the mode of communication to the shoot of changes in the root. However, it frequently fails to take into account the response within the leaf itself and thus is incapable of identifying the precise factor or factors responsible for the control. In the present study, treatments already shown to influence leaf growth were applied to root systems and their effects on root function observed. However, in addition, the detailed effects of treatment on the leaves themselves were also recorded so that the mechanism of control could be investigated at both the level of the whole plant (ie communication between root and shoot) and that of the leaf (ie specific effects on leaf cells).

1.2 THE EFFECTS OF SPECIFIC ROOT TREATMENTS ON THE GROWTH AND PHYSIOLOGY OF THE SHOOT

Several treatments have been employed to inhibit or alter the normal functioning of plant root systems in order to investigate the communication between roots and shoots. Most widely used amongst these have included root excision, root cooling and waterlogging.

1.2.1 Root Excision

Of the different treatments used to alter the normal functioning of the

root system, complete or partial root excision is the most straightforward, and such treatments have been shown to affect the growth of all parts of the shoot including leaves, stems, tillers and buds (eg Veen, 1977; Brouwer and Kleinendorst, 1965; Went, 1938). Depending upon the severity of the treatment and whether it is applied once only or several times, the rate of root growth may also be reduced (Humphries, 1958). Initially root:shoot ratio is reduced by treatment. Subsequently, if regeneration of new roots is allowed to proceed, the original ratio may be restored. However, if root excision is repeated, root:shoot ratio continues to fall, as more dry matter is retained in the shoot and less transported to the roots (Brouwer and Kleinendorst, 1965; Ghobrial, 1983).

In the shoot, the most obvious effects of root excision are frequently observed in the leaves. Lamina expansion is often reduced and both fresh and dry weight increases decline, although dry-weight gain is normally less affected, leading to an increase in leaf dry matter content (Carmi and Van Staden, 1983). Despite a reduction in leaf size, leaf number is frequently unchanged, although rate of leaf emergence may be reduced (Brouwer and Kleinendorst, 1965; Büttrose and Mullins, 1968). Also leaf cell enlargement is usually more affected than cell division (Buttrose and Mullins, 1968; Carmi and Van Staden, 1983) and such reductions in cell growth may lead to the production of leaves substantially thinner than normal (Carmi and Van Staden, 1983).

Excision of the entire root system is not required to alter shoot growth but the extent of the effect, for instance on leaf growth, seems to be related to the severity of treatment (Buttrose and Mullins, 1968). Nor is excision of whole roots necessary since removal of root tips has also been shown to be effective in considerably altering leaf development

(Wareing and Phillips, 1970).

As well as the growth of the shoot, different aspects of its physiology have also been shown to be influenced by roots. Thus, root excision can increase stomatal resistance and reduce chlorophyll content and photosynthetic rate (Humphries and French, 1969; McDavid, Sagar and Marshall, 1973; Carmi and Koller, 1978). Such findings are supported by experiments with rooted leaf cuttings which show roots to be required for the maintenance of photosynthesis and protein metabolism, and the retardation of leaf senescence (Chibnall, 1954; Richmond and Lang, 1957).

1.2.2 Root cooling

In its general effects, root cooling appears to be as effective as root excision, reducing the growth of all plant parts including leaves, stems and roots (Brouwer, 1964; Brouwer and Hoogland, 1964; Skene and Kerridge, 1967). In cereals, leaf growth may be affected directly since the leaf extension zone is close enough to the root system to be cooled by the root treatment (Watts, 1972; Menhennet and Wareing, 1976). However in other plants, communication between the root and shoot must take place.

Generally, roots are more affected by low root temperature than shoots, causing root:shoot ratio to decrease with time (Brouwer, 1964; Stephens, 1981). In the root, elongation of existing roots and proliferation of new ones may be recorded (Atkin, Barton and Robinson, 1971; Stephens, 1981), while in the shoot, many aspects of growth and development are affected including stem elongation (Skene and Kerridge, 1967) and thickening (Brouwer and Hoogland, 1964), and different aspects of the growth of leaves. Once again, leaf number may be relatively unaffected but the emergence of individual leaves is frequently retarded (Atkin,

Barton and Robinson, 1971; Menhennet and Waring, 1976). Also, fresh and dry weight increases are reduced (Brouwer 1964; Unger and Danielson, 1967; Brouwer and Kleinendorst, 1965), the latter rather less so than the former, leading to increased dry matter contents. Lamina expansion is consistently affected, mainly by reductions in leaf cell enlargement, and leaves are generally thinner with fewer intercellular spaces (Brouwer and Hoogland, 1964).

In some aspects of their anatomy and morphology, leaves of root-cooled plants resemble those of xeromorphic species and Brouwer (1974) observed that the effects of root cooling on maize leaves were very similar to those of water stress. Therefore, the finding that root cooling frequently reduces transpiration rate and water uptake may be significant (Bohning and Lusanandana, 1952; Kuiper, 1964). Other observed effects include a reduction in chlorophyll content and increased anthocyanin production in maize (Atkin, Barton and Robinson, 1971) and accumulation of hexose sugars in the leaves of Phaseolus (Unger and Danielson, 1967). One feature of several investigations is the finding that optimum root temperatures exist for root and shoot growth. These vary with plant species and culture conditions, and frequently differ between the root and shoot of the same plant. However, a general finding is that with decreasing root temperature below the optimum the severity of the effects on shoot or root growth increases (Brouwer, 1964).

1.2.3 Waterlogging

The principal effect of waterlogging is to reduce the rate of movement of gases between the root and air, leading to anoxia and the accumulation of waste gases such as carbon dioxide and ethylene in the root zone (Jackson and Kowalewska, 1983). Such conditions cause progressive

degeneration of the roots of susceptible plants leading to inactivation of root apices (Burrows and Carr, 1969) and sometimes causing death of the entire root system (Bradford, 1982). As the stress progresses, several characteristic symptoms develop in the shoot. A general reduction in shoot growth may occur, involving reductions in both stem extension and leaf expansion (Reid and Crozier, 1971). Also leaves may exhibit epinastic curvature, chlorosis and wilting (Railton and Reid, 1973). In certain plants, adventitious roots may form at the point on the stem of the air/water interface, and if these are allowed to develop, relief of the symptoms of waterlogging results (Phillips, 1964). As with the observations of the root excision and root cooling experiments discussed above, these findings support the hypothesis that a living, metabolically active root system is necessary for the maintenance of normal shoot and leaf development.

1.3 EFFECTS OF ROOT TREATMENTS ON THE ROOT SYSTEM

Each of the treatments described above affected the growth and physiology of shoots and leaves despite being applied only to the roots. This implies that mechanisms exist which allow conditions experienced by the roots to be communicated to the shoot, and furthermore, that the roots play a major role in the control of different aspects of shoot growth and physiology. Clues to the nature of the communication between root and shoot can be obtained by considering the effects of the treatments described on root systems and identifying the root functions which might be affected.

1.3.1 General effects of treatments on roots

One consistent effect of root treatments is to reduce the size of the

root system. In the case of root excision, this is brought about by the treatment itself. However, in the other treatments, root size is reduced relative to that of untreated plants by a reduction in growth rate. In addition to the size of the root system, its composition in terms of the types of root it contains may also be affected. Thus, root excision in dicotyledonous plants often involves the removal of lateral roots to leave only a single tap root. One consequence of this is that the majority of the young tissue is removed leaving a root system consisting almost entirely of mature tissue. Also in cereals such as maize, repeated excision of the roots leads to the development of a dense mass of branch roots, quite different from the fibrous root system normally present (Brouwer and Kleinendorst, 1965). Root cooling also alters root morphology, producing root systems consisting of shorter, thicker and less branched roots with substantially fewer root apices (Brouwer and Hoogland, 1964; Skene and Kerridge, 1967; Stephens, 1981). In plants subjected to root excision too, root tip number is reduced, although when the excision is performed once only, regeneration of lateral roots may eventually restore root tip number to its control value (Brouwer and Kleinendorst, 1965). Waterlogging may also cause the inactivation and eventual death of root apices (eg Burrows and Carr, 1969). Since root excision necessarily involves the removal of young tissue, a further consequence of treatment might be a change in the dominant anatomy in the root from juvenile to mature. This would be important if mature roots were more extensively suberised since root excision might then result in a considerable reduction in root permeability (Briggs and Weibe, 1982). Root cooling may also reduce root permeability since it has been found to increase suberisation of the root endodermis, particularly in regions close to the root apex (Brouwer and Hoogland, 1964).

In addition to effects on root size, morphology and anatomy, root treatments might also cause changes in root metabolic activity. Thus, root cooling could reduce the activity of the root per unit weight through a general effect on metabolic processes (Brouwer, 1964) while root excision could have the same overall effect by removing the most active portions of the root system such as the root apices and zones of elongation. By also affecting the types of root comprising the root system, root excision could affect metabolism qualitatively (Brouwer and Kleinendorst, 1964; Obroucheva, 1975).

Because it limits the supply of oxygen to the cells of the roots, waterlogging must considerably reduce root metabolic activity. A further reduction would occur following death of the root apices (Burrows and Carr, 1969).

1.3.2 Effects of treatments on root functions

The implications for root functions of the effects of root treatments on the structure and activity of root systems are considerable. Reductions in root weight and volume would be expected to reduce the amount of tissue actually contributing to root metabolism, thus reducing both the capacity of the root system for synthesis and catabolism, and its sink size. A reduction in root tip number would also reduce the ability of the root to perform synthetic and catabolic functions, although in this case more specific functions, such as the synthesis and interconversion of growth regulators might be affected. By reducing root surface area and permeability, root treatments might lower the capacity of the root for the uptake of minerals and water. Finally, through a general reduction in root metabolism, the sink activity of the root would be reduced. Coupled with the effect of sink size already mentioned, this would lead to a considerable reduction in root sink strength (Warren-Wilson, 1972). Thus the root treatments described above, which have been shown to influence leaf growth can be expected to affect four major functions of the root system; the absorption of water, the uptake of inorganic nutrients, the maintenance of sink strength and specific synthetic and catabolic functions, particularly those of the root apices.

1.3.2.1 Uptake of Water

Low root temperature treatment has been shown to reduce the uptake of water by plants and four distinct factors were identified which might be involved; a decrease in the permeability of root cell membranes, a fall in the rate of root extension, and increases in the viscosity of both water and cytoplasm (Kramer, 1940; Kuiper, 1964). For many plants the rate of water uptake is more-or-less proportional to root temperature. However, in several studies, a critical root temperature has been identified below which the reduction in water uptake is more severe. This point which is thought to coincide with the temperature at which a change in the molecular state of the root cell membranes occurs (McWilliam, Kramer and Musser, 1982) varies considerably with plant species and culture conditions, but for Phaseolus may be approximately 15°C (Bohning and Lusanandana, 1952; Kuiper, 1964; Unger and Danielson, 1967).

Root excision too might be expected to reduce water uptake, either through an effect on overall root surface area or the proportion of root surface comprised of mature and juvenile tissue. Certainly root excision has been found to reduce transpiration rate, often with corresponding effects on leaf expansion (Brouwer and Kleinendorst, 1965; Veen 1977). However, there is some evidence that the permeability of the roots remaining after root excision may increase because of increased demand per unit root area (Briggs and Wiebe, 1983; Sanderson, 1983).

That the effects of root excision and root cooling on leaf growth might be due to an unfavourable water balance in the shoot, caused by reduced water uptake, has been concluded by several groups (eg Brouwer and Hoogland, 1964; Brouwer, 1972). However considerable evidence suggests that a favourable water balance might be maintained, despite reduced

water uptake, if water loss was also controlled (See Section 4.8) A similar conclusion may be drawn from studies of the effects of water-logging. Thus, pea leaf water status was unaffected by water-logging, probably because of reduced stomatal aperture (Jackson, Hall and Kowalewska, 1984). Consequently, the effects of water-logging on leaf growth may be due to some mechanism other than reduced water supply (Jackson et al, 1984; Bradford and Hsiao, 1982b). Evidence from studies of the effects of water and salinity stresses suggest that leaf growth rate is sensitive to changes in the rate of water uptake (eg Takami, Rawson and Turner, 1982; Terry, Waldron and Taylor, 1983). However, other findings (discussed in Section 5.4) confirm that the relationship between leaf water status and growth may may not be a simple one. Therefore a finding that some root treatment reduces water uptake is not sufficient to prove that the same mechanism is responsible for an effect on leaf growth.

1.3.2.2 Uptake of mineral nutrients

One important consequence of reduced water uptake occurring in response to any of the treatments described above might be a reduction in the transport of mineral nutrients from the root to the shoot (Clarkson, 1985). However, treatments might also effect the mineral nutrient content of the whole plant by interfering with uptake into the root. Both active and passive uptake would be reduced by any treatment which reduced root surface area. Also, uptake might be affected by low temperature-induced suberisation of the root endodermis (Brouwer and Hoogland, 1964) or the increase in mature relative to undifferentiated root tissue postulated above. Furthermore, active uptake would be reduced by those treatments lowering root metabolism.

An early theory postulated to explain the effects of root excision on shoot growth was that treatment led to a deficiency of mineral elements throughout the plant and increased competition between root and shoot. Because the roots were the sources of these elements, they were considered to be favoured in the competition, thus depriving the shoot (Humphries, 1958). Brouwer (1964) modified this hypothesis to explain the effects of root cooling, suggesting that low root temperatures reduced the demand of the shoot for mineral nutrients or its ability to utilise them. Later, Davis and Lingle (1961) suggested that root cooling reduced the retention of mineral elements in the shoot by causing the accumulation of some inhibiting substance.

The uptake of phosphorus particularly is affected by root temperature (Atkin, Barton and Robinson, 1971) but nitrogen and potassium are frequently rather less affected (Cooper, 1973). Indeed, a consistent observation is that shoots seldom show visible deficiency symptoms in response to short term root cooling (Brouwer and Hoogland, 1964). Similar observations on *Phaseolus* and *Vitis* plants subjected to root excision led Brouwer and Kleinendorst (1965) and Buttrose and Mullins (1968) to conclude that mineral nutrient deficiency was not the cause of observed reductions in leaf growth. Nor does mineral nutrient deficiency appear to be responsible for reduced leaf growth in water-logged plants (Reid and Crozier, 1971). Instead, findings suggest that elements such as phosphorus may reach toxic concentrations in shoots because of degeneration of the root system (Jackson, Hall and Kowalewska, 1983).

In addition to direct effects on uptake, root treatments might also alter plant mineral nutrient status by affecting processes such as nitrate metabolism (Davis and Lingle, 1961). The implications of any impairment of root nitrogen metabolism are considerable, particularly in the

context of the synthesis of plant growth regulators (Kulaeva, 1962).

1.3.2.3 Root Sink Strength

In the developing seedling, the root system constitutes a major sink for assimilate and other substances synthesised or present in the shoot (Ghobrial, 1983). However, since the strength of any sink is related to its size and metabolic activity (Warren-Wilson, 1972), any of the treatments described above might be expected to reduce it. Unger and Danielson (1967) used this hypothesis to explain the accumulation of hexose sugars in the leaves of root cooled Phasoelus seedlings. Also, Humphries and French (1969) proposed that the strength of the root sink was an important factor in the control of carbon fixation because it controlled the amount of photosynthate which accumulated in the leaf cells and which might reduce photosynthesis by end-product inhibition.

Exactly how a reduction in root sink strength might affect leaf growth is not clear. Conceivably, a lowering of photosynthetic rate might limit growth by reducing the availability of assimilate for energy metabolism and the synthesis of cellular components. However, the effects of reduced root sink strength on plant growth regulator concentrations in the shoot are also likely to be considerable. One example might be the accumulation in the shoot of growth inhibitors normally transported to the root for metabolism (Davis and Lingle, 1961; Henson, 1984).

1.3.2.4 Synthesis and Metabolism of Plant Growth Regulators

That plant root systems might supply specific promoters of shoot and leaf development was first proposed by Went in 1928 (Burrows and Carr, 1969). In subsequent experiments he found that retarded shoot growth

and leaf chlorosis in water-logged tomato plants could be completely reversed by supplying air to a portion of the root system (Went, 1943). The lack of involvement in these effects of any changes in mineral nutrient or water supply was interpreted as showing that healthy, aerated roots produced specific substances which maintained protein metabolism and growth in the shoot. A similar conclusion was reached by Chibnall (1954) using rooted Phaseolus leaves and in 1957, Richmond and Lang showed that the effects of roots in delaying senescence of Xanthium leaves could be reproduced by exogenous cytokinin. Subsequently, plant roots were identified as sites of cytokinin synthesis and the hypothesis proposed that under normal conditions, root-derived cytokinins control protein metabolism and photosynthesis in the shoot (Skene, 1975; Carmi and Koller, 1978; Carmi and Van Staden, 1983).

Gibberellins may also be synthesised or metabolised in roots and regulate different aspects of shoot physiology (for review, see Gollnow and Letham, 1978). However, the involvement of root-derived plant growth regulators in the control of leaf growth is poorly understood (Goodwin and Erwee, 1983)

Several studies have shown that root treatments known to affect shoot and leaf development also profoundly affect the metabolism of plant growth regulators (Gollnow and Letham, 1978). For instance, in response to root cooling, maize plants showed a reduction in leaf growth which was associated with reduced transport of cytokinins and gibberellins to the shoot (Atkin et al, 1971). Meanwhile, the concentrations of several growth inhibitors, one of which co-chromatographed with abscisic acid, all increased. In similar experiments, Skene and Kerridge (1967) found root cooling to affect both the amounts and types of cytokinins in root

exudate of Vitis cuttings, while Menhennet and Wareing (1971) found low soil temperatures to reduce the rates of supply of both gibberellins and cytokinins to Dactylis shoots.

Since the sites of synthesis and metabolism of growth regulators in the root are considered to be the root apices (Van Staden and Davey, 1979) root excision treatment would also be expected to affect the amounts of these substances produced and transported to the shoot (Carmi and Van Staden, 1983). Little direct evidence is available to confirm this hypothesis but support is given by the finding that repeated excision of root apices profoundly alters leaf development in horseradish (Wareing and Phillips, 1972). Also, the conclusion of Buttrose and Mullins (1968) that Vitis root systems produce a promoter of shoot growth in proportion to their size, may indicate a correlation between production of the growth promoter and number of root apices.

Water-logging reduces both the cytokinin and gibberellin contents of xylem sap (Ried and Crozier, 1971, Burrows and Carr, 1969) as well as increasing the amounts of abscisic acid and auxin in the shoot (Wright and Hiron, 1972; Phillips, 1964). Ethylene also increases, although synthesis itself appears to be confined to the shoot, the roots supplying the precurser 1 - aminocyclopropane - 1 - carboxylic acid (Bradford and Yang, 1980). The physiological significance of these effects is confirmed by the findings that exogenous cytokinin or the formation of adventitious roots both greatly alleviate the stress symptoms, including retarded shoot growth, exhibited by water-logged plants (Railton and Reid, 1973; Phillips, 1964).

The involvement of root-derived cytokinins in the mediation of root-shoot communication is further suggested by the finding that water and

salinity stresses appear to influence shoot physiology by lowering the concentration of cytokinins in the xylem sap (Itai, Richmond and Vaadia, 1968). Blackman and Davies (1985) have also implicated cytokinin supply in the induction of stomatal closure by mild localised drying of maize roots, their conclusion being that root-derived cytokinins constitute a sensitive system of communication of soil water status to the shoot.

From the foregoing discussion, it is evident that roots do profoundly affect shoots, and that many factors may be involved. Plant growth regulators clearly must play significant roles in the communication and control processes operating between the root and the shoot. However, although effects on shoot physiology and metabolism are beginning to be understood, few convincing correlations between growth regulator supply and leaf growth have been established.

The failure of many studies to identify the mechanisms by which roots control leaf growth may be due to the complexity of the regulation involved. The use of root treatments to modify shoot and leaf growth has provided some information, particularly relating to the method of communication between root and shoot. However, experiments have frequently failed to investigate the mechanisms by which the communication is received by the leaf cells and translated into an effect on leaf growth. To begin to understand these processes, some aspects of leaf growth and its control, particularly at the level of the cell, must be considered.

1.4 LEAF GROWTH AND ITS CONTROL

The formation of the mature leaf is a complex process involving many structural and physiological developments. Scope for control, both

by environmental and endogenous factors is very great, giving a highly plastic mode of growth and permitting considerable adaption to prevailing conditions (Terry, Waldron and Taylor, 1983). For convenience, leaf growth may be divided into two phases, the primordial phase and the expansion phase, separated by unfolding or emergence (Dale and Milthorpe, 1983). The primordial phase is a time of intense synthetic activity when cells are being formed and the anatomy of the leaf, including its vasculature and mesophyll structure is being laid down (Gemmell, 1969). Many factors may influence primordial growth and because of its importance in dictating final leaf area, much work has been performed to investigate its control. However, the present study is concerned primarily with leaf growth after unfolding and the following comments are confined to that process. In addition, most attention is given to the case in dicotyledonous plants, particularly the primary leaf pair of Phaseolus vulgaris L.

1.4.1 General Features of the Expansion Phase of Leaf Growth

Under optimal conditions, unfolding of the primary leaf pair of Phaseolus vulgaris occurs between six and seven days after germination, and development is complete by day 20 (Dale, 1964). The main structural developments which take place during the phase of expansion are increases in lamina area and thickness. Both involve the processes of cell division and cell enlargement, but in this material, around 50% of the cells in the mature leaf are already formed by unfolding and subsequent growth is mainly by cell enlargement (Dale, 1964). Indeed, mean leaf cell volume may increase by up to 15-fold between unfolding and the completion of leaf expansion (Murray, 1968).

After unfolding, expansion of the leaf is closely correlated with that of the cells making up the epidermal layers (Van Volkenburgh and Cleland,

1984; Morris and Arthur, 1984). However, as well as controlling lamina expansion, enlargement of epidermal cells may also determine leaf anatomy by causing the separation of mesophyll cells and the formation of intercellular spaces (Dale and Milthorpe, 1983). In addition, different tissues within the leaf exhibit different rates and durations of cell enlargement and in this way leaf structure is created (Dengler, Mackay and Gregory, 1975). At the subcellular level, structural and compositional changes taking place during leaf expansion include the deposition of cell wall material and the accumulation of proteins and nucleic acids (Dale, 1967).

During the early part of the expansion phase, the young leaf is completely dependant upon the remainder of the plant for all its nutritional requirements. Carbon substrates must be supplied for the formation of cell wall and cytoplasmic components and for energy metabolism (Morris and Arthur, 1984), while mineral elements, particularly nitrogen and phosphorus for protein and nucleic acid synthesis, and potassium for use as an osmoticum are also required (Dale and Milthorpe, 1983; Terry et al, 1983).

The complexity of the control of leaf growth at this stage is considerable since many factors can influence the supply of the different nutrients. The supply of mineral elements is initially controlled by the amounts stored in the cotyledons and their rate of release. However, subsequently, the ability of the roots to provide these elements, and possibly competition from other sites of growth in the plant, become limiting. Carbon nutrition too is subject to control since external factors such as light, temperature and the supply of carbon dioxide influence the development of photosynthetic capacity, while internal factors such as demand for assimilate regulate photosynthetic rate.

In view of the many factors which could potentially influence leaf growth, any attempt to identify specific control mechanisms would seem impractical. However, a recent approach has been to consider the growth of the leaf as being due to the enlargement of leaf cells. Then its control can be investigated by considering the factors regulating the physical process of cell growth.

1.4.2 The Biophysical Control of Cell Enlargement

The enlargement of leaf cells can be regarded as a physical process in which uptake of water and plastic extension of the cell wall lead to an irreversible increase in cell volume. Then the process can be described by a model which incorporates the various biophysical parameters involved. One such model was proposed by Lockhart (1965) and although developed specifically to describe the elongation of cylindrical cells, has been used with success to investigate the control of growth in the Phaseolus primary leaf and other higher plant systems (Van Volkenburgh and Cleland, 1981; Radin and Boyer, 1982; Davies and Van Volkenburgh, 1983; Matthews, Van Volkenburgh and Boyer, 1984; Taylor and Davies, 1985).

Water supply is a major factor in cell enlargement and Ray, Green and Cleland (1972) have concluded that cell turgor pressure is the driving force for the process. However, two other groups of factors also influence cell growth rate; the yielding properties of the cell wall and the factors influencing the movement of water. The Lockhart model (reviewed recently by Tomos (1985)) takes into account these factors by combining two equations, the first describing the relationship between cell volume and turgor, the second that between cell volume and water movement.

Although the growth rate of plant cells may be linearly related to turgor

pressure, frequently a threshold level of turgor exists below which no growth occurs (Green, Erickson and Buggy, 1971; Bunce, 1977; Van Volkenburgh and Cleland, 1981); this is the wall yield threshold or wall yield stress (Y). The existence of a threshold turgor for growth means that for any value of turgor (P), the pressure effective for growth will be (P-Y). Then the rate of cell enlargement is given by:

$$dV/dt = WEX\ (P-Y) \qquad \text{Eq 1.1}$$

where dV/dt is the rate of irreversible increase in cell volume (V) and WEX is a constant of proportionality termed wall extensibility. Y and WEX are considered to represent rheological properties of the cell wall (Tomos, 1985).

Equation 1.1 has been shown to fit closely data obtained from growing leaves (Hsiao, Acevedo, Fereres and Henderson, 1976; Bunce, 1977) However, its application is limited because it fails to take into account the factors which influence the flux of water into the cell, namely the conductivity of the cell membrane and the water potential gradient between the cell and its surroundings (Tomos, 1985). When these are incorporated, the relationship becomes (Van Volkenburgh and Cleland, 1981):

$$dV/dt = \frac{Lp.WEX\ (\Psi_e - \pi - Y)}{Lp + WEX} \qquad \text{Eq 1.2}$$

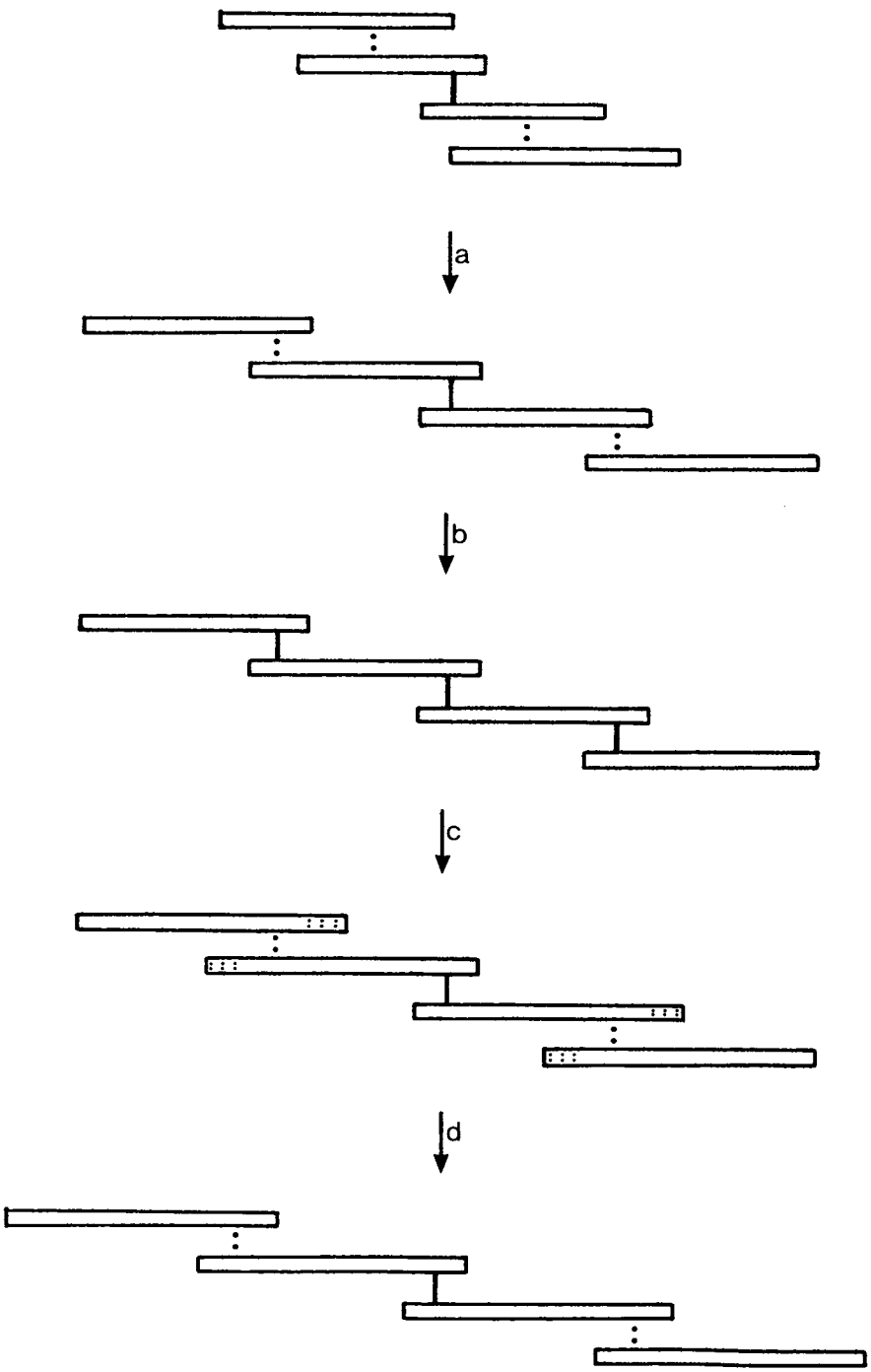
where Lp is the hydraulic conductivity of the pathway of water movement ($cm^3\ s^{-1}\ MPa^{-1}$), π is the osmotic potential of the cell (MPa) and Ψ_e is the water potential of the external medium (MPa). It follows that for any treatment to alter the rate of cell enlargement (dV/dt), it must affect one or more of the four biophysical parameters (Lp, WEX, Y , ($\Psi_e - \pi$)) on the right of the Lockhart equation (Eq 1.2). By considering the basis of each of these parameters, the mechanisms involved in the control of cell enlargement can be investigated.

Wall extensibility (WEx) describes the ability of the cell wall to undergo irreversible or viscoelastic extension (Tomos, 1985). Its biochemical basis is poorly understood, but is probably related to the arrangement of macromolecules in the cell wall and particularly the extent and type of bonding between them (Taiz, 1984). Cell wall extension could occur by the movement, relative to each other, of weakly, or un-linked macromolecules, and be limited by load bearing bonds (Fig 1.1, p 21). Then irreversible extension would depend upon the strain-hardening of the wall in its extended position.

For continuous extension of the cell wall, which is necessary for steady-state growth, the ability of the cell wall to extend must be continuously restored. This process, termed wall loosening, may involve both the breakage of load-bearing bonds and the intussusception of new wall material (Fig 1.1, p 21). Also, for wall loosening to occur, some wall loosening factor (WLF) must be supplied. In some systems, exogenous protons can function as the wall loosening factor (Rayle and Cleland, 1980) and some treatments which increase wall extensibility have been shown to do so by causing proton excretion and the acidification of the wall medium (Rayle and Cleland, 1980; Van Volkenburgh and Cleland, 1980). However, whether the acid conditions cause wall loosening directly or increase the activity of specific enzymes is not known (Taiz, 1984).

Even when the wall loosening factor is supplied, wall extension cannot take place unless the wall has the capacity to respond (Cleland, 1983). In the specific case when the wall loosening factor is low pH, this is termed the capacity for acid-induced wall loosening (CAWL). This may be a function of the composition and cross-linkage pattern of the cell wall or the ability of the cell to synthesis specific wall loosening

Figure 1.1. Proposed model of plant cell wall extension: (a) Extension of cell wall (allowed by weakly or unlinked macromolecules (⊼) but limited by load-bearing bonds (⊥)). (b) Strain - hardening of wall in extended position (to make extension irreversible). (c) Loosening of load-bearing bonds and intussuception of new wall material (▭), ie wall loosening, allowing steady state growth (d) to occur.



enzymes (Van Volkenburgh, Schmidt and Cleland, 1985).

Clearly, because wall extensibility depends on so many factors, a wide variety of mechanisms may be involved in its control. For instance, the formation of the WLF might be controlled by gene expression and protein synthesis (Walker, Legocka, Edelman and Key, 1985) and its activity by the chemical environment in the cell wall (Cleland, 1981). Also, its release into the wall might be regulated by membrane permeability. Since wall loosening is an active process, any factor affecting the energy metabolism of the cell could influence it. Another important factor is the availability of new cell wall material, since if this is limited, sustained wall loosening cannot occur (Cleland, 1981; Brummel and Hall, 1985).

Wall extensibility has been shown to change in response to a large number of factors including light (Van Volkenburgh and Cleland, 1980; Taylor and Davies, 1985) and plant growth regulators (Cleland, 1981). However, because these factors appear to act at different sites or on different processes the mechanisms involved are generally unknown (Tomos, 1985).

The estimation of wall extensibility in plant tissue is by one of three methods, creep, stress relaxation or the Instron technique (for review, see Taiz (1984)). However, none of these gives a direct measurement of WEx since in each case the force vectors employed are uniaxial whereas the force of turgor on the cell wall is multiaxial (Tomos, 1985). Furthermore, since the techniques are usually performed on non-living tissue, they take no account of metabolic processes such as wall loosening and wall synthesis. Nevertheless, correlations have been found between these estimates of wall extensibility and growth rates of plant tissue, particularly in response to treatment with plant growth regulators (Cleland, 1981). Therefore, they appear to provide at least

qualitative information about the effects of treatments on cell wall extensibility. (The validity of Instron-measured plastic extensibility (PEx) as an estimate of WEx is discussed in Section A.1.4).

In theory, wall yield stress (Y) would be expected to be a relatively simple parameter since it defines a threshold below which wall extension ceases (Taiz, 1984). However, Green, Erickson and Buggy (1971) showed that the value of Y for Nitella cells varied with turgor pressure, and concluded that the parameter was subject to complex metabolic control. Values of Y obtained for higher plant tissue are generally less variable, suggesting that wall yield stress in these systems may be of a different non-adjustable type (Taiz, 1984). In leaf tissue, Y may be determined by the thickness, dimensions and stiffness of the cell walls and may be relatively stable, varying only in the long term in response to environment (Bunce, 1977) or with tissue age (Tyree and Jarvis, 1982; Van Volkenburgh and Cleland, 1984).

Plant cell growth depends not only on the ability of the cell wall to extend but also on the presence of cell turgor. In order for turgor to be maintained while wall extension takes place, water must continually enter the cell. The cellular parameters which influence this process and thus, further regulate the rate of cell enlargement are the conductivity of the pathway between the source of water and the cell, and the potential gradient along that pathway.

In whole plants, the pathway between the source of water and the leaf cells is long and complex (Radin and Boyer, 1982). Thus, one problem associated with the measurement of hydraulic conductivity (Lp) is the identification

of the point along the pathway where conductivity might be limiting. In sunflower plants, low nitrogen supply inhibited leaf cell enlargement by reducing the hydraulic conductivity of the roots (Radin and Boyer, 1982). However, Tomos (1985) has proposed that the greatest resistance to the movement of water is in the leaf itself. If this were the case, hydraulic conductivity could be related to tissue anatomy, both at the level of the cell where differentiation and packing might influence water movement, and at the level of organelles where membrane structure, frequency of plasmodesmata, volume of apoplast and other factors could be limiting (Tomos, 1985). Full understanding of the control of cell enlargement by hydraulic conductivity must await identification of the precise pathway by which water moves through plant tissue. However, its regulation by factors such as mineral nutrient supply, growth regulators and metabolism confirm its significance in the control of cell growth (Radin and Boyer, 1982; Boyer and Wu, 1978; Tomos, 1985).

For sustained cell enlargement to take place, the uptake of water associated with growth must be balanced by an accumulation of osmotica, so that the osmotic potential of the cell sap (π) and the water potential gradient between the cell and its surroundings ($\Psi_e - \pi$) are kept constant (Cleland, 1977). This has been recorded in several systems including the primary and trifoliate leaves of Phaseolus (Van Volkenburgh and Cleland 1981; Davies and Van Volkenburgh, 1983). In addition, when osmotic adjustment is inhibited, for instance if potassium supply is insufficient, cell growth rate is reduced (Mengel and Arneke, 1982).

Control of osmotic adjustment must depend to a considerable extent on the supply of osmotica. Thus, any factor which reduces the uptake of potassium or the availability of organic solutes such as sucrose

might be expected to influence growth. Furthermore, Tomos (1984) has proposed that osmotic adjustment may be an important site of regulation of leaf cell enlargement by plant growth regulators.

From the foregoing it is evident that, although the growth of leaf cells can be described by a simple relationship, each of the parameters involved is highly complex, and scope for regulation by endogenous and external factors is considerable. However, if some treatment alters the rate of leaf growth and its effect can be attributed to a change in one of the biophysical parameters described, the mechanism involved may be investigated using knowledge of the basis and control of that parameter.

1.5 PLAN OF INVESTIGATION

The principal aim of the experiments now reported was to investigate the control of leaf growth by roots. The procedure employed was to apply treatments intended to impair normal functioning of the root system and observe their effects on leaf growth. By recording the effects of the treatments on specific root functions, the mechanisms of communication between the root and shoot were investigated. Furthermore by recording the response of the biophysical parameters controlling leaf cell growth, the basis of the response at the level of the leaf cells was also studied.

The treatments employed were root excision and root cooling, both of which had been shown to affect leaf growth in previous studies. (Section 1.2) The plant material used was the Phaseolus vulgaris primary leaf system which had been intensively studied and shown to be amenable to the types of measurements intended (Dale, 1964, 1970; Van Volkenburgh and Cleland, 1979, 1981)

The study was constructed in three parts, the results of which are presented in individual results chapters. Initially, the material was characterised and the effects of root treatments on shoot and root growth recorded. Most attention was given to effects on leaf growth, particularly on the processes of cell division and enlargement. The second part of the investigation considered the effects of root treatments on one aspect of root function, water uptake, considering both the movement of water through the plant and its status in the shoot. Finally the effects of treatments on the yielding properties of the leaf cell walls were recorded so that the basis of the response of the leaf cells to root treatment could be understood.

2. MATERIALS AND METHODS

2.1 PLANT MATERIAL

The plant material used was French bean (Phaseolus vulgaris L.) var. Suttons Selected Canadian Wonder, seed obtained from Charles Sharpe and Co, Sleaford, Lincs. To ensure uniformity, only seeds in the weight range 0.4 to 0.6 g were used, constituting approximately 70% of all seed supplied.

2.2 METHODS

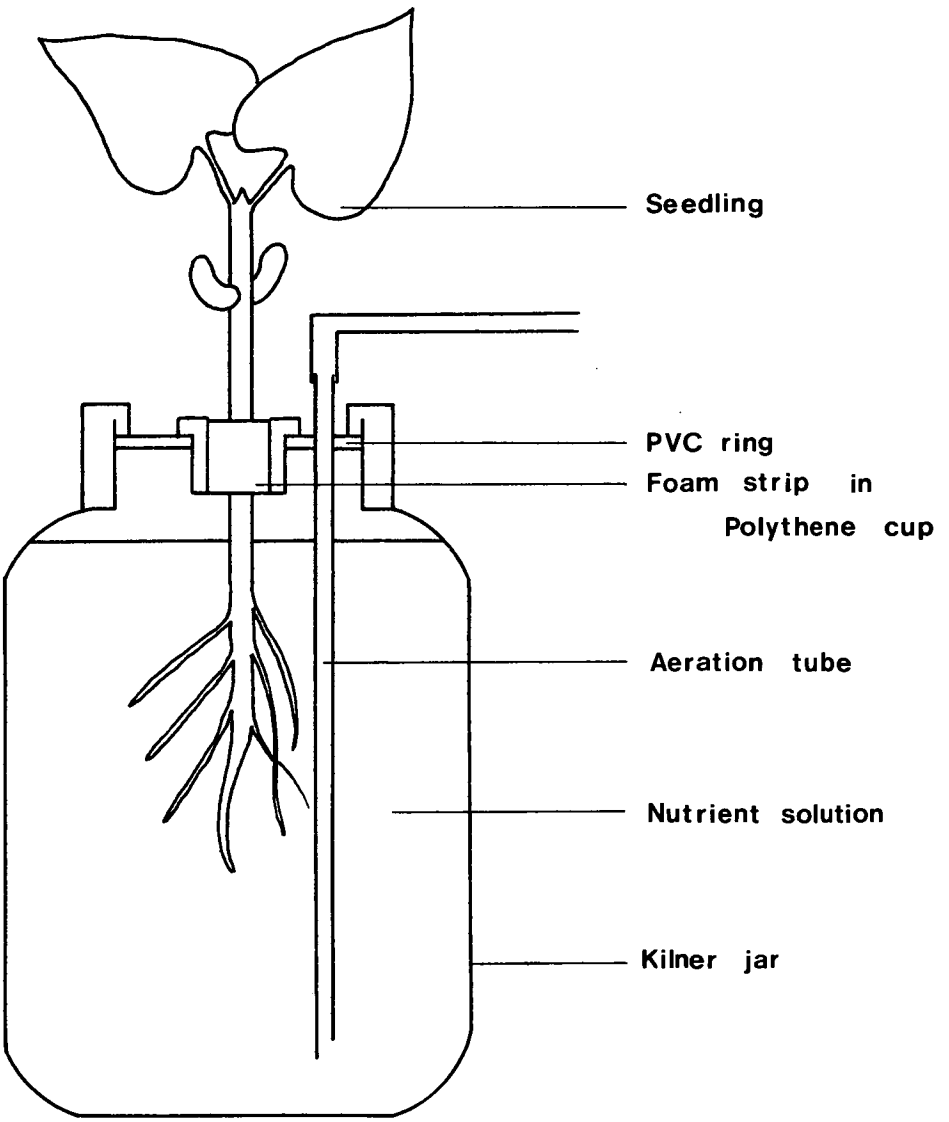
2.2.1 Culture Methods

Experiments were performed at two separate locations, the Department of Botany, University of Edinburgh, and the ARC laboratory at Letcombe Regis. Although every attempt was made to minimise differences in culture methods between the two locations, these were inevitable and are detailed below. In addition, where the location of any particular experiment was of significance, this has been noted in the text.

Seed was sown in trays (30cm x 55cm x 8cm) of vermiculite (Vermiculite-Chatteris Ltd, Chatteris, Camb) in a growth room maintained at 22.5°C. On the day of planting (day 0), and on each of days 3, 5 and 6, each tray was given 500 cm^3 distilled water. On day 7, when the first pair of leaves had begun to unfold, the seedlings were transferred to jars of nutrient solution.

The apparatus used for the solution culture of plants is shown in Fig 2.1 (p 28). Each plant was grown in a 1000 cm^3 Kilner jar painted black to exclude light and filled with nutrient solution; the culture

Figure 2.1


Seedling
PVC ring
Foam strip in
Polythene cup
Aeration tube
Nutrient solution
Kilner jar

solution used was that recommended by the ARC Letcombe Laboratory (Table 2.1, p 31). Plants were held in place by a strip of foam (200mm x 20mm x 4mm) wound around the base of the hypocotyl, and suspended above the nutrient solution by a polythene cup (diameter 35mm) and a disk of PVC sheeting (thickness 3mm). In some experiments, the solution was aerated through a plastic tube at a rate of $180dm^3h^{-1}$.

2.2.2 Growth Room and Glasshouse Conditions

At the Botany Department, University of Edinburgh, jars containing seedlings were placed on an open bench in a growth room. A twelve-hour light period was provided in a twenty-four hour cycle by warm fluorescent tube and incandescent tungsten lighting, giving a mean irradiance at plant level of 215µmol $m^{-2}s^{-1}$. An aerial temperature of 22.5°C was chosen as recommended by Dale (1964).

At the ARC Letcombe Laboratory, jars were placed on benches housed in an air-conditioned glasshouse (Walter, 1983) with an aerial temperature of 20°C ($\pm$2°C). Experiments were performed between April and September, when no artificial light was provided. Instead, only natural light was available, which varied considerably between days and at different times of each day. Blinds were used to shade the glasshouse at very high irradiances (in excess of 1000µmol $m^{-2}s^{-1}$) but no further control of light intensity was possible.

2.2.3 Treatments

Phaseolus seedlings were subjected to treatments seven days after germination when the hypocotyl hook had appeared above the surface of the vermiculite and the primary leaves had begun to unfold. The principal treatments employed were:

Table 2.1. Composition of the nutrient solution used for the culture of seedlings following transfer from vermiculite on day 7.

Major Nutrients	Concentration
calcium nitrate $Ca(NO_3)4H_2O$	3.55×10^{-1} kg m^{-3}
potassium nitrate KNO_3	5.05×10^{-1} kg m^{-3}
potassium di-hydrogen ortho-phosphate KH_2PO_4	1.36×10^{-1} kg m^{-3}
magnesium sulphate $MgSO_4 7H_2O$	3.70×10^{-1} kg m^{-3}
sodium nitrate $NaNO_3$	1.70×10^{-1} kg m^{-3}
ferric EDTA $C_6H_{12}O_8N_2FeNaH_2O$	3.50×10^{-3} kg m^{-3}

Minor Nutrients	Concentration
boric acid H_3BO_3	5.70×10^{-4} kg m^{-3}
cupric sulphate $CuSO_4 5H_2O$	4.00×10^{-5} kg m^{-3}
potassium chloride KCl	1.05×10^{-4} kg m^{-3}
manganese sulphate $MnSO_4 4H_2O$	8.10×10^{-5} kg m^{-3}
ammonium molybdate $(NH_4)_6Mo_7O4H_2O$	2.00×10^{-6} kg m^{-3}
zinc sulphate $ZnSO_4 7H_2O$	2.20×10^{-5} kg m^{-3}

(i) Root excision

(ii) Root cooling

2.2.3.1 Root Excision (RE)

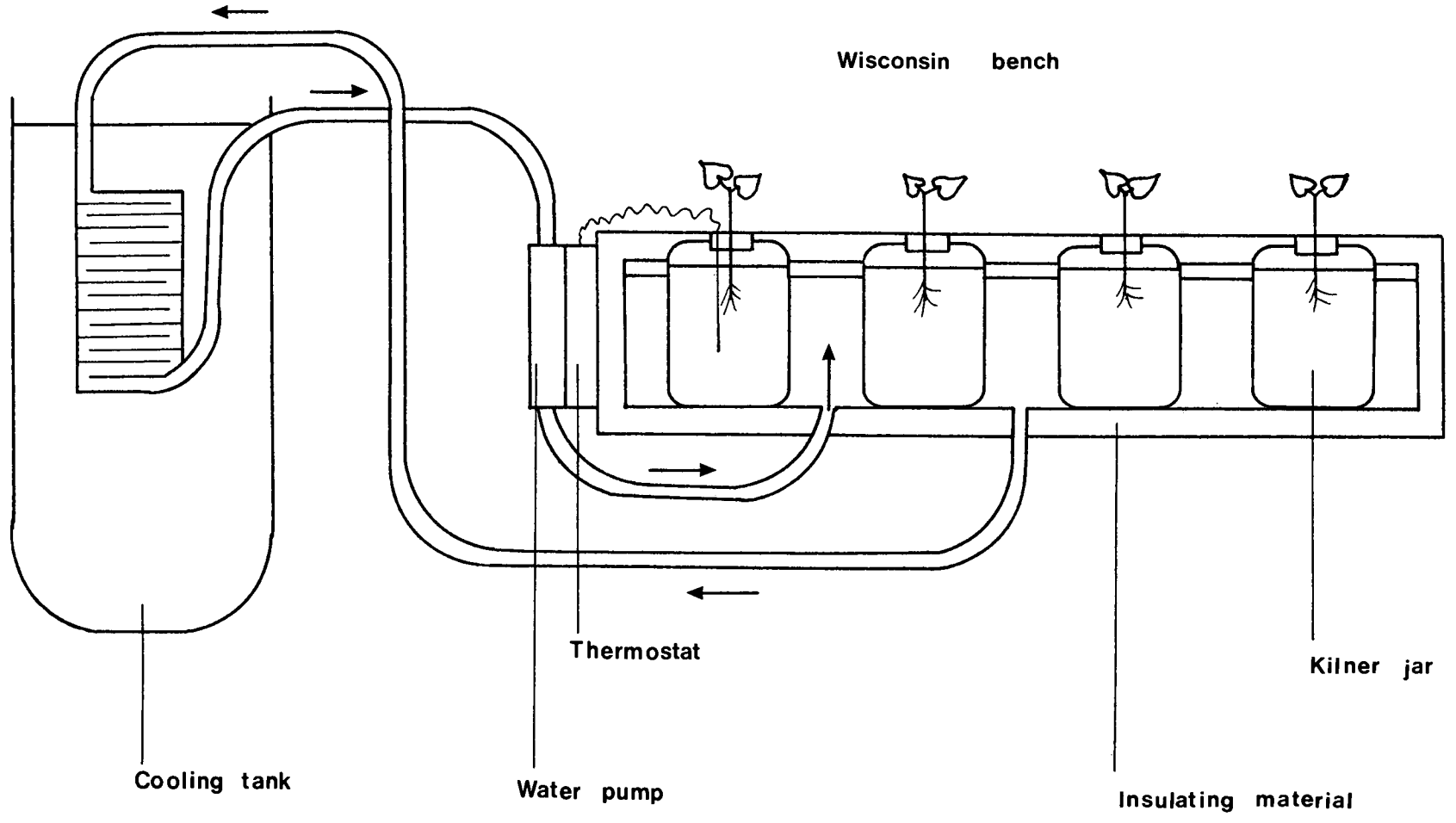
On day 7, each plant was carefully removed fromthe tray of vermiculite and its root system immersed in a basin of water. Then a pair of scissors was used to excise all the lateral roots on the main axis, and to shorten the main axis to approximately 10cm. This treatment resulted in a reduction in total root area of approximately 85 % and a reduction in dry weight of approximately 65 % (Section 3.3.2). Treated plants were then transferred to jars of nutrient solution, as were control plants following rinsing of their root systems to remove any adherent particles of vermiculite.

Less extensive root excision treatments were also used in which one, two or three of the four ranks of lateral roots on the main axis were removed.

2.2.3.2 Root Cooling (RC)

To facilitate root cooling, seedlings were rinsed and transferred intact to jars of nutrient solution, and these cooled to and maintained at the chosen temperature. At Letcombe, where most of the root cooling experiments were performed, jars of nutrient solution were accommodated in custom-designed Wisconsin benches (Fig 2.2, p 33). Each of these consisted of a large (approximately 1.5m x 90cm x 25cm) galvanised steel trough insulated on top, bottom and sides by 5cm of expanded polystyrene. When in use, the bench was filled to a depth of 12cm with water and covered with an aluminium sheet in which holes were cut to accommodate individual Kilner jars. Temperature control was by a Danfoss thermostat located in

Figure 2.2


Wisconsin bench
Thermostat
Kilner jar
Cooling tank
Water pump
Insulating material

one of the jars. When the temperature in the jar increased, a pump was activated which drew water into the bench from a copper coil in an adjacent cooling tank. The system was designed as a closed loop so that water drawn from the coil was replaced from the reservoir within the bench until the required temperature was restored. In this way, jars could be maintained at any temperature between 5°C and ambient (18°C - 22°C). Unless otherwise stated, the root cooling temperature used at Letcombe was 10°C (± 1.5°C). Control plants were accommodated in identical benches without cooling and in which a root temperature of 19°C (± 1.5°C) was maintained.

In Edinburgh, a Grant SB2 water bath and a Grant CC15 cooling unit were used to create root cooling conditions on a smaller scale. Four Kilner jars were accommodated in the water bath and root temperature was maintained at 10°C (± 1°C). The whole apparatus was placed on top of the bench in the growth room described above, and jars containing control plants situated adjacent to it. In the latter group, root temperature was 23.5°C (± 1.0°C).

In all root cooling experiments, plants were transferred from vermiculite to nutrient solution at the control temperature. Thus, the fall in root temperature brought about by placing the jars in the low temperature bath or bench was gradual, taking up to 2 hours. (Fig 2.3, p 36).

2.2.4 Growth Measurements

2.2.4.1 Fresh Weight, Dry Weight, Relative Growth Rate, Net Assimilation Rate

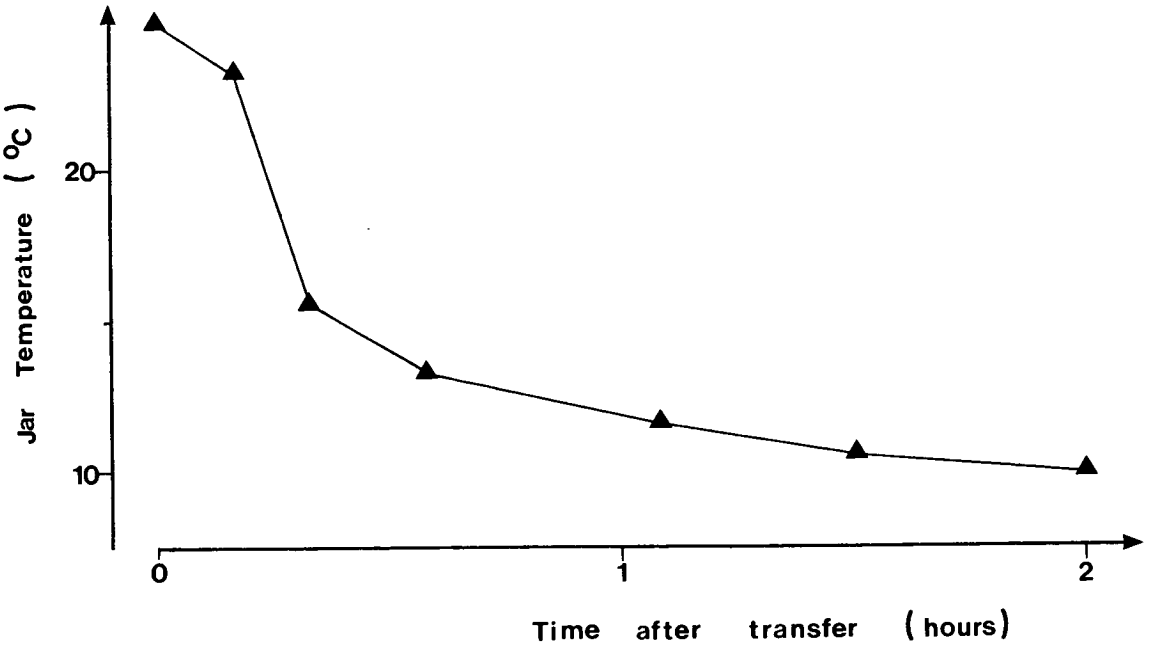
Fresh and dry weights of leaf, root and shoot tissue were measured

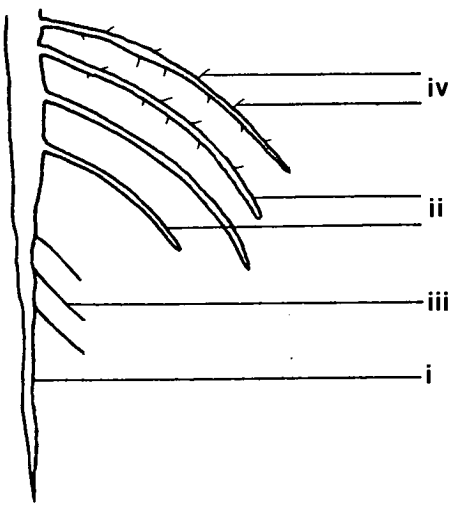
Figures 2.3 and 2.4

Figure 2.3. (top) Effect of transfer of 1-litre kilner jars of nutrient solution at ambient temperature to a Wisconsin bench set to provide a constant root temperature of 10°C; the desired temperature was reached two hours after transfer. Each point represents the mean of 5 values. Standard errors (not shown) ranged from 0.5 to 5% of the mean values.

Figure 2.4. (bottom) Diagram of part of the root system of an 8- to 9-day old Phaseolus vulgaris to show the method of classification of individual roots.

Type of root		Characteristics
i	main axis	seminal root, bearing four ranks of lateral roots
ii	major first-order laterals	situated closest to base of the main axis; may bear second-order laterals
iii	minor first-order laterals	situated towards the apex of the main axis; do not bear second-order laterals
iv	second order laterals	borne on major first-order laterals


Jar Temperature (°C)
20
10
0
1
2
Time after transfer (hours)


iv
ii
iii
i

regularly as required. Dry weight of tissue was determined after material had been weighed fresh, and dried to constant weight at 80°C. Relative Growth Rate (RGR) was calculated as follows:

$$RGR = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1} \qquad (\text{units} \ldots\ldots g g^{-1} d^{-1}) \qquad \text{Eq 1.1}$$

and Net Assimilation Rate (NAR) by the equation:

$$NAR = \frac{(W_2 - W_1)(\log_e A_2 - \log_e A_1)}{(t_2 - t_1)(A_2 - A_1)} \qquad (\text{units} \ldots\ldots g cm^{-2} d^{-1}) \qquad \text{Eq 1.2}$$

Where : $t_2 - t_1$ is the time between successive harvests, W_1 and W_2 are the dry weights at times t_1 and t_2 respectively, and A_1 and A_2 are the leaf areas at these times.

2.2.4.2 Leaf Area

Areas of primary leaves were estimated by a non-destructive method which involved measurement of their maximum linear dimensions (length, l and width, w). The relationship between leaf area (A) and the product of these dimensions (l x w) was determined empirically by regression analysis of 23 points obtained by tracing leaves onto graph paper and calculating their areas from the weight and areas of the tracings. These areas were then plotted against (l x w) and the relationship $A = 0.68 (l \times w) + 0.97$ obtained. Its correlation coefficient was 0.968, and the coefficient of determination 0.937, indicating that more than 90% of the variability in the material had been accounted for.

Similar, non-destructive methods have been used by other groups to estimate Phaseolus primary leaf area. Van Volkenburgh and Cleland (1979) used the same method of correlating the product of linear dimensions with area, while Yagi (1972) obtained a relationship for area based on a model of a leaf as a triangle with semi-circular lobes.

In the present experiments, areas of trifoliate leaves were calculated by treating each leaflet as a diamond-shape with linear dimensions equal to the maximum length and width of each leaflet.

Expansion rates of primary leaves were occasionally calculated, when changes in leaf areas, as measured by the method described above, were expressed per unit time. However, where a more sensitive index of leaf growth rate was required, this was obtained by recording rates of midrib extension (Section 4.6).

2.2.4.3 Leaf Cell Number

Total cell number per leaf was estimated by a procedure similar to that of Brown and Rickless (1949). Following harvest, leaves were weighed and placed in flasks of chromic acid (50g dm^{-3} chromium trioxide in water) at 22.5°C for 20 hours. The volume of acid required for satisfactory separation of cells varied with leaf size. Thus for leaves of area less than $20cm^2$, $100cm^3$ was used, while for larger leaves, up to $500cm^3$ was required. Maceration of the leaf tissue was achieved by first carefully pouring off excess acid, then taking up the tissue six times in a pasteur pippette and six times in a syringe, fitted with a 0.7mm bore needle. The macerates produced were diluted to known volumes with distilled water and aliquots removed for counting of cells on a haemocytometer slide (Hawksley Crystallite, depth 0.2mm). For each sample, six 3 x 3mm fields were counted, to give cell number per $1.8mm^3$. Then cell number per leaf could be calculated, knowing the total volume of the suspension of cells from which the aliquots were drawn.

2.2.4.4 Leaf cell size

Mean volume per leaf cell was estimated by dividing leaf fresh weight by estimated cell number, assuming the density of the tissue to be equal

to that of water (ie 1g per $1cm^3$). No account was taken of the possible effects of particularly large cells such as xylem vessels, so the values obtained may slightly underestimate mean mesophyll or epidermal cell volume.

2.2.4.5 Root measurements

Preliminary calculations revealed that the intersection method (Newman, 1966) of measuring root length in large samples was unsuitable for the smaller root systems of the Phaseolus seedlings studied. Instead, a procedure was developed which involved the counting and measurement of each individual root. Whole root systems were soaked in a dye solution (one tin of Dylon charcoal cold dye in $500cm^3$ of water) for one hour, then transferred to water at $4^{\circ}C$ for storage. At the time of measurement, each root system was dissected in a tray of water to minimise water loss and damage, and individual roots graded according to position, diameter and morphology (Fig 2.4, p 36). The length of each root was measured against a mm scale, and the diameter of a random sample determined with a binocular microscope and eyepiece micrometer. From these data, total length, surface area and volume of each root system, and those of its first- and second-order laterals and main axis were estimated. Also, the number of root tips present, the degree of branching of the root system and any other morphological features were recorded.

2.2.5 Analytical Methods

2.2.5.1 Mineral nutrients

The concentrations of the major nutrients, nitrogen (N), phosphorus (P) and potassium (K) in plant tissue were measured during several experiments at the ARC Letcombe Laboratory. Plant material was dried for 48 hours

at 80°C, then weighed, chopped finely with scissors, and ground in a Ball mill. The remainder of the procedure was carried out by the staff of the Analytical Chemistry section. For phosphorus and potassium analyses, ground plant material was subjected to wet oxidation with nitric and perchloric acids. It was then dried at 230°C and made up to a standard volume with de-ionised water. Sub-samples of the standard volume were used for the analysis as follows. For phosphorus, the sub-sample was mixed with ammonium molybdate/sulphuric acid reagent. Ascorbic acid was then added, and the blue colour produced, measured by colorimetry. Potassium was analysed by flame emission spectrophotometry using a 766nm filter.

For total nitrogen analysis, the ground tissue was placed in a foil cup and heated in a furnace to remove oxygen, water and carbon dioxide. The nitrogen oxides produced were reduced to nitrogen gas and measured by gas chromatography. This procedure was performed using a Carlo Erba 1400 automatic nitrogen analyser.

2.2.5.2 Abscisic acid

The concentration of abscisic acid (ABA) in primary leaf tissue was measured by gas-liquid chromatography, according to a procedure developed by Jackson, Hall and Kowalewska (1983) as follows:

Harvesting : Primary leaves were excised, weighed and placed individually into glass bottles which were immediately plunged into liquid nitrogen. Material was stored in liquid nitrogen until required.

Extraction : Each leaf was homogenised in $5cm^3$ 80% methanol containing 0.02g dm^{-3} (0.02kg m^{-3}) BHT antioxidant, using a Kinematica Polytron macerator at full speed for three minutes. Then each sample was centrifuged at 1250 G for three minutes, the supernatant collected and the pellet

resuspended, homogenised and centrifuged again. The second supernatant was added to the first and the whole sample stored at -18°C.

Purification : Each sample was reduced almost to dryness by heating at 80°C under a stream of nitrogen. Then $3cm^3$ of 5% acetic acid was added and the sample mixed in an ultrasonicator. The first purification procedure involved column chromatography. A C18 reverse-phase 'SEPAK' cartridge was prepared by flushing it with $5cm^3$ 100% methanol, followed by $5cm^3$ 5% acetic acid. The methanol extract containing the ABA was then injected through the SEPAK cartridge, and the eluate discarded. After flushing with a further $5cm^3$ 5% acetic acid and $5cm^3$ distilled water, the ABA was recovered by injecting $3cm^3$ 60% methanol, and collecting the eluate in a small ($5cm^3$) smoked glass reactivial.

Additional purification was by High Performance Liquid Chromatography (HPLC). The column used was 2.5cm x 4.5cm (internal diameter) and the solvents were 1% acetic acid and 100% methanol. Samples collected after column chromatography were dried down to volumes of $2cm^3$ and injected into the HPLC sampling loop. Collection of each sample after separation was during a one minute time-window, determined by running a standard sample of ABA.

Methylation : Samples collected after HPLC were dried and taken up in $20mm^3$ 100% methanol and $50mm^3$ diazomethane. At this stage, an internal standard ($100\ mm^3$ ethyl ABA in cyclohexane) was added to each sample to allow correction for injection errors during gas chromatography. The samples, then containing both methyl (sample) and ethyl (standard) ABA were evaporated to dryness once more, then taken up in $100mm^3$ acetonitrite.

Gas chromatography : Quantitative determination of ABA was by Gas-Liquid Chromatography (GLC). The column used was 1.5m x 0.4mm (internal

diameter) and contained a liquid phase of 12% SE30 plus 0.3% XE60, and a solid phase of diatomite CLQ. Oven temperature was 200°C and the flow rate of the carrier gas (nitrogen) was $4.8cm^3h^{-1}$. Detection was by electron-capture, the current being 10×10^{-10} A. $1mm^3$ of each sample in acetonitrite was injected into the column and sample ABA calculated from the relative areas of the ethyl and methyl ABA peaks.

The addition of a known concentration of ethyl ABA standard to each sample prior to GLC analysis allowed loss of ABA during injection to be corrected for. Loss of ABA during the steps in the procedure before GLC was measured by adding $10mm^3$ tritiated ABA of known radioactivity at the beginning of the extraction process and recording, by scintillation counting, the proportion of that activity remaining in the extracts used for GLC. The percentage recoveries obtained in this way were used to correct the measurement of sample ABA obtained by GLC.

2.2.5.3 Cellulose

Cellulose per unit leaf area was measured for different leaves as a possible index of leaf cross-sectional area (Section 5.2.1). Pieces of leaf tissue of known weight and area were boiled in 100% methanol for three minutes, then transferred to test tubes containing 0.378g dm^{-3} sodium borohydrate and 180g dm^{-3} sodium hydroxide. These were heated at 90°C for one hour, then cooled and centrifuged several times. A few drops of octanol were added at this stage to eliminate air bubbles and accelerate tissue precipitation. After each centrifugation, the supernatant was discarded and replaced with distilled water until it gave a neutral reading with litmus paper. The resultant residues were collected, frozen and freeze-dried, and measured for cellulose using the

Anthrone test for hexoses. Each dried sample was rehydrated in 0.5cm^3 distilled water, and 1.0cm^3 0.2% anthrone in concentrated sulphuric acid (Anthrone reagent) added. Following incubation at 100°C for five minutes, absorbance was determined at 620nm with a bench (EEL) colorimeter. Hexose concentrations were obtained from a calibration curve constructed from the measured absorbances of a range of glucose solutions (5 to 50µg in 0.5cm^3 water).

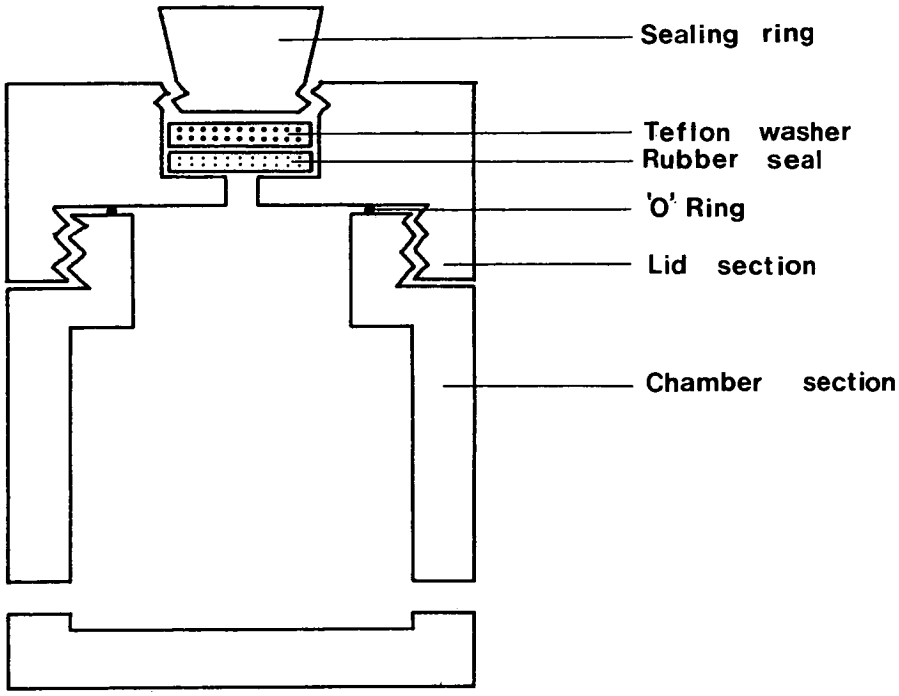
2.2.6 Measurement of Plant Water Relations

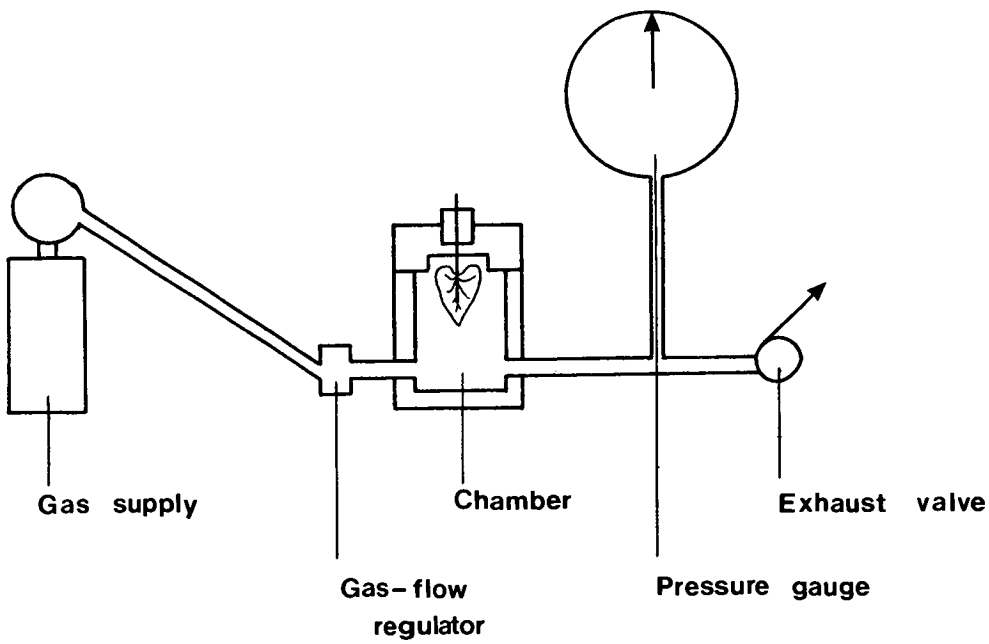
2.2.6.1 Measurement of water potential

The water potentials of primary leaves and whole shoots were measured by the pressure chamber technique. Two pressure chambers were used, one built by the workshop staff at the ARC Letcombe Laboratory, the second borrowed from the Department of Forestry and Natural Resources, University of Edinburgh. Although differing in some details, the basic design of the two instruments was the same, consisting of a chamber, a regulated gas supply and a pressure gauge (Fig 2.5, p 45). The chamber consisted of two parts, the main chamber section which accommodated the leaf or shoot, and a lid section which could be screwed into place and was sealed with a rubber O-ring. The lid section contained an aperture through which the petiole or stem protruded. For efficient sealing, the petiole or stem was also passed through a silicone rubber disk which was compressed by a threaded sealing ring and a teflon washer. Pressurisation of the chamber was achieved by opening a regulator valve, and was displayed on a large gauge marked in divisions of 0.2MPa. The cut end of the petiole or stem was illuminated by fibre-optics (Nachet, E. F. 50s) and viewed with a Vickers binocular microscope (eyepiece x 10, objective x 3).

Figure 2.5

Figure 2.5. Diagram of the Pressure Chamber (top) and associated apparatus (bottom) used in the measurement of shoot and leaf water potential.


Sealing ring
Teflon washer
Rubber seal
'O' Ring
Lid section
Chamber section


Gas supply
Chamber
Exhaust valve
Gas-flow regulator
Pressure gauge

The procedure used for the measurement of leaf or shoot water potential was that of Scholander et al (1965) with modifications to suit the Phaseolus seedlings used. Before the leaf was severed from the plant, the lower part of the chamber was lined with moist filter paper to minimise evaporation of tissue water. Then the silicone rubber and teflon washers were placed in the lid section of the chamber and the sealing ring screwed into place. To prevent damage to the petiole during insertion through the rubber seal, an aluminium sleeve of diameter slightly greater than that of the petiole was inserted in its place. The leaf was then excised from the plant and its petiole inserted into the end of the sleeve protruding from the underside of the lid. When the sleeve was withdrawn, the petiole was left, held in place by the rubber seal. Immediately, the two parts of the chamber were screwed together and pressure within the chamber increased. At first, gas flow was regulated to raise the chamber pressure at a rate of 0.004MPa s^{-1}. Simultaneously the sealing ring was tightened slightly to prevent leakage of gas. When chamber pressure had risen to within 0.25MPa of the expected balance pressure, the rate of increase in pressure was reduced to 0.0025MPa s^{-1}. The balance pressure (ie the chamber pressure necessary to drive sap from the leaf cells, and thus equivalent to leaf water potential) was determined by observing the cut surface of the petiole and switching off the gas supply as soon as sap began to exude. Errors caused by gas passing through the leaf and forcing sap from cells damaged during excision out of the petiole (Turner, 1981) were avoided by swabbing the cut surface with soft tissue throughout the first minute of pressurisation. Balance pressures were obtained in this way for both whole shoots and individual primary leaves.

2.2.6.2 Measurement of Osmotic Potential

2.2.6.2.1 Analysis of pressure-volume curves

Pressure volume curves or water potential isotherms were obtained for individual primary leaves using the pressure chamber technique (Tyree and Hammel, 1972). Each leaf was excised and rehydrated overnight by immersing the cut end of its petiole in freshly boiled and cooled water. It was then weighed and sealed into the pressure chamber, and its balance pressure determined as described previously (Section 2.2.6.1). A tared vial, lined with absorbent paper was placed over the end of the petiole to collect expressed sap, and the pressure within the chamber raised by 0.2MPa. After fifteen minutes, the pressure was reduced to the value of the previous balance pressure and the leaf allowed to equilibrate for ten minutes. Meanwhile, the vial was weighed and the weight of the sap expressed from the leaf recorded. After the equilibration period, a new balance pressure was determined and the whole procedure of overpressurisation, collection of sap, equilibration, determination of weight of sap expressed and establishment of new balance pressure repeated several times. Then the leaf was removed from the chamber and its final fresh and oven-dry weights determined.

To obtain the relationship between water content and water potential, the fresh weight of the leaf at each balance pressure had to be determined. This was done by calculating the cumulative weight of water expressed at each balance point and subtracting it from the initial weight of the leaf. Undetected water loss, probably due to evaporation into the chamber (Turner, 1981) was calculated by subtracting the final fresh weight, and comparing this value with the cumulative weight of water expressed at the final balance pressure determined. The error, which according to Turner (1981) was not

to exceed 5% of the total water content of the leaf, was then divided by the number of measurements of weight of water expressed, and the result added to each. The corrected values thus obtained were then summed to give the cumulative weight of water expressed at each balance pressure and thus, the leaf fresh weight at each leaf water potential.

The turgid weight (TW) of the leaf (ie its weight at relative water content = 1) was obtained by plotting the first three or four pairs of balance pressure and leaf fresh weight results and extrapolating the line to the point at which balance pressure was zero (Ladiges, 1975). From the turgid (TW) and dry (DW) weights of the leaf, and its weight at each balance pressure (FW) relative water content (RWC) was obtained, ie:

$$RWC = \frac{FW - DW}{TW - DW}$$

Pressure-volume curves were drawn by plotting relative water content (RWC) against reciprocal balance pressure ($1/P_B$). A line drawn through the linear portion of the curve gave the bulk osmotic potential of the leaf at any relative water content. The points at which the line crossed the axes corresponding to RWC = unity and $1/P_B = 0$ gave, respectively, the leaf osmotic potential at full turgor and the volume of apoplasmic water. (see Fig 4.5, p 127).

The pressure-volume data obtained were also used to construct Höfler-type diagrams according to the procedure of Tyree (1976). These allowed leaf turgor pressure to be determined from any measurement of water potential or relative water content. In addition, the bulk modulus of elasticity could be estimated by comparing incremental changes in leaf turgor and relative water content (Tyree, 1976; see also Section 4.2.2)

2.2.6.2.2 The cryoscopic technique

Osmotic potential was measured by the cryoscopic technique using a digital micro-osmometer (Camlab, Cambridge, Camb). Leaves were excised at the point of attachment to the petiole, weighed, wrapped in foil and plunged immediately into liquid nitrogen for thirty seconds. Then each was ground with 1.0ml distilled water in a mortar and pestle and the macerates separated by centrifugation using a bench centrifuge at full speed for three minutes. The supernatants were then drawn off and stored at $4^{\circ}C$. Osmotic potential was measured on 50µl aliquots of each supernatant. The osmometer was switched on and allowed to stand for thirty minutes before calibration was checked using 50µl samples of distilled water and a standard solution of osmotic potential 300mOsmol. Then each sample of supernatant was transferred to a plastic vessel and placed into the cooling chamber of the osmometer until supercooling had occurred (indicated by an audible signal). Freezing of the sample of supernatant was initiated by inserting a cooled needle into the sample vessel. Then the osmotic potential (in mOsmol) of the solution was obtained from the plateau value shown on the digital readout. The bulk osmotic potential of the leaf ($\bar{\pi}$) was obtained by correcting for the dilution performed during the maceration stage, and converted to MPa, assuming that 1mOsmol = 0.22MPa.

2.2.7 Measurement of Plant Cell Wall Parameters

2.2.7.1 Measurement of Cell Wall Extensibility

Cell wall extensibility of Phaseolus leaf tissue was measured by the Instron technique (Cleland, 1967) using a tensiometer built in the Department of Botany, University of Edinburgh to the design of Van Volkenburgh, Hunt and Davies (1983). Full details of the construction and operation of the instrument are provided in Appendix 1.

Primary leaves were excised three to four hours after the beginning of the light period and a knife, consisting of two scalpel blades held exactly 5mm apart, used to cut strips of lamina from regions free of major veins. These were boiled in methanol for three minutes, then transferred to cold methanol for storage. For measurement of cell wall extensibility, the strips of leaf tissue were cut into 15mm lengths and inserted between the clamps of the tensiometer, so that the distances between the clamps was exactly 5mm. Then the tissue was rehydrated and extended at a constant rate to a load of 20g. At this point, the clamps were returned to their original positions and the tissue extended a second time in the same way. Load on the tissue, measured by a force transducer, was recorded against time on a y-t chart recorder. However, since displacement of the clamps (and extension of the tissue) occurred at a constant and known rate, the curves could be treated as load-extension relationships. Total extensibility of the tissue was calculated from the slope of the first curve, while elastic extensibility was given by that of the second. Plastic extensibility, an estimate of cell wall extensibility (Cleland, 1984) was calculated by subtracting the elastic extensibility from the total, and was expressed as a relative (percentage) change in length per 10g increase in load (Davies and Van Volkenburgh, 1983).

To avoid errors caused by differences in leaf thickness or cross-sectional area, some methanol-treated leaf pieces were dried and weighed, and the results obtained used to correct measured extensibilities as recommended by Cleland (1967) (Section 5.2.1).

2.2.7.2 Measurement of Wall Yield Threshold

Wall yield threshold (Y), the value of turgor below which plant cell growth ceases, was determined by comparing the rates of elongation of leaf pieces maintained at different turgor pressures. Twelve petri - dishes were filled with polyethylene glycol (PEG) solutions (average Mwt = 4000) in a range of concentrations giving solution osmotic potentials (π soln) of zero to -1.75 MPa. Strips of lamina, measuring 10mm x 2mm, were excised from freshly cut leaves and floated abaxial surface downwards, on the PEG solutions. Generally leaves from six plants of each treatment were used and one strip of lamina tissue from each leaf floated on each solution. After six to seven hours at 22.5°C, the leaf pieces were removed, surface-dried with soft tissue, and the length of each measured to an accuracy of 0.1mm with a graduated eyepiece.

To determine Y, the turgor pressures of the leaf pieces on each PEG solution had to be determined. Tissue water potential (Ψ) was assumed to be equal to the osmotic potential of the external solution (π soln) and tissue osmotic potential ($\bar{\pi}$) was obtained from Höfler plots drawn from pressure-volume data for control and treated leaves. Then the turgor pressure (P) of each group of tissue strips was obtained from the difference between the osmotic and total potentials ($P = \bar{\pi} - \Psi$). When tissue turgor (P) was plotted against final strip lengths, all treatments gave a relationship with a plateau and linear portion. The tissue strip length at the plateau was assumed to be the starting length, and the value of P at which the linear portion intersected the plateau, taken as the value of Y.

The slope of the linear portion of the turgor - final strip length relationship was also recorded and used as an index of plastic extensibility

(ie increase in tissue strip length per unit increase in turgor pressure).

2.2.8 Other Measurements

2.2.8.1 Transpiration Rate

Transpiration was monitored by recording weight-loss of Kilner jars containing plants and correcting for loss due to direct evaporation of the nutrient solution. Plants were transferred to Kilner jars and the whole assemblies weighed to an accuracy of 0.01g. Several jars containing nutrient solution but no plants were also set up. These were weighed at the same times and in the same way as those containing plants. After each time interval, jars were re-weighed and loss of water through transpiration calculated by subtracting the mean weight-loss of jars without plants from each of the weight-losses from jars with plants. Leaf areas were also measured at the beginning and end of each time interval, and transpiration rate (T) given by:

$$T = \frac{Q\ (\log_e A_2 - \log_e A_1)}{(t_2 - t_1)\ (A_2 - A_1)} \qquad \text{(units....} g cm^{-2} h^{-1})$$

Where $t_2 - t_1$ was the duration, in hours, of the time interval, Q was the total (corrected) weight of water lost and A_1 and A_2 were respectively the total leaf areas at the beginning and end of the time interval. No correction was made for changes in plant dry weight since these were insignificant in relation to the weights of water lost.

2.2.8.2 Stomatal Resistance

Stomatal resistance was estimated by measuring leaf diffusive resistance using an automatic diffusion porometer (Delta-T Devices, Burwell, Camb).

The sensor head of the instrument, which consisted of a chamber containing a hygrometric sensor, was clamped onto the leaf and the rate of humidification of the chamber measured. Leaf resistance was then obtained by reading from a calibration curve. The instrument was calibrated by measuring rates of humidification of the chamber when clamped to porous surfaces of known and different resistances. Care was taken to ensure that calibration was carried out under the same conditions of temperature and humidity as actual measurements of leaf resistance. Also, calibration drift was prevented by performing two separate calibrations, one before and one immediately after each group of measurements.

The measurement of stomatal resistance was carried out on the abaxial surface of the primary leaf, the sensor head being positioned half-way between the centre of the midvein and the leaf margin. Usually a consistent reading was obtained within thirty seconds but if the reading fluctuated after one minute, no result was recorded. Errors caused by handling were prevented by changing the plants used for stomatal resistance measurements. Thus even when hourly readings were required, no individual plant was used more than once in four hours.

2.2.9 Other Methods

2.2.9.1 Preparation of Plant Material for Light Microscopy

The effects of root treatments on leaf anatomy were investigated by viewing transverse sections of primary leaves under the light microscope. Samples of leaf tissue were excised from a position midway between the centre of the midvein and the leaf margin, and avoiding any major veins. These samples were fixed in Craf solution (chromic acid, acetic acid and neutral formalin) for at least ten hours, then dehydrated in an ethanol/tertiary butanol series. Next the tissue pieces were infiltrated, first with paraffin oil, then with paraffin wax, and embedded in blocks of the latter.

After seven to ten days, the blocks were sectioned using a manually operated microtome to give a tissue section thickness of 6 to 10μm.

Sections of lamina obtained in this way were collected on microscope slides and stained with safranin and fast green before being viewed at a magnification of x 250. Sections were drawn from photographs taken with a 35mm camera and microscope adaptor.

2.2.9.2. Statistical Methods

Most of the results presented represent means of samples drawn from larger populations. Standard errors are provided as measurements of the extent of deviation of individual values from those means. In the figures, plus and minus standard errors are shown by a vertical bar through each point. However, where the proximity of other points made this impossible, only one standard error (either plus or minus) is shown. Occasionally standard errors were too small to represent on the figures but this is noted where it occurs.

Where the significance of a difference between two means was of particular importance, the student-t test was used. This gives the probability (p) that a difference is due to some treatment or other effect and not to random variation. In the figures where this test was used, significances of differences between means are shown as follows:

***	significant at p = 0.001
**	significant at p = 0.01
*	significant at p = 0.05
-	not significant

3. THE EFFECTS OF ROOT EXCISION AND ROOT COOLING ON PLANT GROWTH AND DEVELOPMENT

3.1 INTRODUCTION

The initial aims of this study were to characterise the plant material used and record the general effects of the major root treatments. Although the development of Phaseolus vulgaris seedlings had been described in great detail already, (Dale, 1964) it was necessary to repeat some of the measurements to determine if culture on mineral nutrient solution had any spurious effects. Also, although later experiments were planned to investigate effects on leaf growth in more detail, it was necessary to record the effects of root excision and root cooling on whole plant growth to provide a frame of reference for those later results.

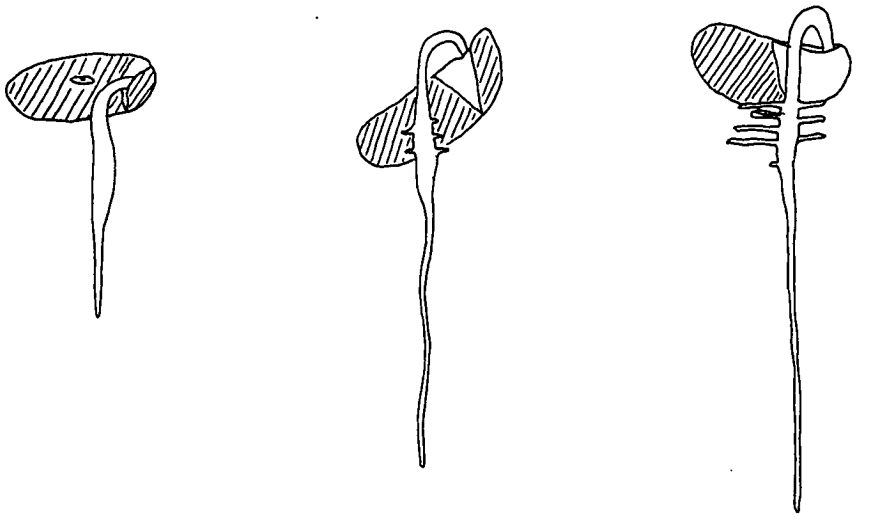
Initially, the early growth of the plant material, including any effects of transfer from the germinating medium to solution culture were studied. Then effects of treatments on long term growth of the shoot and root, distribution of dry matter and root/shoot ratio were investigated. Subsequent studies dealt with the specific effects of root treatments on the size and structure of the root system, and the pattern of cell division and growth in the primary leaves. Finally, three short experiments were performed to show the effects of other root treatments on leaf growth.

3.2 PLANT DEVELOPMENT UNDER STANDARD CONDITIONS

The appearance of seedlings of Phaseolus vulgaris germinated and grown on vermiculite is shown in Figure 3.1 (p 57). Germination occurred 2 to 3 days after planting, with the first lateral roots appearing on day 4.

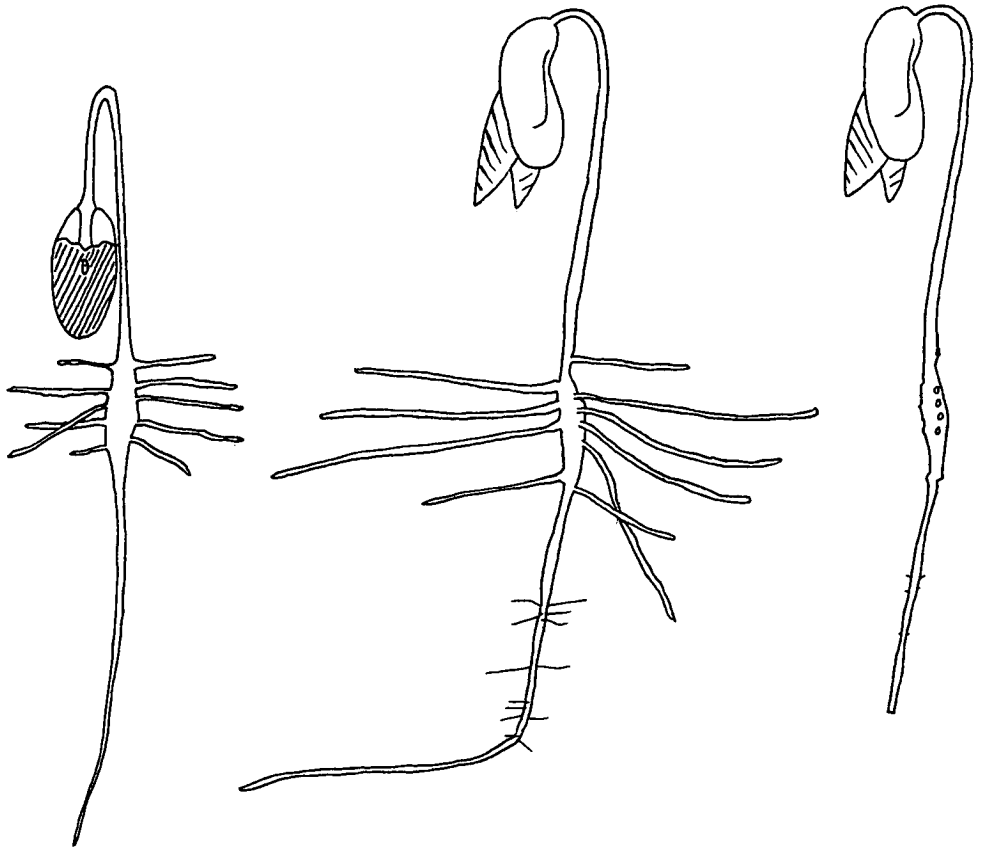
Figure 3.1

Fig 3.1. Germination of Phaseolus vulgaris seedlings grown on vermiculite. Seeds were planted on day 0 and transferred to solution culture on day 7. The plant marked 7 RE has been subjected to the root excision treatment discussed in the text. All plants are drawn approximately life-size and are representative individuals. For clarity, only two of the four ranks of lateral roots actually present are shown.



Day 3 4 5

6 7 7 RE



By day 6, the hypocotyl hook had emerged above the surface of the vermiculite and by day 7 it had begun to straighten. Around the same time, the primary leaf pair began to unfold from within the cotyledons and the seed coat was sloughed off. At this stage, the seedlings were transferred to solution culture so that their root systems could be observed and manipulated. Although transfer earlier than day 7 would have been preferred, this was not possible because the length of the hypocotyl was limiting (see Section 2.2.1). However transfer from the solid medium to solution had no detectable effect on primary leaf growth (Fig 3.2, p 60).

Following transfer to solution culture, rapid expansion of the primary leaves continued (Fig 3.3, p 60), growth in area following a typical sigmoid time course with near-exponential increase occurring up to day 8 and the asymptote being reached by day 20.

3.3 EFFECTS OF ROOT TREATMENTS ON PLANT GROWTH AND DEVELOPMENT

3.3.1 Major effects of root excision and root cooling on shoot and root growth

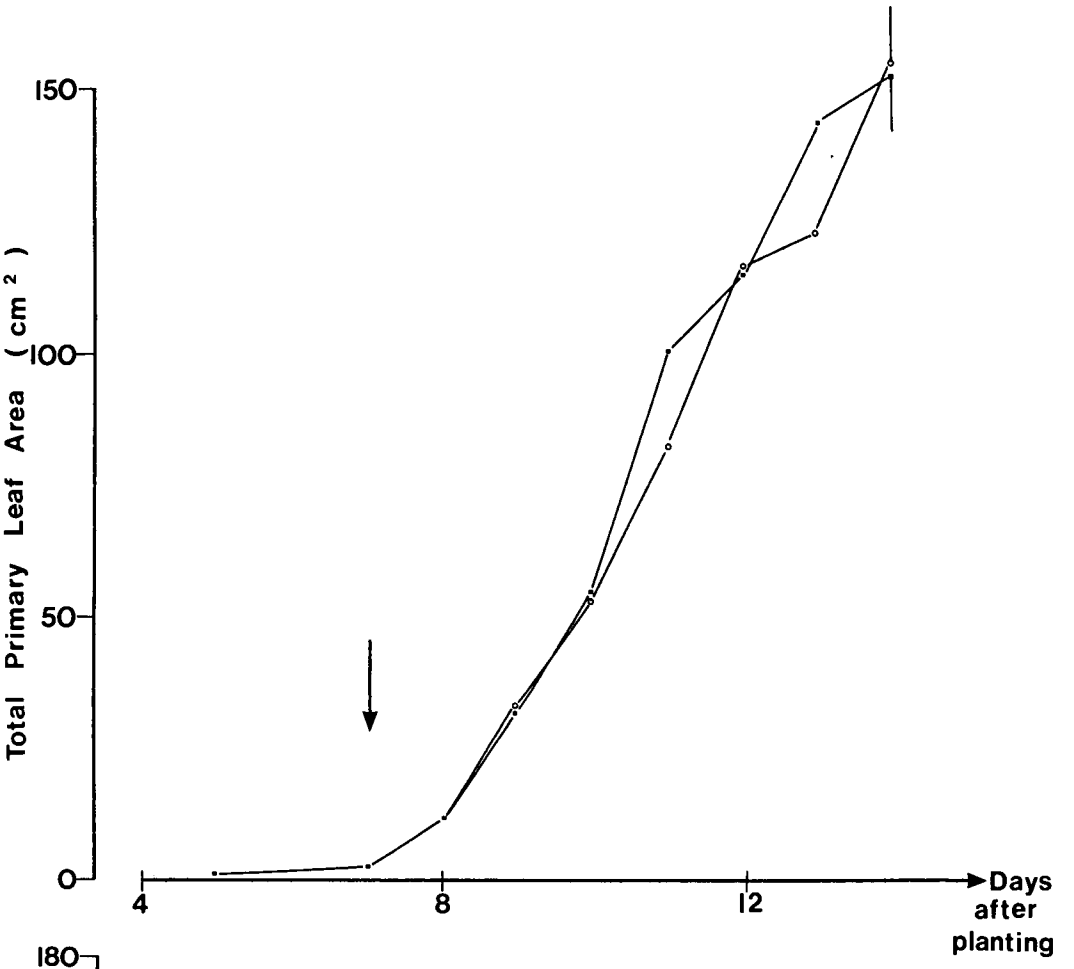
Both root excision (RE) and root cooling (RC) profoundly affected the growth and appearance of the shoot and root. In one long-term study, measurements of changes in area of the primary leaf pair showed that both final area and rate of expansion of each leaf were reduced (Fig 3.4, p 62) Also, in plants of both treatments, unfolding of trifoliate leaves 1, 2 and 3 was delayed and leaves 4, 5 and 6 failed to reach measureable size by the end of the experiment.

For the primary leaf pair, final area was reduced by both treatments by approximately 60%. The duration of the period over which expansion of

Figures 3.2 and 3.3

Fig 3.2. Effects of continual culture on vermiculite (⟜) or of transfer on day 7 (↓) to solution culture (⟝) on the time course of Total Primary Leaf Area per plant. The vertical bars on the final points represent one standard error.

Fig 3.3. Typical time course of the expansion of the primary leaves of Phaseolus seedlings. The arrow (↓) indicates the point at which, in the main experiments, plants were transferred to solution culture and subjected to root treatments.


150
100
50
0
Total Primary Leaf Area (cm 2)
4
8
12
Days after planting

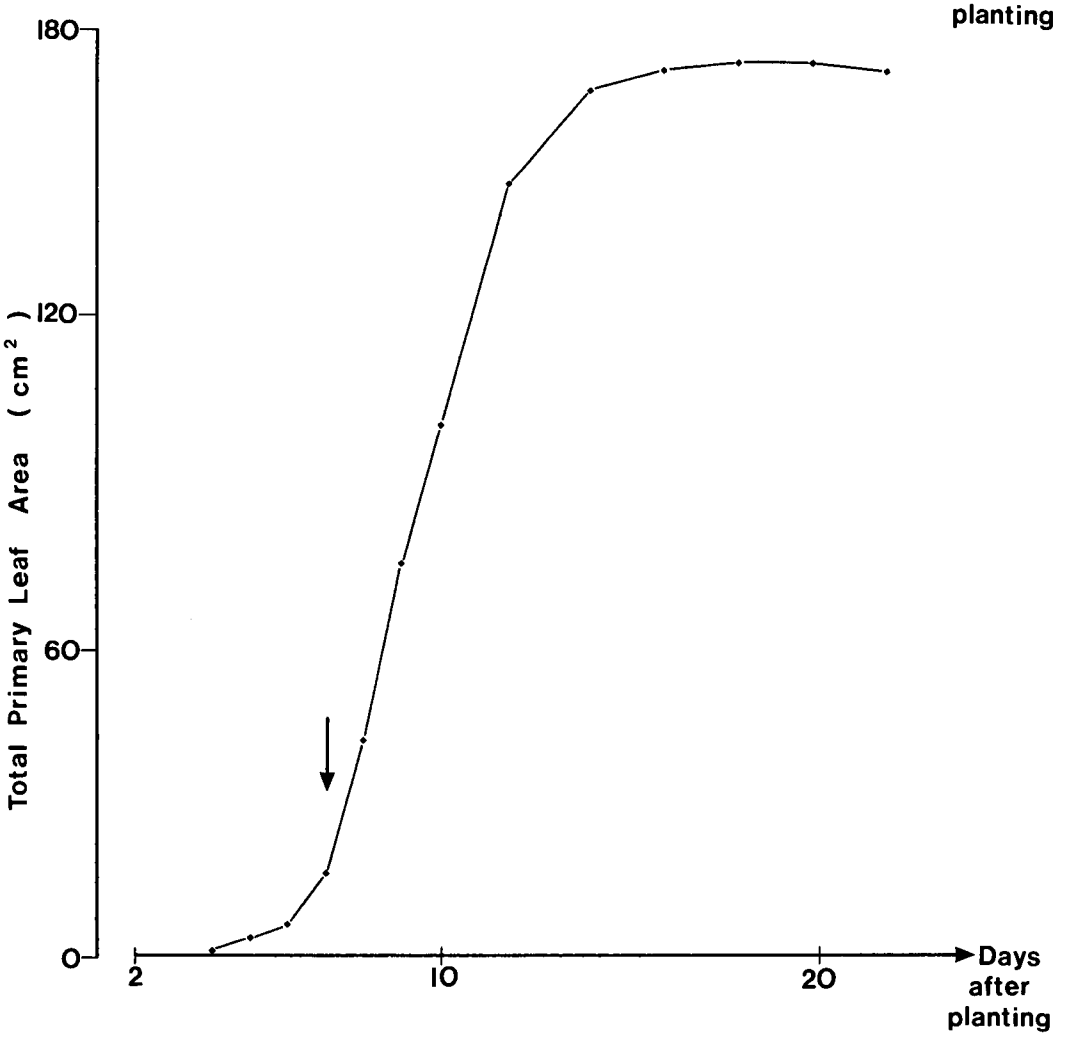

180
120
60
0
Total Primary Leaf Area (cm 2)
2
10
20
Days after planting

Figure 3.4

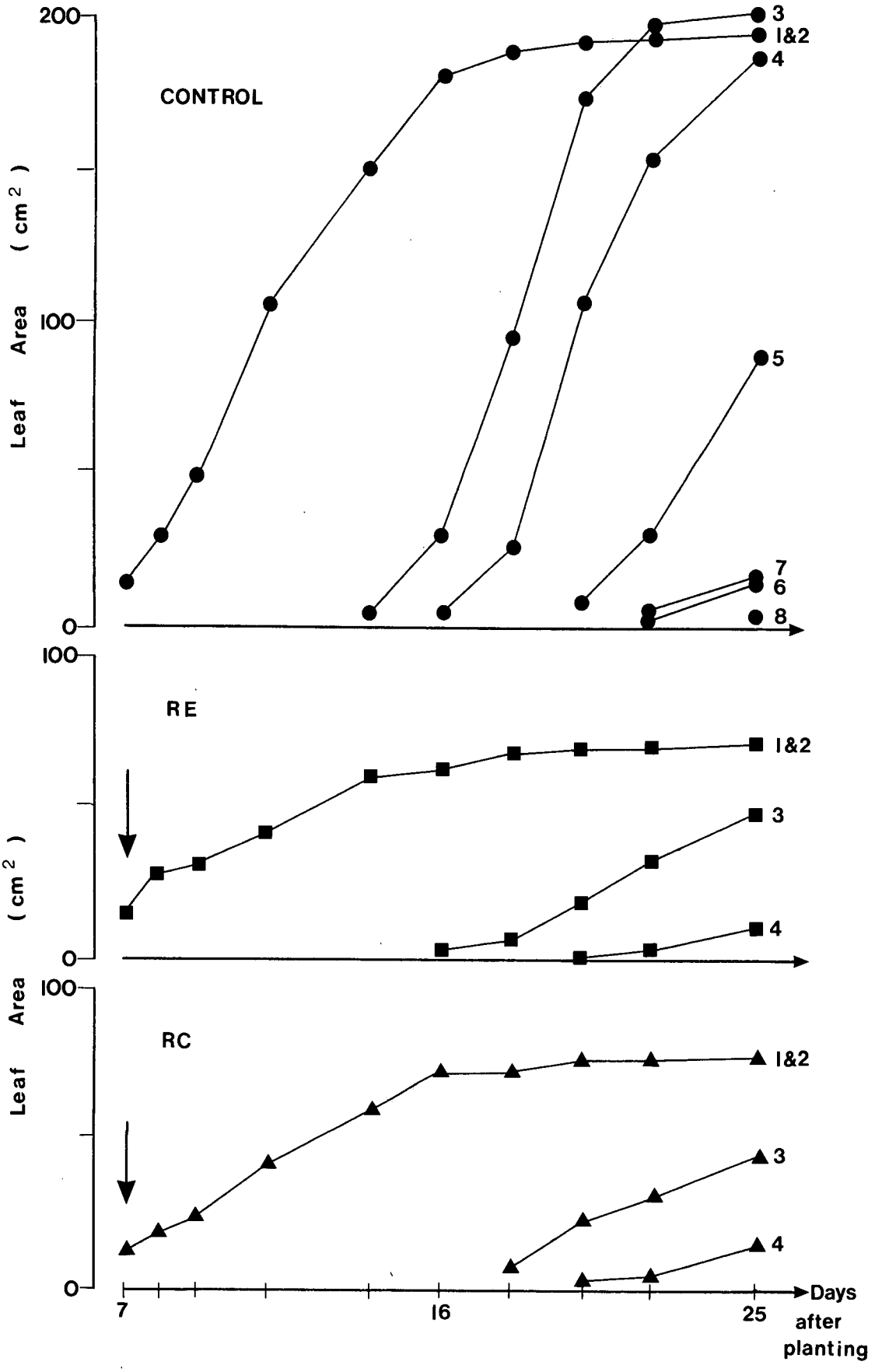
Fig 3.4 a) (opposite). Typical time-courses of the expansion of different leaves of Phaseolus seedlings subjected to root excision (RE), root cooling (RC) or no treatment (CONTROL). The onset of both treatments is indicated by the arrow and the leaves numbered as shown in Fig 3.4 b). Note that although leaves 5 to 8 were present on plants subjected to root excision and root cooling, they were too small to measure and are not included here.

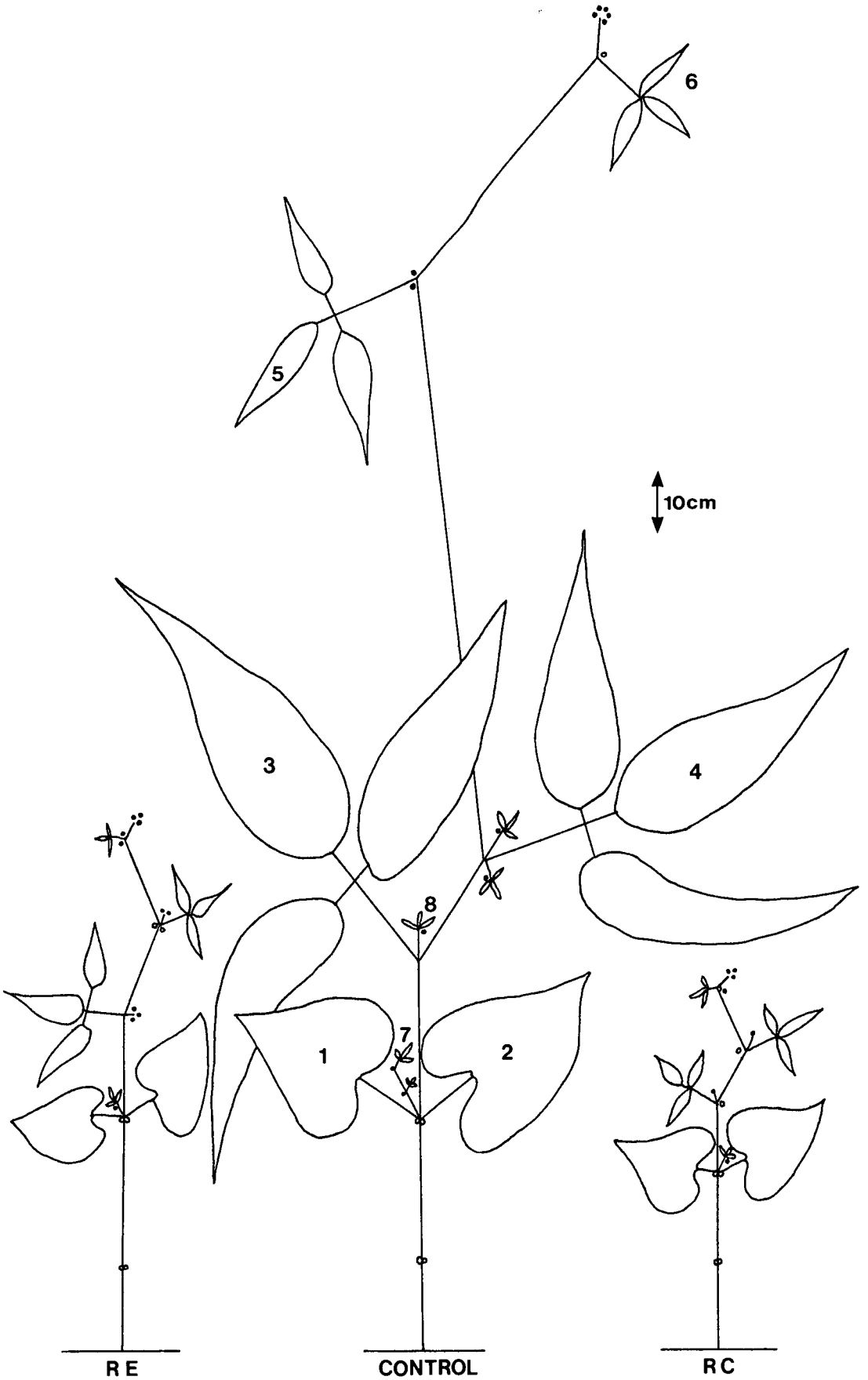
Fig 3.4 b) (overleaf). Stylised drawings of the shoots of 25-day-old Phaseolus seedlings subjected to root excision (RE), root cooling (RC) or no treatment (CONTROL). Numbers show the designation of leaves used in Fig 3.4 a) as follows:

1, 2	primary leaves
3	1st trifoliate leaf
4	2nd trifoliate leaf
5	3rd trifoliate leaf
6	4th trifoliate leaf
7	trifoliate leaf in axil of primary leaf
8	trifoliate leaf in axil of 1st trifoliate leaf

All plants are drawn approximately one tenth life-size, structures too small to draw being represented by symbols (∘ = unfolding bud containing leaves, • = developing flower.

a)


200
100
0
Leaf Area (cm 2)
CONTROL
3
I&2
4
5
7
6
8
100
0
RE
I&2
3
4
100
0
RC
I&2
3
4
7
16
25
Days after planting


b)
6
5
10cm
3
4
8
1
7
2
RE
CONTROL
RC

these leaves took place however was unaffected by root treatment, suggesting that this aspect was under local control, residing in the leaves.

In addition to effects on lamina expansion, root treatments also affected the morphology of individual leaves. By day 9, primary leaves of RC and RE plants were darker in colour than those of controls, and by day 14 the leaves of RC plants had developed chlorotic mottling, particularly at the margins. A similar trend was recorded in the first trifoliate leaves, those of RC plants rapidly becoming chlorotic while those of RE plants remained darker in colour than the controls.

In general appearance, plants subjected to either root treatment were quite different from the controls; whole plant weight was reduced, as were the weights of the root and shoot (Table 3,1, p 66), internodes were shorter and in the case of root cooling, substantially fewer lateral roots were present.

The distribution of dry matter between the root system and the shoot, which was clearly affected by both root treatments (Table 3.1, p 66) was studied further in two experiments. In the first, the effects of the standard root excision treatment (RE) were compared to those of one in which regeneration of new root tissue was permitted (RR). Following root excision on day 7, new laterals appeared on the main root axis around day 11. In RE plants, these were removed immediately and this process repeated at two-day intervals as additional roots emerged.

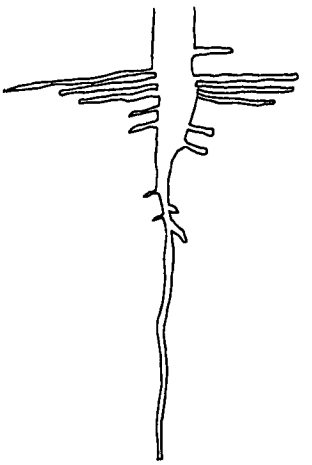
In RR plants, the lateral roots which appeared on day 11 were allowed to continue to grow (Fig 3.5, p 67) and by day 21 had formed substantial root systems, similar in structure although generally smaller than those of control plants.

Table 3.1. Effects of root excision (RE), root cooling (RC) or no treatment (CONTROL) on total lamina area, total internode length (from the root/shoot interface to the petiole of the ultimate leaf primordium), total shoot dry weight and total root dry weight. Figures presented are means of four measurements with standard errors in brackets. (* The mean dry weight of root tissue excised from individual RE plants over the entire period between days 7 and 25 was 0.071g).

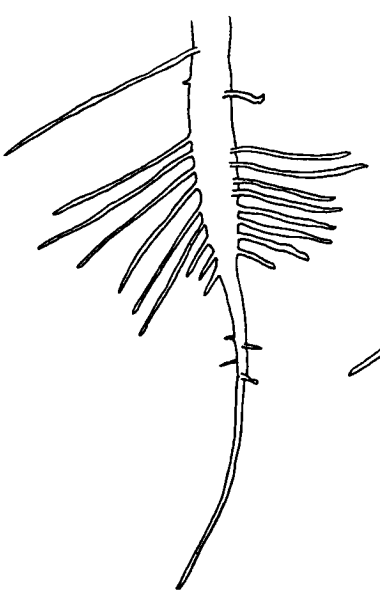
Day	Treatment	Total lamina area (cm^2)	Total internode length (cm)	Total shoot dry weight (g)	Total root dry weight (g)
7	CONTROL	15.1 (0.5)	7.48 (0.44)	0.313 (0.017)	0.033 (0.001)
	RE				0.012 (0.001)
25	CONTROL	700.9 (54.6)	26.75 (2.84)	2.268 (0.175)	0.314 (0.016)
	RE	130.5 (3.9)	9.55 (0.49)	0.586 (0.021)	0.043* (0.004)
	RC	136.0 (20.0)	6.23 (0.55)	0.682 (0.066)	0.074 (0.008)

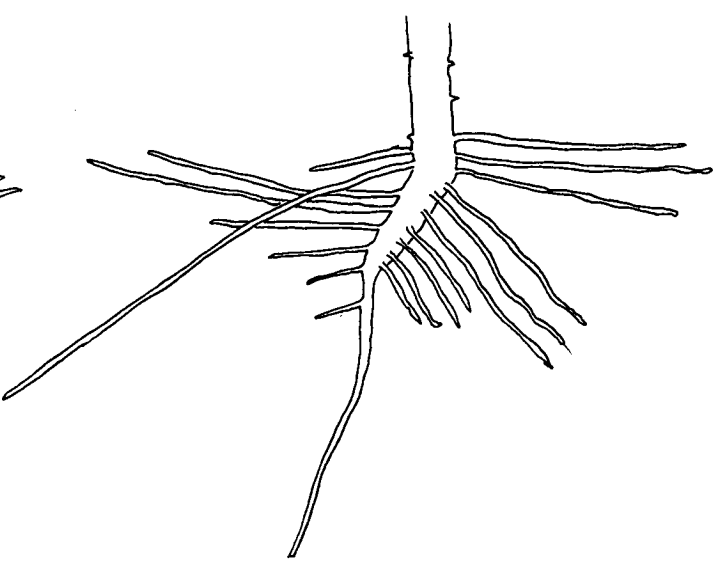
Figure 3.5

Fig 3.5. Regeneration of lateral roots on plants subjected to a single root excision treatment on day 7 (ie corresponding to treatment RR). All drawings are approximately life-size and depict representative root systems. For clarity however, only two of the four ranks of lateral roots actually present are shown.



Day 7 10 12





13 14

Repeated root excision (RE) caused a reduction in mean seedling dry weight which was detected at the first harvest (day 10) after treatment (Fig 3.6, p 70). At subsequent harvests, plant dry weight remained low relative to the control value and by day 21, total dry matter production had been reduced by over 70%. In RR plants, increase in dry weight was initially reduced to the same extent as in RE plants, but by day 13, mean plant dry weight was significantly greater than that of the RE group and by day 21 more than twice as much dry matter had been produced, equivalent to over 60% of the control production.

The effects of repeated and single root excision treatments on the rates of increase in whole plant, shoot and root dry weights are shown in Table 3.2 (p 72). In control plants, growth rates generally increased with time. However, in RE plants, growth rates fell in response to the initial root excision treatment and remained low throughout the period studied. In RR plants, root growth rate began to increase around day 11 and was followed by considerable increases in shoot and primary leaf growth (Fig 3.7, p 73).

The effects on root and shoot growth described above caused considerable differences in root/shoot ratio (Fig 3.8, p 75). In control plants, the relative growth rate of the root generally exceeded that of the shoot, causing the R/S ratio to increase with time. In RE plants, the ratio was reduced by the initial treatment and remained low as long as excision was repeated. However, in RR plants root/shoot ratio began to increase when new roots first appeared and exceeded the control value by day 13, although falling below it by day 21. Thus, when regeneration of lateral roots was allowed to proceed, plants tended to re-establish the same weight balance between root and shoot as existed in the control plants.

Figure 3.6

Fig 3.6. Time courses of the effects of repeated root excision (RE, ■), of a single root excision treatment (RR, x) or of no treatment (CONTROL, ●) on Total Plant Dry Weight. The points represent means of 5 values with standard errors shown by vertical bars. The beginning of the repeated root excision treatment and the day of the single root excision are indicated by the arrow .

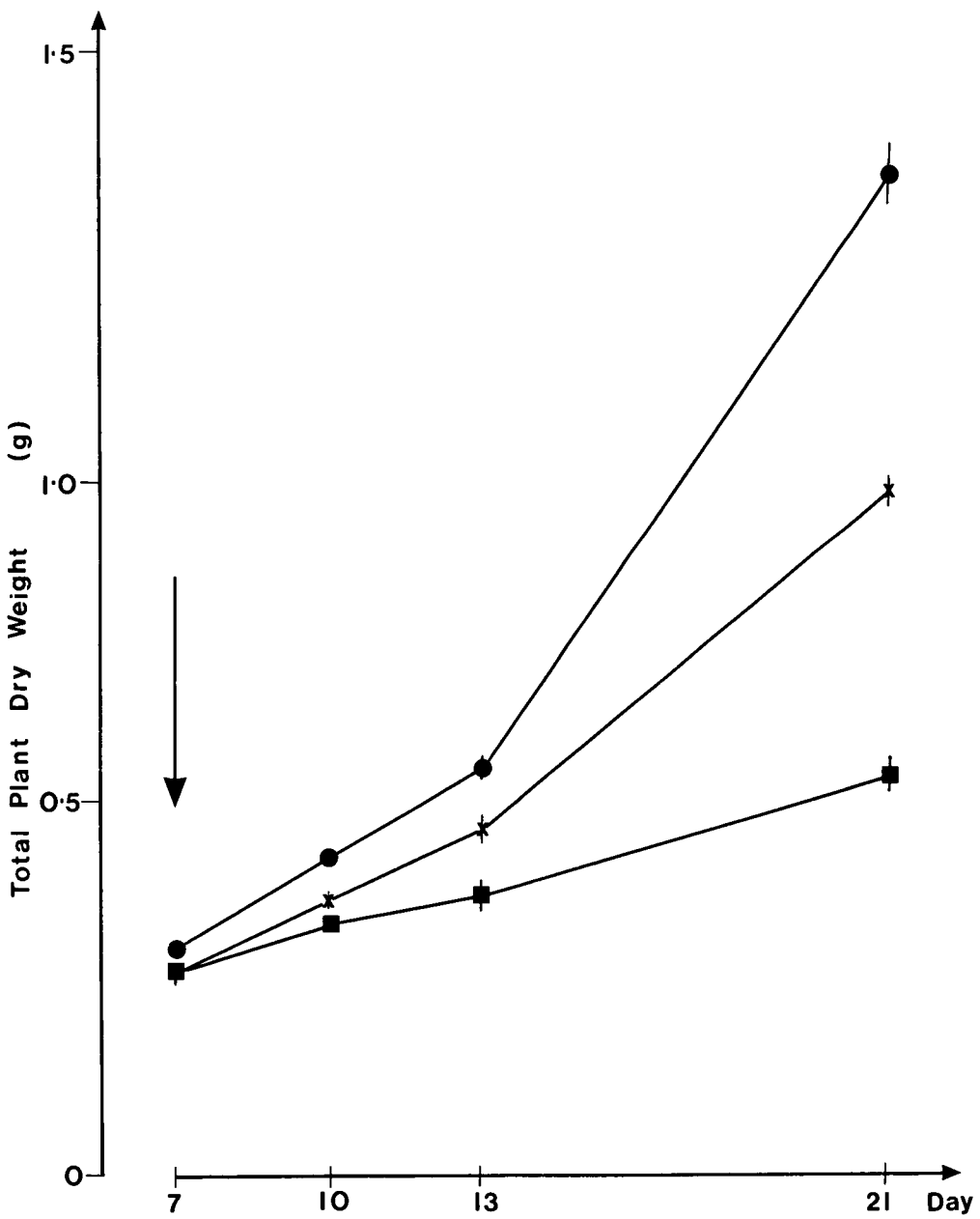

1·5
1·0
0·5
0
Total Plant Dry Weight (g)
7
10
13
21
Day

Table 3.2. Effects of repeated root excision (RE), a single root excision treatment (RR) or no treatment (CONTROL) and time on the absolute and relative rates of increase in dry weight of whole plants, shoots and roots. Results were calculated from treatment means determined by harvesting between three and six plants of each treatment on days 7, 10, 13 and 21.

Absolute growth rate (gd^{-1})

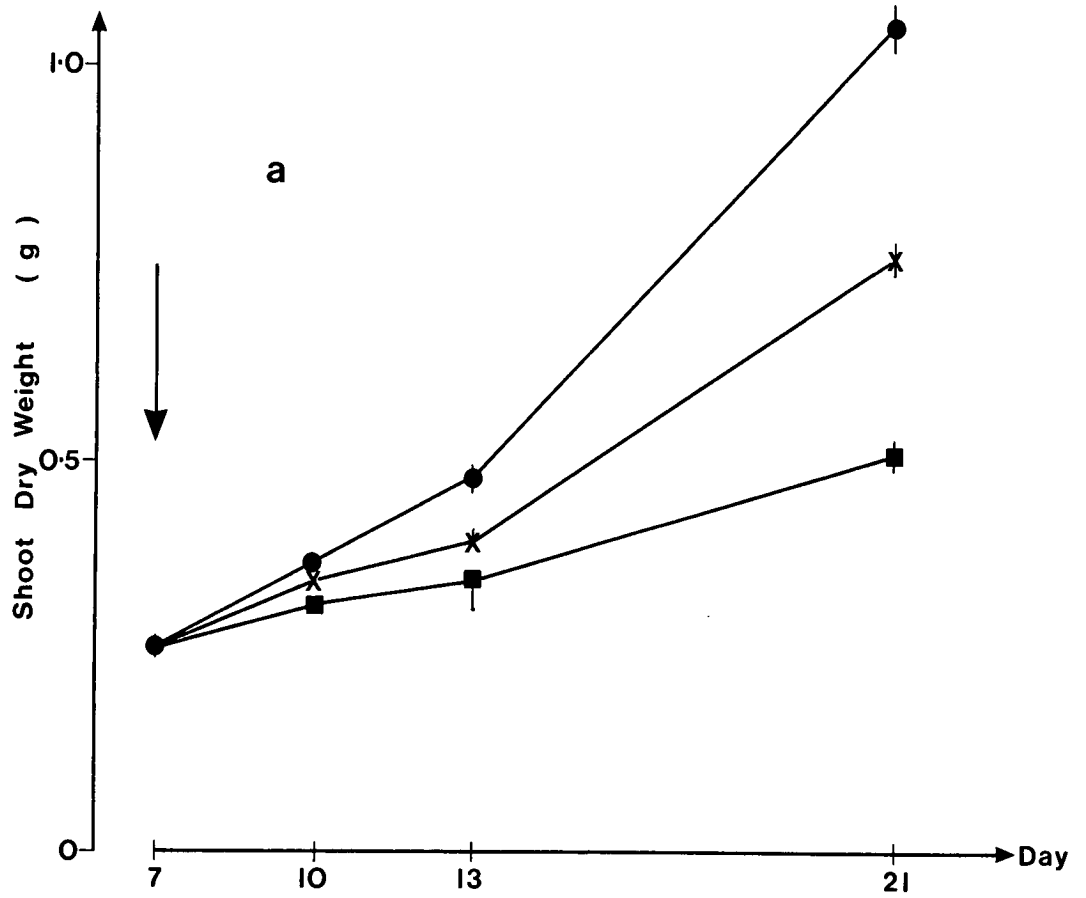
Period	Treatment	Whole plant	Shoot	Root
Days 7-10	CONTROL	0.041	0.032	0.0084
	RE	0.017	0.015	0.0028
	RR	0.026	0.025	0.0035
Days 10-13	CONTROL	0.041	0.036	0.0048
	RE	0.012	0.010	0.0018
	RR	0.033	0.016	0.0137
Days 13-20	CONTROL	0.099	0.077	0.0285
	RE	0.021	0.020	0.0011
	RR	0.057	0.045	0.0129

Relative growth rate ($gg^{-1}d^{-1}$)

Period	Treatment	Whole plant	Shoot	Root
Days 7-10	CONTROL	0.113	0.100	0.235
	RE	0.056	0.049	0.237
	RR	0.081	0.081	0.279
Days 10-13	CONTROL	0.085	0.085	0.084
	RE	0.033	0.030	0.094
	RR	0.080	0.043	0.390
Days 13-21	CONTROL	0.112	0.097	0.189
	RE	0.046	0.046	0.041
	RR	0.087	0.080	0.126

Figure 3.7

Fig 3.7. Time courses of the effects of repeated root excision (RE, ■), of a single root excision treatment (RR, X) or of no treatment (CONTROL, ●) on a) Shoot Dry Weight and b) Total Primary Leaf Area. The points represent means of 5 values with standard errors shown by vertical bars. The beginning of the repeated root excision treatment and the day of the single root excision are indicated by the arrow.


a
1·0
0·5
0
Shoot Dry Weight (g)
7
10
13
21
Day

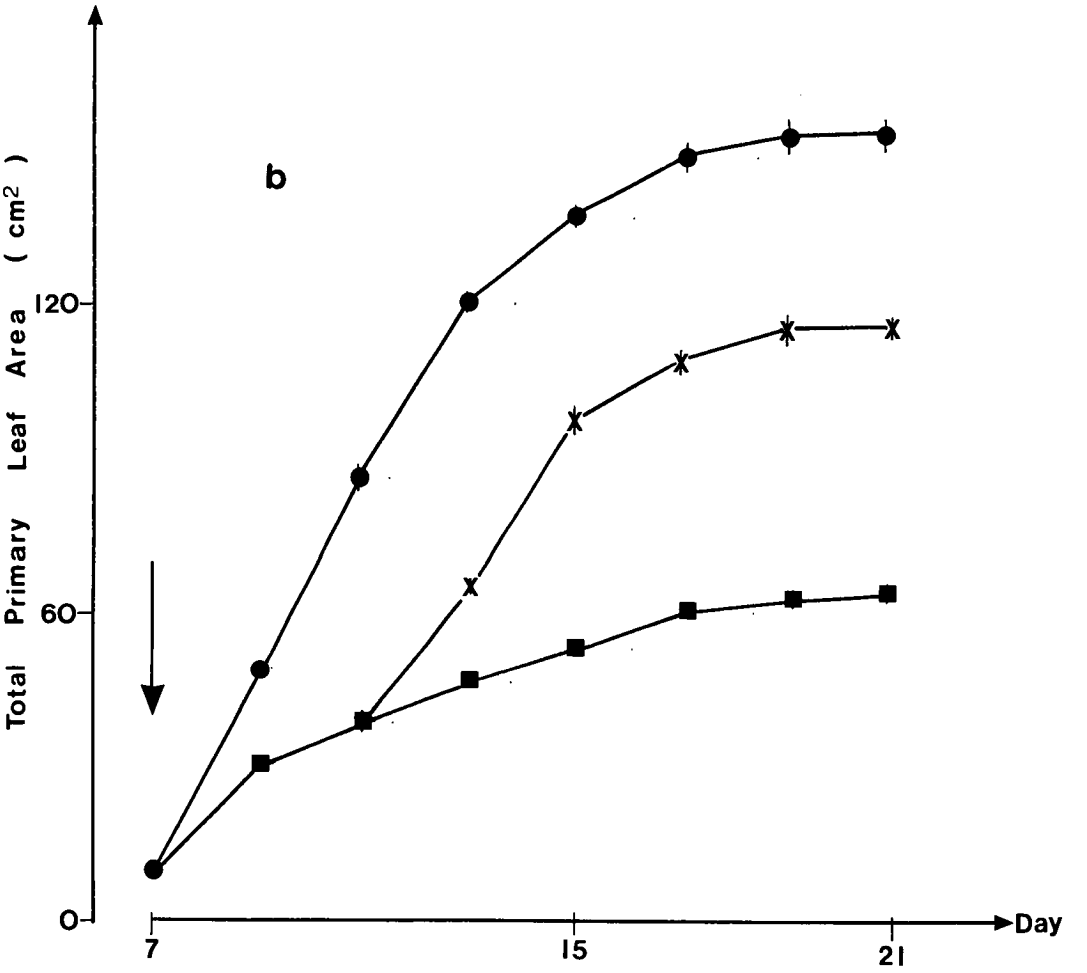
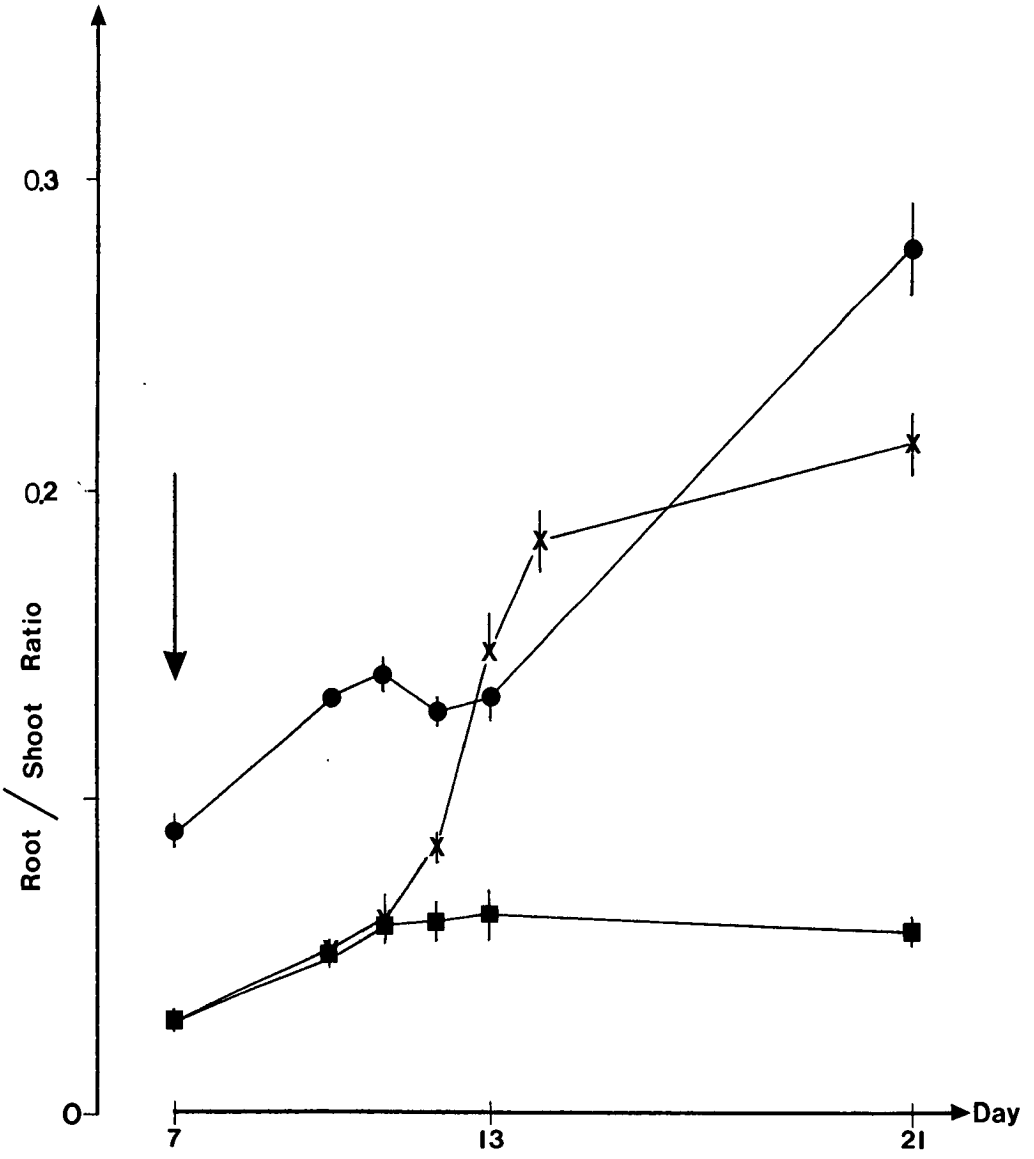

b
120
60
0
Total Primary Leaf Area (cm²)
7
15
21
Day

Figure 3.8

Fig 3.8. Time courses of the effects of repeated root excision (RE, ■), of a single root excision treatment (RR, X) or of no treatment (CONTROL, ●) on Root/Shoot Ratio. The points represent means of 5 values with standard errors shown by vertical bars. The beginning of the repeated root excision treatment and the day of the single root excision are indicated by the arrow.


0.3
0.2
0
Root / Shoot Ratio
7
13
21
Day

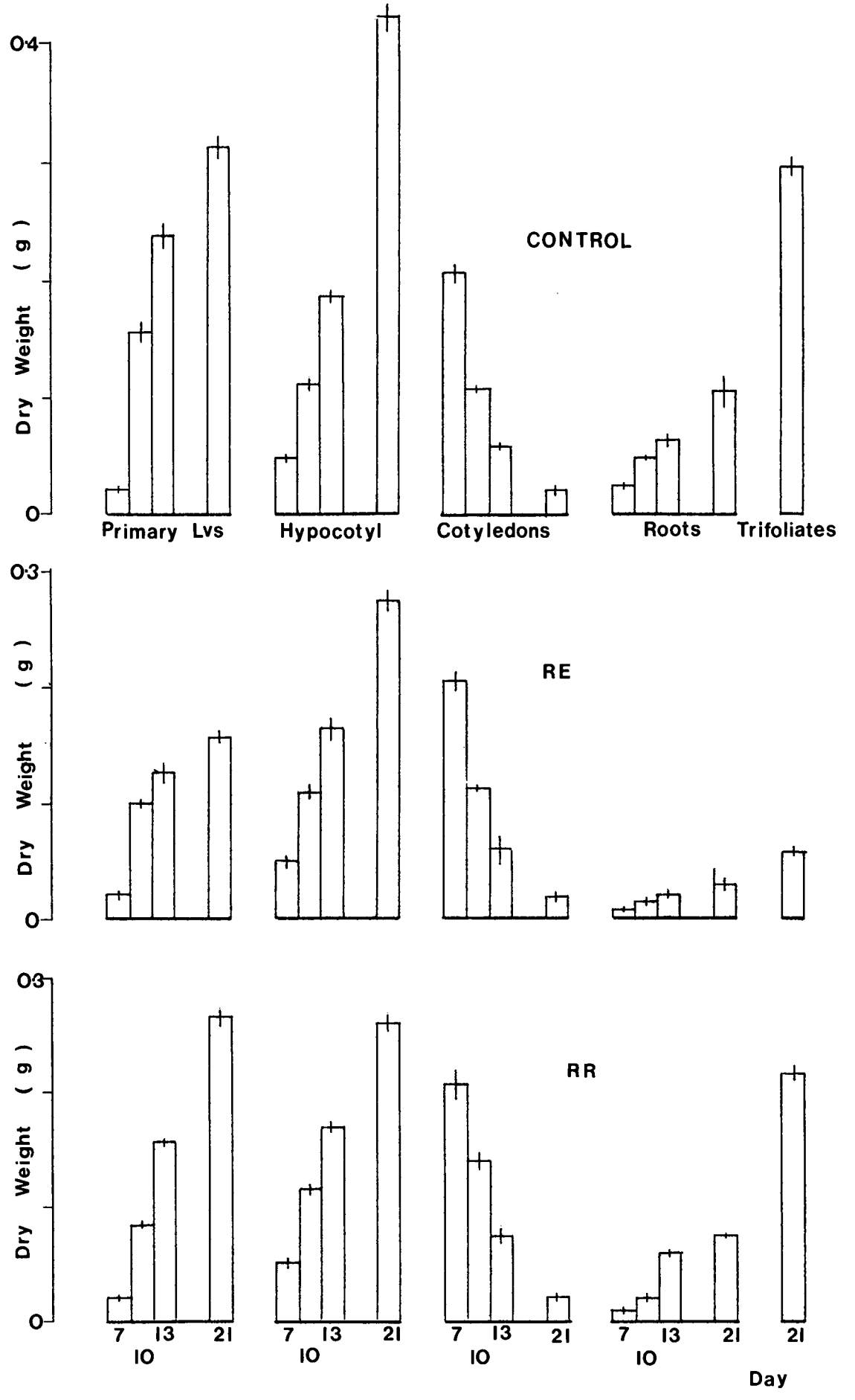
The effects of the two root excision treatments on changes in dry weight of all plant parts are summarised in Fig 3.9 (p 78). Repeated root excision (RE) reduced the growth of the root and all shoot parts except cotyledons. Up to day 14, the primary leaves were most affected but subsequently, the greatest effect was on the first trifoliate leaf. The proportion of shoot dry weight in the hypocotyl was significantly increased by the same treatment. In RR plants, particularly at the last harvest, distribution of dry matter was similar to that of control plants.

The effects of root cooling on plant growth and the distribution of dry matter were investigated in a parallel experiment. Increase in seedling weight was substantially reduced by root cooling (Fig 3.10, p 80) a significant effect being recorded by the second harvest (day 11). In control plants, absolute and relative growth rates of the whole plant increased with time (Table 3.3, p 82) but in RC plants, rates changed little, except for an increase between day 13 and 15,and were always significantly lower than the control values. Similar trends were seen in the growth rates of both root and shoot (Table 3.3, p 82 ; Fig 3.11, p 83). In control plants, relative growth rate of the root exceeded that of the shoot, so that root/shoot ratio increased with time. In RC plants, root and shoot relative growth rates were approximately equal, and root/shoot ratio remained constant except for a decline at the end of the experiment (Fig 3.12, p 80).

The promotion of seedling growth which occurred in RC plants between days 13 and 15 was caused by an increase in shoot, and particularly primary

Figure 3.9

Fig 3.9. Time courses of the effects of repeated root excision (RE), of a single root excision treatment (RR) or of no treatment (CONTROL) on the Dry Weight of different plant parts. Each vertical block represents the mean of 5 values with standard errors shown by vertical bars. With the exception of the trifoliate leaves which were of negligible weight for much of the experiment, all plant parts were harvested on days 7 (the first day of treatment), 10, 13 and 21.


0·4
Dry Weight (g)
0
CONTROL
Primary Lvs
Hypocotyl
Cotyledons
Roots
Trifoliates
0·3
Dry Weight (g)
0
RE
0·3
Dry Weight (g)
0
RR
7
10
13
21
Day

Figures 3.10 and 3.12

Fig 3.10. Time courses of the effects of root cooling (RC, ▲) or no treatment (CONTROL, ●) on Total Plant Dry Weight. The points represent means of 5 values with standard errors shown by vertical bars. The beginning of treatment is indicated by the arrow.

Fig 3.12. Time courses of the effects of root cooling (RC, ▲) or no treatment (CONTROL, ●) on Root/Shoot Ratio. The points represent means of 5 values with standard errors shown by vertical bars. The beginning of treatment is indicated by the arrow

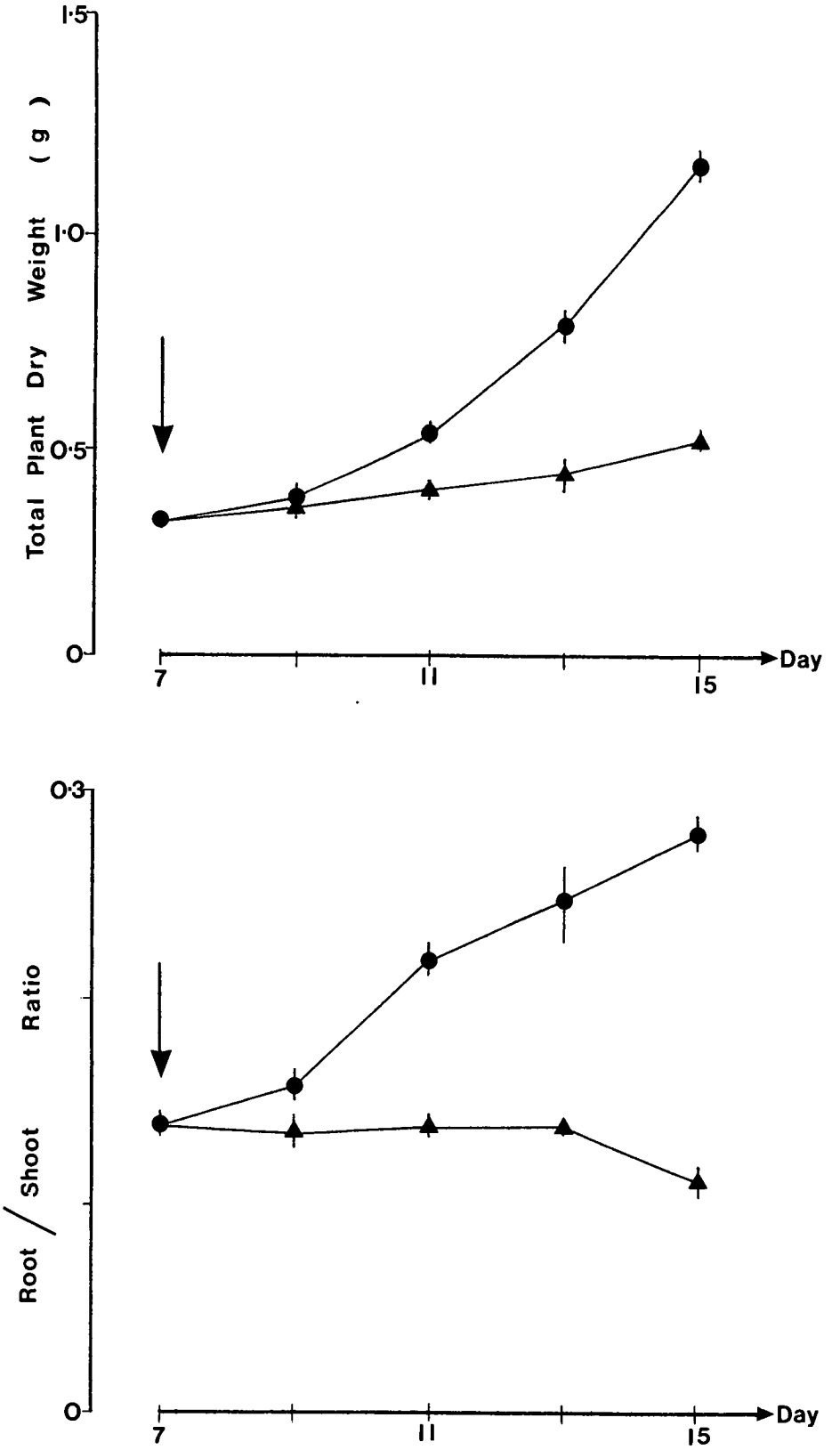

Total Plant Dry Weight (g)
1·5
1·0
0·5
0
7
11
15
Day
Root / Shoot Ratio
0·3
0
7
11
15
Day

Table 3.3 Effects of root cooling (RC) or no treatment (CONTROL) and time on the absolute and relative rates of increase in dry weight of whole plants, shoots, primary leaves and roots. Results were calculated from treatment means determined by harvesting five plants of each treatment at two-day intervals.

Absolute growth rate (gd^{-1})

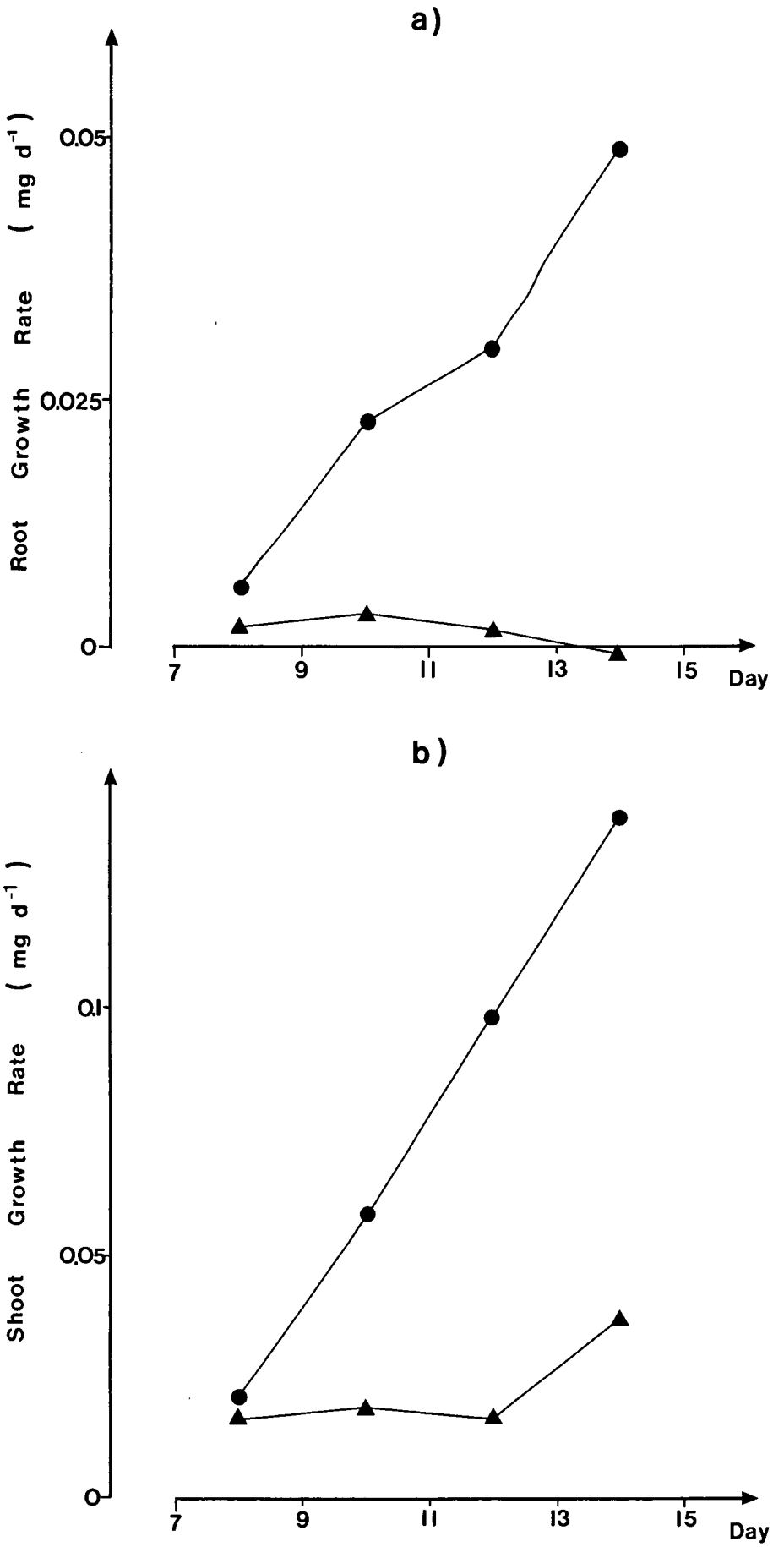
Period	Treatment	Whole plant	Shoot	Primary leaves	Root
Days 7-9	CONTROL	0.026	0.020	0.053	0.006
	RC	0.019	0.016	0.030	0.002
Days 9-11	CONTROL	0.080	0.057	0.067	0.023
	RC	0.021	0.018	0.028	0.003
Days 11-13	CONTROL	0.128	0.098	0.074	0.030
	RC	0.018	0.016	0.004	0.002
Days 13-15	CONTROL	0.189	0.139	0.108	0.049
	RC	0.042	0.036	0.032	-0.001

Relative growth rate ($gg^{-1}d^{-1}$)

Period	Treatment	Whole plant	Shoot	Primary leaves	Root
Days 7-9	CONTROL	0.075	0.066	0.800	0.133
	RC	0.056	0.054	0.583	0.044
Days 9-11	CONTROL	0.178	0.151	0.347	0.321
	RC	0.054	0.055	0.251	0.070
Days 11-13	CONTROL	0.196	0.185	0.219	0.243
	RC	0.042	0.043	0.030	0.034
Days 13-15	CONTROL	0.196	0.182	0.209	0.242
	RC	0.087	0.087	0.174	-0.014

Figure 3.11

Fig 3.11. Time courses of the effects of root cooling (RC, ▲) or no treatment (CONTROL, ●) on the absolute rate of increase of a) Root and b) Shoot Dry Weight. Points were obtained from treatment means determined by harvests of 5 replicates of each treatment at two-day intervals. Root cooling commenced on day 7.


a)
Root Growth Rate (mg d^{-1})
0.05
0.025
0
7
9
11
13
15
Day
b)
Shoot Growth Rate (mg d^{-1})
0.1
0.05
0
7
9
11
13
15
Day

leaf growth (Table 3.3, p 82). A similar effect was recorded around the same time in several other experiments and suggests that some adaptation to low root temperature may have taken place, permitting leaf growth to recover from the initial inhibitory effects of low root temperature. However, since later studies were concerned only with seedling growth up to day 12, experiments to test this hypothesis were not performed.

The effects of root cooling and seedling age on the distribution of dry matter within the plant were also studied (Fig 3.13, p 86). Root cooling reduced the dry weight increases of all growing parts, particularly the roots and primary leaves. Also, compared to the control plants, a disproportionately large amount of dry matter accumulated in the hypocotyl.

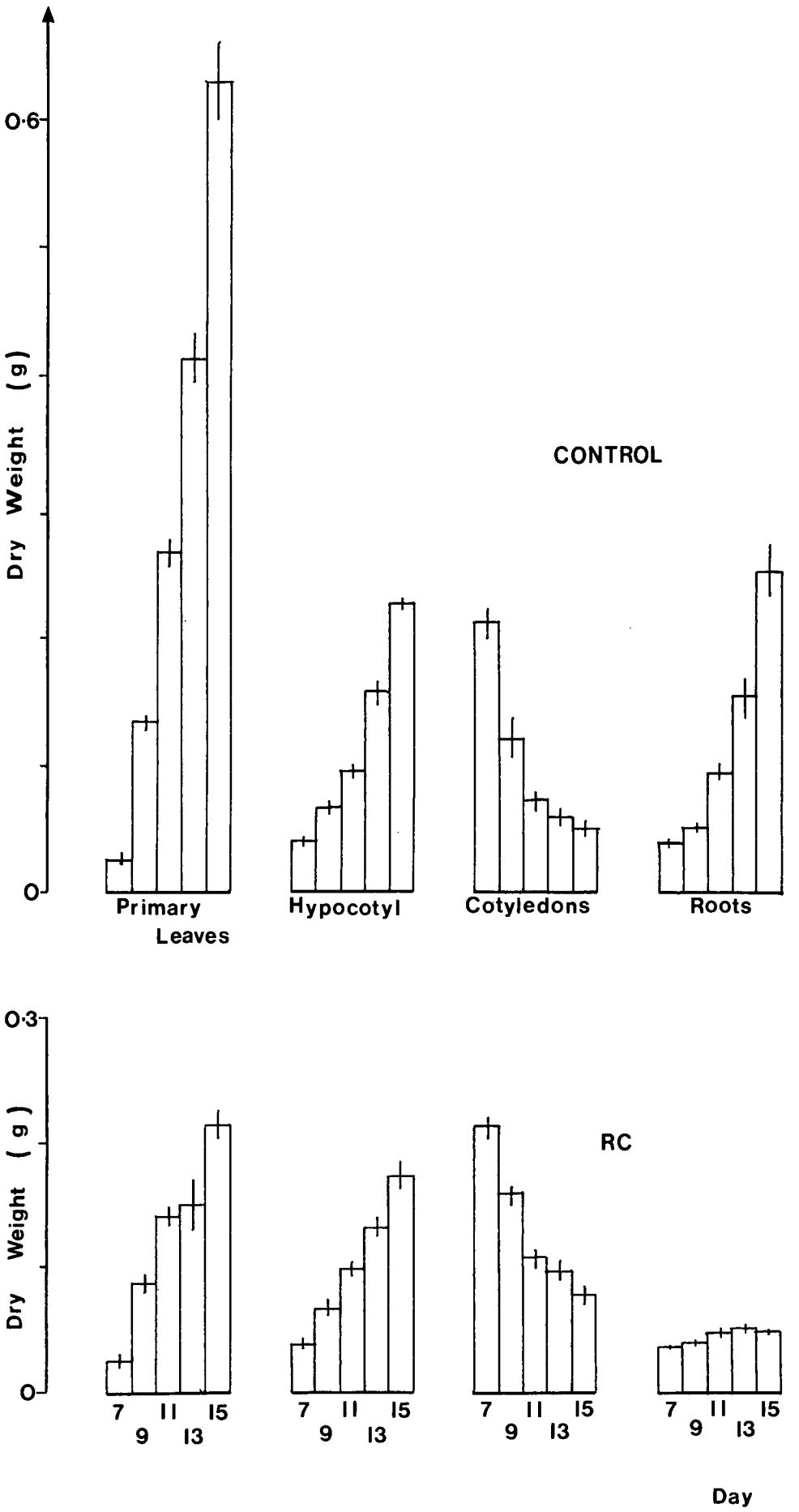
3.3.2 Effects of root excision and root cooling on the growth and morphology of the root system

The general effects of the major root treatments on root growth have already been indicated. However, to allow more thorough understanding of the possible mechanisms involved in the inhibition of leaf growth, a more detailed study of the effects on roots, particularly on root surface area and root tip number was undertaken.

The root excision treatment applied on day 7 reduced total root surface area by 85% (Fig 3.14, p 88). Subsequently, while control root areas increased more or less linearly with time (mean growth rate = 1.10 x 10^3 mm^2 d^{-1}), the areas of RE root systems showed no significant change and by day 12 total area was only 6% of the control value. Root cooling also greatly reduced the expansion of the root surface. On day 7 the root systems of the control and RC groups were of equal size, but by day 12 RC root area was only 40% of the control value.

Figure 3.13

Fig 3.13. Time courses of the effects of root cooling (RC) or no treatment (CONTROL) on the Dry Weight of different plant parts. Each vertical block represents the mean of 5 values with standard errors shown by vertical bars. Plants were harvested on days 7 (first day of treatment), 9, 11, 13 and 15.

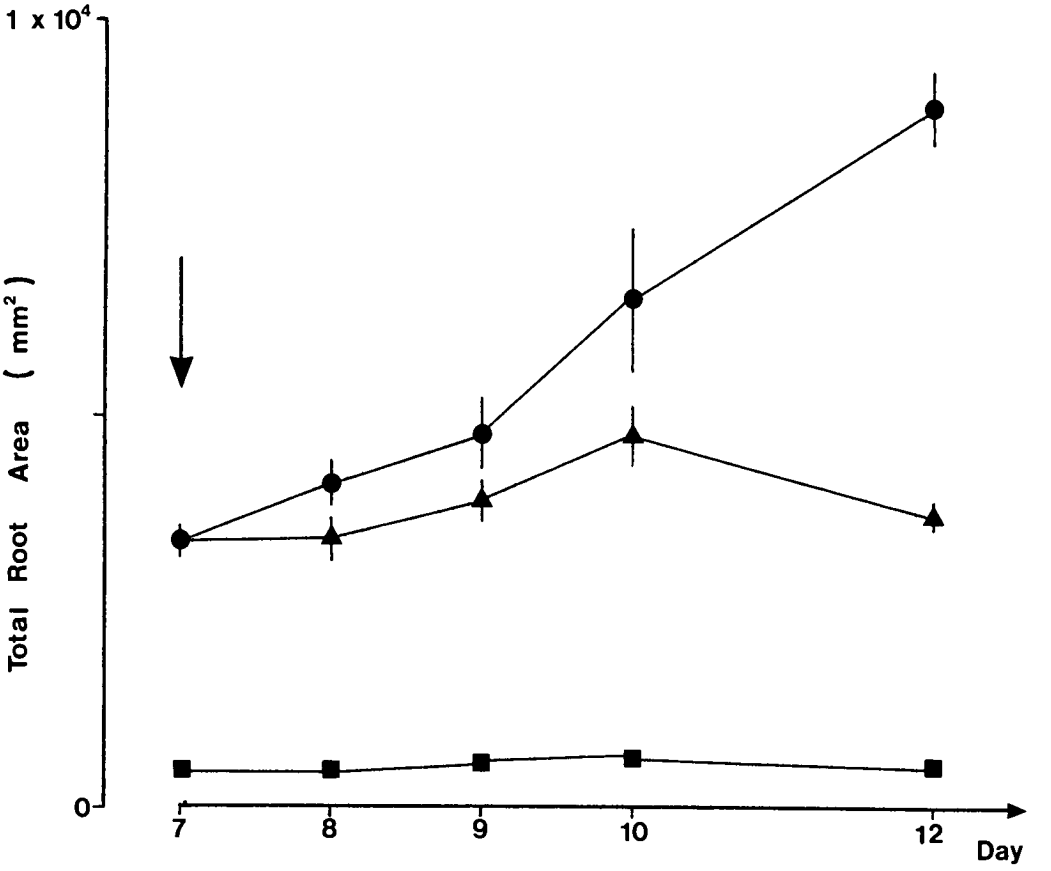

0·6
0
Dry Weight (g)
CONTROL
Primary Leaves
Hypocotyl
Cotyledons
Roots
0·3
0
Dry Weight (g)
RC
7
9
11
13
15
Day

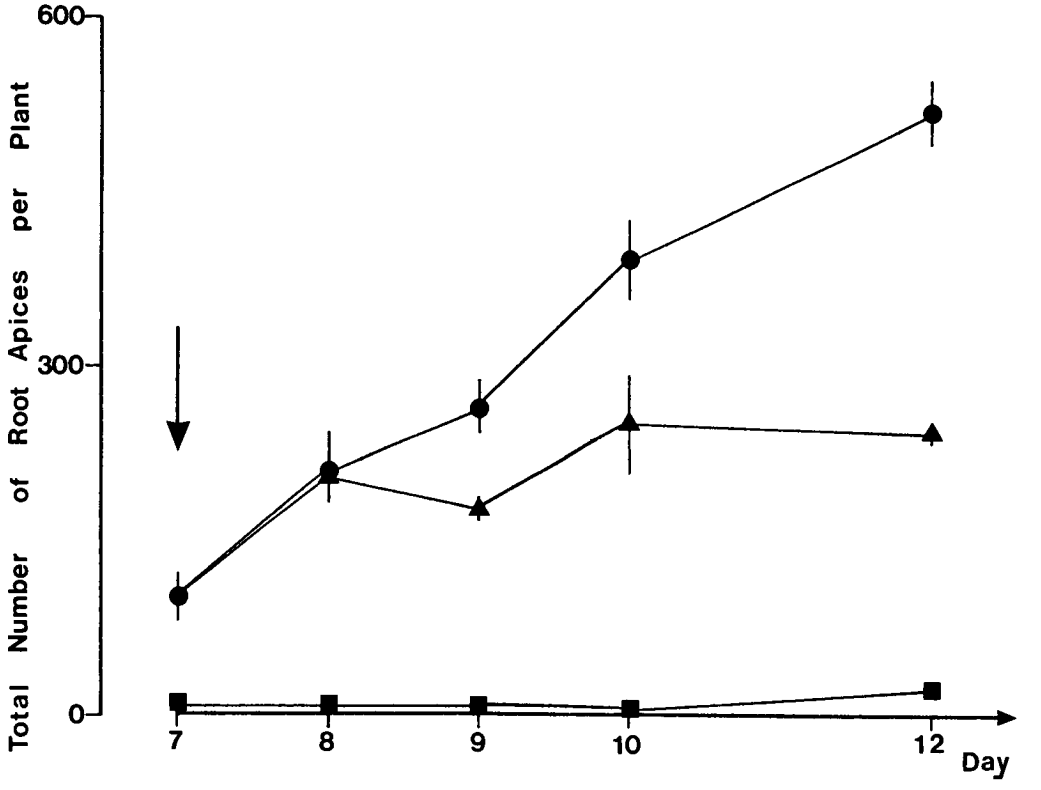
Figures 3.14 and 3.15

Fig 3.14. Time courses of the effects of root excision (RE, ■), root cooling (RC, ▲) or no treatment (CONTROL, ●) on Total Root Area per plant. The points represent means of 4 or 5 values with standard errors shown by vertical bars. The beginning of treatment is indicated by the arrow.

Fig 3.15. Time courses of the effects of root excision (RE, ■), root cooling (RC, ▲) or no treatment (CONTROL, ●) on the Total Number of Root Apices per Root System. The points are means of 4 or 5 values with standard errors shown by vertical bars. The beginning of treatment is indicated by the arrow.

Note: In both figures, standard errors of the mean values for the root excision treatment were too low to be represented but were approximately 10% of each mean value plotted.


1 x 10⁴
Total Root Area (mm²)
0
7
8
9
10
12
Day


600
300
0
Total Number of Root Apices per Plant
7
8
9
10
12
Day

In addition to effects on root area, root treatments also reduced the number of root tips per root system (Fig 3.15, p 88). On day 7 about 100 intact roots were present, but root excision reduced that number to approximately 5, the roots which remained being too small to remove by cutting. In control plants number of root tips increased linearly with time but in RE plants, where root excision was repeated on days 10 and 12, the number of intact roots present was always less than 20. In RC plants, a rapid increase in root tip number was recorded over the first 24 hours of treatment. This increase was largely due to the appearance of second order laterals, although these may have been present and too small to detect on day 7. Subsequently, however, a slower increase in number of root tips occurred (about 28 per day, compared to control production of about 84 per day) and by day 12, RC plants had less than half the number present in the control group.

As well as reducing the rates of root area increase and root formation root cooling also reduced the elongation of laterals, causing a reduction in mean length from day 8 onwards (Table 3.4, p 91).

The proportions of total root area contributed by different types of root (Section 2.2.4.5) varied considerably with treatment (Fig 3.16, p 92). In control plants, major first order laterals made up 64% of the total root area on day 7, the remainder being contributed roughly equally by the main axis and minor first order laterals. For the next five days, the proportion contributed by major first order laterals decreased only slightly, but the contributions of the other roots fell sharply as second order laterals increased, and by day 12 the latter constituted more than 45% of the total area. In RC plants, a completely different pattern was seen in which the proportions recorded on day 7 hardly changed and second

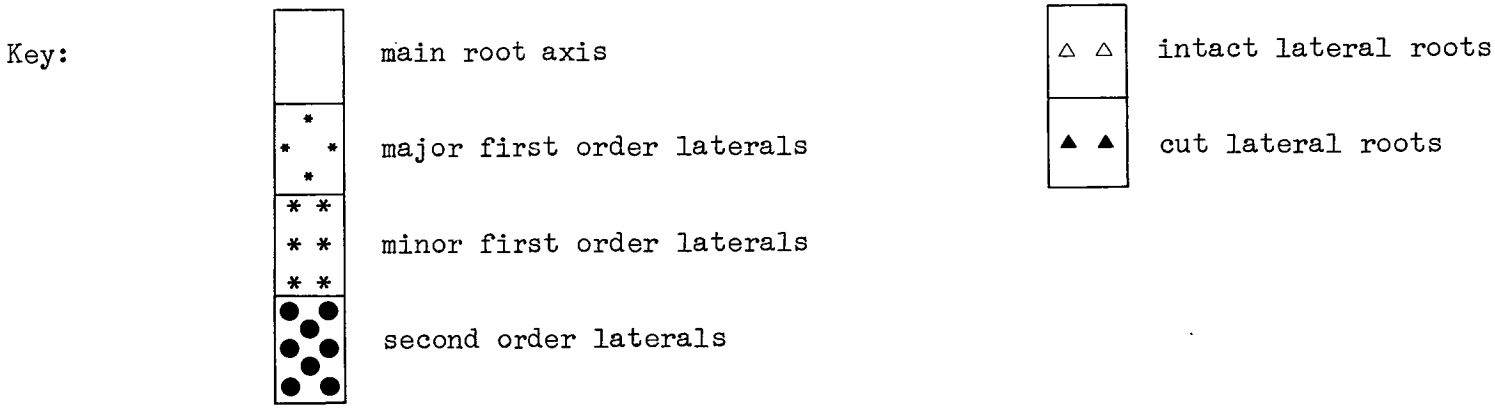
Table 3.4. Effects of root cooling (RC) or no treatment (CONTROL) and time on the length of second order laterals. Figures presented are means of values obtained from four replicate plants with standard errors in brackets.

Mean length of second order laterals (mm)

Day	CONTROL	RC
7	1.39 (0.07)	
8	2.48 (0.44)	2.01 (0.11)
9	4.35 (0.31)	1.54 (0.05)
10	6.39 (2.01)	1.73 (0.14)
12	14.37 (1.32)	1.60 (0.23)

Figure 3.16

Fig 3.16. Time courses of the effects of root excision (RE), root cooling (RC) or no treatment (CONTROL) on the relative contributions to Total Root Area of different types of root. The data were obtained from measurements on four to six root systems per treatment harvested on each of days 7 (first day of treatment), 8, 9, 10 and 12.



Contribution to Total Root Area (%)
0
50
100
CONTROL
RC
RE
7
8
9
10
12
Day

order laterals never contributed more than 2% of total root area (see also Table 3.4, p 91). In RE plants the main axis was the major component of the root system, making up between 75% and 90% of its total area. Only on day 10 did intact laterals make up more than 15% of the total root area, since on that day new roots had begun to emerge but were too small to be removed.

3.3.3 Effects of repeated root excision and root cooling on the development of the primary leaves

The expansion of the primary leaf pair of Phaseolus seedlings was considerably reduced by both root treatments (Fig 3.4, p 62). To investigate these effects in more detail, time courses of changes in primary leaf fresh and dry weight and the contributions made to leaf growth by cell division and cell enlargement were recorded. Since reductions in leaf growth had been detected in previous experiments within two to four days of the commencement of treatment, measurements of these parameters were concentrated on the period between days 7 and 13.

Both root treatments had considerable effects on all aspects of primary leaf growth (Table 3.5, p 95) although of the two, repeated root excision was most effective. In all plants, leaf area and fresh weight initially increased at similar rates so that by day 11 neither treatment had affected fresh weight per unit leaf area. Subsequently, this parameter increased in control plants, but remained constant in plants subjected to root excision and increased only slightly in response to root cooling. In contrast dry weight per unit leaf area was significantly higher for treated than control plants. These trends were reflected in percentage water content which increased steadily

Table 3.5. Effects of root excision (RE), root cooling (RC) or no treatment (CONTROL) on aspects of the growth of the primary leaves. Figures presented are means of measurements on six replicates with standard errors shown in brackets.

Day	Treatment	Total area (cm^2)	Total fresh weight (g)	Total dry weight (g)	F wt per unit area (gcm^{-2})	D wt per unit area (gcm^{-2})	Leaf water content (% of F wt)
7	CONTROL	14.52 (1.35)	0.269 (0.035)	0.045 (0.006)	0.0182 (0.0007)	0.0030 (0.0001)	83.50 (0.31)
11	CONTROL	102.55 (2.18)	2.060 (0.068)	0.230 (0.007)	0.0201 (0.0004)	0.0022 (0.0001)	88.82 (0.22)
	RE	33.98 (1.31)	0.670 (0.002)	0.123 (0.004)	0.0198 (0.0005)	0.0036 (0.0001)	81.68 (0.13)
	RC	40.65 (3.78)	0.793 (0.081)	0.148 (0.001)	0.0194 (0.0003)	0.0036 (0.0001)	81.33 (0.17)
13	CONTROL	164.33 (7.31)	3.899 (0.250)	0.405 (0.027)	0.0237 (0.0007)	0.0025 (0.0001)	89.60 (0.12)
	RE	34.55 (1.69)	0.690 (0.035)	0.123 (0.005)	0.0200 (0.0001)	0.0036 (0.0001)	82.06 (0.34)
	RC	55.85 (4.58)	1.185 (0.101)	0.201 (0.016)	0.0212 (0.0003)	0.0036 (0.0001)	83.02 (0.28)

in control plants but remained low and constant in plants of the other treatments.

Since expansion of the primary leaves up to day 9 involves both division and expansion of leaf cells (Dale, 1964; Verbellen and De Greef, 1979), effects of root treatments begun on day 7 could conceivably have involved reductions on both processes. To test this, two experiments were performed in which daily measurements of leaf cell number and mean cell volume were carried out. The first, which investigated the effects of repeated root excision was conducted under growth cabinet conditions while the second, showing the effects of root cooling was performed in a glasshouse (Section 2.2.2). Although leaf growth rate and final cell number of control plants were slightly higher under glasshouse conditions, general trends in growth, and effects of treatment were unchanged.

Consistent with the findings of other experiments (Table 3.5, p 95), root excision reduced primary leaf growth within one to two days of the beginning of treatment (Table 3.6, p 97). No effect of treatment on leaf cell number was detected, however, and the effect on leaf growth was entirely attributable to a reduction in leaf cell enlargement. Thus, mean cell volume was significantly lower in RE plants by day 8, and by day 11 was less than half the control value. The decreases in measured leaf cell number which occurred between days 9 and 11 have been recorded elsewhere (Dale, 1964; Wignarajah, Jennings and Handley, 1975) and may have been caused by destruction of large cells during the maceration process (Section2.2.4.3).

The effects of root cooling on leaf cell division and enlargement

Table 3.6. Effects of root excision (RE) or no treatment (CONTROL) on the fresh weight, area, fresh weight per unit area, total cell number and mean cell volume of primary leaves harvested on the first day of treatment (day 7) and on each of days 8, 9 and 11. Figures are means of five values with standard errors in brackets.

Day	Treatment	Total area (cm^2)	Total fresh weight (g)	F wt per unit area (gcm^{-2})	Cells per leaf (10^6 cells)	Mean cell volume ($10^{-6}mm^3$)
7	CONTROL	10.67 (0.50)	0.198 (0.007)	0.0186 (0.0003)	21.38 (1.38)	4.78 (0.28)
8	CONTROL	16.10 (0.40)	0.300 (0.009)	0.0186 (0.0002)	25.29 (1.02)	5.91 (0.39)
	RE	14.76 (0.59)	0.270 (0.012)	0.0183 (0.0003)	26.21 (2.12)	5.10 (0.36)
9	CONTROL	36.62 (2.11)	0.654 (0.049)	0.0178 (0.0003)	31.27 (0.90)	10.75 (0.79)
	RE	30.80 (1.28)	0.546 (0.028)	0.0177 (0.0003)	31.93 (2.07)	8.53 (0.29)
11	CONTROL	85.96 (2.54)	1.581 (0.050)	0.0184 (0.0004)	28.70 (2.05)	26.43 (1.24)
	RE	39.06 (2.11)	0.679 (0.038)	0.0174 (0.0003)	25.92 (1.41)	12.95 (0.90)

(Table 3.7, p 99) were similar to those of excision, although the results suggest a slight, though statistically insignificant reduction in final cell number. As before, a considerable effect of root treatment was seen in mean cell volume which was reduced to half its value in control leaves by day 11.

That root treatments had little effect on leaf cell number but a considerable one on mean cell volume is consistent with the finding that leaf dry weight was less affected by treatment than fresh weight (Table 3.5, p 95) and suggests that the leaves may have been thinner than those of controls and contained smaller, more densely-packed cells. Transverse sections of primary leaves of 11-day-old seedlings (Fig 3.17, p100) confirmed that mesophyll thickness and mean cell size were reduced by root treatment. Intercellular space formation was also reduced, possibly through reductions in epidermal cell growth.

3.3.4 Effects of other root treatments on plant growth

Although repeated root excision and root cooling were the standard root treatments used in these and subsequent experiments, the effects of several other treatments, particularly on primary leaf expansion were also examined.

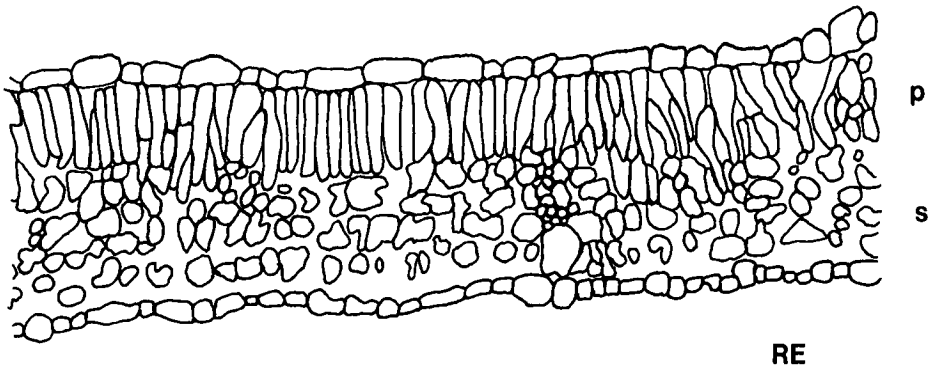
To investigate further the relationship between leaf growth and the size of the root system, an experiment was conducted in which root systems were pruned to different extents on day 7 and maintained at similar relative volumes until primary leaf growth had ceased. As well as a control group and plants subjected to the standard root excision treatment (RE), a third and fourth group of plants were included in which respectively two (RE2) and three (RE3) of the four ranks of lateral roots on the

Table 3.7. Effects of root cooling (RC) or no treatment (CONTROL) on the fresh weight, area, fresh weight per unit area, total cell number and mean cell volume of primary leaves harvested on the first day of treatment (day 7) and on each of days 8, 9, 10 and 11. Figures are means of five or six values with standard errors in brackets.

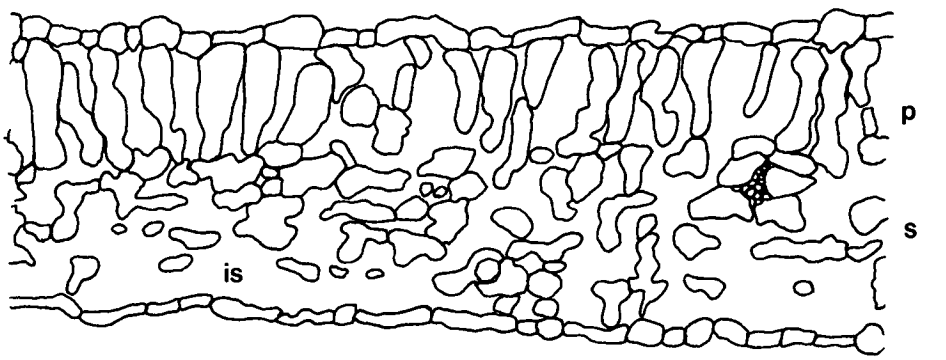
Day	Treatment	Total area (cm^2)	Total fresh weight (g)	F. wt per unit area (gcm^{-2})	Cells per leaf (10^6 cells)	Mean cell volume ($10^6 mm^3$)
7	CONTROL	12.43 (0.71)	0.224 (0.018)	0.0179 (0.0005)	29.49 (1.32)	3.77 (0.17)
8	CONTROL	16.27 (2.57)	0.268 (0.047)	0.0164 (0.0003)	29.11 (2.59)	4.58 (0.56)
	RC	14.87 (1.05)	0.257 (0.018)	0.0171 (0.0013)	27.49 (2.82)	4.70 (0.15)
9	CONTROL	30.60 (2.27)	0.544 (0.039)	0.0178 (0.0003)	30.48 (0.77)	8.88 (0.43)
	RC	26.36 (0.77)	0.442 (0.018)	0.0168 (0.0004)	34.11 (1.11)	6.48 (0.10)
10	CONTROL	60.62 (3.63)	1.130 (0.093)	0.0185 (0.0004)	35.86 (2.82)	16.04 (0.71)
	RC	32.22 (2.48)	0.560 (0.044)	0.0174 (0.0002)	31.16 (2.04)	8.97 (0.37)
11	CONTROL	96.64 (7.45)	1.930 (0.133)	0.0200 (0.0005)	39.19 (2.78)	24.41 (1.47)
	RC	42.64 (1.31)	0.758 (0.029)	0.0178 (0.0004)	33.18 (1.37)	11.29 (0.55)

Figure 3.17

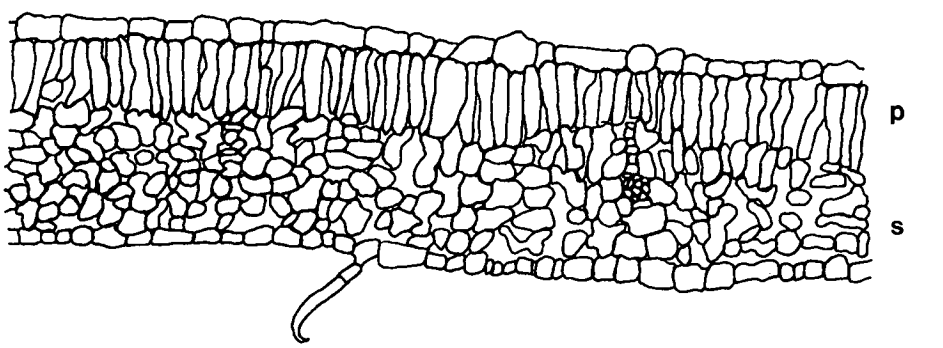
Fig 3.17. Transverse sections of primary leaves of 11-day-old plants subjected to root excision (RE), root cooling (RC) or no treatment (CONTROL). All sections were taken from the same area on the leaf (midway between the midvein and the leaf margin) and are to the same scale. Note the greater density of packing of cells in the treated material and the associated reduction in intercellular space. (p = palisade mesophyll, s = spongy mesophyll, is = intercellular space).



RE



CONTROL



RC

100 µm

seminal axis were excised (see Section 2.2.3). Excision was repeated whenever new roots appeared on the excised regions and leaf growth was monitored by area measurements at two to three day intervals. Within two days of treatment, the primary leaves of control plants were significantly larger than those of RE plants and by day 12 a relationship between leaf area and root size had emerged, such that leaf area was proportional to the amount of root tissue remaining (Fig 3.18, p 103). This relationship persisted until day 20, by which time primary leaf expansion had ceased in all plants. Although some compensatory growth of roots on the ranks of laterals not removed did occur (Table 3.8, p 105), the method of root excision used was successful at maintaining root systems of different sizes, and the results confirm that a positive correlation exists between root size and the growth of both the whole shoot and the primary leaves.

A similar experiment was carried out to investigate the effects of a range of root temperatures on primary leaf growth. Over the period studied, rate of leaf expansion was related to root temperature (Fig 3.19, p 106) as were the dry weight increases of the root and shoot (Table 3.9, p 108). Generally, root growth was more sensitive to root temperature than that of the shoot, so root/shoot ratio was highest for control plants and lowest for the group grown at the lowest root temperature.

The final experiment in this series considered the effect of day of root excision on shoot growth, again with emphasis on primary leaf expansion, Control plants were grown as normal but on day 10 a group were subjected to a root excision treatment identical to that performed on RE plants on day 7. Root excision on day 10 completely arrested primary leaf expansion, no change in area being detected over the subsequent three

Figure 3.18

Fig 3.18. Time courses of the effects of repeated excision of two (·) three (*) or all four (■) ranks of lateral roots (corresponding to treatments RE2, RE3 and RE respectively) or of no treatment (●) on Total Primary Leaf Area. The points represent means of 5 values with standard errors shown by vertical bars. The beginning of all treatments is indicated by the arrow

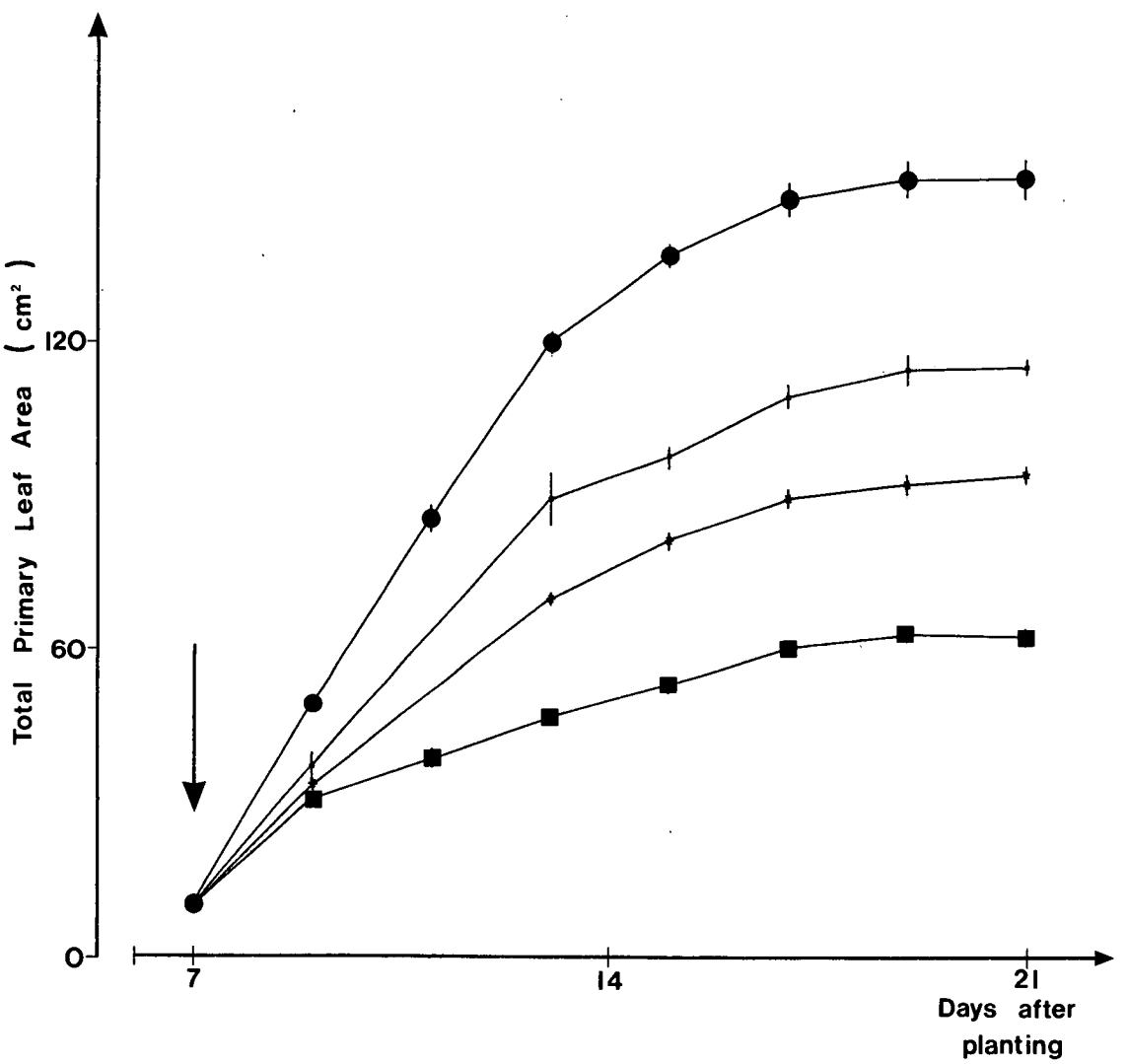

Total Primary Leaf Area (cm²)
120
60
0
7
14
21
Days after planting

Table 3.8. Effects of repeated excision of two (RE2), three (RE3) or all four (RE) ranks of lateral roots, or of no treatment (CONTROL) on total primary leaf area, leaf, shoot and root dry weights and root/shoot ratio. Figures presented are means of five (or in the case of day 7 data, eight) values with standard errors in brackets.

Day	Treatment	Total primary leaf area (cm^2)	Total primary leaf dry weight (g)	Total shoot dry weight (g)	Total root dry weight (g)	R/S ratio
7	CONTROL	10.7 (0.5)	0.031 (0.001)	0.327 (0.004)	0.033 (0.001)	0.100 (0.004)
22	CONTROL	152.2 (5.6)	0.305 (0.013)	1.081 (0.028)	0.262 (0.028)	0.242 (0.017)
22	RE2	123.7 (7.9)	0.243 (0.016)	0.881 (0.038)	0.157 (0.011)	0.179 (0.012)
22	RE3	94.8 (2.5)	0.197 (0.005)	0.725 (0.020)	0.113 (0.011)	0.155 (0.013)
22	RE	62.2 (2.0)	0.142 (0.005)	0.483 (0.021)	0.036 (0.003)	0.075 (0.007)

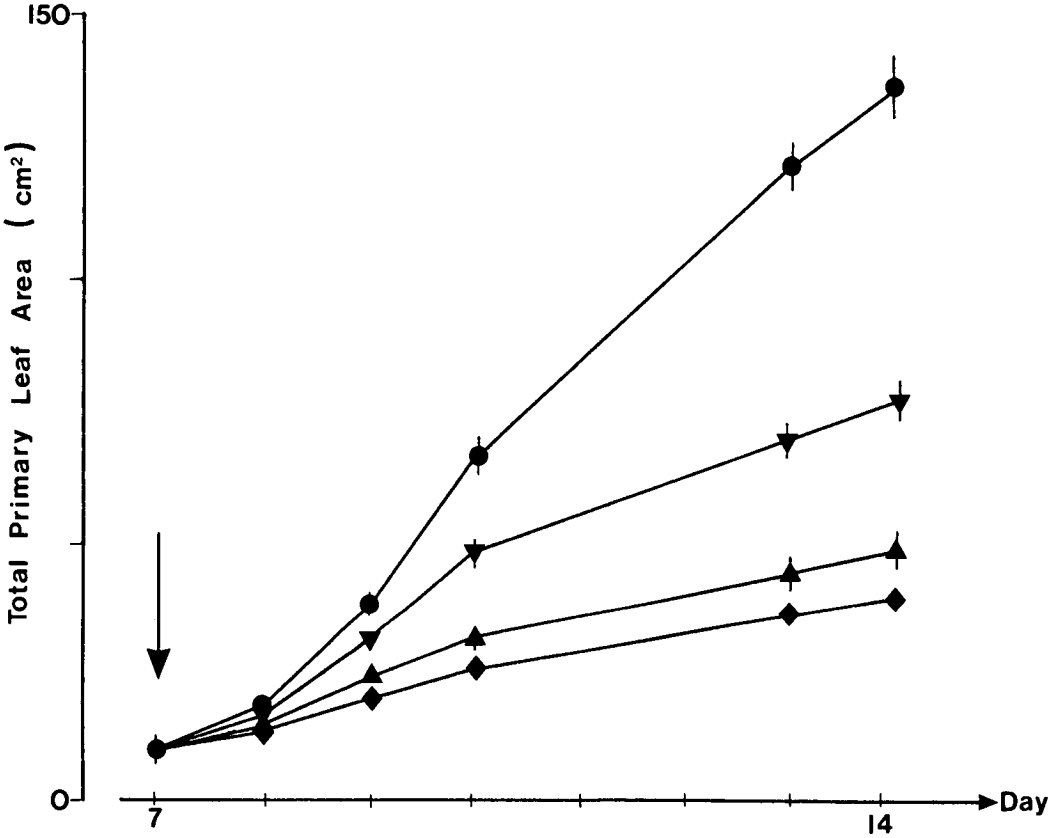
Figures 3.19 and 3.20

Fig 3.19. Time courses of the effects of different root temperatures on Total Primary Leaf Area. The points represent means of 5 values with standard errors shown by vertical bars. Transfer from the control temperature is indicated by the arrow.

Key: ● = 19°C (CONTROL) ▼ = 12.5°C

▲ = 10°C (RC) ◆ = 8°C

Fig 3.20. Time courses of the effects of repeated root excision commenced on either day 7 (■) or day 10 (◆), or of no treatment (●) on Total Primary leaf area. The points represent means of 5 values with standard errors indicated by the vertical bars. Arrows mark the first days of each of the two treatments.


150
Total Primary Leaf Area (cm²)
0
7
14
Day

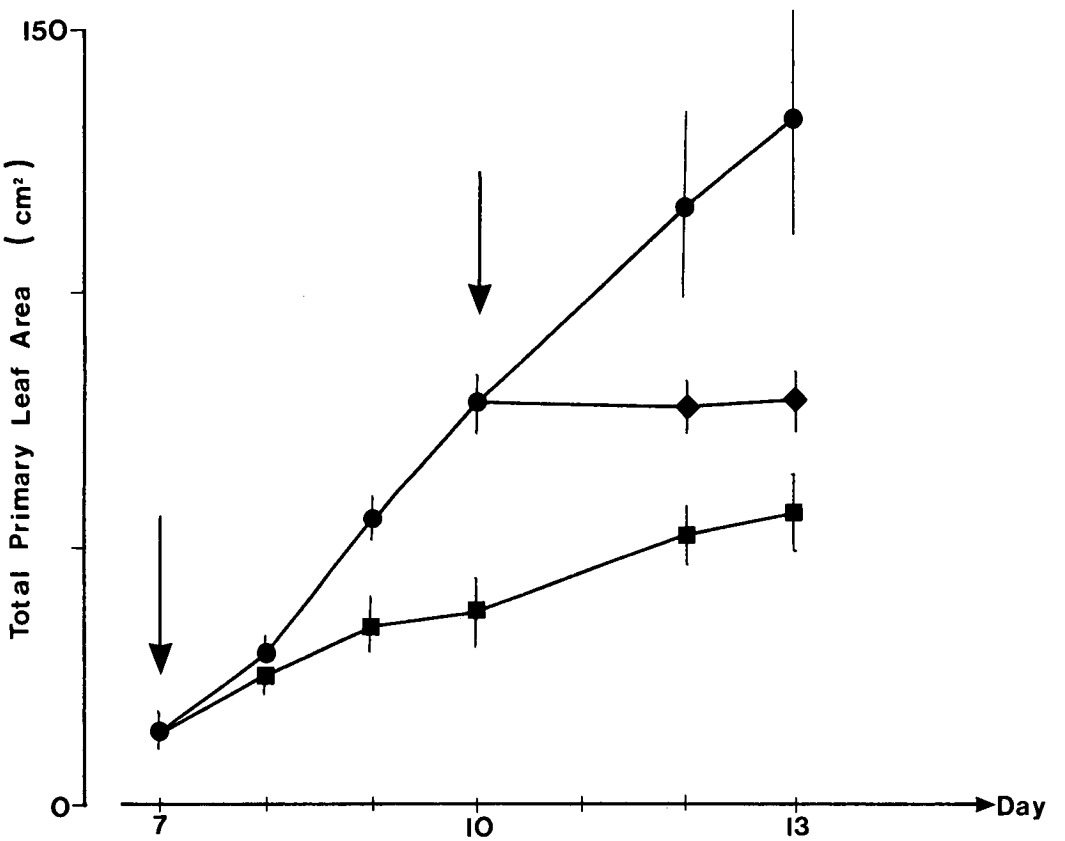

150
Total Primary Leaf Area (cm²)
0
7
10
13
Day

Table 3.9. Effects of different root temperatures on primary leaf area, leaf, shoot and root dry weight and root/shoot ratio. Treatment was commenced at the time of transfer to solution culture (day 7). All results are means of five values with standard errors in brackets.

Day	Root Temperature (°C)	Total primary leaf area (cm^2)	Total primary leaf d wt (g)	Total shoot d wt (g)	Root d wt (g)	R/S ratio
7	19	10.1 (0.29)	0.027 (0.003)	0.283 (0.015)	0.039 (0.002)	0.139 (0.005)
14	19	136.9 (5.50)	0.427 (0.022)	0.695 (0.021)	0.109 (0.005)	0.158 (0.005)
	8	39.0 (2.06)	0.188 (0.017)	0.460 (0.024)	0.040 (0.006)	0.085 (0.009)
	10	48.9 (3.40)	0.233 (0.019)	0.503 (0.026)	0.051 (0.003)	0.100 (0.003)
	12.5	76.9 (3.82)	0.339 (0.027)	0.658 (0.043)	0.067 (0.006)	0.101 (0.005)

Table 3.10. Effects of root excision (RE), root cooling (RC) or no treatment (CONTROL) on net assimilation rate (NAR). Results were calculated from mean values of total plant dry weight and leaf area determined by destructive harvests of five plants of each treatment at two- to three-day intervals.

Period	Treatment	NAR ($g\ cm^{-2}\ d^{-1}$)
Days 7-10	CONTROL	13.18×10^{-4}
	RE	8.70×10^{-4}
Days 10-13	CONTROL	4.50×10^{-4}
	RE	2.95×10^{-4}
Days 7-9	CONTROL	9.70×10^{-4}
	RC	10.30×10^{-4}
Days 9-11	CONTROL	10.60×10^{-4}
	RC	6.86×10^{-4}
Days 11-13	CONTROL	9.13×10^{-4}
	RC	4.06×10^{-4}

days (Fig 3.20, p 106). Shortly after root excision, the primary leaves wilted, remaining flaccid for the rest of day 10 and during much of the photoperiods of the next three days. It may be that failure of the leaves to grow was the result of this sudden and drastic loss of turgor. Significantly, no such visible loss of turgor was recorded in response to root excision on day 7.

3.4 DISCUSSION

Both root excision and root cooling inhibited shoot growth in Phaseolus seedlings, their effects being generally consistent with published accounts (for discussion, see Sections 1.2.1 and 1.2.2). Over the first four to five days, the visible effects of the two treatments were strikingly similar; the primary leaves were smaller, thinner and darker in colour than those of control plants, and internode extension was reduced. Subsequently the primary leaves of RC plants increased in area, weight and thickness relative to those of the RE group and became lighter in colour due to the development of chlorotic patches. These differences suggest that either the effects on the plant of root cooling or the sensitivity of the primary leaves to those effects decreased after several days of treatment. However, between days 7 and 12, when the influence on leaf growth was first recorded, the effects of the two treatments on leaf growth and morphology were sufficiently similar to suggest that similar mechanisms may have been responsible.

Effects of root treatments on the roots

The most obvious effect of treatment on the root system was to reduce its size, both in terms of surface area and weight. When different proportions of the root system were removed, a correlation was found between the weight

of root tissue remaining and leaf growth. A similar result for Vitis vinifera has been interpreted as evidence that roots provide the shoot with a growth promoting factor, the supply of which is related to the amount of root tissue present (Buttrose and Mullins, 1968). In the present study, the relationship between root size and leaf growth did not hold when intact root systems were maintained at the cooling temperature. Instead, leaf growth was substantially lower than might have been predicted, being approximately the same as that of plants grown at the control root temperature with excision of all lateral roots. The implication is that leaf growth is dependent upon some or all of the various functions (synthetic, catabolic, uptake-related) of the root which are probably reduced at low temperatures. Thus it can be concluded that leaf growth depends not only on the size of the root system but also its metabolic activity.

One particular aspect of root growth markedly reduced by root excision and root cooling, and which seemed to correlate with the effects on leaf growth was root tip formation. Although considerably fewer root tips were present on RE than RC root systems, once again the similarity between the effects of the two treatments on leaf growth can be explained if it is assumed that root cooling also reduced the metabolic activity of the root tips. The possible involvement of viable root apices in the control of leaf growth is illustrated by the observation that in plants which received only a single root excision treatment (RR), leaf growth rate increased very shortly after new root tips emerged. Thus, the presence of growing root tips was sufficient to promote leaf growth, a large increase in root area being unnecessary.

An investigation of the effects of root apices on shoot growth was not attempted in this work because of the considerable practical difficulties involved; indeed few published accounts of the specific effects of root

apices are available. However, leaf shape in Horseradish (Armoracea lapathifolia) was altered by the removal of root tips (Gollnow and Letham, 1978) and the formation of new root primordia in pea seedlings was associated with increased export of cytokinins from the roots (Forsyth and Van Staden, 1981). These two results raise the intriguing possibility that root treatments might affect leaf growth by altering the supply of growth promoters reaching the shoot (Carmi and Van Staden, 1983; see also general discussion, Chapter 6).

Effects of root treatments on the development of the primary leaves

Primary leaf growth was highly sensitive to the root treatments used and a study of the cellular basis of the effect showed that while cell division was relatively unaffected by treatment, considerable reductions in cell enlargement occurred. In all plants, regardless of treatment, final cell number per leaf was lower than might have been expected on the basis of published results (Dale, 1964; Dale and Murray, 1968), possibly reflecting variation between batches of seed. However, the sequence of developmental events was normal, cell division ceasing around day 9 with lamina expansion proceeding subsequently by cell enlargement alone (Dale, 1964; Morris and Arthur, 1984).

The considerable effects of root treatments on mean cell volume led in RE and RC plants to the formation of small, thin leaves with small, densely-packed cells and high stomatal frequencies (results not shown). Such morphology is reminiscent of the effects of stress conditions such as drought (Quarrie and Jones, 1977) and salinity (Waldron et al, 1985), on leaves, and suggests that water deficit may have occurred in response to treatment (Brouwer, 1964). In support of this hypothesis leaf cell enlargement is generally found to be more sensitive than cell division

to water deficit (Hsiao, 1973; Clough and Milthorpe, 1975), although the converse has occasionally been shown (Berlin, Quisenberry, Bailey, Woodworth and McMichael, 1982). In fact, no visible signs of water stress were observed in response to any of the treatments used, except when root excision was applied to 10-day old plants in which lamina area was much greater than on day 7; in these plants, severe wilting occurred and lamina expansion was completely arrested. Nevertheless, the published finding, that root cooling to 10°C reduces water uptake in Phaseolus (Bohning and Lusanandana, 1952; Kuiper, 1964), suggests that in RC plants at least, water supply may have been limiting. In RE plants, water supply is less likely to have been reduced because excision of laterals may have allowed direct entry of water through the open ends of cut xylem vessels. This, and other aspects of the water relations of RE and RC plants are discussed in Chapter 4.

Effects of root treatments on the nutritional status of the shoot

The appearance of chlorotic patches on the primary leaves of RC plants after five to six days of treatment indicates that by that time, mineral nutrient supply may have become limiting. However prior to that, no symptoms of mineral deficiency were observed in either RC or RE plants. The primary leaves were dark green in colour, an observation interpreted in Vitis vinifera (Buttrose and Mullins, 1968) and Lolium perenne (Brouwer and Kleinendorst, 1965) to indicate that mineral supply was not limiting for leaf growth. Also, cell division in the primary leaves and initiation of trifoliate leaves were relatively unaffected by treatment, again suggesting that mineral supply was adequate. Although both root cooling and root excision could conceivably reduce mineral uptake (Section 1.3.2.2), concentrations in the shoot could be maintained firstly by reduced demand due to reduced shoot growth, and secondly by utilisation of cotyledonary

reserves (Brouwer, 1964). In fact, measurements carried out on days 7, 11 and 13 (see Appendix 2) confirm that the concentrations of the major nutrients (nitrogen, phosphorus and potassium) in the shoot were considerably reduced by both treatments. However in the short term, when leaf expansion was not reduced (days 7 to 9), shoot concentrations may not have fallen sufficiently to have reached limiting levels.

Calculations on the results presented in Section 3.3.1 reveal that both root excision and root cooling caused significant reductions in net assimilation rate (Table 3.10, p 109). While this may have been due to stomatal closure (see Section 4.3), a similar result in root-pruned bean plants was associated with a reduction in the activity of the enzyme system necessary for the fixation of carbon dioxide (Carmi and Koller, 1978). Later work showed that the root pruning treatment which reduced photosynthesis also reduced the supply of cytokinins moving from the roots to the shoot and it was concluded that root-derived cytokinins were necessary for the maintenance of shoot photosynthesis (Carmi and Van Staden, 1983). In the present work it is possible that root excision and root cooling inhibited photosynthesis by affecting the supply of cytokinins or other plant growth regulators to the shoot. However, an important observation is that despite the effect on net assimilation rate, RE and RC plants showed relative increases in the amount of dry matter in the shoot, particularly the hypocotyl. This may have been due to reduced translocation of assimilate from the shoot due to a decrease in the sink strength of the roots (Ghobrial, 1983). According to Warren-Wilson (1972), the ability of any plant tissue to attract assimilate or any other substance (ie its sink strength) is the product of its size and (metabolic) activity. In the present experiments, both treatments reduced the size of the root sink while root cooling may also

have reduced its activity. Perhaps photosynthesis was inhibited by an accumulation of assimilate through this mechanism (Humphries and Thorne, 1964 ; Burt, 1964).

However photosynthesis was reduced, whether by stomatal closure, a reduction in enzyme activity or an accumulation of assimilate, it is unclear whether leaf growth was inhibited as a direct result or for some other reason. Since dry matter accumulated in the shoot, it is unlikely that leaf growth was limited by availability of assimilate. Instead, the ability of the leaf cells to utilise that stored potential for growth may have been impaired, possibly through a shortage of one or more root-derived factors. Alternatively, low root sink strength may have caused the accumulation in the shoot of factors inhibitory to leaf growth (see Section 4.7 and Chapter 6).

In conclusion, root excision and root cooling profoundly affected the growth of the primary leaves, apparently by reducing leaf cell enlargement. According to Ray, Green and Cleland (1972), cell turgor is the driving force for cell growth. Also root excision and root cooling have both been shown to reduce water uptake (Brouwer and Kleinendorst, 1965; Kuiper, 1964). Therefore the next series of experiments were planned to investigate the effects of root treatments on plant water relations with particular attention paid to the water status of the leaf cells.

4. THE EFFECTS OF ROOT EXCISION AND ROOT COOLING ON WATER UPTAKE AND PRIMARY LEAF WATER RELATIONS

4.1 INTRODUCTION

Having shown the principal effect of both major root treatments on leaf growth to be a reduction in leaf cell enlargement, the hypothesis was proposed that this effect came about through a reduction in leaf turgor caused by reduced water uptake. This idea was supported by previous findings that leaf cell expansion was highly sensitive to changes in leaf turgor (Acevedo, Hsiao and Henderson, 1971; Bunce, 1977), and that both root excision and root cooling could lead to decreases in plant water uptake (eg Veen, 1977; Bohning and Lusanandana, 1952). However, in the present study, no visible signs of water deficit were observed, except after two or three days of treatment, when effects on leaf growth had already appeared. The experiments now described were carried out to answer three central questions: whether root excision and root cooling affected the water relations of the leaves of Phaseolus seedlings, whether they caused any changes in the uptake of water by the root systems and whether effects on leaf growth could be correlated with changes in leaf turgor. The investigation concentrated on the period between days 7 and 11, since previous experiments had shown effects on leaf growth to develop over that time.

The main part of the work considered the effects of root treatments on primary leaf water relations and involved direct measurement of leaf water and osmotic potentials, and calculation of leaf turgor. Changes in leaf diffusive resistance caused by root treatments were then recorded to determine effects on stomatal aperture and its control. Effects of treatments on transpiration rate were also determined to provide data necessary for the calculation of root permeability. Concurrently,

detailed measurements of leaf growth rate were made so that correlations with the other parameters could be investigated. Finally the possible involvement of abscisic acid (ABA) in the effects on growth and water relations of treated and control plants was investigated by measuring the effects of one of the treatments, root cooling, on leaf ABA content.

Most of the work reported in this chapter was carried out under growth room conditions, because a well defined light/dark cycle and relatively constant aerial conditions were required. However, the data presented in section 4.7 were obtained under glasshouse conditions.

4.2 EFFECTS OF ROOT EXCISION AND ROOT COOLING ON THE WATER RELATIONS OF THE PRIMARY LEAVES

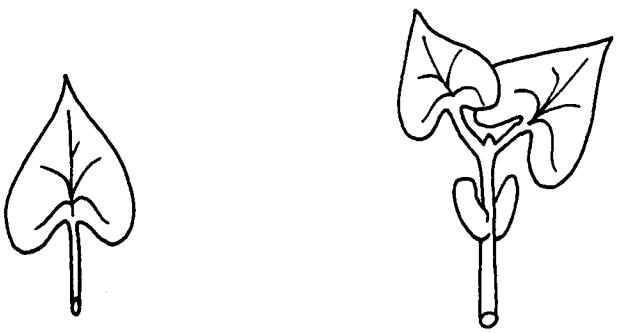
4.2.1 Effects of root treatments on leaf water potential

Leaf water potential (Ψ_l) was measured by the pressure chamber technique according to the procedure described in 2.2.6.1. The psychrometric method was also used, although with limited success because of difficulties in preventing temperature fluctuations and thermal induction within the apparatus. Consequently, only results obtained with the pressure chamber are reported here. Generally measurements were made on single, primary leaves. However, leaves of seven-day old plants were unsuitable because their petioles were too short to be accommodated in the sealing assembly (Fig 2.5, p 45). Instead, whole shoots excised 1 - 2cm below the cotyledons were used. Comparison of leaf and shoot water potentials of identical groups of slightly older plants showed no effect of method of measurement on water potential (Fig 4.1, p 118).

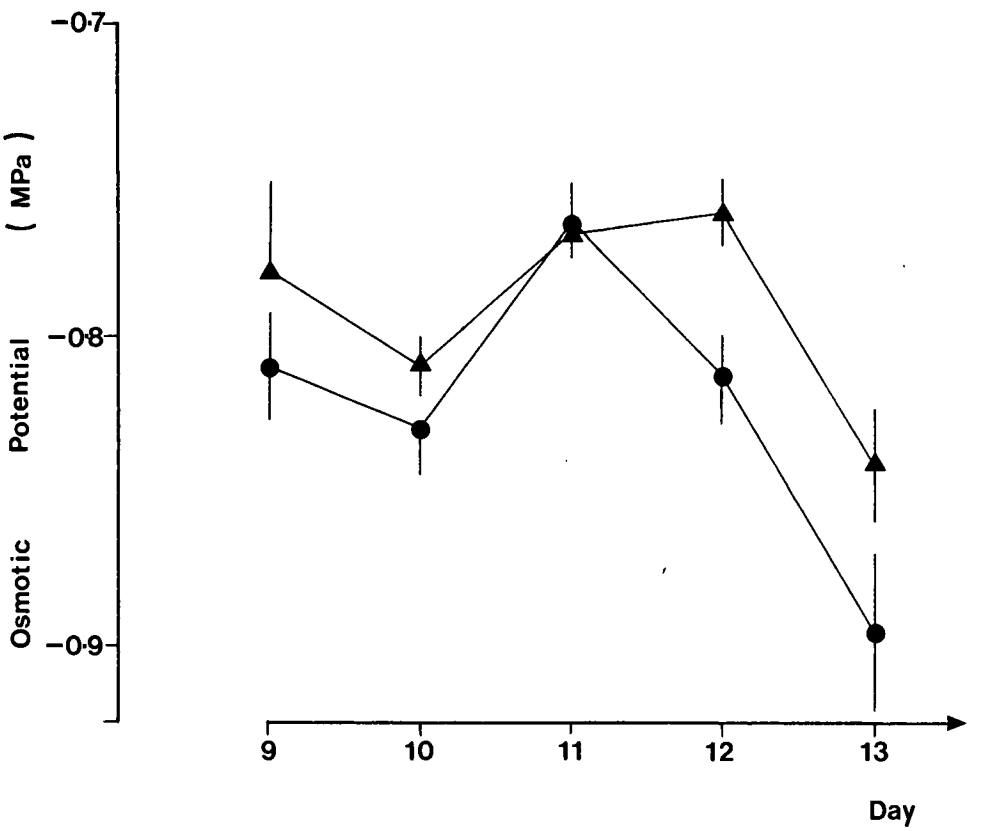
Figures 4.1 and 4.4

Figure 4.1. (top) Comparison of measurements of water potential (Ψ) on primary leaves or whole shoots of identical 8 day old seedlings.

Figure 4.4. (bottom) Time courses of the effects of root cooling (▲) or no treatment (●) on primary leaf Osmotic Potential. All points are means of 5 values with standard errors shown by vertical bars.



Ψ leaf		Ψ shoot	
−0·216	mean	−0·206	MPa
0·006	se	0·004	
6	n	6	



Leaf water potentials of control and RE plants were measured at two to three hour intervals during the photoperiods of days 7 to 10, and both shortly after the beginning and shortly before the end of each dark period. For RC plants, less frequent measurements were made because the number of plants available was limited.

Within one hour of treatment, leaf water potential in RE plants was significantly higher than that of the control group (Fig 4.2, p 121). This difference persisted throughout the remainder of the photoperiod, although, because of increasing variability in the plant material, it was statistically not significant after three hours. In RC plants, treatment was associated with an immediate lowering of leaf water potential by approximately 0.08 MPa (Fig 4.3, p 121). However, after three hours, the difference between the RC and control groups was no longer significant. Notably, the act of transferring plants from vermiculite to solution culture in itself caused only a small and not significant reduction in leaf water potential.

During the dark period following day 7, leaf water potentials of control and RE plants fell slightly, but no difference between the two groups was evident. Nor were any differences between RE and control plants recorded during the photoperiod of day 8, all plants showing a gradual decline in leaf water potential followed by a gradual increase. In RC plants, leaf water potentials measured three hours after the beginning of the photoperiod of day 8 were not significantly different from those of the control group.

In the dark period between days 8 and 9, leaf water potentials in control and RE plants decreased significantly. This effect is unlikely to have been due to water deficit since leaf water content , particularly of

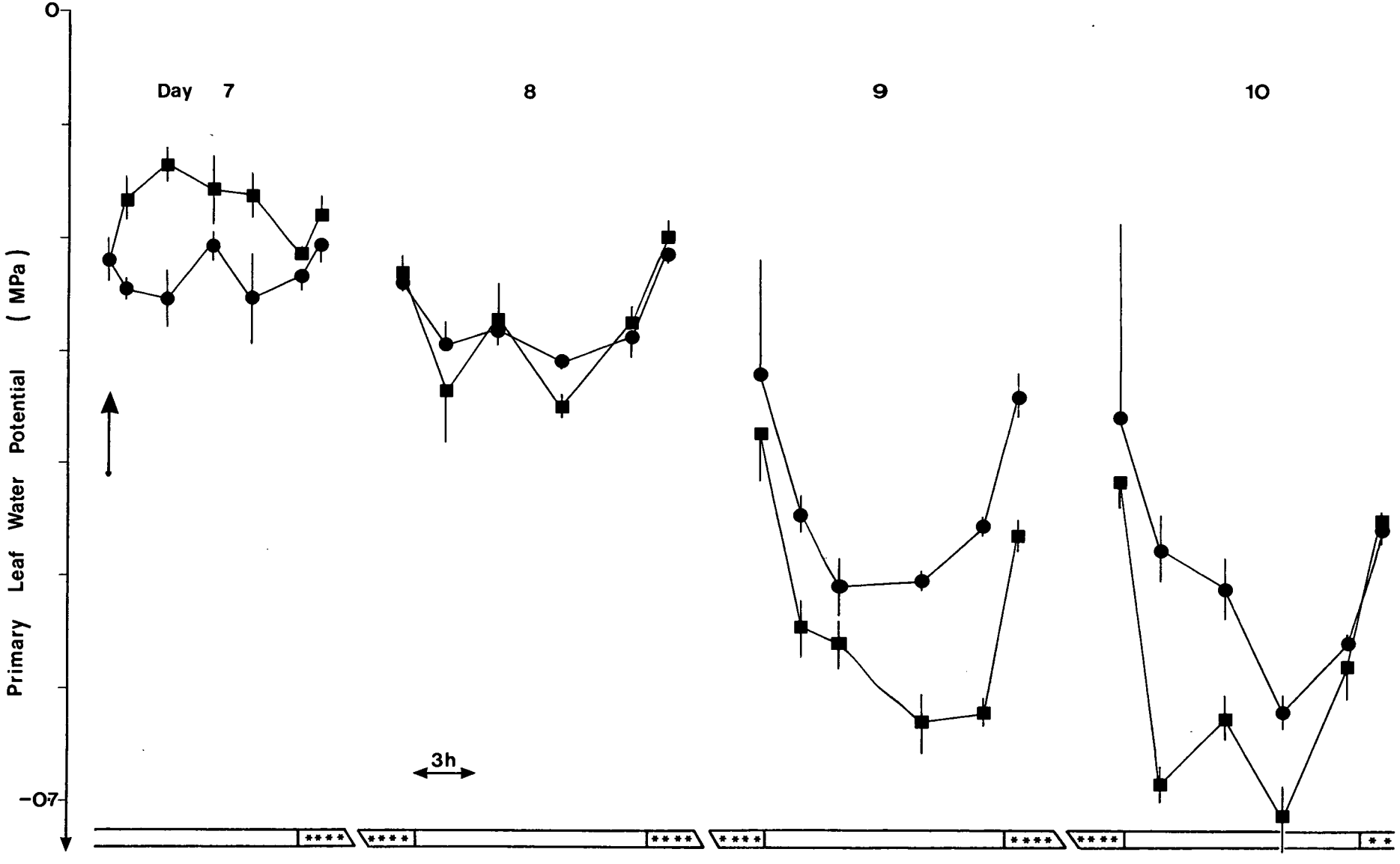
Figures 4.2 and 4.3

Figure 4.2. (opposite) Time courses of the effects of root excision (RE, ■) or no treatment (CONTROL, ●) on Primary Leaf Water Potential.

Figure 4.3. (overleaf) Time courses of the effects of root cooling (RC, ▲) or no treatment (CONTROL, ●) on Primary Leaf Water Potential.

In both Figures the time of transfer of plants to nutrient solution is indicated by the arrow.

All points represent means of 5 or 6 values with standard errors shown by vertical bars.


0
Day 7
8
9
10
Primary Leaf Water Potential (MPa)
3h
-07
****
****
****
****
****
****
**

0
Day
7
8
9
10
Primary Leaf Water Potential (MPa)
3h
-0·7

solution grown plants, generally increases during the dark period (Davies and Van Volkenburgh, 1983). Instead, the reductions were probably part of the general downward trends in leaf water potential which were recorded in plants of all treatments with increasing age. The causes of these gradual reductions in leaf water potential were not investigated, but may have been related to slight decreases in cell osmotic potential or reductions in cell wall elasticity (Tyree and Jarvis, 1982).

Within two hours of the beginning of the photoperiod of day 9, leaf water potentials of control and RE plants showed significant reductions (Figs 4.2 and 4.3, pages 121 to 123). However, the reduction in the RE group was more pronounced, and for the first time, leaf water potential was significantly lower than its control value. Throughout the remainder of that photoperiod, and during a large part of the next, RE plants showed significantly lower leaf water potentials than those of the control group. Fluctuations in Ψ_l values, particularly during day 10, suggest that stomatal closure occurred in these plants, apparently when leaf water potential dropped below -0.65 to -0.70 MPa. In RC plants measurements of leaf water potential around midday on days 9 and 10 were not significantly different from those of control plants. While not ruling out the possibility that water potential decreased in the latter halves of the photoperiods, these results show firstly that RC plants were less affected by treatment, in terms of reductions in leaf water potential than RE plants, and secondly, that for at least part of days 9 and 10, root cooling had no effect on leaf water potential.

4.2.2. Effects of root treatments on leaf osmotic potential, turgor and bulk modulus of elasticity

The cryoscopic and pressure chamber or pressure-volume curve techniques were used to determine leaf osmotic potential, the former on control

and RC plants grown under greenhouse conditions, the latter on control and RE plants from a growth room (Section 2.2.6.3). Although all possible precautions were taken to minimise error, both methods presented considerable practical difficulties. The cryoscopic technique has been criticised because it fails to take into account the possible dilution of cell sap by apoplasmic water and because a further error may be introduced by the formation of low molecular weight compounds during the extraction process (Turner, 1981). The second source of error was minimised here by maintaining all leaf extracts below $4^{\circ}C$ and measuring osmotic potential as soon as possible after harvest of the plant tissue. However, dilution with apoplasmic water could not be avoided, so the results obtained by this technique may overestimate true osmotic potential values by up to 20% (Tyree and Jarvis, 1982).

The pressure-volume curve technique is not subject to dilution errors, but because its completion takes up to one hour, loss of water through evaporation into the chamber may be considerable. In the present experiments, only 3% - 8% of total water content of the leaf was lost in this way. However, this sometimes resulted in up to 15% of the total water expressed being lost, making the interpretation of the PV curves obtained more difficult. A second problem encountered was that only large leaves with petioles greater than 15mm in length gave satisfactory PV curves; smaller leaves yielded volumes of sap which were too small to measure accurately, and since their shorter petioles often barely protruded through the seal of the chamber, it was often impossible to collect all the sap expressed. Consequently, the use of this technique was confined to plants over ten days old.

Root cooling of glasshouse-grown plants had no significant effect on bulk leaf osmotic potential ($\bar{\pi}$) as measured by the cryoscopic tech-

nique until five days after the beginning of treatment (Fig 4.4, page 118). No measurements of osmotic potential were made on seven or eight day old plants because their leaves were too small to yield the minimum volume of sap required. However, significant changes in $\bar{\pi}$ are unlikely to have taken place over such a short period (Tyree and Jarvis, 1982). Between days 9 and 11 inclusive, $\bar{\pi}$ of control and RC plants varied between -0.74 and -0.84 MPa but showed no significant effect of treatment. However, on day 12, $\bar{\pi}$ was approximately 0.05 MPa higher ($p = 0.05$) for RE than control plants. On day 13, the difference between treatments was again not significant but interestingly both RC and control plants showed a marked lowering of $\bar{\pi}$ since the previous day. Overall, bulk leaf osmotic potential fell by approximately 0.07 MPa in both groups and remained within the range -0.75 to -0.88 MPa, although considering the possibility of a 20% dilution error, this range may have been closer to-0.94 to -1.10 MPa. Osmotic potential was generally lower although not significantly so, in control material and no evidence of osmotic adjustment in the leaves of RC plants was found.

A similar pattern emerged from the data for the effects of root excision, treatment resulting in slightly increased osmotic potential values at any given water potential (Fig 4.5, p 127; Table 4.1, p 129). Thus at full turgor, leaf osmotic potential was 0.02 MPa higher in the RE material on day 11 and 0.10 MPa higher on day 14. The same parameter at zero turgor was also higher in the RE material, the difference increasing from 0.02 MPa on day 11 to 0.05 MPa on day 14.

Although several pressure-volume curves were obtained for both RE and control plants on both days studied, only the most complete example of each is used in the present analysis. Thus the significance of the differences just described is doubtful. Nevertheless, once again no evidence was

Figure 4.5

Figure 4.5. A typical pressure-volume relationship for a single intact leaf (actually harvested on day 14 from a plant subjected to root excision treatment). The relationship was obtained by plotting inverse balance pressure against relative water content (RWC) and fitting a straight line to the linear portion of the curve (Section 2.2.6.2.1).

i → inverse of osmotic potential at full turgor (RWC = 1)

ii → inverse of osmotic potential at the turgor-loss point

iii → relative water content at the turgor-loss point

iv → fraction of total leaf water contained in the apoplasm

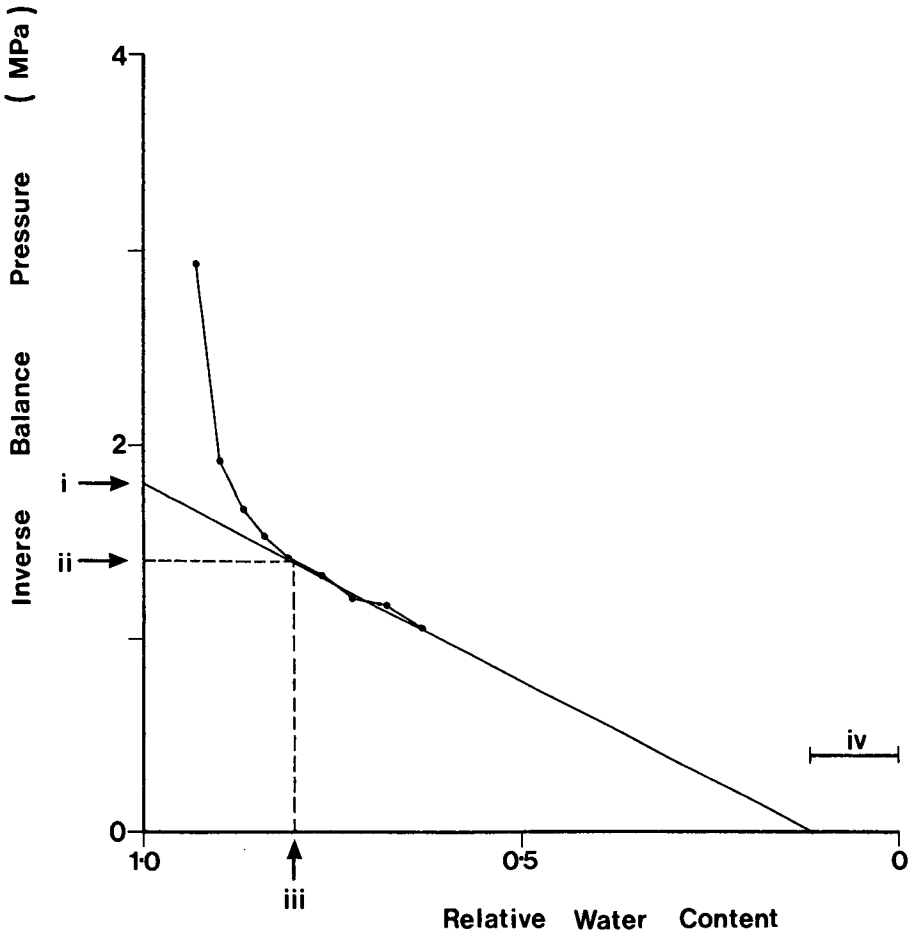

Inverse Balance Pressure (MPa)
4
2
0
i
ii
iii
iv
1·0
0·5
0
Relative Water Content

Table 4.1. Summary of data obtained by pressure-volume analysis of single, intact primary leaves of plants subjected to root excision (RE) or no treatment (CONTROL). Treatment was commenced on day 7 and leaves harvested for analysis on days 11 and 14. (Note that the same pressure-volume curves were also used to construct the Höfler diagrams shown in Figure 4.5.)

		Osmotic potential (MPa)		RWC
Day	Treatment	at full turgor ($-\pi = P$)	at turgor-loss point ($\pi = \psi$)	at turgor-loss point
11	CONTROL	-0.621	-0.758	0.83
11	RE	-0.591	-0.735	0.81
14	CONTROL	-0.651	-0.758	0.85
14	RE	-0.553	-0.709	0.80

(π = Osmotic potential, ψ = Water potential, P = Turgor, RWC = Relative water content)

found to suggest osmotic adjustment took place in response to treatment.

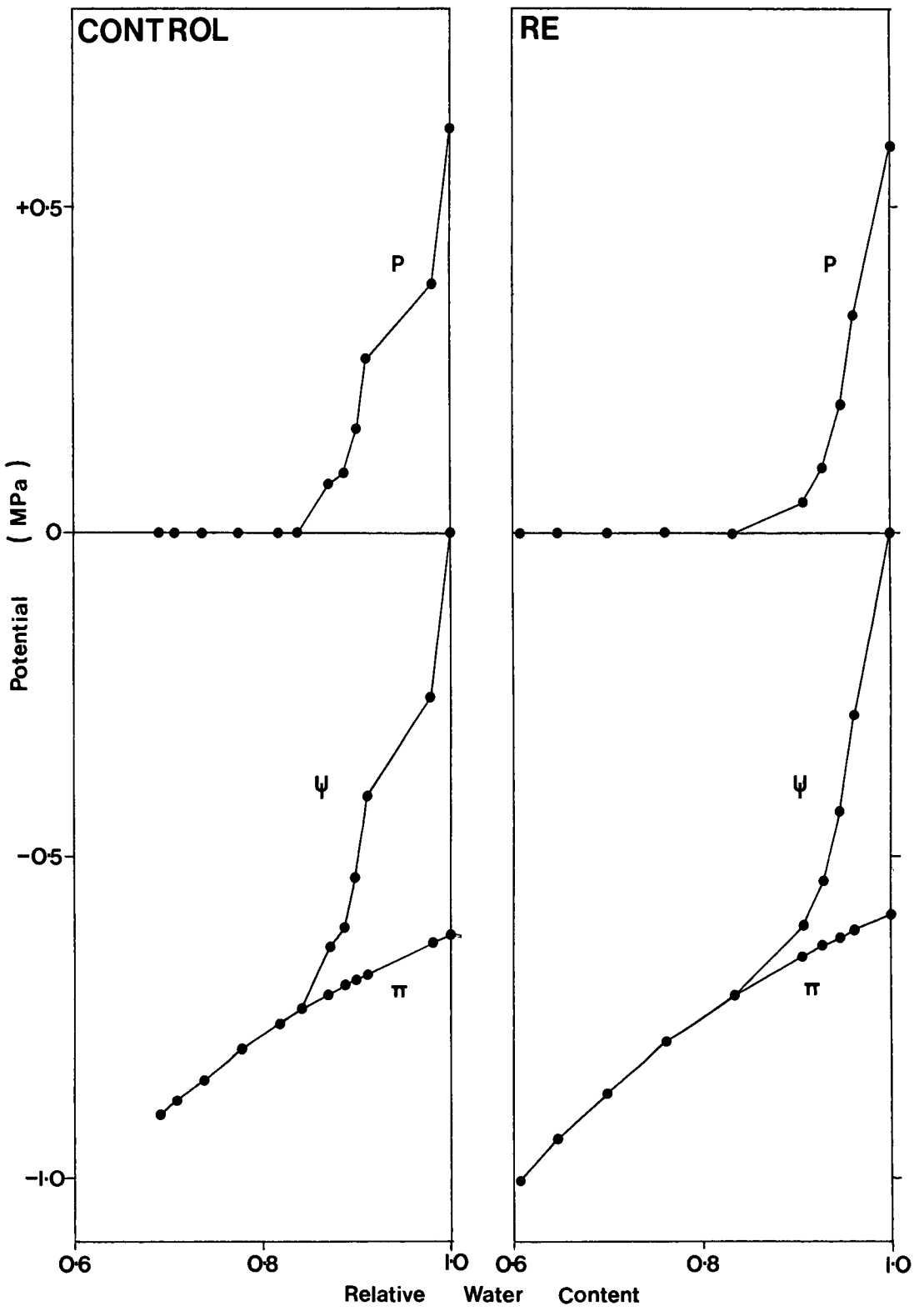
Data from the PV curves described above were used to construct the Höfler diagrams shown in Fig 4.6 (p 131). These allowed values of turgor pressure to be read off from known values of water potential and used in the determinations of wall yield stress (Section 5.3) and the relationship between leaf turgor and leaf extension rate (Section 4.8).

The bulk modulus of elasticity (ε) relates changes in cell volume to those in turgor pressure and thus reflects the elasticity of the cell wall. In the present study, a weight-averaged bulk elastic modulus ($\bar{\varepsilon}$) was calculated from paired values of leaf turgor and leaf relative water content obtained from the Höfler diagrams described above (Fig 4.6, p 131). Since it is not constant but varies with turgor pressure, $\bar{\varepsilon}$ was estimated at a number of relative water contents (Fig 4.7, p 134). Although the results obtained were quite variable, they do suggest that $\bar{\varepsilon}$ may have been higher (ie that tissue elasticity may have been lower) in leaves of control plants, particularly at high RWC values. For plants of both treatments, on both days, $\bar{\varepsilon}$ increased with increasing RWC and turgor pressure although in three of the four relationships obtained, the value of $\bar{\varepsilon}$ corresponding to the maximum RWC was slightly lower than that corresponding to the next highest RWC. On day 11, $\bar{\varepsilon}$ was consistently higher for the control leaf than the RE one, except for one reading (at RWC = 94.5%) which was also considered suspect because of an inconsistency in the corresponding Höfler diagram. On day 14, however, $\bar{\varepsilon}$ was only highest for the control leaf at RWC values above approximately 94%. At lower relative water contents, $\bar{\varepsilon}$ of the RE leaf was consistently the highest. Of significance here, because it reflects the success with which cell wall elasticity can maintain turgor as tissue water content falls, is the

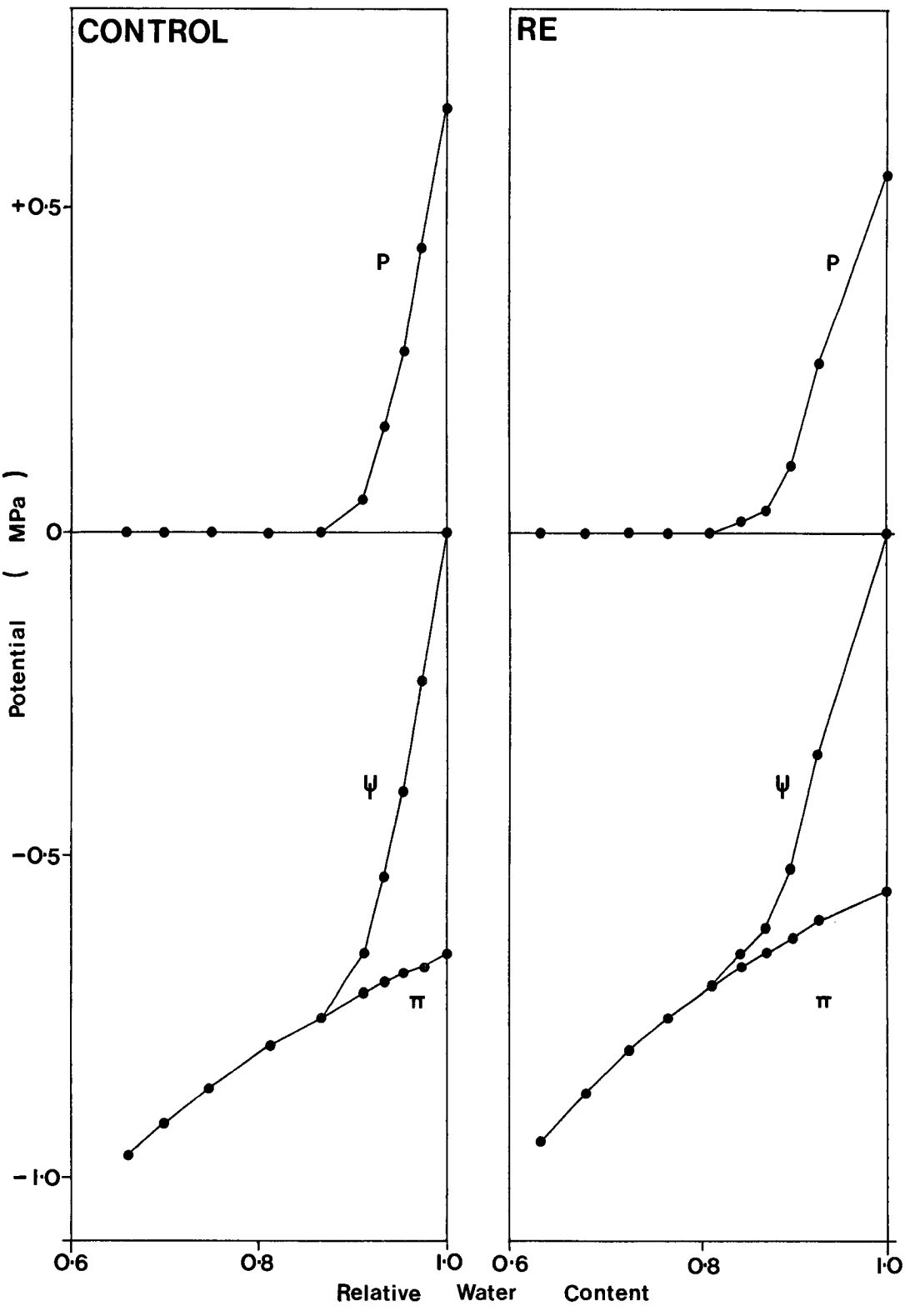
Figures 4.6 a) and b)

Figure 4.6. The total water (Ψ), osmotic (π) and turgor (P) potentials of single, intact primary leaves plotted as functions of relative water content (Höfler diagrams). The data were obtained by pressure-volume analysis of single leaves harvested on days 11 (a), opposite) and 14 (b), overleaf) from plants subjected to root excision (RE) or no treatment (CONTROL).

a)

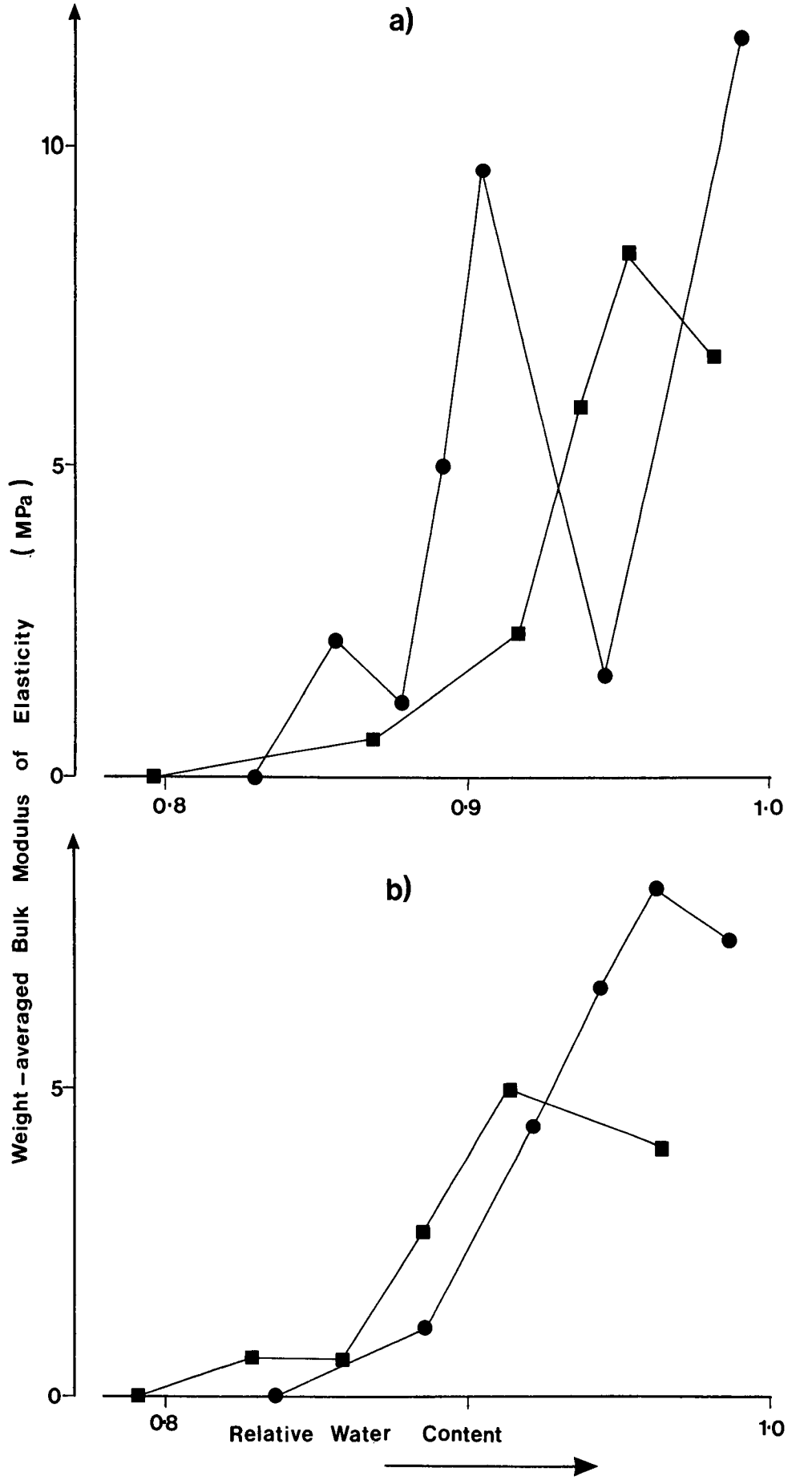

DAY 11
CONTROL
RE
+0·5
0
-0·5
-1·0
Potential (MPa)
P
Ψ
π
0·6
0·8
1·0
Relative Water Content

b)


DAY 14
CONTROL
RE
+0·5
0
-0·5
-1·0
P
Ψ
π
Potential (MPa)
0·6
0·8
1·0
Relative Water Content

Figures 4.7 a) and b)

Figure 4.7. Relationship between weight-averaged Bulk Elastic Modulus and Relative Water Content for single, intact primary leaves harvested on days 11 (a), top) and 14 (b), bottom) from plants subjected to either root excision (■) or no treatment (●).


a)
10
5
0
0·8
0·9
1·0
Weight-averaged Bulk Modulus of Elasticity (MPa)
b)
5
0
0·8
Relative Water Content
1·0

relative water content at the turgor-loss point. On both days, this was highest (ie turgor was maintained at lower relative water contents) for the RE leaf.

As already stated, leaf turgor pressures were obtained when required by reading from the Höfler plots shown in Fig 4.6 (p 131). However, since osmotic potential was only slightly affected by either treatment (Figs 4.4 and 4.5, pages 118 and 127 ; Table 4.1, p 129) and because these effects took several days to develop, it can be concluded that over the critical period for leaf growth between days 7 and 10, the effects of treatment on leaf turgor are unlikely to have differed substantially from those on leaf water potential (Figs 4.2 and 4.3, pages 121 to 123). Thus leaf water potential may be regarded as a reliable index of leaf turgor, any change in the former being indicative of a change in the latter of approximately equal magnitude and direction.

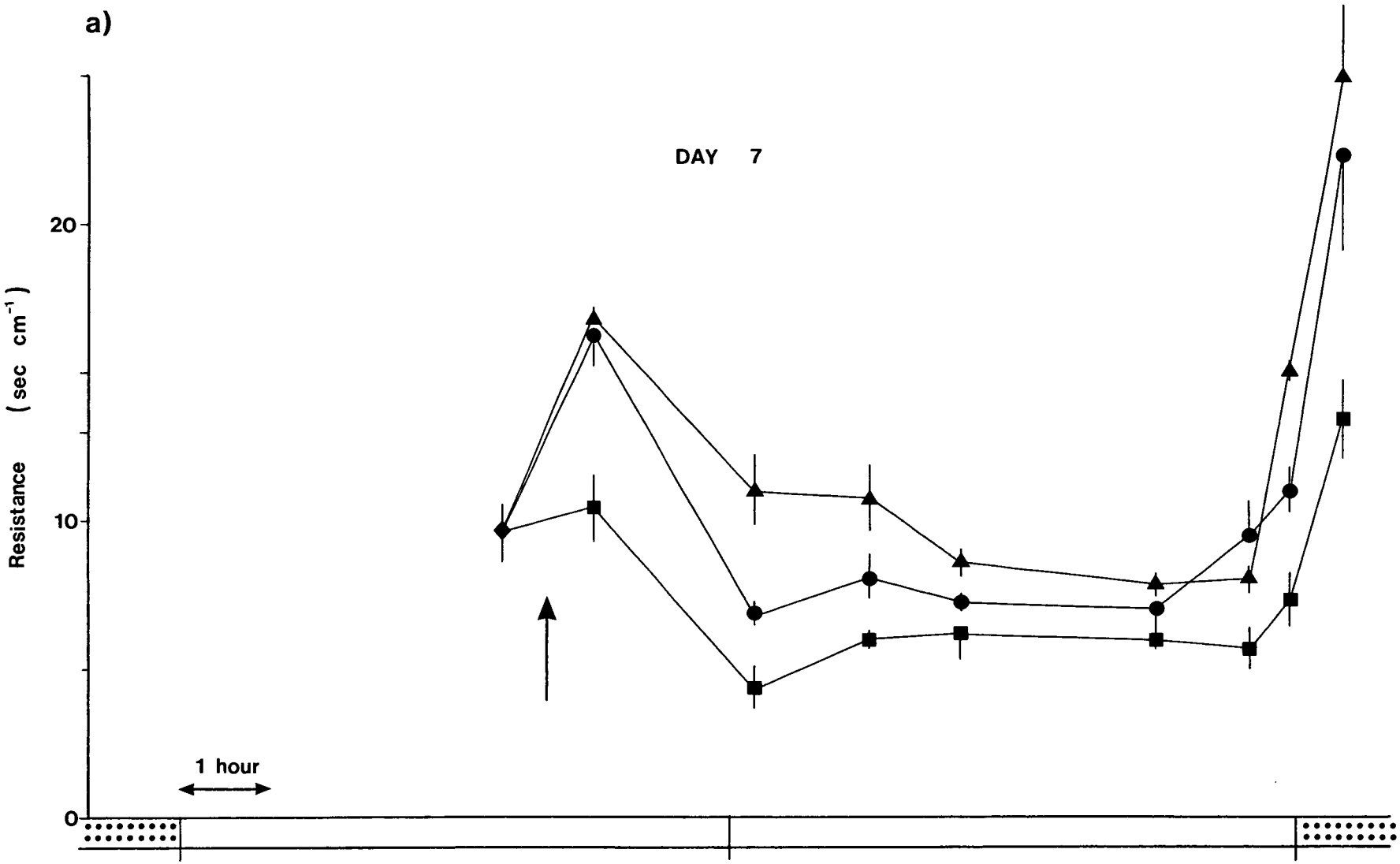
4.3 EFFECTS OF ROOT EXCISION AND ROOT COOLING ON DIFFUSIVE RESISTANCE OF THE PRIMARY LEAVES

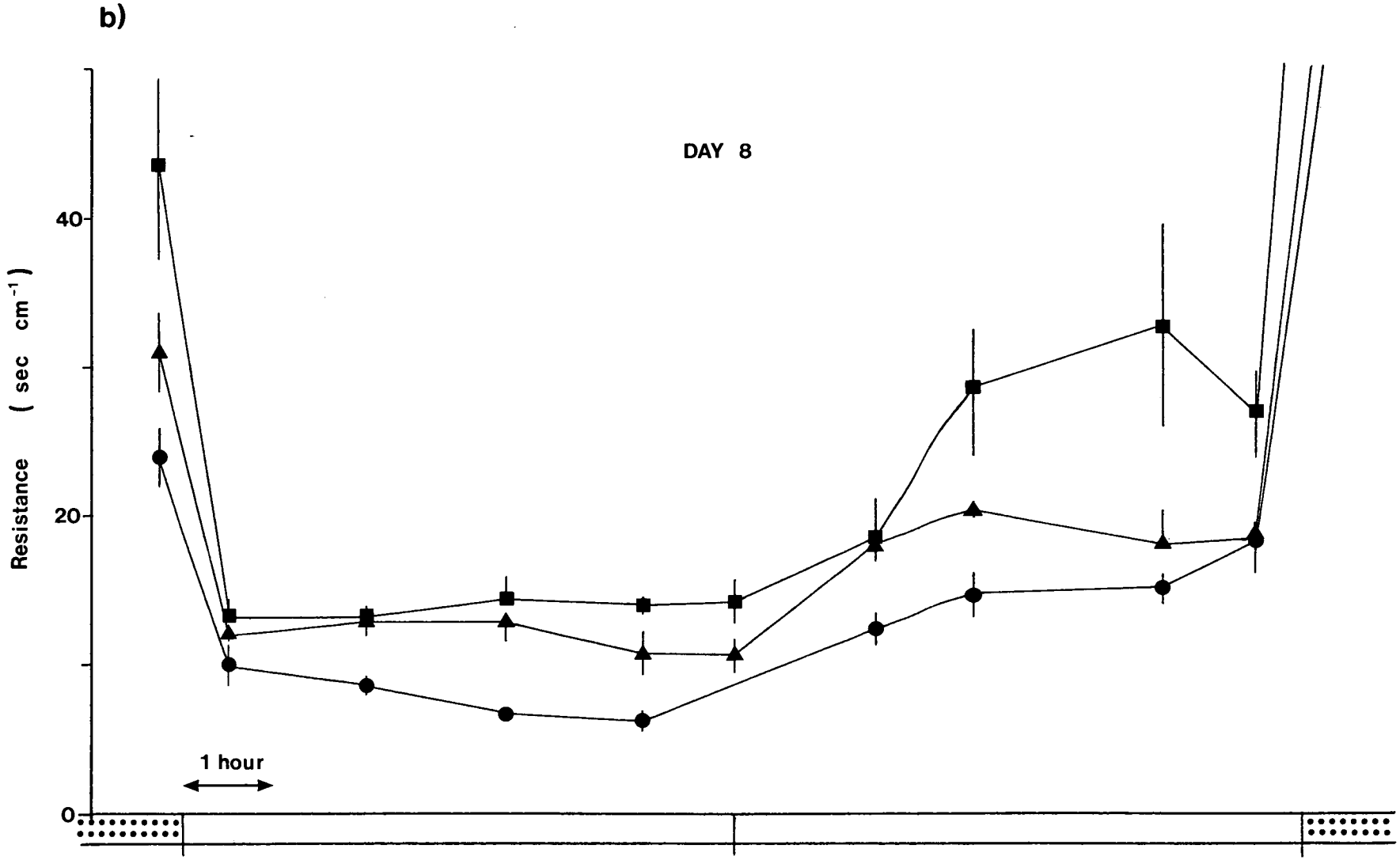
Plants subjected to either root cooling or root excision differed from those of the control group in both the extent and timing of their stomatal movements on all days studied. The two treatments also differed markedly from each other in the details of their effects. Within one hour of transfer from vermiculite to nutrient solution on day 7, plants of the control and RC groups showed small but significant increases in leaf diffusive resistance, while those of the RE group showed no effect (Fig 4.8a, p 137). After a further two hours, leaf diffusive resistance in the control group returned to its original value and remained at that level until shortly before the end of the photoperiod. The same parameter in RC plants remained significantly greater than the control

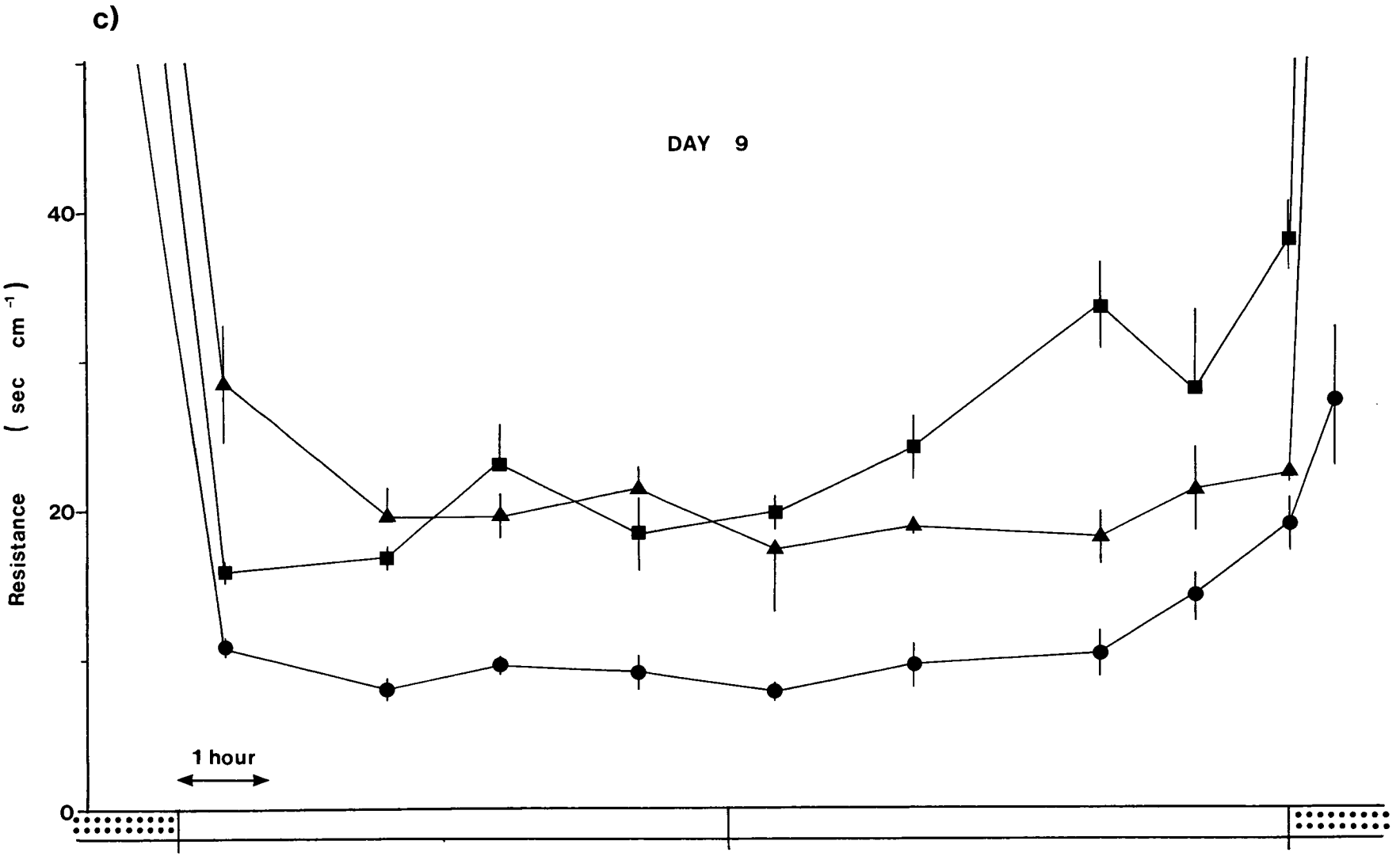
Figures 4.8 a), b) and c)

Figures 4.8 a), b) and c). Daily time courses (days 7, 8 and 9) of the effects of root excision (■), root cooling (▲) or no treatment (●) on the measured Resistance of attached primary leaves. All points represent means of 4 to 6 values with standard errors shown by vertical bars.

Each day consists of a 12-hour light period. In Figure 4.8 a) the time of treatment of plants and transfer to solution culture is indicated by the arrow.


a)
DAY 7
Resistance (sec cm^{-1})
20
10
0
1 hour


b)
DAY 8
Resistance (sec cm^{-1})
40
20
0
1 hour


c)
DAY 9
Resistance (sec cm -1)
40
20
0
1 hour

value for much of the day, while it remained significantly lower in the RE group. Towards the end of the photoperiod, measured diffusive resistances of plants of all treatments converged, but stomatal closure appeared to begin in RC plants before the other groups.

By thirty minutes after the beginning of the photoperiod on day 8, substantial stomatal opening had occurred in all plants, regardless of treatment (Fig 4.8b,p 137). However, for much of the remainder of the photoperiod, RC and RE plants exhibited significantly higher resistances than the control groups. All plants showed increased resistances after the middle of the photoperiod but these were by far most pronounced in the RE group, suggesting significant premature closure of stomata. No such effect was recorded in RC plants.

Large decreases in leaf diffusive resistance occurred in all plants at the beginning of day 9, although those of the RC and RE groups failed to reach the very low values exhibited by control plants (Fig 4.8c,p 137). Subsequently RC and control plants showed more or less constant resistance values, although the former took significantly longer to reach stable levels, implying delayed stomatal opening. Also, an increase in measured leaf resistance occurred in control plants two hours before the end of the photoperiod, perhaps indicating some water stress-induced stomatal closure, possibly related to the relatively large size of the control leaves. Leaf resistance in RE plants during day 9 was more variable, showing significant increases after three and a half, eight and eleven hours of illumination. These data are thus in agreement with the findings of Section 4.2.1 which showed that RE plants experienced a significant drop in leaf water potential during day 9.

During the photoperiods of days 10, 11 and 12, control plants showed

similar patterns of changes in stomatal resistance to those recorded on day 9 (eg Fig 4.9, p 143). However, on the later days, stomatal closure commenced some two to three and a half hours before the beginning of the dark period, suggesting that leaf water deficit had developed by then. In RE plants, leaf resistance, which was always higher than the control value, increased significantly some three to four hours after the beginning of the photoperiods of days 10, 11 and 12, decreasing again after a further five to six hours before increasing once more prior to the beginning of the dark period. These apparent reductions in stomatal aperture which occurred around midday and later, probably took place to counteract developing water deficit, evidence for which was recorded on day 10 (Fig 4.2, p 121).

The effects of root cooling on leaf resistance after day 9, unlike those on previous days, were recorded under glasshouse conditions. Consequently, close control of the light/dark cycle was not possible. However, the results obtained show that in common with root excision, root cooling prevented leaf resistances from reaching the low values exhibited by control plants. Instead, measured resistances, although quite constant in comparison to those of RE plants, were significantly higher than those of control plants throughout each of days 10, 11 and 12 (Fig 4.10, p 143). The data shown were obtained on day 11, but similar trends were recorded on days 10 and 12.

4.4 EFFECTS OF ROOT EXCISION AND ROOT COOLING ON TRANSPIRATION RATE

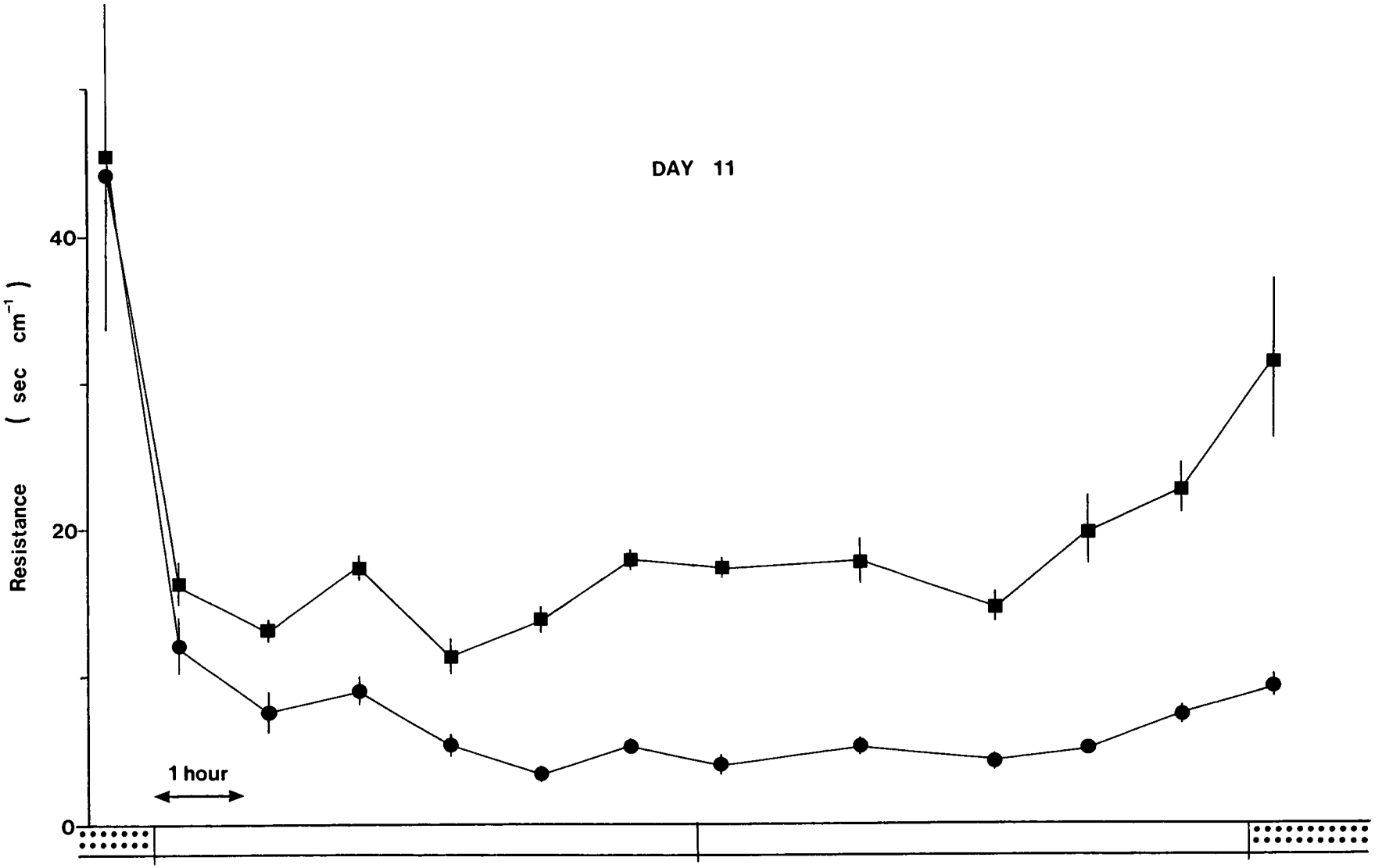
Transpiration rates were calculated from measurements of water-loss over periods of two to four hours during the photoperiods and over each of the twelve-hour dark periods. Measurements of water-loss over whole photoperiods were obtained from the first and last weighings on each day.

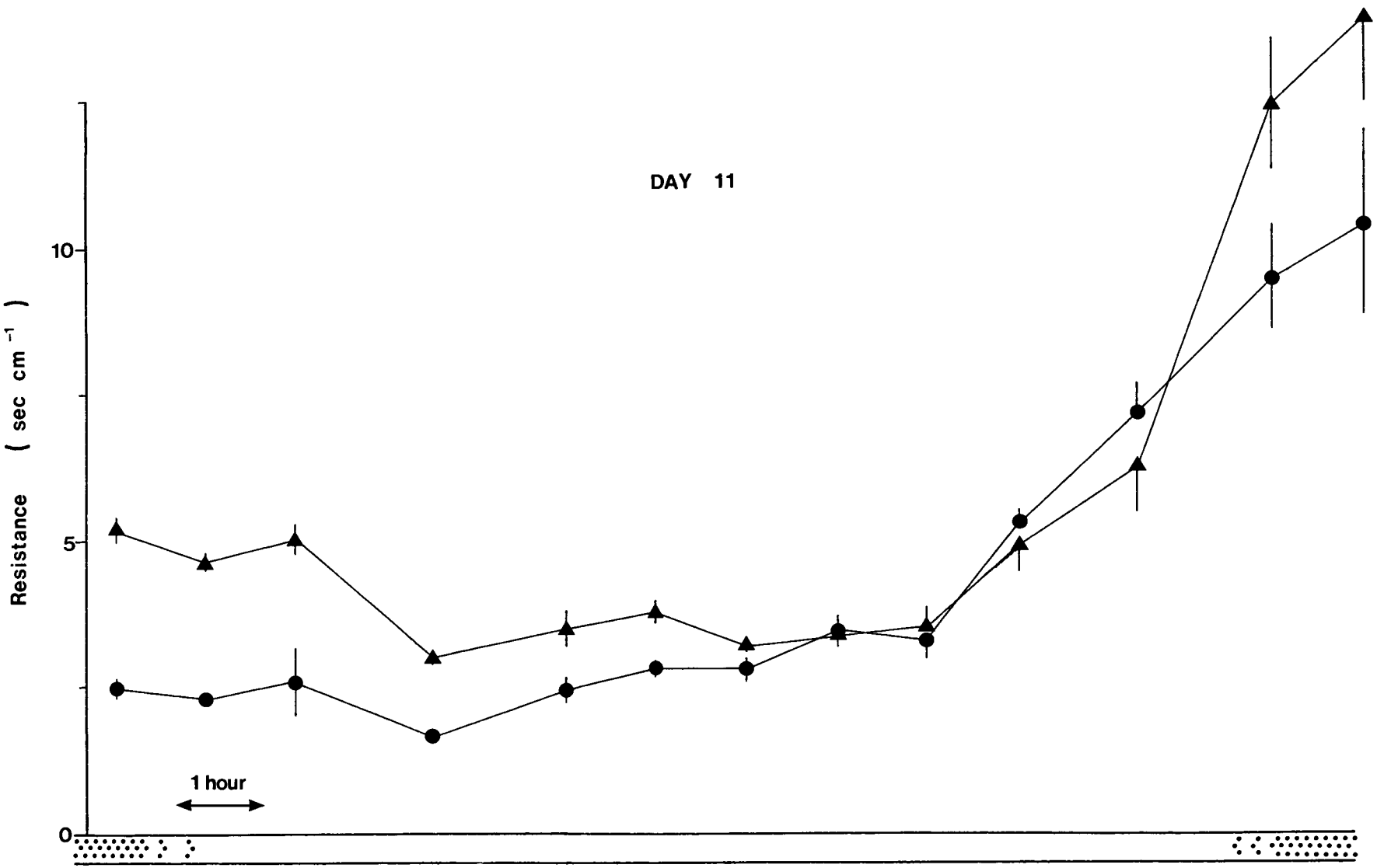
Figures 4.9 and 4.10

Figure 4.9. (opposite) Time courses of the effects of root excision (■) or no treatment (●) on the Resistance of attached primary leaves measured at approximately one hour intervals throughout day 11. The light period was 12 hours in duration.

Figure 4.10. (overleaf) Time courses of the effects of root cooling (▲) or no treatment (●) on the Resistance of attached primary leaves measured at approximately one hour intervals throughout day 11. These plants were grown under natural light but the period of direct illumination was approximately 12 hours in duration.

In both figures, the points represent means of 4 to 6 values with standard errors shown by vertical bars.


DAY 11
Resistance (sec cm^{-1})
40
20
0
1 hour


DAY 11
Resistance (sec cm $^{-1}$)
10
5
0
1 hour

For all plants, daytime transpiration rates exceeded those recorded during the dark periods by a factor of two or more (Table 4.2, p 147). Daytime transpiration rates of control plants generally exceeded those of the treated groups, although no significant difference was found between RE and control transpiration rates on day 7. Maximum RE transpiration rates were recorded on day 7, maximum control and RC rates on day 10.

The detailed effects of root excision and root cooling on transpiration rate are shown in Figures 4.11 and 4.12 respectively (pages 148 and 150). Following transfer to solution culture on day 7, untreated plants had high transpiration rates which decreased as the photoperiod progressed. During the dark period between days 7 and 8, transpiration rate was approximately one third of its maximum rate on the previous day, but it rose again at the beginning of the next photoperiod and reached a maximum after five hours. For the next three photoperiods (days 8, 9 and 10) transpiration rate in control plants followed the same pattern, increasing to a maximum around midday, then decreasing to reach a minimum during the dark period. On day 11 however, transpiration rate increased for the first three hours of the photoperiod, then began to decrease, suggesting that an increase in stomatal resistance, possibly in response to developing water deficit had taken place.

In RE plants, the act of root excision had no immediate effect on transpiration rate, which was not significantly different from that of the control group throughout day 7. However, from three hours after the beginning of day 8, RE transpiration rates were significantly lower than those of control plants and this difference was recorded for the remainder of that day, and throughout each of the next three photoperiods (days 9 to 11). Generally, therefore, transpiration rates reflected the changes in

Table 4.2. Effects of a) root excision (RE) or no treatment (CONTROL) and b) root cooling (RC) or no treatment (CONTROL) on Transpiration Rate (weight of water transpired per unit leaf area per unit time) averaged over each 12 hour light or dark (N) period. All treatments were commenced on day 7, and the results presented are means of 4 values with standard errors in brackets.

a) Transpiration Rate (10^{-2} g cm^{-2} h^{-1})

Day	CONTROL	RE
7	1.105 (0.020)	1.088 (0.041)
7N	0.383 (0.027)	0.404 (0.023)
8	0.759 (0.045)	0.455 (0.027)
8N	0.166 (0.017)	0.155 (0.016)
9	0.962 (0.044)	0.667 (0.075)
9N	0.149 (0.012)	0.121 (0.018)
10	1.166 (0.045)	0.571 (0.060)
10N	0.207 (0.012)	0.121 (0.018)
11	0.993 (0.031)	0.529 (0.042)

b) Transpiration Rate (10^{-2} g cm^{-2} h^{-1})

Day	CONTROL	RC
7	0.983 (0.043)	0.727 (0.039)
7N	0.317 (0.027)	0.382 (0.078)
8	0.956 (0.045)	0.584 (0.052)
8N	0.158 (0.019)	0.188 (0.016)
9	1.206 (0.025)	0.737 (0.019)
9N	0.181 (0.005)	0.253 (0.006)
10	1.339 (0.036)	0.827 (0.007)

Figure 4.11

Figure 4.11. Time courses of the effects of root excision (■) or no treatment (●) on Transpiration rate (weight of water transpired per unit leaf area per unit time). All points represent means of 4 to 6 values with standard errors shown by vertical bars.

(NB. For Figures 4.11 and 4.17 inclusive, light and dark periods were each 12 hours in duration, but for clarity, the latter are shown condensed.
In all Figures the time of transfer to solution culture is indicated by a vertical arrow.)

Transpiration Rate (g cm⁻² h⁻¹)
0·01
0
DAY 7
8
9
10
11

Figure 4.12

Figure 4.12. Time courses of the effects of root cooling (▲) or no treatment (●) on Transpiration rate (weight of water transpired per unit leaf area per unit time). All points represent means of 4 values with standard errors shown by vertical bars.

Transpiration Rate (g cm -2 h -1)
0·01
0
DAY 7
8
9
10

leaf resistance recorded in Section 4.3.

RC plants showed a significant decline in transpiration rate within three to six hours of transfer to low temperature conditions, and this decrease continued during the remainder of day 7. On day 8, transpiration rate was slow to rise, not reaching its maximum value until six to nine hours after the beginning of the photoperiod, and decreased sharply during the three hours prior to the beginning of the dark period. A similar pattern was recorded on each of days 9 and 10, with approximately the same maximum rate being reached on each occasion. Once again, particularly in timing of increases and decreases, transpiration rates correlated well with the stomatal resistance measurements already given (Section 4.3).

4.5 EFFECTS OF ROOT EXCISION AND ROOT COOLING ON ROOT PERMEABILITY

Root permeability was calculated from the transpiration data discussed in Section 4.4 as follows. Each measurement of water loss was expressed in terms of net uptake of water per unit time (gh^{-1}) and divided by the total area of the root system at that time to give a volume flux of water ($gh^{-1}\ cm^{-2}$). Dividing volume flux by the difference in water potential between the shoot and the root medium gave volume flux per unit water potential difference ($gh^{-1}\ cm^{-2}\ MPa^{-1}$) or root permeability. To permit these calculations, a number of assumptions about the plant material and the conditions of the experiment had to be made. Firstly, it was assumed that the uptake of water into the plant and its loss from the whole system (determined by weighing) were equal. A second assumption was that water uptake proceeded at the same rate across the entire surface of the root system, and that this arrangement was unaffected by changes in water flux. This is unlikely to be the case, because it has been shown

that young, unsuberised roots are more permeable to water, and that with increasing water flux, the proximal parts of root systems become more important as absorbing surfaces (Brouwer, 1953; Sanderson, 1983). However, measurements to assess the relative contributions of different parts of the root system to water uptake were not possible within the constraints of these experiments, so volume fluxes and root permeabilities are expressed per cm^2 of total root area (as given in Section 3.3.2). When a root area measurement for a particular time was not available, the required value was obtained by extrapolation of the previous and following known values, assuming that root area increase over the intervening period was linear with time. A similar process was employed to determine leaf water potentials at specific times, although by far the majority of these values were obtained by direct measurement, the exceptions being those corresponding to the middle of each dark period. A final assumption made was that the water potential of the root medium was zero. The water potential difference between the primary leaf and the root medium was then equal in magnitude to the leaf water potential value for that time. It will be noticed that permeability calculated in this way is that of the whole system between the root medium and the leaf mesophyll. However the resistance of the root is likely to be the greatest within the entire pathway, so it is permissible to refer to the permeabilities calculated as those of the root (Stoker and Weatherley, 1971; Aston and Lawlor, 1979; Kramer, 1983).

In all plants, net water uptake was highest at, or shortly after, the middle of each light period and lowest in the dark. In control plants, net uptake increased considerably with plant age up to day 10 (Fig 4.13, p 154). In treated plants, actual values were always lower than those of the control group, and smaller increases occurred. When these data were expressed as volume fluxes (ie per unit root surface area), large differences between

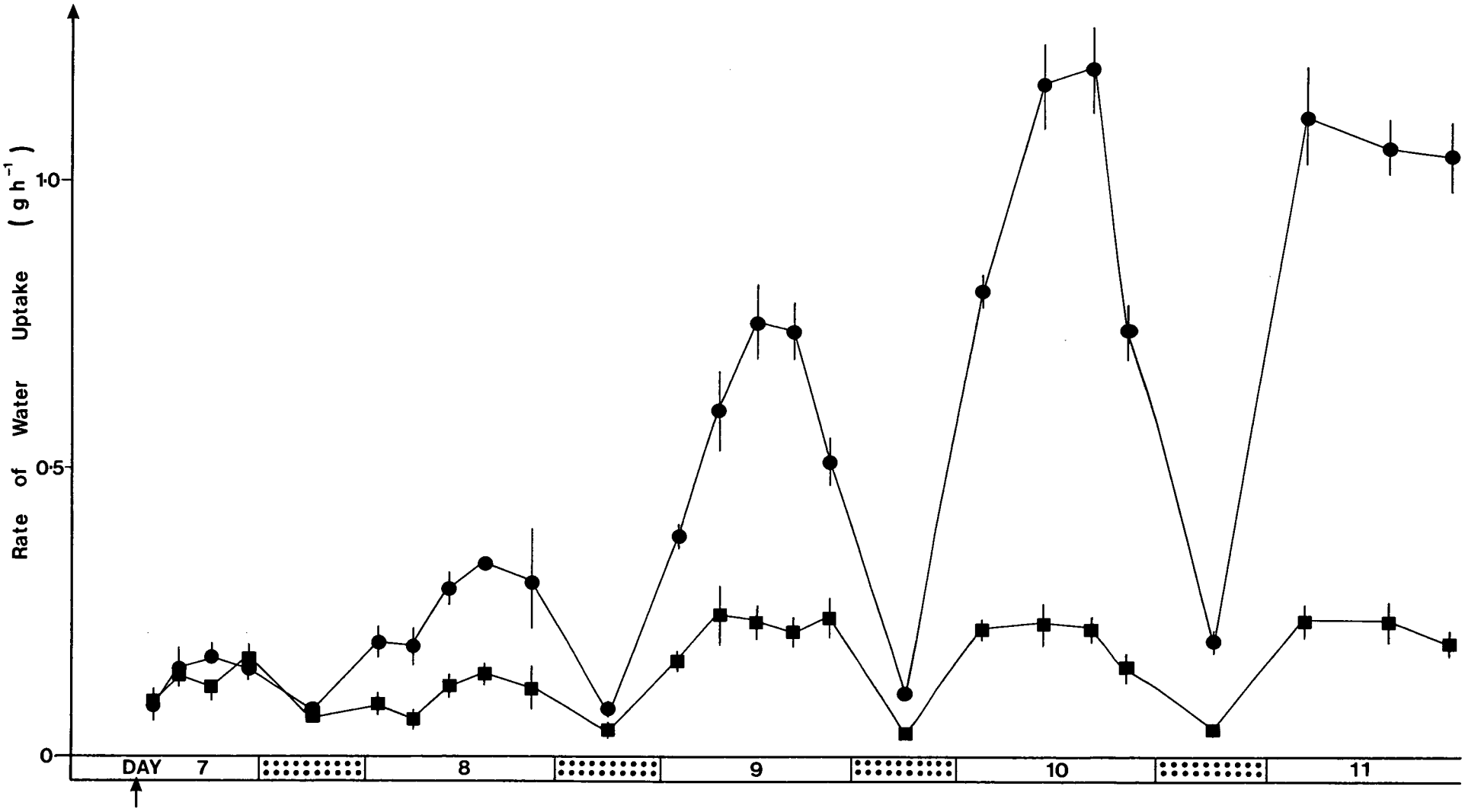
Figures 4.13 a) and b)

Figure 4.13 a). (opposite) Time courses of the effects of root excision (■) or no treatment (●) on absolute Rate of Water Uptake (weight of water transpired per unit time).

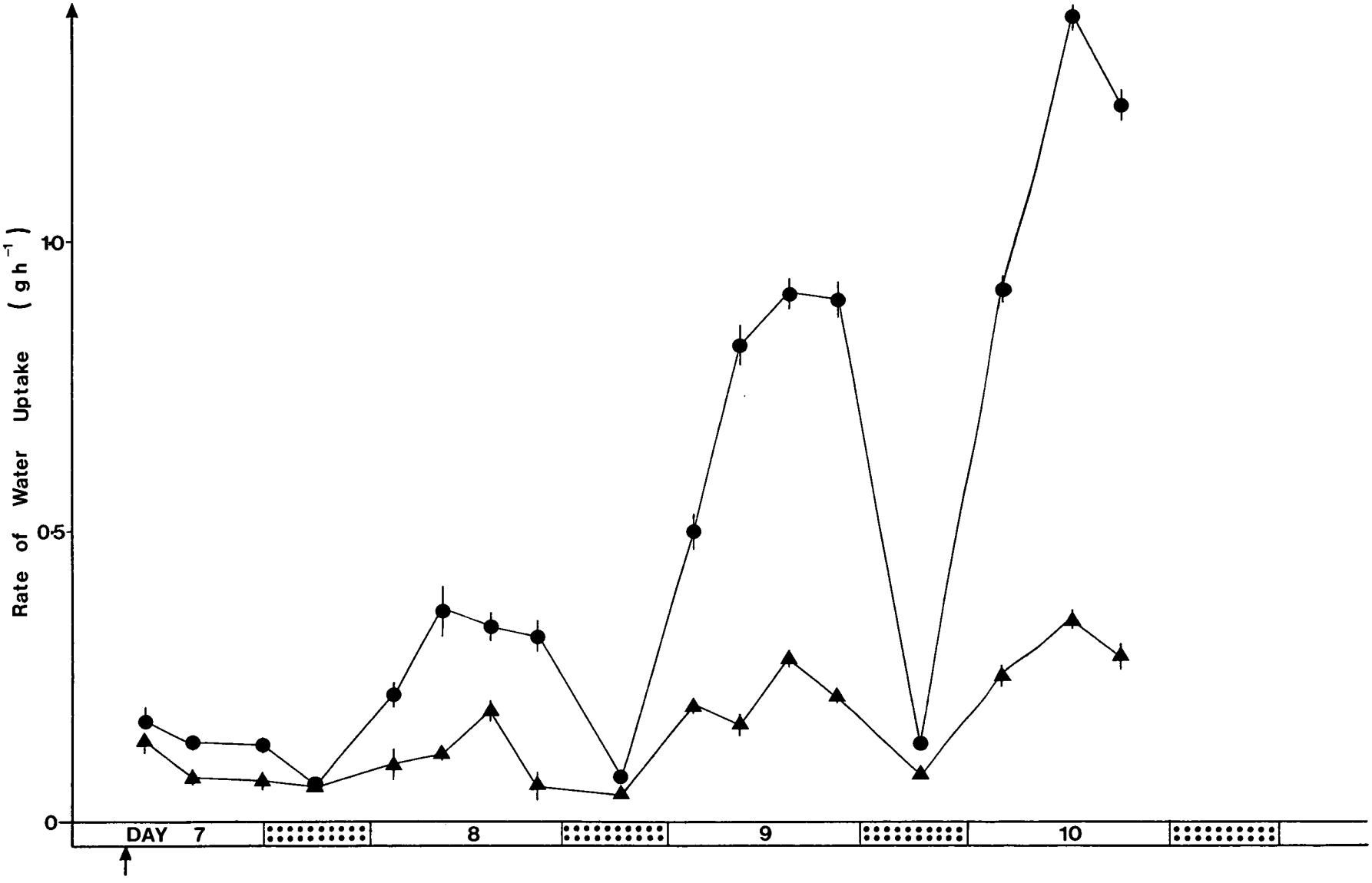
Figure 4.13 b). (overleaf) Time courses of the effects of root cooling (▲) or no treatment (●) on absolute Rate of Water Uptake (weight of water transpired per unit time).

In both Figures, points represent means of 4 values with standard errors shown by vertical bars.

a)



b)

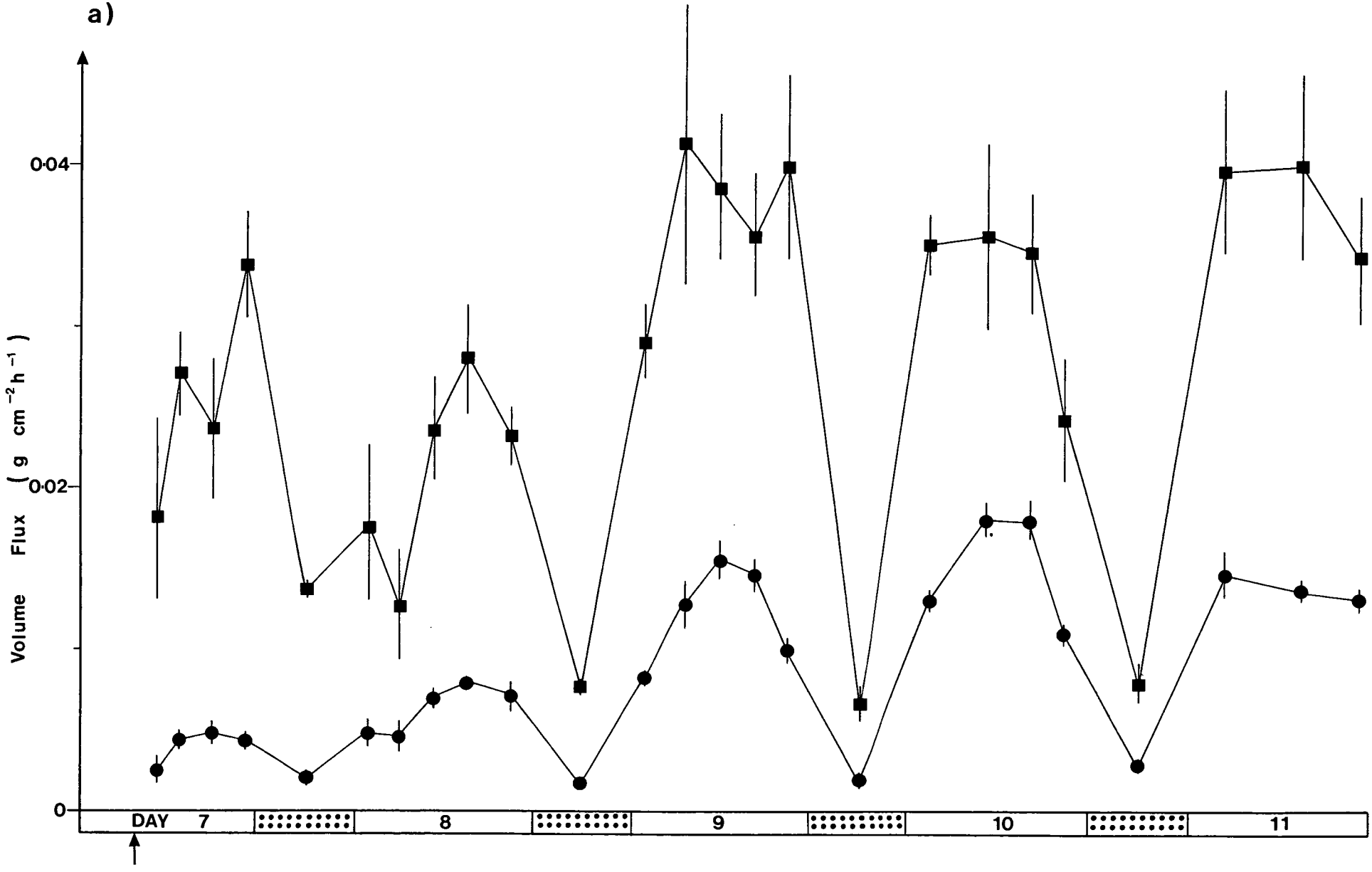


Figures 4.14 a) and b)

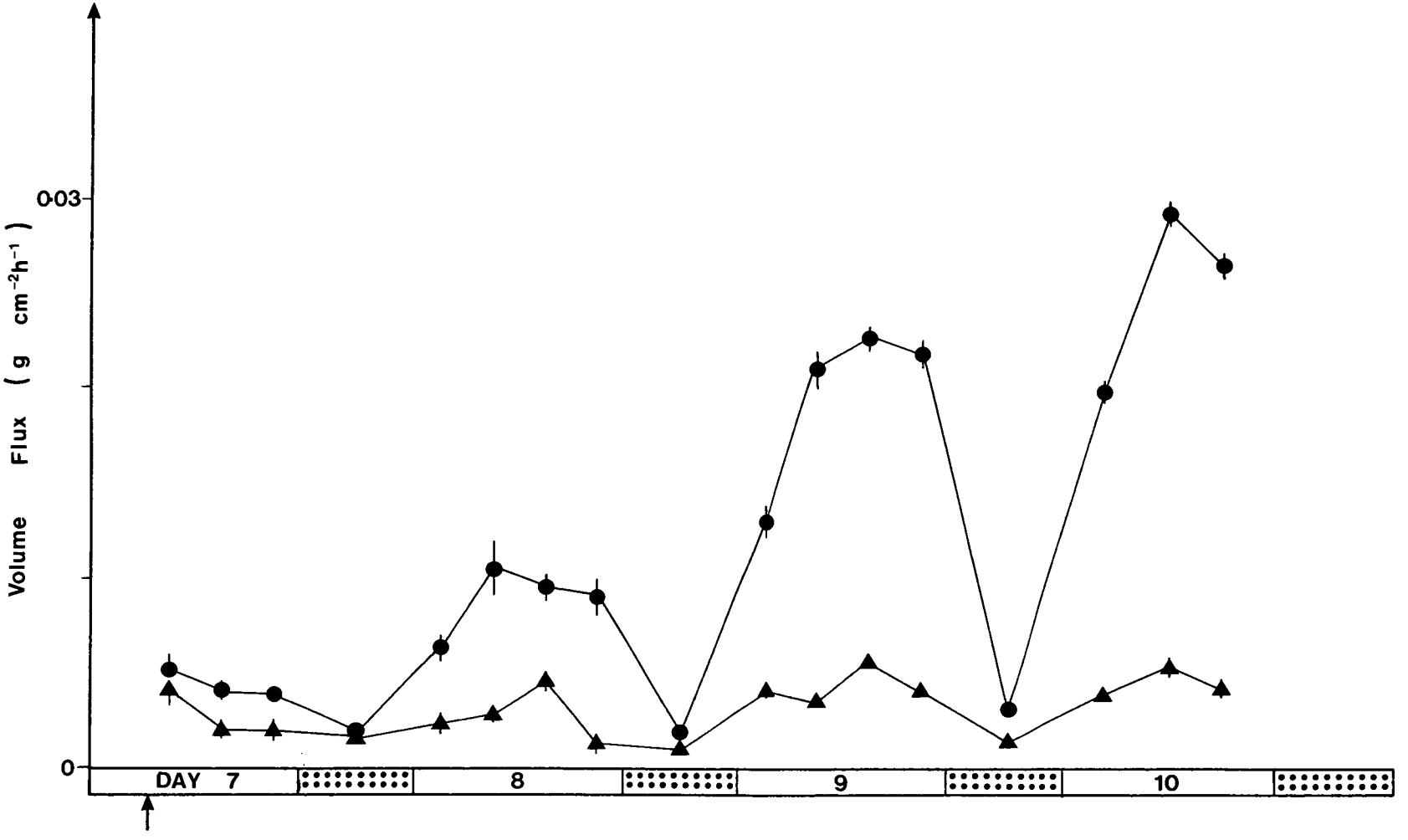
Figure 4.14 a). (opposite) Time courses of the effects of root excision (■) or no treatment (●) on Volume Flux (weight of water transported per unit root area per unit time).

Figure 4.14 b). (overleaf) Time courses of the effects of root cooling (▲) or no treatment (●) on Volume Flux (weight of water transported per unit root area per unit time).

In both Figures, points represent means of 4 values with standard errors shown by vertical bars.


a)
Volume Flux (g cm⁻² h⁻¹)
0·04
0·02
0
DAY 7
8
9
10
11

b)



treatments were observed (Fig 4.14, p 157). In control plants, volume flux showed the same daily pattern as net uptake (highest values around midday, lowest during the dark period); however, the large increases observed in net uptake associated with increasing plant age were not so evident, due to concomitant increases in root area. In RE plants, volume flux showed the same diurnal pattern, but was always greater than the control value. Thus, on average, water crossed the same area of root of RE plants at more than twice the control rate. The opposite effect was recorded in plants subjected to root cooling where volume fluxes were much lower than those of the control root systems, particularly during the photoperiods.

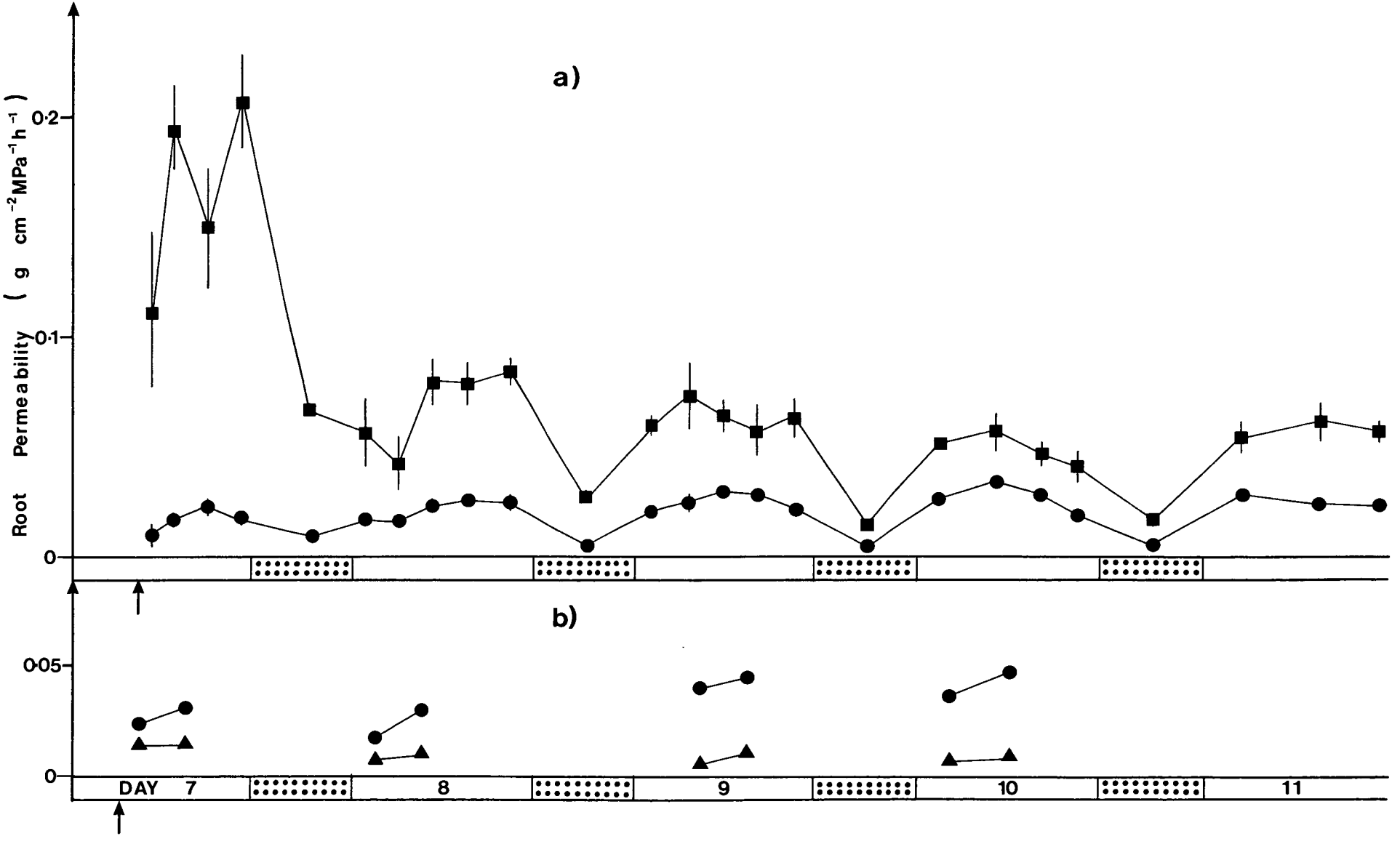
As mentioned above, dividing volume flux by the water potential gradient along the pathway gives the inverse resistance or permeability of that pathway. Using the water potential data described in Section 4.2.1, a detailed time course of the effects of root excision on root permeability was obtained (Fig 4.15 (a), p 161). Because of lack of sufficient water potential measurement, the effects of root cooling could not be shown in such detail. However, the calculations which were possible show the general effects of this treatment (Fig 4.15 (b), p 161).

In control plants, root permeability to water increased during the first half of each photoperiod, then decreased and reached its lowest level during the dark periods. In this, the data resemble those for transpiration rate (Section 4.4) and imply that root permeability may increase as water flow through the plant increases. Plants subjected to the root excision treatment showed a similar pattern of changing permeability with time of day. However, while permeability increased slightly with seedling age in control plants, it declined in the RE group. Nevertheless,

Figure 4.15

Figure 4.15. Time courses of the effects of a) root excision (■) or no treatment (●) and b) root cooling (▲) or no treatment (●) on Root Permeability (weight of water transported per unit root area per unit pressure difference between root medium and shoot per unit time.) All points represent means of 4 values with standard errors shown by vertical bars.

(The standard error bars for the mean values plotted in Figure 4.15 b) (and several of those in Figure 4.15 a)) are too small to show in these diagrams but were approximately 5 to 10% of the means.)


a)
Root Permeability (g cm^{-2}MPa^{-1}h^{-1})
0·2
0·1
0
b)
0·05
0
DAY 7
8
9
10
11

the mean permeability of the RE root systems was always significantly greater than that of the control group.

The actual act of root excision was followed by a considerable rise in root permeability to over ten times its control value (Fig 4.15 (a), p161). This, in turn, was followed by a gradual decline which became more pronounced with the onset of the dark period between days 7 and 8. However, permeability increased at the beginning of day 8 and was over three times the control value by the middle of the photoperiod. Nevertheless, this value was less than half the day 7 maximum for the RE group, and midday values on days 9 and 10 showed a continued, although substantially smaller decline.

In RC plants, the onset of treatment on day 7 was associated with a significant reduction in root permeability relative to the control value (Fig 4.15 (b), p 161). On subsequent days, while the permeability of control plants increased slightly, that of the RC group remained low. Nor was there any evidence for fluctuations in root permeability with transpiration rate, as was found for the control and RE groups, although the data available are insufficient to allow firm conclusions on this point.

4.6 EFFECTS OF ROOT EXCISION AND ROOT COOLING ON THE GROWTH OF THE PRIMARY LEAVES

Results of previous experiments showed that both root excision and root cooling reduced leaf growth after one to two days, and that the effects on leaf growth persisted as long as treatment continued. However, such data provided little information on the detailed effects of the treatments, particularly diurnal trends and effects of seedling age, which must be

known if the mechanisms responsible are to be more fully understood. Nor were previous methods sufficiently accurate to highlight differences between the effects of the two treatments. For these reasons, detailed measurements of leaf expansion and the effects of root excision and root cooling on the process were undertaken.

The most suitable method of measuring short-term changes in leaf growth rate involves the use of a leaf extension meter, incorporating a linearly variable displacement transducer or similar device. Such instruments have been used with success to monitor growth rate changes in cereals and elongate dicot leaves such as Phaseolus trifoliate leaflets (Sharp, Osonubi, Wood and Davies, 1979; Davies and Van Volkenburgh, 1983). However, the technique was found to be unsuitable in the present study because of errors caused by diurnal changes in leaf orientation. Instead, leaf growth was monitored by measuring changes in midrib length with a ruler.

Measurements of midrib length were performed on preselected primary leaves at two to three hour intervals throughout each photoperiod, and at the beginning and end of each dark period. Results were expressed as relative rates of midrib extension (RRME) with units of cm cm^{-1} h^{-1}. Two separate studies of midrib extension were performed, the first recording the effects of root excision, the second those of root cooling. However, since both were conducted under identical (growth room) conditions, their results are considered together.

The general trends recorded in relative rate of midrib extension are summarised in Table 4.3 (p 166). In control plants, highest values were recorded on day 8, agreeing with the findings of other studies that this is the time of most rapid relative leaf growth in this species (Dale, 1964;

Morris and Arthur, 1984). Subsequently RRME decreased gradually with leaf age and showed no evidence of any significant diurnal trend. In contrast, RE plants showed a marked diurnal pattern with significantly higher leaf growth rates occurring during the dark periods, particularly between days 8 and 11. In RC plants, dark period relative extension rates exceeded those during the photoperiods on days 7 and 8, but later, no significant effect was evident. In all plants, regardless of treatment, relative rates of midrib extension showed a general decline with increasing plant age.

When shown in full (Figs 4.16 and 4.17, pages 167 and 169) the RRME results show several interesting effects not apparent from the summary in Table 4.3 (p 166). In control plants, relative rate of midrib extension on day 7 showed no significant change during the five to six hours immediately after transfer but increased significantly over the last two to three hours of the photoperiod, possibly in response to an increase in leaf turgor caused by stomatal closure. A similar, although smaller increase was recorded in RC plants at the same time, but the main effect of root cooling on day 7 was to reduce leaf growth significantly within three to six hours of transfer. In contrast to these effects, root excision failed to reduce leaf growth on day 7 and may even have promoted it, although differences between control and RE rates were not significant.

Plants of all treatments showed slightly higher relative rates of midrib extension during the dark period following day 7, although for the RC and RE groups, these increases were not significant. Notably, control plants had much higher rates than those of the other treatments. At the beginning of the photoperiod corresponding to day 8, all plants showed increases in relative midrib extension rate although once again, the change was not significant in the RC group. During the remainder of the

Table 4.3. Effects of a) root excision (RE) or no treatment (CONTROL) and b) root cooling (RC) or no treatment (CONTROL) on the Relative Rate of Midrib Extension averaged over each 12-hour light or dark (N) period. All treatments were commenced on day 7, and the results presented are means of 4 values with standard errors in brackets.

a) Relative Rate of Leaf Extension (10^2cm $cm^{-1}h^{-1}$)

Day	CONTROL	RE
7	0.783 (0.069)	0.808 (0.025)
7N	2.138 (0.042)	1.483 (0.037)
8	2.122 (0.083)	1.285 (0.111)
8N	1.831 (0.085)	1.563 (0.063)
9	1.029 (0.102)	0.214 (0.045)
9N	0.998 (0.038)	0.904 (0.046)
10	0.476 (0.075)	0.009 (0.019)
10N	0.710 (0.019)	0.853 (0.042)

b) Relative Rate of Leaf Extension (10^2cm $cm^{-1}h^{-1}$)

Day	CONTROL	RE
7	1.211 (0.059)	0.919 (0.295)
7N	2.031 (0.031)	1.137 (0.042)
8	1.883 (0.069)	0.942 (0.081)
8N	1.535 (0.023)	0.989 (0.114)
9	0.804 (0.069)	0.554 (0.036)
9N	0.865 (0.019)	0.463 (0.038)
10	0.300 (0.027)	0.394 (0.031)

Figure 4.16

Figure 4.16. Time courses of the effects of root excision (RE) or no treatment (CONTROL) on the Relative Rate of Midrib Extension of primary leaves. Each pair of vertical blocks represent the means of respectively four CONTROL and four RE values measured over the same 2 or 3 hour period during the day, or 12 hour dark period.

Standard errors are indicated by the vertical bars.

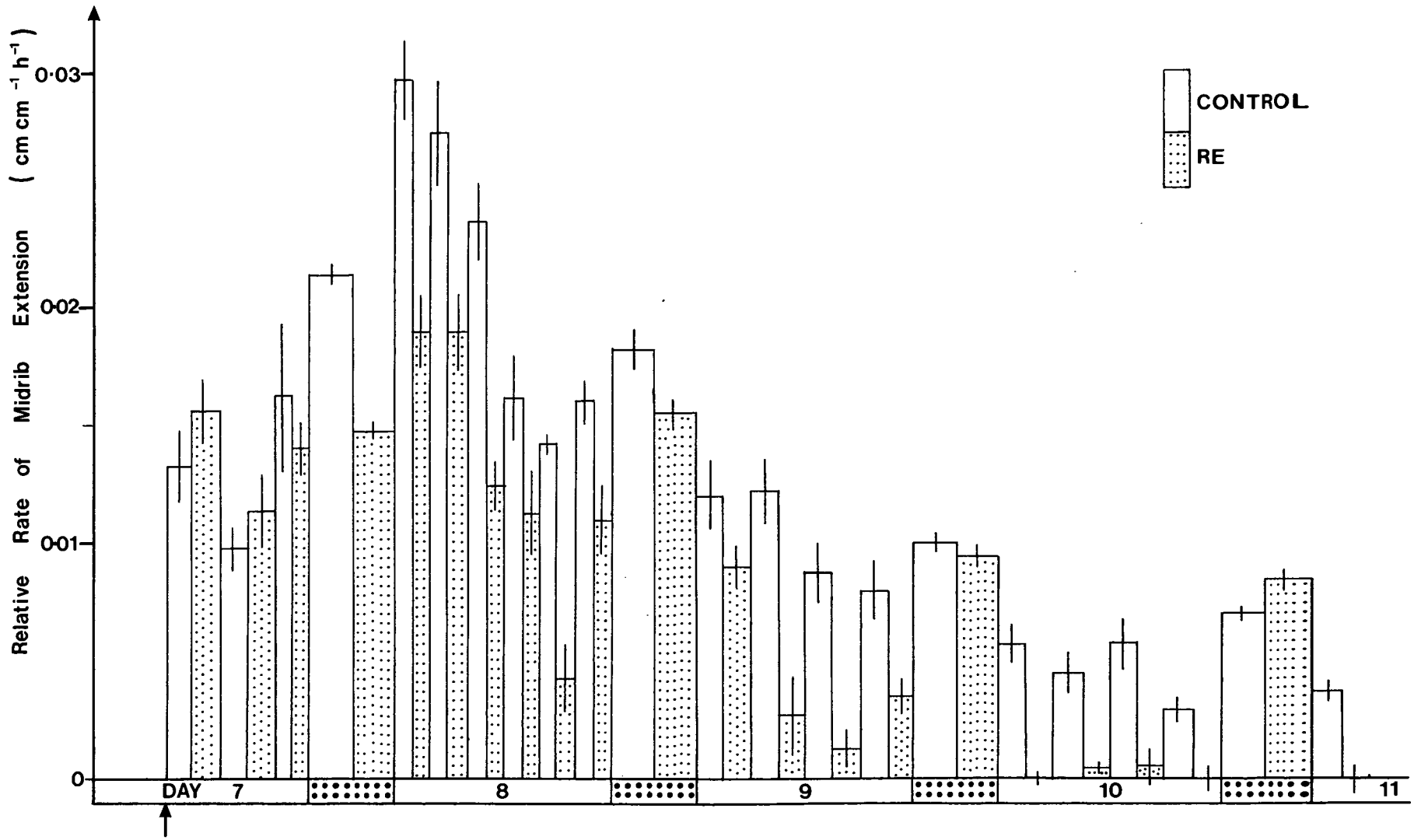

Relative Rate of Midrib Extension (cm cm -1 h-1)
0·03
0·02
0·01
0
CONTROL
RE
DAY 7
8
9
10
11

Figure 4.17

Figure 4.17. Time courses of the effects of root cooling (RC) or no treatment (CONTROL) on the Relative Rate of Midrib Extension of primary leaves. Each pair of vertical blocks represent the means of respectively four CONTROL and four RC values, measured over the same 3 to 4 hour period during the day or 12 hour dark period.

Standard errors are indicated by the vertical bars.

Relative Rate of Midrib Extension (cm cm $^{-1}$ h $^{-1}$)
0·02
0·01
0
DAY 7
8
9
10
CONTROL
RC

photoperiod, rates decreased steadily in RE and control plants but were always higher in the latter group. In RE plants, the minimum rate was reached two to four hours before the end of the photoperiod but was followed by a significant increase, again suggesting the involvement of stomatal regulation of leaf turgor. In RC plants, relative rate of midrib extension fluctuated considerably during day 8 and could not be linked with changes in stomatal aperture.

During subsequent photoperiods, control plants showed significantly higher RRME values than those of the RE group and on day 10, no leaf growth was recorded in RE plants. During the dark periods however, relative midrib extension in RE plants equalled that of the control group. In RC plants, day and night RRME values were approximately the same, and generally lower than control rates over the same periods.

4.7 EFFECTS OF ROOT TEMPERATURE ON THE ABSCISIC ACID (ABA) CONTENT OF THE PRIMARY LEAVES

Because of its suspected involvement in the control of stomatal behaviour, root permeability and leaf cell growth, and because it has been shown to increase in plants in response to a variety of treatments, abscisic acid (ABA) was considered likely to be involved in some of the effects of root treatments described here (Hiron and Wright, 1973; Davies, Mansfield and Wellburn, 1980; Van Volkenburgh and Davies, 1983; Eze, Dumbroff and Thompson, 1983). To examine this hypothesis, the effects of one of the root treatments, root cooling, on ABA content of Phaseolus primary leaves was investigated. Measurement of ABA was according to a well-established method (Jackson, Hall and Kowalewska, 1983; Section 2.2.5.2) in which losses during the extraction and purification processes are monitored by the

inclusion of a tritiated ABA internal standard. Using this practice, average recovery was found to be 53.0%, lower than the 60% - 70% achieved by Pierce and Raschke (1981) using a similar technique, but similar to that of Jackson et al (1983).

Plants were grown on Wisconsin benches in an air-conditioned glasshouse (Section 2.2.2) and harvested for measurement of primary leaf growth and ABA content. In the first experiment reported, the first harvest was conducted at the time of transfer (day 7) and the second five days later, four hours after the beginning of the photoperiod on day 12. The two main temperatures were 18°C (control) and 10°C (RC) but plants maintained at an intermediate root temperature of 14°C were also included. As found in previous experiments (3.3.3), root cooling caused significant reductions in leaf area and fresh weight increases (Table 4.4, p 173). Once again, the lowest root temperature was associated with the greatest inhibition of leaf growth, this trend also being evident in the ABA data. After five days at the root temperature of 10°C, plants showed a six-fold increase in total ABA per leaf, corresponding to more than a doubling of the day 7 concentration (Table 4.4, p 173). In control plants, total ABA per leaf showed a 40% increase in total content over the same period, but ABA concentration fell by over 75%. Thus, leaves of twelve day old RC plants had ABA concentrations ten times those of control plants of the same age. The intermediate root temperature caused a smaller rise in total ABA per leaf and led to ABA concentrations intermediate between those of the other temperatures and not significantly different from the control value on day 7.

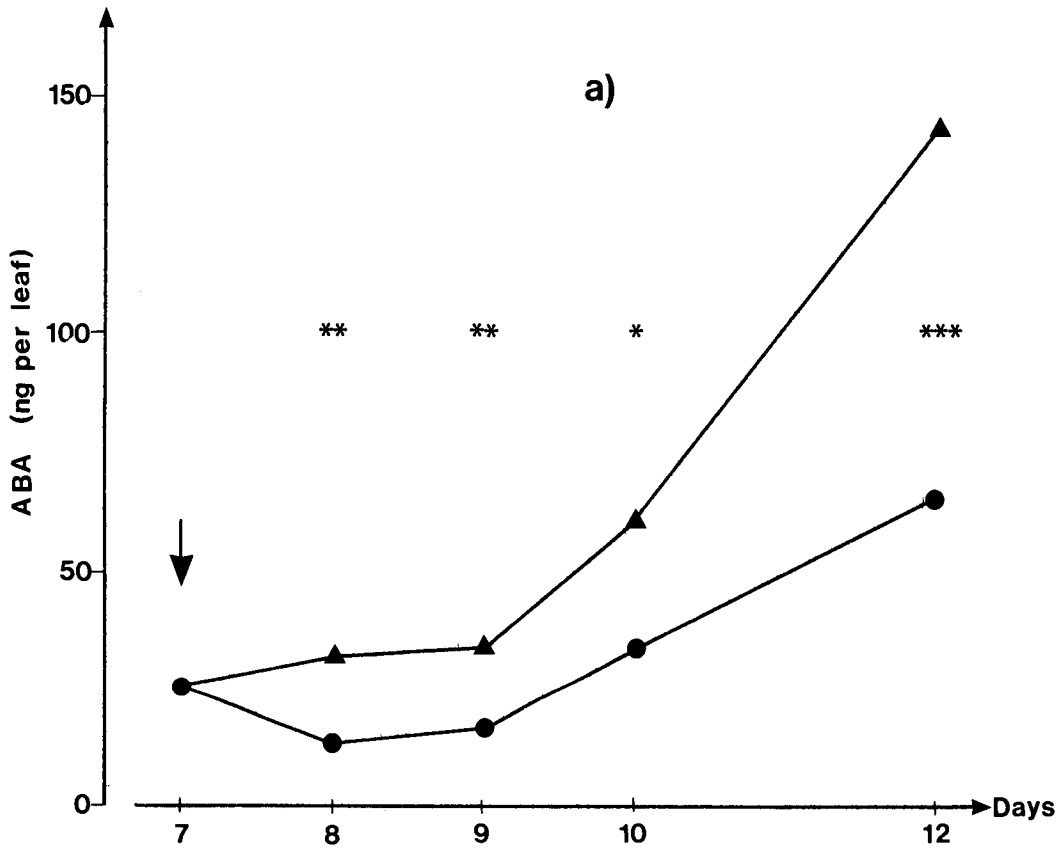
To investigate the time-courses of changes in primary leaf ABA, a second experiment was conducted in which daily measurements of ABA per leaf and per unit fresh weight were obtained. The control root temperature was 18°C and the low root temperature 14°C. Plants at both root temperatures showed overall increases in leaf ABA content during the experiment but on all days, control contents were significantly lower than those of RC plants

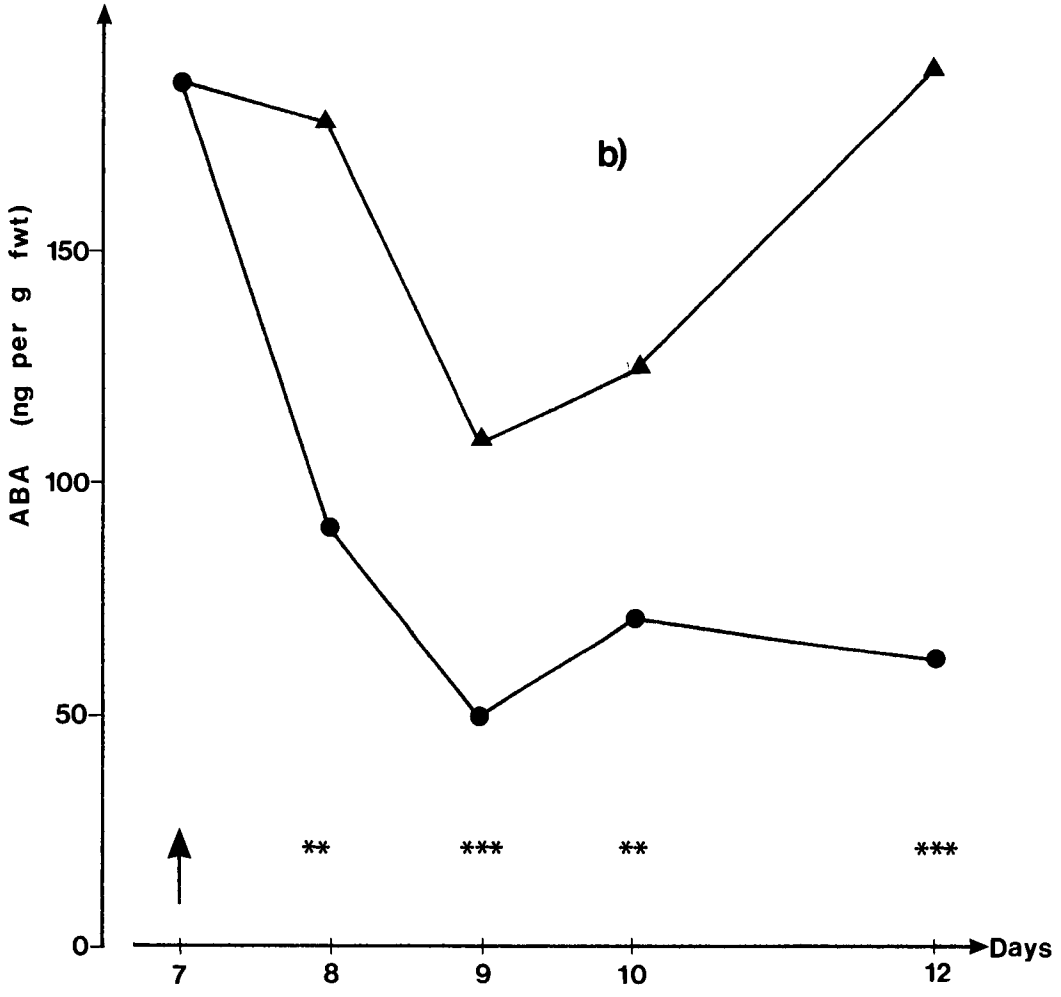
Table 4.4. Effects of different root temperatures on Leaf Area and abscisic acid (ABA) content. The root temperatures used were 18°C (CONTROL), 14°C and 10°C (RC). Plants were harvested on the day of transfer (day 7) to nutrient solution at the chosen temperature and after five days of treatment (day 12). All results are means of 5 values with standard errors in brackets.

Plant age (days)	Root temp. °C	Leaf area (cm^2)	ABA per leaf (ng)	ABA per unit Fwt (ng g^{-1})
7	18	8.1 (0.5)	26.0 (6.8)	184.7 (61.1)
12	18	96.6 (7.4)	43.3 (5.3)	44.3 (3.4)
12	14	54.8 (3.1)	97.4 (5.7)	207.2 (19.9)
12	10	43.2 (1.3)	167.5 (11.6)	441.3 (43.5)

Figure 4.18

Figure 4.18. Time courses of the effects of root cooling (▲) or no treatment (●) on the abscisic acid (ABA) content of primary leaves expressed a) per leaf and b) per gram leaf fresh weight. Each point represents the mean of 4 or 5 values with the significance of differences between means indicated as described in Section 2.2.9. The beginning of the root cooling treatment is indicated by the arrow.


a)
150
100
50
0
ABA (ng per leaf)
**
**
*
***
Days
7
8
9
10
12


b)
150
100
50
0
ABA (ng per g fwt)
**
***
**
***
Days
7
8
9
10
12

(Fig 4.18 (a), p 174). Within one day of transfer, leaf ABA content showed a slight decrease in control plants but no change in those with roots at 14°C. The same results were recorded on day 9, but on subsequent days, considerable increases were recorded in plants with roots at both temperatures. The increase in ABA content of the leaves of low root temperature plants occurred at approximately twice the control rate and by day 12, their leaves contained more than twice as much ABA. In terms of concentrations of ABA, highest values for control plants were recorded in the youngest leaves (day 7). Over the next two days, ABA concentration fell rapidly before reaching a plateau (Fig 4.18 (b), p 174). RC plants also showed an initial decline in ABA concentration, although this was sufficiently delayed for a significant difference between the concentrations in control and RC plants to develop on day 8. The decrease in ABA concentration was also smaller than in control plants and after day 9 was reversed, so that by day 12, the concentration recorded on day 7 had been restored. Consequently, ABA concentration was more than three times higher than the control value by the end of the experiment (Fig 4.18 (b), p 174).

4.8 DISCUSSION

The main aims of the experiments reported in this chapter were to examine the effects of root treatments on plant water uptake and leaf water relations, and to establish whether they could be responsible for observed changes in leaf growth rate. By also considering effects on leaf resistance and ABA content, an understanding of the integrated responses of RE and RC plants to each treatment was sought.

Both root excision and root cooling were found to reduce water uptake.

However, changes in root permeability and particularly stomatal aperture, possibly induced by increased abscisic acid, prevented the development of water deficit, at least over the first forty-eight hours of treatment. Nevertheless, leaf growth rates were reduced throughout that period, suggesting that a simple relationship between leaf water status and growth did not exist.

Effects of root treatment on leaf water relations

In order to avoid such consequences of loss of leaf turgor as impaired photosynthesis, reduced cell growth and structural damage, the mesomorphic plant must maintain a balance between water uptake and water loss (Hsiao, 1973). This might be expected to be a problem for plants subjected to treatments such as root excision and root cooling which might limit the availability of water by reducing root metabolism and the area of root surface available for absorption (Brouwer, 1964; Briggs and Weibe, 1982). The results obtained show that maintenance of favourable water balance, as indicated by leaf water potential, was achieved to varying extents depending upon plant age, time of day and treatment. Within a few hours of treatment, RE plants showed increased leaf water potential, associated with a fall in leaf resistance and unchanged transpiration rate. In RC plants, an immediate reduction in leaf water potential was observed but the control value was restored within a further three hours, possibly through reduced transpiration caused by closure of stomata. On day 8, neither root treatment affected leaf water potential, despite the considerable transpiration rates exhibited by all plants. In fact, no difference between the leaf water potentials of control and RE plants was recorded until the photoperiod of day 9. This finding is consistent with the observation that visible loss of turgor in leaves of RE plants did not occur until late on that day. It was also observed that leaves of RC

plants remained turgid throughout the entire period studied and where available, measurements of leaf water potential confirm this. In RE plants, however, significant reductions in leaf water potential were recorded during the photoperiods of days 9 and 10.

These observations suggest that although root treatments may have reduced water uptake, a favourable water balance was maintained in the leaves of both RE and RC plants for much of the period studied. In this respect, the results differ from several published accounts where root excision and particularly root cooling caused water loss to exceed uptake and leaf water potential to fall and remain below its control value (eg Veen, 1977; Bohning and Lusanandana, 1952). However, these studies differed from the present one in that treatments were applied to mature plants with large leaves and high transpiration rates. In addition, other workers have shown leaf water potential and leaf turgor to be hardly affected by root treatments. Thus Carmi and Koller (1978) found no evidence of water deficit in the leaves of Phaseolus plants subjected to partial root pruning; nor was water stress detected in root-pruned peas (McDavid, Sagar and Marshall, 1973), vines (Buttrose and Mullins, 1968) or sunflowers (Briggs and Weibe, 1982). In the case of root cooling, water potential may be initially reduced but is frequently found to return to its control value within a few days (McWilliam, Kramer and Musser, 1982; Davies and Van Volkenburgh, 1983).

In order to maintain a favourable leaf water balance when water loss threatens to exceed uptake, a number of control processes may be invoked within the plant. Most important amongst these, particularly in the short term, are likely to be reductions in stomatal aperture and increases in root permeability (Aston and Lawlor, 1979). The present evidence, discussed in the following sections, suggests that both processes may have contributed significantly to the avoidance of water deficit, particularly

in RE plants. However, the possibility also exists that specific turgor-maintaining processes took place.

At the level of the leaf cells, turgor maintenance may be brought about by three processes: an accumulation of osmotic solutes, a reduction in cell size or an increase in the elastic properties of the cell wall (Tyree and Jarvis, 1982). By accumulating osmotica and causing osmotic potential to fall, leaf cells can retain turgor while maintaining sufficiently low water potentials to drive water uptake. The advantage of this adaption seems to be that stomatal closure can be prevented and thus photosynthesis maintained throughout the stress period (Hsiao, 1973). In the present experiments, no evidence for such osmotic adjustment in response to either treatment was found. Instead, taking the initial (day 7) value to be approximately -1.0 MPa (J E Dale, unpublished result), leaf osmotic potentials, in common with those of control plants actually increased following treatment. It is conceivable that root treatments inhibited osmotic adjustment by reducing the availability of inorganic ions, particularly potassium (Mengel and Arneke, 1982; Delane, Greenway, Munns and Gibbs, 1982). However, since dry matter accumulated in the leaves of RE and RC plants (Section 3.3.1), organic osmotica may have been available instead (Ackerson, 1981).

That reduced cell size might improve leaf survival under conditions of restricted water supply is suggested by the finding that leaves of stress-adapted plants frequently consist of small, densely packed cells (eg Brouwer and Hoogland, 1964; Quarrie and Jones, 1977). Because a higher proportion of their volume is likely to be composed of non-osmotic solution, small cells can retain turgor at lower water contents than larger ones (Cutler, Rains and Loomis, 1977; Tyree and Jarvis, 1982). In addition

if cell wall elasticity is high, that is, if bulk modulus of elasticity is low, leaf cells can shrink as leaf water content decreases, thus concentrating solutes and further depressing the turgor-loss point (Elston, Karamanos, Kassam and Wadsworth, 1976). Results presented in this and the previous chapter show that both root treatments reduced leaf cell size and that root excision at least also increased cell elasticity. Thus these structural adaptions may have increased the tolerence of leaves of RE and RC plants to fluctuating water contents caused by reduced water supply.

The above mechanisms probably represent long-term adaptions to the detrimental effects of reduced water supply. However, in the short term, the establishment and maintenance of a favourable water balance is more likely to be due to changes in resistance, particularly of the roots and stomata, to the movement of water.

Effects of root treatments on root permeability

In control plants, the pattern of changes in root permeability resembled that of changes in transpiration rate, suggesting that root permeability increased with rate of water flow. If this were not the case, leaf water potential would be expected to fluctuate much more than was observed. Indeed, changing permeability in response to transpiration rate has been proposed as a mechanism by which the plant may maintain a relatively constant leaf water potential under changing atmospheric conditions (Aston and Lawlor, 1979). In plants subjected to root excision, a similar pattern of changing permeability with transpiration rate was observed. However, although RE permeabilities always exceeded those of the control group, they showed marked fluctuations with time.

The very large increase in permeability recorded on day 7 may have been caused by the cutting open of xylem vessels during the excision process which would have eliminated the resistance normally encountered in the cortex and particularly the endodermis (Brouwer, 1961). The relatively unimpeded uptake of water made possible in this way would explain the increase in leaf water potential recorded in RE plants on day 7. Also, increased leaf water content may have been responsible for the observed increase in stomatal conductance through hydropassive movement of guard cells (Millburn, 1979).

The gradual decline in root permeability of RE plants which occurred after day 7 may have been caused by blockage of the open ends of cut xylem vessels by debris or callus (Briggs and Wiebe, 1982). Another contributing factor may have been the development of suberisation of the endodermis associated with root maturation (Brouwer and Hoogland, 1964.) In control plants, such maturation of the basal parts of the root system would be expected to be compensated for by formation of new lateral roots, and the shifting of maximum uptake to these regions (Brouwer, 1953). A similar conclusion was reached by Fiscus and Markhart (1979) who measured root-system hydraulic conductivity of solution-grown bean plants, and found the parameter to increase between days 7 and 12, then to decrease with increasing plant age. They concluded that the increase was due to the formation of first- and second-order laterals, and that the subsequent decrease was caused by the gradual suberisation of these new roots.

The slight increase in root permeability to water recorded in RE plants between days 10 and 11 coincided with the first appearance of new lateral primordia on the seminal root surface. While the lateral roots themselves are unlikely to have contributed significantly to water uptake.

they may have increased root permeability indirectly by rupturing the endodermis during their emergence and thus creating low-resistance pathways for water movement.

The finding that root cooling caused an immediate and prolonged reduction in root permeability is in agreement with the observations of several other groups. Kuiper (1964) showed that a more-or-less linear relationship exists in Phaseolus between water uptake and root temperature, over the range 10°C to 35°C. Initially, the rate of uptake is controlled by the viscosity of water at that temperature. However, within one hour of the onset of root cooling, a change in root membrane permeability takes place and begins to reduce uptake still further. In other plants adapted to warm/temperate environments, a critical temperature exists, below which root permeability is severely reduced (Markhart, Fiscus, Naylor and Kramer, 1979(a)). In Phaseolus, indirect evidence suggests that this may also be the case. Thus pre-treatment of plants by growing at intermediate temperatures, or use of a slow rate of cooling of the root medium both increased plant tolerance to root cooling, apparently by shifting downwards the temperature at which root permeability begins to decrease (Kuiper, 1964; Bohning and Lusanandana, 1952). Apart from effects on root membrane permeability, root cooling might also reduce water uptake by inhibiting or reducing the accumulation of ions in the xylem sap and thus reducing the driving force for entry of water by osmotic flow (Kuiper, 1964).

The foregoing results indicate that particularly on day 7, changes in root permeability played an important role in maintaining leaf water status in RE plants. In RC plants however, no such mechanism existed, since root permeability was reduced by treatment and appeared not to respond to changes in rate of water flux. Instead, the balance between water uptake and loss

in these plants, and in the RE group after day 7, appeared to be maintained primarily by stomatal movements.

Effects of root treatments on stomatal behaviour

Evidence for a major role of stomatal movements in the control of water balance in treated plants is considerable. Thus, although diffusive resistance of RE plants was reduced on day 7, it was higher than the control value on all subsequent days, and premature closure of stomata was suspected on each of days 8 and 11. In RC plants, the onset of root cooling was associated with an immediate increase in leaf diffusive resistance. Throughout the remainder of day 7, and during the photoperiods of all subsequent days, diffusive resistances were consistently higher in RC than in control plants. In addition, root cooling caused delayed opening of stomata on day 9, and premature closure on days 7 and 9. As confirmation of these data, corresponding changes in transpiration rate were also observed.

It should be noted at this point that leaf diffusive resistance has several components, and that a change in measured resistance does not necessarily indicate a change in stomatal aperture. An important component of leaf resistance is mesophyll resistance, which is affected particularly by the extent of intercellular space within the leaf (El-Sharkawy and Hesketh, 1965). Since leaves of RC and RE plants were shown to have more densely packed cells and smaller intercellular spaces than those of the control group (3.3.3) , a difference in leaf resistance from the control value might be due entirely to this difference in leaf anatomy. However, since these changes took several days to develop, they may be of little significance here. Another possible cause of misinterpretation of diffusive resistance results might be a

difference between treated and control plants in stomatal pore size. Thus, if leaves of RC or RE plants had smaller stomata than those of the control group, measured diffusive resistances might be found to be higher in the treated plants even when stomata were fully open. However, stomatal density would be expected to rise in RE and RC plants because of reduced leaf area and possibly cancel out such an effect of reduced pore size. In the absence of further data, therefore, it must be concluded that an effect of treatment on leaf diffusive resistance probably does represent a change in stomatal aperture.

The mechanisms by which stomatal aperture was controlled in RC and RE plants are not immediately evident from the data obtained. It seems likely that premature closure of stomata may have been caused by hydropassive movements of guard cells in response to falling leaf turgor; certainly premature closure was frequently associated with a preceding decrease in leaf water potential. However, the sustained high resistances recorded must be due to some other mechanism since, particularly on day 8, no associated changes in leaf water status were recorded. In similar experiments, Aston and Lawlor (1979) found that cooling or excision of roots of solution-grown sunflower plants caused rapid stomatal closure which was not associated with changes in leaf water status. Their conclusion was that stomata responded directly to changes in the flow of water across the root, although no specific mechanism was proposed. Partial stomatal closure in the absence of a change in leaf water status was also observed in maize plants subjected to localised drying of the root system (Blackman and Davies, 1984). Here the conclusion was that full stomatal opening depended upon the continuous supply of cytokinin from the roots. Interference with that supply through root drying caused a rapid and sustained increase in stomatal resistance. While such a mechanism is unlikely to operate in Phaseolus because of the known insensitivity of

the stomata of dicotyledonous plants to cytokinins, at least when applied exogenously (Willmer, 1983), the general concept that treatment reduced the supply of some factor necessary for the maintenance of stomatal aperture is conceivable. Alternatively, stomatal aperture may have been regulated by some substance produced or accumulated in response to root treatments. In view of its proposed role in the control of stomatal aperture in water stressed plants (Wright and Hiron, 1969) abscisic acid would seem likely to be involved.

The effects of root temperature on the abscisic acid (ABA) content of the primary leaves

Root cooling caused a substantial increase in the ABA content of the primary leaves, the effect being evident within one day of the beginning of treatment and persisting throughout the experiment. In addition, the leaves of RC plants had the highest concentrations of ABA on all days. Two important questions are raised by these data: whether the ABA was synthesised in the leaves themselves or accumulated by some other mechanism, and whether the increased concentrations of ABA were responsible for any of the recorded effects of treatment on shoot development and physiology. In view of its proposed role in the mediation of the developmental responses of plants to water deficit and other stresses (Davies, Mansfield and Wellburn, 1980), the possibility exists that ABA performed a similar function in RC (and RE) plants.

The finding that ABA accumulated over the entire period of root cooling rules out the possibility that the increase was due solely to an immediate response at the beginning of treatment. Instead, ABA may have accumulated in the primary leaves through (i) increased synthesis or a change in the balance between synthesis and breakdown, (ii) increased import from other

plant organs or (iii) increased retention in the leaves, of ABA normally exported to the remainder of the plant.

Although increased synthesis of ABA has been recorded in response to a number of stress treatments (eg Mizrahi, Blumenfeld and Richmond, 1972; Jackson, Hall and Kowalewska, 1983; Henson, 1984) most studies have considered the effects of water deficit (eg Wright and Hiron, 1969; Walton, Galson and Harrison, 1977; Eze, Dumbroff and Thompson, 1981). Indeed, it has been proposed that lowering of tissue water potential or turgor is a pre-requisite for increased synthesis of ABA, regardless of the stress treatment applied (Eze, Dumbroff and Thompson, 1983). In the present experiments, the accumulation of ABA recorded on day 8 in RC plants may have been due to synthesis induced by a reduction in leaf turgor on day 7. However, the same mechanism is unlikely to have been responsible on subsequent days since no significant change in leaf turgor was recorded.

In the absence of local synthesis, primary leaf ABA in RC plants may have risen through increased synthesis elsewhere in the plant and subsequent transport to the shoot. In maize plants, for instance, root cooling has been shown to increase the concentrations of growth inhibitors in the xylem sap (Atkin, Barton and Robinson, 1971). Also, root tips of Phaseolus coccineus have been shown to possess the ability to synthesise ABA (Hartung and Abou-Mandour, 1980). Thus, the ABA which accumulated in the primary leaves of RC plants may have originated in the root tips and been synthesised in response to the low temperature, prior to transport in the transpiration stream.

The third way in which root cooling may have caused the accumulation of ABA in the primary leaves is by reducing its export from them. One

effect of low root temperature is to reduce the sink strength of the root system and to lower its demand for assimilate and other substances originating in the shoot (see Discussion, Chapter 3). Davis and Lingle (1961) proposed such a mechanism to explain the effects of root cooling on shoot growth in tomato, suggesting that growth was inhibited by the accumulation of material normally metabolised in the roots. Such accumulation of normally translocated material within source leaves was induced in soybean plants by pod removal or petiole girdling and found to result in a considerable increase in leaf ABA which was due to reduced translocation rather than increased synthesis (Setter, Brun and Brenner, 1980 a, b). Similar experiments with millet gave the same findings and confirmed that leaf water deficit was not necessary for leaf ABA to rise (Henson, 1984).

An interesting interpretation of the data obtained in the present study is that the accumulation of ABA which occurred in response to root cooling may have contributed to the maintenance of shoot water balance. One important way in which this might have been brought about is through an effect on stomatal behaviour. No correlation appeared to exist between stomatal resistance and leaf water status in RC plants because while leaf turgor probably fluctuated in the course of each photoperiod, stomatal resistance although substantially higher than the control value remained relatively unchanged. Instead, the sustained high values of stomatal resistance may have been caused by the elevated concentrations of ABA. That the concentrations of ABA in the leaves of RC plants were sufficient to affect stomatal aperture is confirmed by the finding that in fully expanded leaves of Phaseolus, stomatal closure was initiated at leaf ABA concentrations of 99 ng g^{-1} fresh weight (Walton et al, 1977), similar to those recorded here. Furthermore, other instances of correlations

between stomatal resistance and leaf ABA concentration but not leaf water status have been found, for example in waterlogged peas (Jackson et al, 1983) and soybean and millet plants with lowered rates of translocation from source leaves (Setter et al, 1981; Henson, 1984). A second way in which increased ABA may have contributed to the maintenance of shoot water balance is through increased root permeability (Davies, Mansfield and Wellburn, 1980). In studies with exogenous ABA, several groups have shown the growth regulator to enhance water flux through plant root systems by affecting ion flux and root membrane permeability (Karmoker and Van Steveninck, 1978; Markhart, Fiscus, Naylor and Kramer, 1979(b)). In the present experiments, however, such a mechanism probably did not operate since no detectable increase in root permeability of RC plants was found.

Another way in which increased leaf ABA may have affected shoot water balance is through reductions in leaf cell enlargement and lamina expansion. Apart from the reduced rate of water-loss likely to result from a reduction in lamina area, reduced cell size would also benefit the plant by contributing to turgor maintenance (Tyree and Jarvis, 1982). Two possible mechanisms have been proposed by which ABA might reduce leaf cell growth: a direct effect on leaf cell wall plasticity or an indirect one via stomatal closure on the availability of assimilate (Van Volkenburgh and Davies, 1983). Another proposed effect of ABA, that of reducing phloem loading (Vreugdenhill, 1983) could also contribute to turgor maintenance in the shoot by causing assimilate to accumulate in leaf cells with a resultant reduction in osmotic potential. The present finding that leaf growth and specifically leaf cell enlargement were inhibited by root cooling in the absence of associated changes in leaf water status but with an associated increase in leaf ABA suggests that ABA

may be involved in the control of leaf growth. In support of this hypothesis, Quarrie and Jones (1977) found that the effects of water deficit on leaf morphology in wheat, notably reduced cell size, could be reproduced by exogenous ABA, apparently in the absence of any reduction in leaf turgor.

The relationship between leaf growth and leaf water status in control, RE and RC plants

The central aim of the work reported in this chapter was to determine the extent to which the reductions in leaf growth caused by root excision and root cooling were due to effects on leaf water status. Certainly leaf growth in other systems has been shown to be highly sensitive to leaf water potential and turgor pressure (Boyer, 1970; Barlow, Boersma and Young, 1976; Hsiao, Acevedo, Fereres and Henderson, 1976; Bunce, 1977). In addition, leaf cell expansion has been identified as the component process of leaf growth most affected by water deficit (Hsiao, 1973; Clough and Milthorpe, 1975, Bradford and Hsiao, 1982a) although in a few studies cell division was equally or more affected (Terry, Waldron and Ulrich, 1971; McCree and Davies, 1974). Thus the general effects of root excision and root cooling on leaf growth and leaf cell expansion recorded in the present study are consistent with the known effects of plant water deficit.

Faced with similar evidence to the above, several groups have concluded that root treatments such as excision and cooling inhibit leaf growth solely by altering the water status of the leaf cells. In a classic study, Brouwer (1964) subjected Phaseolus seedlings to a range of root temperatures and concluded that reduced shoot and leaf growth were caused by an unfavourable water balance. A similar conclusion was reached by Unger and Danielson (1967) with the same plant material, but neither

group performed sufficiently detailed measurements of leaf water status to confirm their conclusions. Also, in contrast to the previous results, Davies and Lingle (1961) found that low root temperatures which reduced leaf growth in tomato had no effect on leaf water relations. In the case of root excision too, the involvement of altered leaf water status in the effects on leaf growth is incompletely established. Thus only in studies where leaf area was particularly high was reduced leaf turgor considered to be the sole cause of reduced leaf growth (Troughton, 1974) and in many accounts, little or no change in leaf water status was found (Buttrose and Mullins, 1968; McDavid, Sagar and Marshall, 1973; Carmi and Koller, 1978; Briggs and Wiebe, 1982).

A common deficiency in published work is that measurements of leaf water relations and growth are not performed on the same material, or are too infrequent to permit reliable determination of correlations. Often the index of leaf water status used is leaf water potential or relative water content, when in view of its role as the driving force for cell expansion, leaf turgor would be the most appropriate (Green, Erickson and Buggy, 1971; Ray, Green and Cleland, 1972). In the absence of measurements of turgor, leaf water potential (Ψ_l) may be used, provided that the relationship between that parameter and leaf turgor (P) is established. In the present experiments, this was achieved by measuring osmotic potential ($\bar{\pi}$) and assuming $P = \Psi_l - \bar{\pi}$ (Turner, 1981).

Ideally, leaf extension rate and turgor should be measured simultaneously on the same leaf; the latter possibly by micro-pressure probe (Van Volkenburgh and Cleland, 1984). In the work reported, this was not possible but care was taken to ensure uniformity of treatment and conditions, thus allowing general conclusions to be drawn from the leaf water relations

and growth data obtained (Sections 4.2 and 4.6). Viewing these results together it is clear that although leaf turgor, as represented by leaf water potential was an important factor in the control of leaf growth, the relationship between turgor and growth was not a simple or constant one, but varied, particularly with treatment. This was most evident in the case of root excision.

Initially, the effects of root excision on leaf growth and turgor (estimated from water potential) were closely correlated. Within two to three hours of treatment, leaf water potentials of RE plants were slightly higher than those of the control group and a small, although not statistically significant, increase in leaf extension rate also occurred. However, during the dark period following day 7, when leaf turgors of the control and RE groups were considered to be approximately equal, leaf extension rates of control plants were substantially higher. During the photoperiod of day 8 and the subsequent dark period a similar pattern of comparable leaf turgors but higher control leaf extension rates was found. In fact, between the end of the photoperiod of day 7 and the beginning of that of day 9, leaf extension rates of RE plants were approximately 30% lower than those of the untreated group. On later days too, evidence suggests that leaf growth in RE plants was limited by some factor other than leaf turgor. Thus on day 10, leaf growth continued in control plants at leaf water potentials which caused complete cessation of growth in the treated group. The relationship between leaf growth and water status in RC plants is less clear because fewer measurements of leaf water relations parameters were made. However, leaf extension rates were consistently lower in the RC group even when leaf turgors of control and RC plants were shown to be the same, again suggesting that treatment altered the sensitivity of leaf growth to turgor pressure.

That the relationship between leaf growth and turgor might be variable and affected by different treatments or environmental factors has only recently become fully recognised (Van Volkenburgh and Cleland, 1984). Previously most attention had been given to the direct effects of treatments on leaf turgor (Boyer, 1968; Acevedo, Hsiao and Henderson, 1971). However, recent work has shown the sensitivity of leaf extension to turgor to be a major factor in the control of leaf growth. Bunce (1977) showed that the response of leaf growth to turgor in soybean could be altered by mild water stress and suggested that in addition to its effect on turgor, water deficit also altered the turgor/growth relationship. More recently, Michelena and Boyer (1982) found leaf extension in maize to be reduced by water stress even though osmotic adjustment was sufficient to maintain turgor in the growing regions. Also, Davies and Van Volkenburgh (1983) showed that trifoliate leaves of Phaseolus plants subjected to low root temperatures had turgor pressures similar to those of control plants, but exhibited substantially reduced growth rates. Considering the model of cell growth proposed by Lockhart (1965; see Chapter 1 for discussion), the conclusion from these data is that drought and root cooling may affect leaf growth not only by reducing leaf turgor but also by affecting the other biophysical parameters controlling leaf cell growth (Van Volkenburgh and Cleland, 1984). Evidence from the present study suggested that the root excision and root cooling treatments used might also have such effects. Therefore, the next series of experiments was conducted to determine the effects of these treatments on the other parameters shown to influence leaf cell growth, particularly cell wall extensibility and wall yield stress.

5. THE EFFECTS OF ROOT EXCISION AND ROOT COOLING ON LEAF CELL WALL PARAMETERS

5.1 INTRODUCTION

The theoretical basis of our understanding of plant cell enlargement was discussed at length in Chapter 1. It was shown then that in addition to plant turgor, plant cell growth is also affected by factors controlling the flux of water into the cell and the plastic deformation of the cell wall. The experiments already described indicate that root cooling and root excision inhibit lamina expansion and leaf cell enlargement in Phaseolus seedlings, but that these effects cannot be correlated with changes in leaf turgor. The work now described was carried out to test the effects of the two major root treatments on the rheological properties of the leaf cell walls.

The two growth parameters investigated were wall extensibility (WEx) and wall yield threshold (Y). According to Cleland (1984), wall extensibility can be usefully approximated by the plastic compliance or plastic extensibility (PEx) obtained by the Instron technique. In the present experiments, estimates of this parameter were obtained using an instrument built in the Department of Botany of the University of Edinburgh and based on a previous design of Van Volkenburgh, Hunt and Davies (1982). (For a full description of the instrument and of the procedure employed in its use, see Appendix 1).

The second growth parameter, wall yield threshold (Y) is the threshold level of turgor, below which no cell enlargement can occur (Tomos, 1985). Here it was measured using an established technique in which the rates of growth of tissue strips of different turgor values are recorded, and the lowest turgor permitting growth taken to be equivalent to Y (Van Volkenburgh and Cleland, 1981; Davies and Van Volkenburgh, 1983). In addition to

providing information on the rheological properties of the tissues being investigated, the data obtained allowed critical appraisal of two widely used techniques.

As discussed previously, (Section 1.4.2), the rheological properties of plant cell walls appear, under certain conditions, to limit cell growth. Since the two parameters already implicated (ie WEx and Y) are subject to control by a range of factors, a major role for each in the fine control of tissue and organ growth seems likely. In this knowledge, the present experiments were performed to establish whether leaf cell wall rheological properties changed in response to root treatments and whether they could then be implicated in the observed effects on leaf growth.

5.2 EFFECTS OF ROOT EXCISION AND ROOT COOLING ON TOTAL, ELASTIC AND PLASTIC EXTENSIBILITIES OF PRIMARY LEAF TISSUE

Total, elastic and plastic extensibilities of leaf tissue were estimated from load-extension curves obtained by the Instron technique described in Appendix 1. Since in this technique, extensibility is related to the cross-sectional area of tissue over which the load is applied, some measurement of leaf thickness was also required (Cleland, 1967). This was obtained by recording lamina weight per unit area for each of the leaves used. Measured extensibilities, which were obtained for a standard load (20g), were then corrected and expressed per unit increase in load per unit cross-sectional area.

5.2.1 Effects of root excision and root cooling on primary leaf weight per unit leaf area

At each harvest for measurements of extensibility, separate pieces of lamina of standard area ($0.5cm^2$) were excised and boiled in methanol before

drying and weighing. In this way, time courses for the effects of both root excision and root cooling on lamina piece weight (LPW) after methanol treatment were obtained (Figs 5.1 and 5.2, p196). In control plants, mean LPW decreased steadily between days 7 and 10. On later days, the control group in one experiment showed a further decrease while that of a second showed a slight increase. This discrepancy may reflect differences in the precise timing of events of lamina expansion between the two control groups. However, it also brings into question the reliability of such figures as correction factors.

Root excision, predictably, had no significant effect on LPW by day 8, since by that time no significant effect on leaf growth could be detected. However, between days 8 and 10, the mean weight of lamina pieces was unchanged and over the following five days, it showed only a relatively slow decrease (Fig 5.1, p196). A comparison of this time course and that for control plants in the same experiment shows that RE plants had significantly higher values of LPW over the period between days 9 and 11.

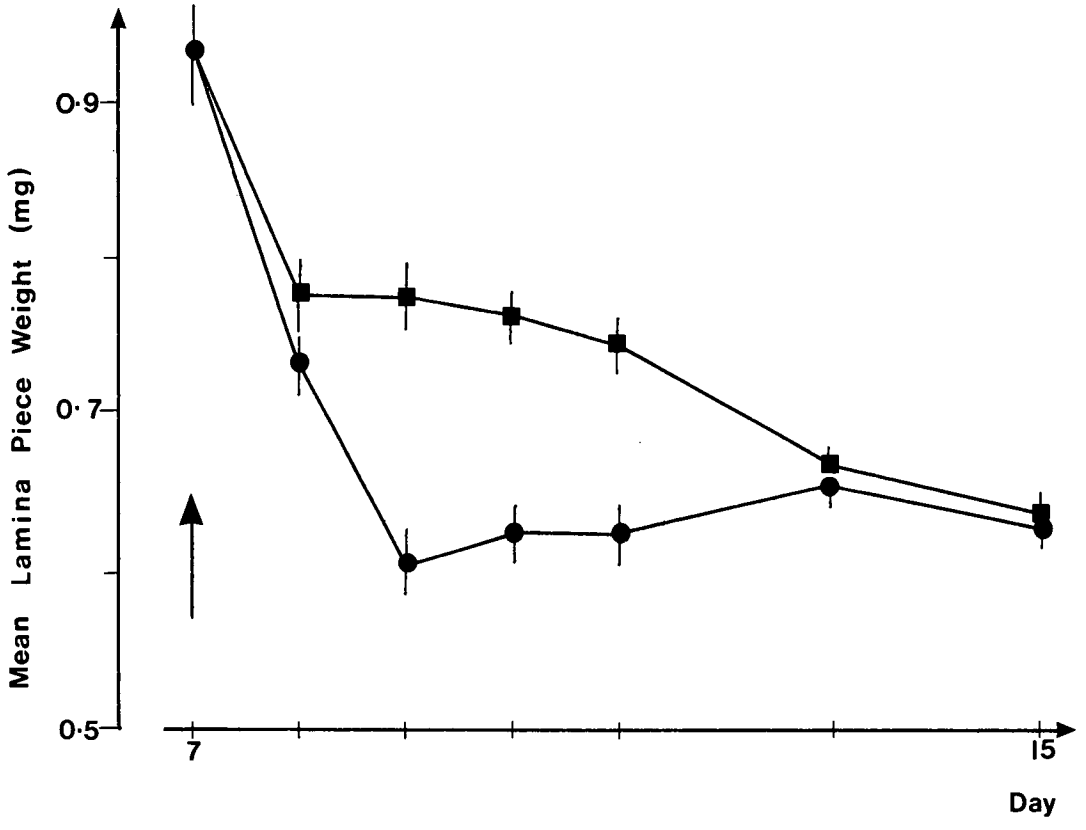
In RC plants, treatment caused a significant increase in LPW by day 8. At all subsequent harvests, LPW was significantly higher in the RC group, although in common with that of root excision, the effect of root cooling decreased around day 11.

In a supplementary experiment, the relationship between dry weight and cellulose content of pieces of lamina boiled in methanol was investigated. Leaves of seven, eight and nine day old plants were used and both measurements performed on the same pieces of tissue. Data were not obtained for eight day old plants subjected to root cooling, but root excision had no significant effect on either parameter by that time (Table 5.1, p 196). However, by day 9, mean LPW and cellulose content of both RE and RC plants

Figures 5.1 and 5.2

Fig 5.1. Time courses of the effects of root excision (RE, ■) or no treatment (CONTROL, ●) on Mean Lamina Piece Weight (following boiling in methanol). The time of first excision of roots is indicated by the arrow and plus and minus standard errors of means (n = 8) shown by the vertical bars.

Fig 5.2. Time courses of the effects of root cooling (RC, ▲) or no treatment (CONTROL, ●) on Mean Lamina Piece Weight (following boiling in methanol.) The beginning of the root cooling treatment is indicated by the arrow and plus and minus standard errors of means (n = 8) shown by the vertical bars.


Mean Lamina Piece Weight (mg)
0·9
0·7
0·5
7
15
Day

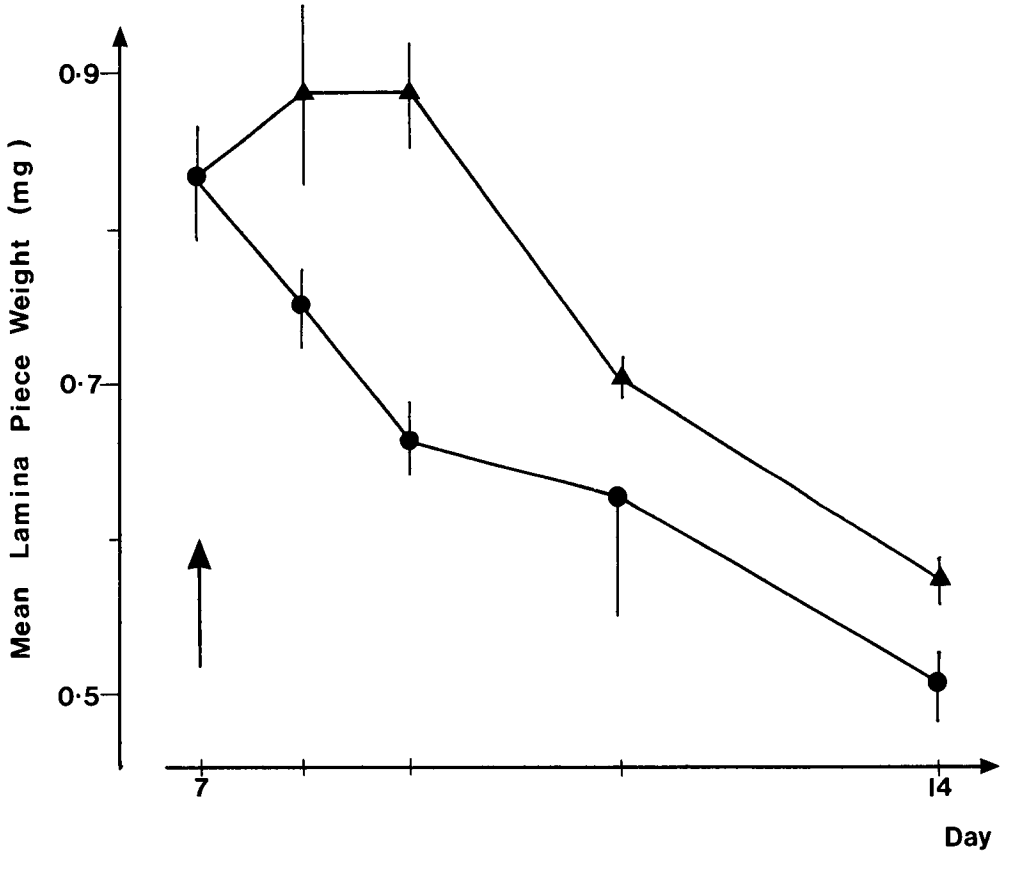

Mean Lamina Piece Weight (mg)
0·9
0·7
0·5
7
14
Day

Table 5.1. Effects of root excision (RE), root cooling (RC) or no treatment (CONTROL) on the dry weight after boiling in methanol, and cellulose content of pieces of primary leaf tissue, and on the ratio of cellulose to dry weight. Figures shown are means of 8 values with standard errors in brackets.

Day	Treatment	Dry weight (mg cm^{-2})	Cellulose content (mg x 10^{1} cm^{-2})	Cellulose dry weight (g g^{-1})
7	CONTROL	1.877 (0.055)	1.398 (0.065)	0.071 (0.006)
8	CONTROL	1.468 (0.053)	1.312 (0.142)	0.090 (0.011)
	RE	1.554 (0.059)	1.342 (0.114)	0.086 (0.006)
9	CONTROL	1.176 (0.044)	1.087 (0.028)	0.094 (0.001)
	RE	1.541 (0.048)	1.408 (0.064)	0.092 (0.005)
	RC	1.519 (0.048)	1.303 (0.068)	0.086 (0.004)

were significantly higher than the control values. Over the two-day period studied, LPW of RE, RC and control plants decreased while cellulose content increased in both treated groups but decreased in the control plants. The ratio between the two parameters (g cellulose, g^{-1} dry weight) increased in plants of all three groups but on any one day showed no significant effect of either treatment. It may be concluded that mean dry weight of methanol boiled leaf pieces (LPW) is a reasonably accurate index of cellulose content on any one day between days 7 and 9. However, the possibility remains that root treatments affect the relationship between the two parameters on subsequent days and may be responsible for the high level of variability in the data.

5.2.2 Effects of root excision and root cooling on total, elastic and plastic extensibilities of primary leaf tissue

Primary leaf tissue was harvested at one or two day intervals between days 7 and 15 so that the effects of root treatments on leaf tissue extensibilities and the changes in these parameters associated with leaf maturation could be observed. All harvests were conducted approximately four hours after the beginning of the photoperiod so that any changes in plastic extensibility (PEx) caused by the transition from darkness to light could be avoided (Davies and Van Volkenburgh, 1983). Effects of root cooling and root excision were investigated in separate experiments and the results of each corrected for possible effects of leaf thickness using separate measurements of lamina weight (Section 5.2.1) However, because of the high variability in the lamina weight data, the original extensibility measurements are also presented in the uncorrected form.

5.2.2.1 Effects of root excision on measured and corrected extensibilities of primary leaf tissue

In all plants, total extensibility (TEx) remained between 6.0% and 7.5%

throughout the period studied (Fig 5.3a, p 201). However within one day of treatment, TEx of RE leaf tissue had decreased significantly ($p = 0.01$) and it remained significantly ($p = 0.001$) lower than the corresponding control value until day 10; subsequently, no effect of treatment was detected. An identical pattern was exhibited by plastic extensibility (PEx), except that all values were between 2% and 4%, and the increased variability in the data reduced the significance of the day 8 difference to $p = 0.05$ (Fig 5.3c, p 201). Elastic extensibility (EEx) which remained between 3.5% and 4.2% was unaffected by root excision up to day 9 (Fig 5.3b, p **201**). However, between days 10 and 13, leaf pieces of RE plants had significantly higher EEx values than those of the control group.

Corrections of extensibility measurements for possible differences in leaf thickness (Appendix 1, Section A1.3.5) profoundly affected the trends and effects exhibited by the data (Fig 5.4, p 203). For tissue from control and RE plants, TEx decreased considerably between days 7 and 10, then remained relatively unchanged (Fig 5.4a, p 203). This decrease was more pronounced in tissue from control plants so that TEx was significantly higher in RE material between days 9 and 15. A similar pattern was found for EEx which was considerably higher in RE than control plants between days 8 and 13 (Fig 5.4b, p 203).

Correcting for cross-sectional area further added to the already considerable variability in the PEx data and consequently, few of the effects of treatment actually recorded were statistically significant (Fig 5.4c, p 203). Treatment did cause a significant ($P = 0.05$) reduction in corrected PEx by day 8, but on days 9 and 10 this effect, although still detectable was not significant ($p = 0.1$). On days 11 to 15, RE plants had slightly higher LPW-corrected PEx values than the controls, but again these

Figure 5.3

Fig 5.3. Time courses of the effects of root excision (RE, ■) or no treatment (CONTROL, ●) on a) Total, b) Elastic and c) Plastic Extensibilities of primary leaf tissue. The time of first excision of roots is indicated by the arrow and the significance of differences between means shown as described in Section 2.2.9.

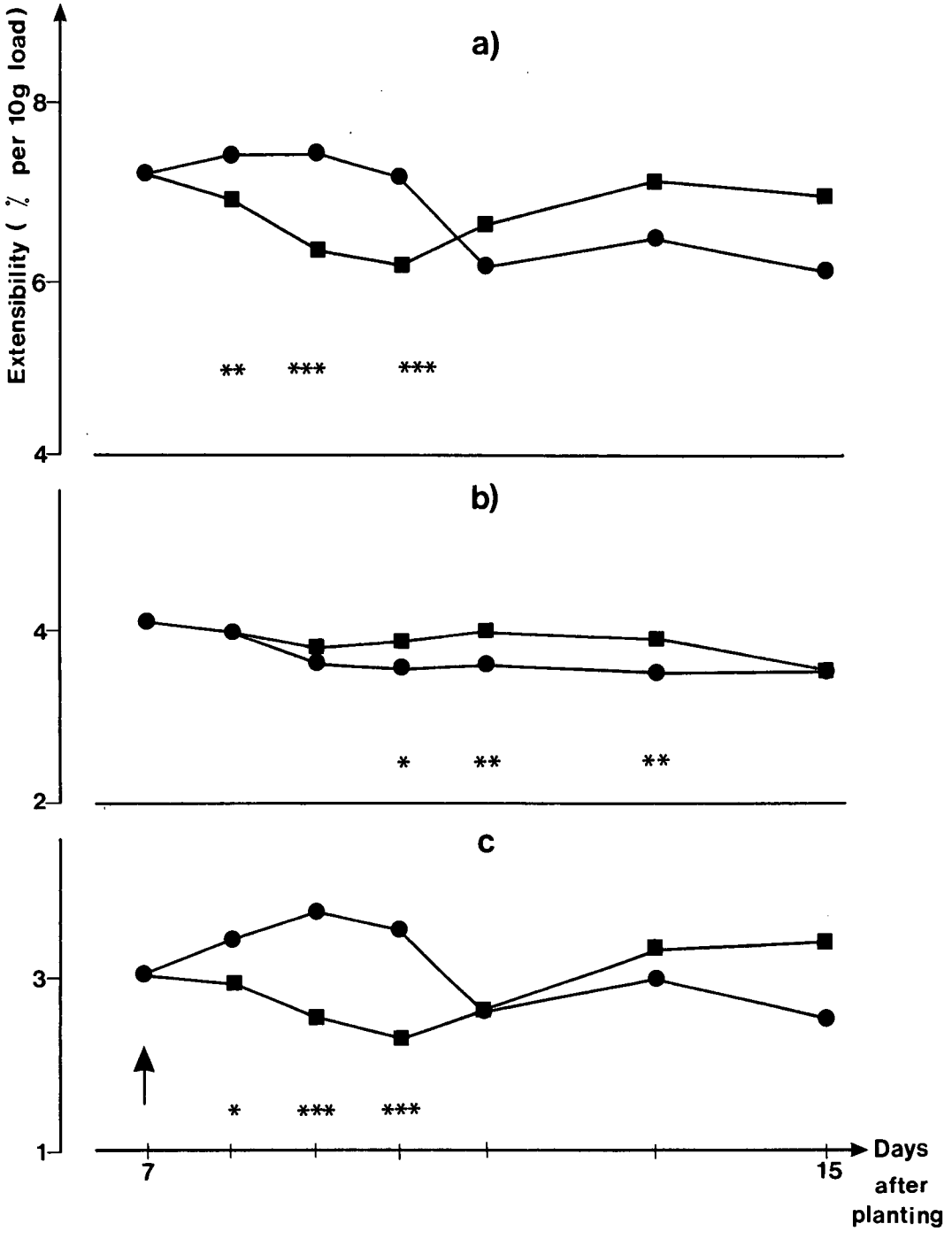
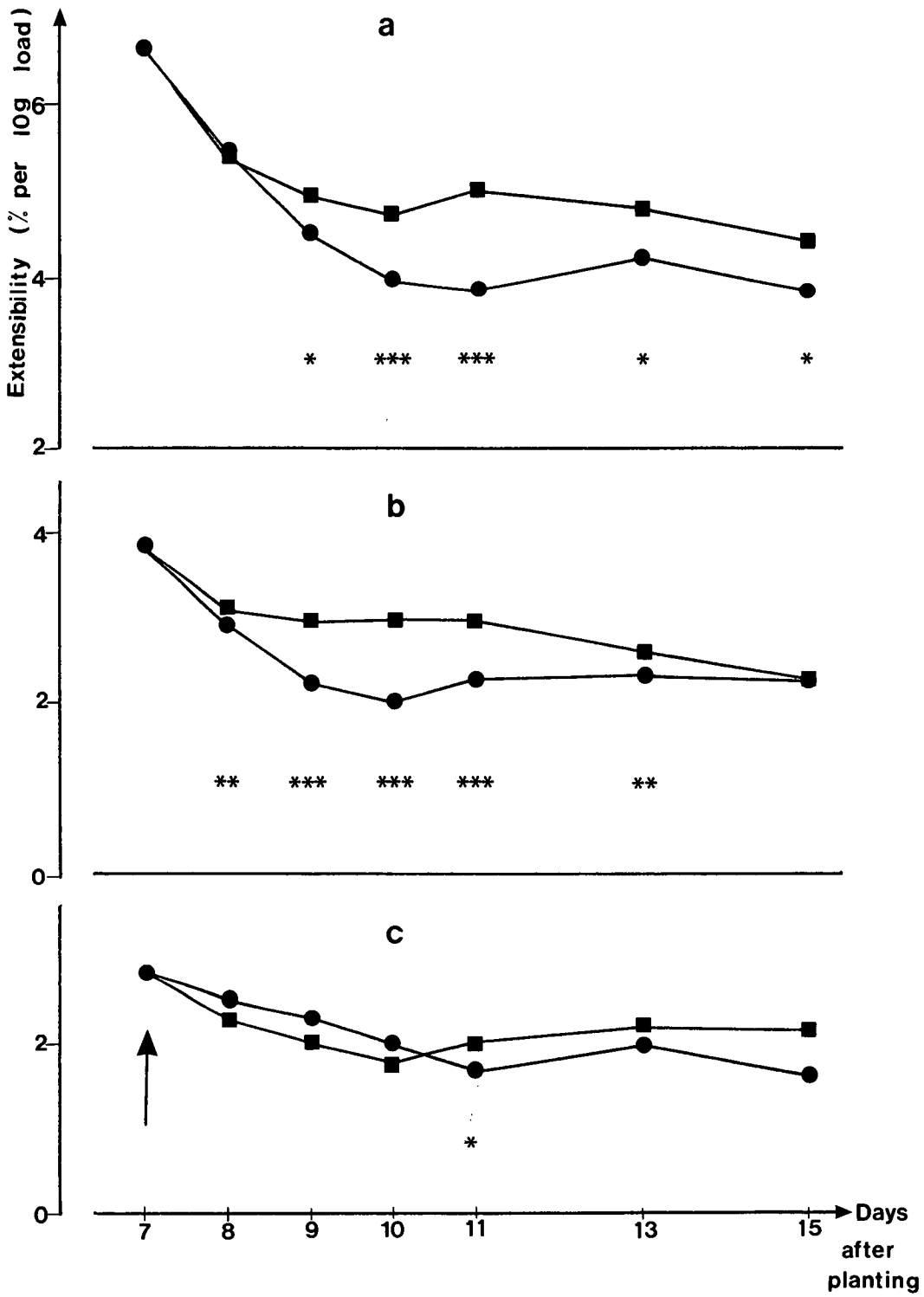

Extensibility (% per 10g load)
a)
8
6
4
**
***
***
b)
4
2
*
**
**
c
3
1
*
***
***
7
15
Days
after
planting

Figure 5.4

Fig 5.4. Time courses of the effects of root excision (RE, ■) or no treatment (CONTROL, ●) on a) Total, b) Elastic and c) Plastic Extensibilities of primary leaf tissue following correction of measured extensibilities (Fig 5.3) for differences in mean lamina piece weight (Fig 5.1). The time of first excision of roots is indicated by the arrow and the significance of differences between means shown as described in Section 2.2.9.


Extensibility (% per 10g load)
a
6
4
2
*
***
***
*
*
b
4
2
0
**
***
***
***
**
c
2
0
*
7
8
9
10
11
13
15
Days after planting

differences were only slightly, or not, significant ($p = 0.05$ to 0.1).

5.2.2.2 Effects of root cooling on measured and corrected extensibilities of primary leaf tissue

Measured extensibilities were generally slightly greater in this experiment than the previous one; in control plants total extensibility remained between 7.0% and 8.3% throughout. Root cooling considerably reduced TEx within one day of treatment and the same effect was evident on day 9 (Fig 5.5a, p 206). However, on days 10 and 11, no significant difference between treatments was observed, and by day 14 tissue pieces from leaves of RC plants had much higher TEx values than those of the control group. An identical pattern was evident in the PEx data with treatment first reducing, then considerably increasing measured extensibility (Fig 5.5c, p 206). However elastic extensibility was consistently higher in RC than control plants on all days (Fig 5.5b, p 206).

Once again, correction for tissue thickness considerably altered the data obtained, although several of the effects observed in the raw data were still evident (Fig 5.6, p 208). Total, elastic and plastic extensibilities of control leaf tissue all showed similar gradual decreases with increasing age as were recorded in the previous experiment. In RC plants, similar decreases occurred but were less pronounced and in the cases of total and plastic extensibilities, were reversed by day 14 (Figs 5.6a and b, p 208). Total extensibilities of RC leaf tissue decreased until day 11, then increased considerably by day 14. However between days 9 and 14 it was consistently higher than the corresponding control values. Plastic extensibility was initially reduced by treatment ($p = 0.05$ on day 8) but between days 9 and 11 was unaffected, and by day 14 was almost twice the control value (Fig 5.6c, p 208). Elastic extensibility was consistently

Figure 5.5

Fig 5.5. Time courses of the effects of root cooling (RC, ▲) or no treatment (CONTROL, ●) on a) Total, b) Elastic and c) Plastic Extensibilities of primary leaf tissue. The beginning of the root cooling treatment is indicated by the arrow and the significance between means shown as described in Section 2.2.9.

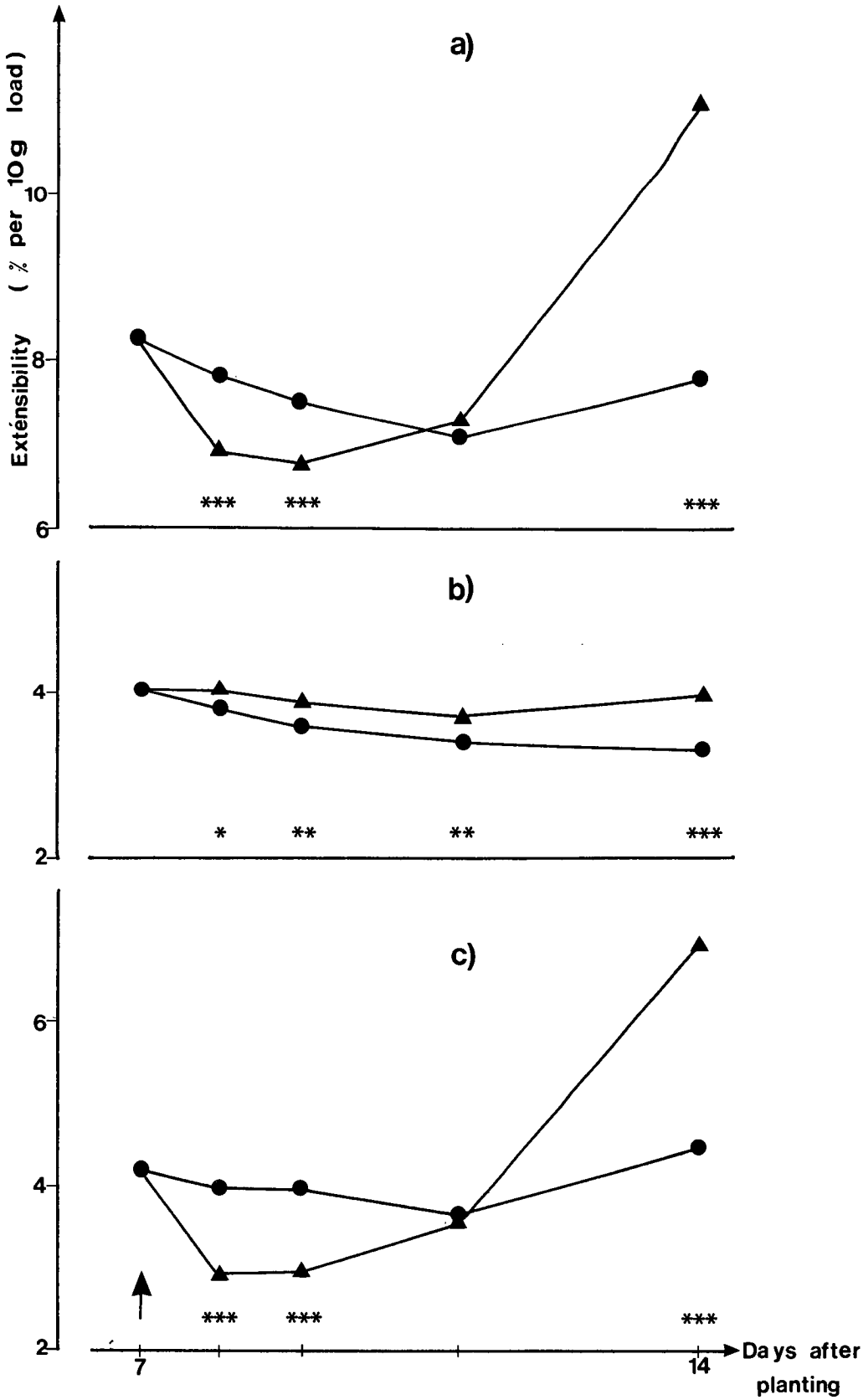
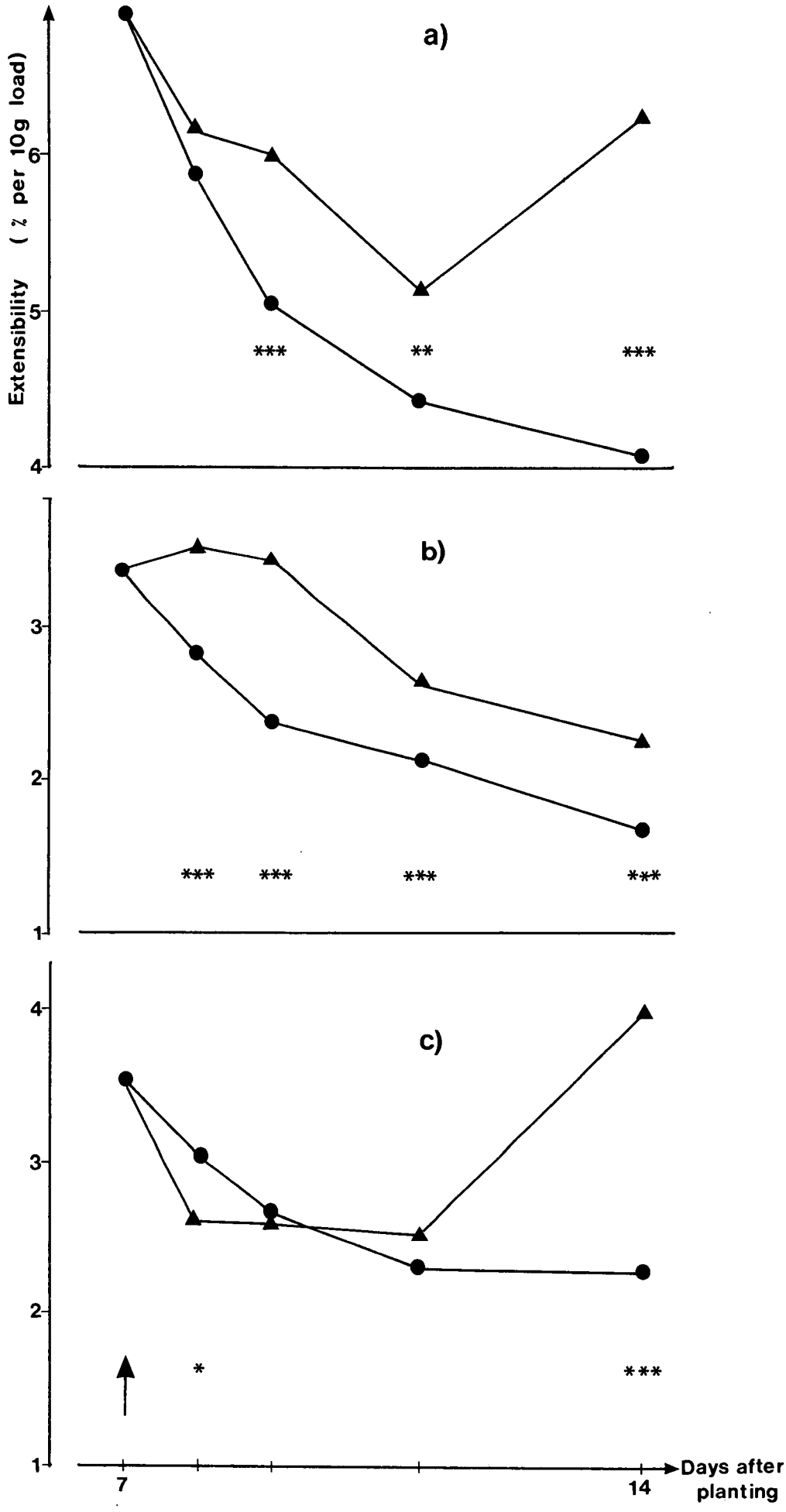

a)
Extensibility (% per 10g load)
10
8
6
***
***
***
b)
4
2
*
**
**
***
c)
6
4
2
***
***
***
7
14
Days after planting

Figure 5.6

Fig 5.6. Time courses of the effects of root cooling (RC, ▲), or no treatment (CONTROL, ●) on a) Total, b) Elastic and c) Plastic Extensibilities of primary leaf tissue following correction of measured extensibilities (Fig 5.5) for differences in mean lamina piece weight (Fig 5.2). The beginning of the root cooling treatment is indicated by the arrow and the significance of differences between means shown as described in Section 2.2.9.


a)
Extensibility (% per 10g load)
6
5
4
***
**
***
b)
3
2
1
***
***
***
***
c)
4
3
2
1
*
***
7
14
Days after planting

higher in RC than control tissue throughout the period studied, the correction for leaf thickness actually increasing the magnitude of the difference (Fig 5.6b, p 208).

5.3 EFFECTS OF ROOT EXCISION ON THE WALL YIELD THRESHOLD OF PRIMARY LEAF TISSUE

The technique used to estimate the wall yield threshold (Y) of leaf tissue (Section 2.2.7.2) is performed on strips of lamina. In these experiments, insufficient material was available from RC plants to allow the necessary level of replication. In addition, control and RE material less than 10 days old proved unsuitable because the area of lamina between major veins was too small to allow excision of adequate quantities of leaf strips. Consequently this study was confined to the effects of one treatment, root excision, on the wall yield threshold of primary leaf tissue between days 10 and 14.

In control plants, Y was remarkably constant remaining between 0.15MPa and 0.18MPa over the entire duration of the experiment (Table 5.2, p 211). In RE tissue, the same parameter was considerably more variable, ranging from 0MPa to 0.14MPa with no apparent pattern or trend. Consequently Y in control plants was consistently higher than that of RE plants, suggesting that the leaf cells of RE tissue required less turgor to commence growth than did those of the control leaves.

The relationship between tissue strip length and turgor pressure above the value corresponding to Y (ie P - Y) generally yielded a straight line, the slope ($\dot{m}$) of which gave the rate of irreversible extension of the tissue with increasing turgor pressure (Table 5.2, p 211). As such, this parameter was considered analogous to the plastic extensibility of the leaf

Table 5.2. Effects of root excision (RE) or no treatment (CONTROL) on the wall yield threshold (Y) and growth per unit turgor pressure (m) of strips of primary leaf tissue. Each value of Y or m given represents a single measurement but similar results were obtained in identical experiments.

Day	Treatment	Y (MPa)	m ($mm \times 10^{-1}$ MPa^{-1})
10	CONTROL	0.18	7.63
	RE	0.09	9.31
11	CONTROL	0.16	7.23
	RE	0.00	5.33
12	CONTROL	0.12	9.70
	RE	0.14	9.88
13	CONTROL	0.17	7.80
	RE	0.12	7.62

cell walls. In the leaves of control plants, m changed little over the duration of the experiment, but for tissue from RE plants it was considerably more variable. However, no correlation between m and either plant age or treatment was found and differences in the data were generally not significant (Table 5.2, p 211).

5.4 DISCUSSION

Assessment of the Edinburgh Tensiometer and the Instron technique for measuring leaf tissue extensibilities

In the course of measurement of tissue extensibilities by the Instron technique, three possible sources of error were identified: (i) the actual measurement of extensibilities and the interpretation of load/extension curves, (ii) artefacts arising from the treatment of tissue prior to extensibility measurement and (iii) correction of measured extensibility values for differences in tissue thickness or internal structure.
A fourth doubt about the technique, concerning the relationship between Instron-measured plastic extensibility (PEx) and the growth parameter wall extensibility (WEx) is discussed in Appendix 1.

One possible source of error during measurement of extensibilities may have been a lack of homogeneity in tissue structure. Prominent veins, particularly when these ran parallel to the direction of movement of the tensiometer clamps, considerably reduced the extensibilities recorded. Ideally, all tissue strips containing prominent vascular tissue would have been excluded but this was not always possible, particularly when the leaves were small (days 7 and 8). Another problem most often encountered with very young material was slippage of tissue strips during the extension runs. This happened when tissue pieces were too short to allow adequate overlap within the tensiometer clamp jaws and was revealed by an irregularity

in the corresponding load-extension curve.

The actual procedure involved in extensibility measurement was relatively straightforward. The incorporation of a fast reverse function to quickly return the clamps to their original positions reduced the time tissue was subjected to significant load between each pair of extensions. However, the length of the tissue strips after the second extension was frequently slightly greater than that after the first, implying that this precaution may not have been entirely successful. Nevertheless, this source of error was considered small enough to be ignored in the final analysis.

The interpretation of load extension curves and the calculation of extensibilities provided no further problems. However, the fact that plastic extensibility was not measured directly but derived from the other two parameters (total and elastic extensibilities) increased the variability in these data and made effects of leaf age and root treatment more difficult to ascertain. Generally, however, the instrument gave satisfactory results, anomalous measurements being easily recognised and excluded from the final data.

The second major cause of doubt relating to the Instron technique used was the treatment of leaf tissue prior to extensibility measurement. Boiling in methanol is carried out primarily to kill tissue so that cell turgor and enzyme effects, both possible sources of error, are eliminated (Olsen, Bonner and Marre, 1965). This treatment also permits long term storage so that extensibility measurements can be performed on tissue several weeks after its collection. Because of these advantages, methanol-boiling of tissue is now standard practice (Cleland, 1981; Davies and Van Volkenburgh, 1983). However Taiz (1984) has suggested that such treatment might considerably alter the structure of the cell wall.

The principal effects of boiling in methanol on leaf tissue are likely to be the disruption of cell membranes and the loss of low molecular weight compounds such as free sugars, amino acids and some inorganic salts (Selvendran, 1975). Proteins, polysaccharides and nucleic acids, including the structural components of the cell wall are likely to be retained (Cleland, 1967; Selvendran, 1975) but the possibility exists that some breakdown and loss of pectic polysaccharides might occur as a result of the high temperature used (Jarvis, Logan and Duncan, 1984). Olsen, Bonner and Marre (1965) found no effect of boiling in methanol on measured total extensibility of Avena coleoptiles, but Cleland (1967) found a 160% increase in plastic extensibility using the same material and treatment. Although the latter finding implies that some disruption of the cell wall may have taken place, it is significant that the state of the tissue (boiled or unboiled) had no effect on the relative changes in extensibility recorded in response to pre-treatment with auxin. This implies that the component of wall extensibility involved in cell growth is not significantly altered by boiling of tissue in methanol.

A second criticism of the methanol boiling treatment is that it may lead to an increase in wall stiffness through the deposition of denatured cytoplasmic proteins on the surfaces of isolated cell walls (Selvendran, 1975). This might explain the finding that treatment with Pronase, which has no effect on the structural glycoproteins of the cell wall, nevertheless increases the measured extensibility of methanol-boiled tissue (Cleland, 1967). Perhaps the Pronase treatment removes deposited cytoplasmic proteins from the cell walls. Once again, however, although Pronase treatment affects the absolute extensibilities recorded, it has no effect on the relative effect of auxin. Thus, it would appear that methanol-boiled tissue retains the component of extensibility which is affected by auxin

treatment and which appears to be involved in the control of cell growth.

An important consideration which may be overlooked in the determination of tissue extensibility is the effect of tissue thickness or internal structure. Comparable extensibility measurements of different samples of material should ideally be obtained using a constant force per unit cross-sectional area of material. Failing this, a correction can be made after extensibilities have been determined, provided that the area of material exposed to the force is known. For leaf tissue, a simple measurement of lamina thickness is unsuitable because of the complicated structure of the leaf mesophyll. Instead, some index of the area of tissue being subjected to force, for example the cross-sectional area of cell wall, must be obtained.

In the present experiments, correction of measured extensibilities for differences in leaf internal structure were made using dry weights of tissue pieces following boiling in methanol (Van Volkenburgh, Hunt and Davies, 1983). Such measurements give gross values for tissue density but may be unsuitable indices of tissue area subjected to force because much of the material which is left after methanol treatment (notably starch and protein) is either not part of the cell wall or does not contribute to the stiffness of the tissue as measured by the Instron technique. Cleland (1967) sought a more accurate index of cell wall area by subjecting Avena tissue pieces to Pronase treatment prior to weighing. This practice removed over 98% of the protein content of the tissue as well as all detectable starch and RNA. However, this was not attempted in the work reported here.

To test the value of dry weight of methanol-boiled leaf tissues as an index of cell wall content, the relationship between lamina dry weight and cellulose content was investigated (Section 5.2.1). The ratio of dry

weight to cellulose content was not significantly affected by treatment on any of days 7, 8 or 9, implying that methanol-insoluble dry weight may be a useful index of cell wall content, and the area of tissue likely to be subjected to force. However, the high level of variability in the data makes this conclusion tentative. Furthermore, the force exerted during Instron-extension may not be distributed evenly over the cross-sectional area of the leaf pieces, but may be primarily concentrated in the upper and lower epidermal layers. If this were the case, the cross-sectional area of epidermal cell wall would be a more appropriate index of the area subjected to force. Although this parameter was not measured here, it might be expected to differ only negligibly between treatments, thus eliminating the necessity for correction of measured extensibilities on the basis of differences in leaf internal structure.

In addition to doubts concerning its necessity or value as an index of tissue area subjected to force (p233), the accuracy of the lamina weight data obtained and used to correct measured extensibilities is also suspect. Variability, both within and between experiments was high and no account was taken of variation in lamina weight between different parts of the leaf. Therefore, at best, the data presented in Section 5.2.1 can provide only a rough guide to changes in lamina weight per unit area, and extensibility values corrected using these data must be interpreted with caution.

Effects of root treatments on total, elastic and plastic extensibilities of leaf tissue

Total, elastic and plastic extensibilities could all be measured with sufficient accuracy to clearly show effects of root treatments and leaf age. Despite the errors inherent in the technique, obvious trends and consistently-detected effects confirm that the method provides at least qualitative estimates of the rheological properties of effectively isolated

cell walls (Tomos, 1985).

For reasons discussed in Appendix 1, the irreversible or plastic component (PEx) of Instron-measured extensibility is regarded as the parameter which most closely represents the wall extensibility term used in contemporary models of cell growth (Lockhart, 1965; Cleland, 1981; Tomos, 1985). In the present experiments, possible errors associated with its calculation from measured values of TEx and EEx and with correction for differences in leaf thickness, make its interpretation more difficult. However, clear trends were consistently observed. In control plants, uncorrected PEx (which would appear to be the most reliable estimate of WEx, given the considerable doubt regarding the necessity for and accuracy of any correction (p233)) was initially high but showed a general decline with increasing leaf age, particularly around days 9 to 11 (fig 5.3, p201; fig 5.5, p206). This finding might suggest that the slowing of leaf cell wall expansion associated with the normal course of maturation of the leaf involves a fall in cell wall extensibility (Van Volkenburgh, Schmidt and Cleland, 1985). In RE and RC plants, reductions in uncorrected PEx with increasing leaf age were also recorded, although these were more pronounced between days 7 and 10, and tended to be reversed between days 10 and 15.

In RC plants, the increase in plastic extensibility which occurred between days 11 and 14 coincided with a slight enhancement of leaf growth recorded in several experiments (Section 3.3.1). In Chapter 4, acclimatisation of roots to the cooling temperature was proposed as the factor responsible for this slight increase in leaf growth. The present results suggest that the mechanism behind the effect may have involved an increase in leaf cell wall extensibility.

The long-term changes in plastic extensibility discussed above may reflect alterations to leaf cell wall geometry or gross structure which take several days to develop and involve changes in the rate or pattern of deposition of wall material (Davies and Van Volkenburgh, 1983; Hsiao, Silk and Jing, 1985). However, evidence suggests that wall extensibility may also change in the short term, possibly through changes in the frequency or nature of cross-linkages between cell wall macromolecules (Cleland, 1981; Taiz, 1984; Cleland, 1984). In the present study, both excision and root cooling caused significant reductions in uncorrected plastic extensibility of leaf tissue by the first harvest (20 hours) after the beginning of treatment, In RE plants, this effect persisted until day 10, and in RC plants, at least until day 9. Following correction of measured values of PEx for differences in tissue thickness, some of these effects were not significant. However they were recorded consistently and may have been significant had the variability in the data been reduced.

It may be significant that these apparent changes in leaf cell wall extensibility coincide in timing with reductions in leaf growth rate recorded in previous experiments (Section 4.6). On later days, when wall extensibility was not reduced by treatment, leaf growth rate appeared to be limited by leaf turgor (Section 4.2). However, on days 8 and 9, particularly in RE plants, turgor was sufficient to sustain the control rate of leaf growth and yet this was not reached. The present results suggest that on days 8 and 9 in RE plants and on day 8 in RC plants, wall extensibility may have been the limiting factor for leaf cell growth.

Whether cell wall elasticity affects steady state growth of plant cells is not known (Tomos, 1985), but the present data show no correlation between the elastic component (EEx) of Instron-measured estensibility and cell

growth rate. Instead,while both root treatments slowed leaf cell growth they increased $\dot{E}\dot{E}\dot{X}$. Measurements of the bulk modulus of elasticity (ε) in a previous experiment (Section 4.2.2) also showed that root excision increased leaf cell elasticity. The mechanisms by which such changes might be brought about are discussed in Chapter 6. How they might benefit the plant subjected to root excision or root cooling might be related to the effects of those treatments on the water status of leaf cells. As shown in Section 4.4, root excision and root cooling, particularly in the long term, cause considerable fluctuations in the supply of water to the leaf. Under such conditions, highly elastic cell walls capable of a rapid response to changes in cell water content might allow the maintenance of turgor (Tyree and Jarvis, 1982). In this way. structural damage resulting from loss of turgor and possibly detachment of the plasmalemma from the cell wall could be avoided.

Effects of root excision on the wall yield threshold of leaf tissue

A number of techniques have been employed by different groups to estimate the wall yield threshold (Y) of plant cells and tissues. Green, Erickson and Buggy (1971) measured the decrease in turgor (P) necessary to prevent growth of Nitella internode cells and obtained Y by subtracting this quantity from the original values of P; an analogous procedure was used to determine Y for higher plant leaf tissue from paired measurements of leaf growth rate and turgor (Bunce, 1977; Davies and Van Volkenburgh, 1983). Recently, the micro-pressure probe has been used to obtain Y from the value of P reached in plant tissue deprived of a continuous supply of water following stress-relaxation of its cell walls (Cosgrove, Van Volkenburgh and Cleland, 1984). However, the simplest and most widely employed method is the one used in the present experiments in which the growth rates of pieces of tissue floated on different osmotic solutions are plotted

against their estimated turgor pressures and Y estimated from the value of P below which no growth occurs (eg Van Volkenburgh and Cleland, 1981; Davies and Van Volkenburgh, 1983).

Despite its frequent application, the tissue strip method of determining Y presented several practical problems which may have affected the results obtained. Although the polyethylene glycol (PEG) used was of a high molecular weight (approximately 400), that value represents an average, and very much smaller molecules may have been present in the floating solutions used. If these low molecular weight fragments were taken up by the leaf stips, tissue osmotic potential may have risen and the threshold value of P (ie Y) been underestimated. Alternatively, osmotic solutes may have been lost to the medium from the tissue, causing an increase in tissue osmotic potential and an overestimation of Y. It is also possible that PEG interfered with the process of cell enlargement indirectly through some toxic effect on cell metabolism, while considerable drifts in the value of Y may have occurred during the five to six hour floating period (Taiz, 1984). The actual measurement of strip length and the identification of those solutions in which no tissue growth occurred also proved difficult since some shrinkage of tissue occurred, particularly in the solutions of lowest osmotic potentials. The results obtained were suffiently consistent to suggest that artefactual effects may have been small, and that any treatment effects observed were at least qualitatively accurate. However, the possibility remains than an effect observed may simply reflect a difference in susceptibility to artefact between treatments.

Measured values of Y ranged from zero to 0.18 MPa for all plants but were usually higher for control than RE material. In magnitude these values are substantially lower than those recorded in published

work on the same or similar material. For instance, rapidly growing primary leaves of Phaseolus had values of Y close to 0.45 MPa (Van Volkenburgh and Cleland, 1981) while trifoliate leaf values ranged from 0.55 to 1.00 MPa (Davies and Van Volkenburgh, 1983). Values of Y close to zero have been recorded for leaves of other species (Bunce, 1977; Takami, Rawson and Turner, 1982) and in Phaseolus trifoliates using a different method (Davies and Van Volkenburgh, 1983). However, the data presented here were obtained from relatively mature, slow-growing leaves, and since Y may be so high as to limit growth in older leaves (Tyree and Jarvis, 1982; Van Volkenburgh and Cleland, 1984), they are substantially lower than might be expected.

Despite the likelihood that the data obtained underestimate the true values of Y, root excision treatment does seem to cause a reduction, since a consistent effect was detected. How that effect might have come about however, is unclear, since so little is known about the factors which determine Y. It seems likely that Y is a function of at least three factors:- the area of the cell wall, its thickness and its molecular structure (Taiz, 1984). Thus, lower values of Y would be expected for tissues composed of small cells with thin cell walls and a relatively easily-deformed wall structure. In the present study, the cells of RE tissue were smaller than those of the control material but may have had thicker cell walls; nothing is known about their molecular structure. In the absence of further data, it must be concluded that root excision caused a reduction in Y, probably as a consequence of a decrease in mean leaf cell size and possibly also involving a change in the molecular composition or arrangement of the leaf cell walls. What is also suggested is that the reduction in leaf cell growth caused by root excision cannot be attributed to a rise in wall yield threshold since, at least between

days 10 and 14, the values of that parameter decreased.

The involvement of cell wall parameters (WEx, Y) in the effects of root treatments on leaf growth

Although the principal factor controlling leaf cell enlargement has been identified as plant water status, or more specifically leaf cell turgor, increasing evidence suggests that the growth rate of cells may also be affected by the rheological properties of their cell walls (Van Volkenburgh and Cleland, 1984 and references therein). The results presented in this chapter lend further support to this hypothesis by suggesting that root treatments such as excision and cooling affect leaf growth by altering the plastic extensibility of leaf cell walls.

Variation in the response of plant cell growth rate to the driving force, turgor, has now been shown in response to a number of factors. In soyabean, the relationship between leaf extension rate and leaf turgor shows marked seasonal variation (Bunce, 1977) while that of several cereal species has been found to fluctuate with time of day (Acevedo, Fereres, Hsaio and Henderson, 1979). One study showed light intensity and quality to affect the response of leaf growth to water status in tree seedlings (Taylor and Davies, 1985) and leaf age has also been shown to cause variation (Van Volkenburgh, Schmidt and Cleland, 1985). Of considerable interest in the context of the present findings are the observations that the water status of the leaf, or its prior exposure to water deficit, can alter its sensitivity in terms of cell growth rate to turgor (Bunce, 1977; Cutler, Shahan and Steponkus, 1980; Matthews et al, 1984). In numerous studies water deficit has been shown to reduce leaf growth directly by affecting either leaf water potential or turgor (Boyer, 1970; Acevedo,

Hsiao and Henderson, 1971). However, in several cases such a mechanism was not responsible since turgor was maintained by osmotic adjustment (eg Michelena and Boyer, 1982). Also, in maize plants subjected to salinity stress, restoration of the control level of leaf turgor by artificial means failed to restore leaf growth rate (Termatt, Passioura and Munns, 1985). Such observations suggest that treatments such as exposure to drought or salinity influence leaf growth by affecting factors other than, or in addition to, cell turgor (Van Volkenburgh and Cleland, 1984)

In several such experiments, where some treatment altered the response or sensitivity of leaf growth to turgor, a change in the rheological properties of the leaf cell walls was either suspected or proven. Generally, treatments caused leaf growth to be less sensitive to turgor, implying that either wall yield threshold (Y) was increased or wall extensibility (WEx) reduced. In some studies, predicted changes in Y and WEx were indeed found (Meyer and Boyer, 1972; Cutler, Shahan and Steponkus, 1980; Matthews, Van Volkenburgh and Boyer, 1984). However in others, treatment was found either to reduce Y or increase WEx (Bunce, 1977; Davies and Van Volkenburgh, 1983). The disagreements in these data may be due to errors in the methods of measurement, for instance if corrections of PEx values for differences in leaf thickness were not made, or if incorrect osmotic potentials were obtained for tissue used in the measurement of Y. However it also highlights the dangers of drawing simple conclusions from such results. At present, the control of the rheological properties of plant cell walls, their effects on cell growth and interactions with other factors are poorly understood (Hsiao, Silk and Jink, 1985). One factor, for example, which is frequently neglected is cell wall elasticity. As yet, the relationships between elastic and plastic extensibility in plant tissue has not been determined (Tomos, 1985) and

one treatment might have quite different effects on the two parameters (Uhrstrom, 1974; present data, Section 5.2). Also, the same treatment may have different effects on cell wall elasticity depending upon the conditions and plant species used. Thus, while water stress has been shown to increase leaf tissue elasticity (Elston, Karamanos, Kassam and Wadsworth, 1976) it may also reduce it (Turner, 1981; Osonubi and Davies, 1981). While such doubts remain about the control and role of fundamental biophysical parameters, the scope of conclusions based on measurements of one or two parameters is inevitably limited.

What can be concluded from the data presented in this chapter is that treatments applied to plant roots may affect leaf growth by altering the rheological properties of leaf cell walls. This implies two processes, the communication of a signal from root to shoot and the response of the leaf cells to that signal. If treatment were associated with a decrease in water uptake, leading to leaf water deficit, and if sustained turgor was necessary for the development of normal cell walls, the properties of the leaf cell walls might be altered in this way. Loss of cell turgor could result in the deposition of new cell wall material without concomitant expansion of the wall surface, leading to the formation of small, thick-walled cells which might have reduced plasticity. Furthermore, if wall structure was dependant upon close contact between the plasmalemma and cell wall during development, a cell experiencing fluctuating water content might develop a cell wall different in structure from normal.

In the present study, decreases in plastic extensibility were frequently recorded in the absence of corresponding decreases in leaf turgor. The finding that the rheological properties of plant cell walls can be affected by certain plant growth regulators (Cleland, 1981) suggests an

alternative hypothesis; that root excision and root cooling reduced leaf cell wall extensibility and growth rate by affecting the supply of growth promoters or inhibitors to the leaf cells. In the previous chapter (Section 4.7) root cooling was shown to increase the abscisic acid content of the primary leaves; it is also possible that root treatments could reduce the concentrations of growth promoters such as certain cytokinins in the leaf cells (Blackman and Davies, 1984; Termatt, Passioura and Munns, 1985). The possible involvement of plant growth regulators in these effects is discussed in Chapter 6.

The data presented above show that root treatments such as root excision and root cooling affect the wall extensibility of leaf cells, and suggest that the inhibition of leaf growth brought about by such treatments may be caused in this way. While the biochemical basis of these effects and the factors mediating them have not been established, the results suggest the existence of a highly sensitive and flexible means by which leaf growth can be adjusted to suit the conditions experienced by, and physiological state of, the root.

6. GENERAL DISCUSSION

The principal aim of the work reported in this thesis was to investigate the control of leaf growth and development by plant roots. The procedure employed was to subject plants to root treatments known to inhibit leaf growth, and record the effects of those treatments both on the growth of leaves and on the various functions of the root system. By considering also the biophysical basis of the response in the leaf, it was possible to speculate on which of the aspects of root functions affected by treatment were responsible for the inhibition of leaf growth.

6.1 EFFECTS OF ROOT TREATMENT ON THE PRINCIPAL FUNCTIONS OF THE ROOT SYSTEM

In Chapter 1, the four main aspects of root function likely to be affected by root excision and root cooling were identified as water uptake, mineral nutrient uptake, sink behaviour and the synthesis and metabolism of organic substances including plant growth regulators. It was postulated that an effect on one or more of these functions could be responsible for the observed effects on leaf growth, and thus, could be the mechanism by which changes in the state of the root are communicated to the shoot.

Both root treatments were found to substantially reduce the flux of water through the plant. In the case of root cooling, this appeared to be due to a reduction in root permeability which occurred within a few hours of the beginning of treatment. In the case of root excision, the permeability of the root tissue which remained after treatment actually increased considerably. However, because root area was substantially reduced, the total volume of water transported was low. The effects

of these reductions in water supply on leaf water relations were less than might have been expected because stomatal closure occurred to reduce water loss. Consequently RE plants showed no reduction in leaf water potential until two days after treatment, while RC plants showed only a transitory reduction immediately after transfer to low temperature conditions. These data suggest that reduced water supply, although it was an effect of both major treatments, did not inhibit leaf growth, at least on days 7 to 9, specifically by causing leaf water deficit. Instead, the leaf cells may have responded directly to the change in the flux of water (Aston and Lawlor, 1979; Davies and Van Volkenburgh, 1983) or the inhibition of leaf growth may have come about because the flux of some xylem-borne promoter was reduced (Beevers, 1972).

That both root treatments reduced the uptake of mineral nutrients is in keeping with published accounts (eg, Power, Grunes, Reichman and Willis, 1970; Veen, 1977; Clarkson, 1985). However, indirect evidence obtained here suggests that particularly in the short term, no shortage of minerals was experienced by the shoot. No visible symptoms of nutrient deficiency were detected except in the case of root cooling after six or seven days of treatment. Nor was primary leaf cell division or the unfolding of the first trifoliate leaf substantially affected by either treatment, suggesting that the synthesis of proteins and nucleic acids proceeded as normal. No attempt was made to ascertain whether the absence of deficiency symptoms indicated a fall in leaf demand for mineral nutrients or the fulfilment of that demand by some other source. However if the latter were the case, additional supplies may have been drawn from substantial reserves contained in the cotyledons (Yagi, 1972).

By the actual excision of tissue, or the inhibition of extension growth,

both root treatments reduced root size relative to that of control plants. In addition, both root cooling through its general effect on metabolism and root excision through its removal of the most actively growing tissues, must also have reduced root metabolic activity and hence root sink strength. Since plant roots normally constitute a major sink for assimilated carbon, root excision and root cooling would be expected to cause the accumulation of carbohydrate in the shoot (Thorne and Evans, 1964; Humphries and French, 1969). This was confirmed by the finding that the proportion of dry weight relative to fresh weight in the shoot was substantially increased by both treatments, and suggests that leaf cell growth was not limited by the availability of assimilate for energy metabolism or the synthesis of structural polysaccharides. However the accumulation of photosynthate may itself have inhibited leaf growth by altering the normal course of carbohydrate metabolism (Humphries and Thorne, 1964; Ghobrial, 1983). Alternatively, the effects of treatment on the root sink may have caused the accumulation of other factors inhibitory to leaf growth (Davis and Lingle, 1961).

An effect of treatment on the strength of the root sink may have been responsible for the accumulation of abscisic acid (ABA) recorded in response to root cooling. In control plants, leaf ABA concentration was initially high but decreased with increasing seedling age. However, in RC plants, ABA concentration fell only slightly and was always higher than the corresponding control value. In the absence of new synthesis ABA probably accumulated in the leaves of RC plants because transport out of the shoot was restricted (Setter, Brun and Brenner, 1981; Henson, 1984).

The investigation of the effects of root cooling on the ABA content of

the primary leaves was the only attempt made in the present study to determine the possible involvement of plant growth regulators in the effects of root treatments on leaf growth. However, evidence from other sources suggests that effects on the other groups of plant growth regulators may have been considerable. Since both treatments reduced the number of root apices per root system, and since the root apices are considered to be major sites of synthesis and/or metabolism of cytokinins and gibberellins, it is probable that both root excision and root cooling reduced the amounts of these substances reaching the shoot (Carr and Reid, 1968; Gollnow and Letham, 1978; Van Staden and Davey, 1979). Furthermore, since the root is an important sink for shoot-derived auxin, both root treatments could have caused an accumulation of that substance in the primary leaves (Phillips, 1964). According to Phillips (1964), auxin accumulation in the shoots of waterlogged pea plants was associated with an increase in the ratio of dry weight to fresh weight in the shoot, and the formation of adventitious roots at the base of the stem. Both phenomena were recorded in the present experiments, providing further indirect evidence for an accumulastion of auxin.

From the observations made, it is evident that root excision and root cooling may have profoundly affected several aspects of root function including the uptake of water and mineral nutrients, root sink strength and the synthesis and metabolism of different plant growth regulators. However, such findings fail to reveal the precise mechanisms by which the root treatments affected leaf growth. The findings that the effects on leaf growth were approximately proportional to the severity of treatment and largely reversed by the regeneration of root tips suggests that the critical function of the root system for leaf growth is proportional to

its size and metabolic activity and may reside in the root apices. However once again, several aspects of root function fulfil these criteria. It was because of this dilemma that the study of the effects of root treatments on the biophysical parameters controlling leaf cell growth was undertaken; so that the basis of the response at the level of the leaf cell could be elucidated and used to predict the specific mechanisms of root-shoot communication.

6.2 EFFECTS OF ROOT TREATMENTS ON THE BIOPHYSICAL PROPERTIES OF THE LEAF CELLS

As shown in Section 3.3.3, root excision and root cooling had their greatest effects on leaf cell enlargement, cell division being only slightly reduced. Consequently, the use of the Lockhart model (Lockhart, 1965) in the analysis of the observed effects was considered appropriate. According to that model, for a treatment such as root excision or root cooling to reduce the rate of leaf cell enlargement, it must either reduce turgor (P), wall extensibility (WEx) or hydraulic conductivity (Lp) or increase wall yield threshold (Y) or osmotic potential (π). Although criticised as being incomplete and unsuitable for general application, (Ortega, 1985; Tomos, 1985) the use of the Lockhart model here allowed the identification and testing of those parameters likely to involved in the effects on leaf cell growth.

Cell turgor (P), effectively the driving force for cell enlargement, might be expected to change in response to root excision or root cooling. In the present study, this was initially the case for root cooling, the onset of treatment being associated with a significant fall in leaf turgor. However, on all subsequent days, although leaf growth rate decreased substantially, leaf turgor was maintained at or close to its control value,

primarily it was concluded by stomatal closure. In the case of root excision, reductions in leaf growth on days 9, 10 and 11 were associated with decreased leaf turgor. However, the same mechanism could not account for the reduced growth rates exhibited on day 8 or during the dark periods between later days.

Interpretation of the turgor data obtained is complicated by doubts about the accuracy of the values quoted. One criticism is that they were obtained from measurements of osmotic and total potentials and so include two sources of measuring error. In addition, the values obtained relate to the leaf as a whole, probably representing a mean value for all the leaf cells. Such measurements would be inappropriate if only some of the cells, for instance those in the upper and lower epidermes were contributing to lamina expansion (Hsiao, Silk and Jing, 1985). However, in the absence of sophisticated techniques for the measurement of turgor in individual cells, (Zimmerman and Steudle, 1978), the bulk leaf measurements obtained here must be relied upon; the conclusion from these is that although turgor plays an important role in the control of leaf growth, other biophysical parameters may have been limiting in RE and RC plants, particularly during the critical period up to day 9.

No determinations of hydraulic conductivity (Lp) of leaf tissue were attempted in the present work, but reductions in this parameter could conceivably have caused the effects on leaf turgor discussed above. For instance in RE plants, root permeability decreased from day 7 onwards and may have resulted in reduced transport of water to the leaf cells.

Because of doubts about its basis, particularly its location in the plant hydraulic conductivity is frequently neglected in studies of leaf cell

growth. However, it may play an important limiting role, particularly in complex tissues (Tomos, 1985).

Bulk leaf osmotic potential ($\bar{\pi}$) may have slightly decreased with time in control and RC plants but exhibited no obvious treatment affect (fig 4.4, p119). However as was the case for cell turgor, the osmotic potential measurements obtained were whole-leaf values and no account was taken of possible variation between tissues and cells. Nevertheless they provided no evidence that the solute relations of the leaf cells were affected by either treatment (Fig 4.4, p119; Table 4.1, p129).

In several published studies, leaf cell wall extensibility (WEx) has been shown to be sensitive to a variety of environmental factors and to be altered sufficiently to significantly affect leaf growth rate (for discussion, see Van Volkenburgh and Cleland, 1984). In the present study, similar effects were suspected but were largely obscured by the considerable variation introduced by the methods used to measure and calculate extensibilities. The original measurements were highly variable, partly because they were obtained indirectly from measurements of total and elastic extensibilities, but probably also because of spatial heterogeneity in the leaf (Hsiao, Silk and Jing, 1985). The first of these sources of error was inherent in the technique and could not be reduced. However, the second may have been minimised by using tissue strips excised from identical positions on each leaf instead of from the same general area. Correction of the

original data for differences in tissue cross-sectional area also contributed to the variability in the final results. In Chapter 5, the question of which correction factor was most appropriate was discussed. However, doubt remains as to whether any correction at all was required. The procedure actually used was to correct measured extensibilities for differences in tissue weight per unit area, however much of that weight may have been located outwith the cell walls. Furthermore, it is possible that the walls of the epidermal cells contributed most to Instron-extensibility. If this were the case, the difference in effective cross-sectional area between treatments would probably have been negligible and any correction entirely inappropriate.

In view of the above, the raw, uncorrected data would appear to provide the most accurate impression of the effects of root treatments on leaf cell wall extensibility. These data show that root excision and, to a lesser extent, root cooling both reduced the plastic extensibility (PEx) of leaf tissue, and that these effects were discernible within one day of the beginning of treatment. Also suggested is that over the first two days of treatment, leaf cell growth in treated plants was limited by this parameter. Thus, over the critical period between days 7 and 9, root treatments may have inhibited leaf growth by lowering wall extensibility and reducing the sensitivity of leaf cells to turgor pressure.

The measurements of wall yield threshold (Y) obtained in this study are of limited application because they relate to a period (days 10 to 14) some time after the main effect on leaf growth rate had occurred. Although no significant effect was revealed by these measurements, there is indirect evidence that wall yield threshold may have been limiting for leaf growth in RE and RC plants. Thus on several occasions, mainly

during the dark periods, leaves of control and treated plants had identical turgor values but only the control leaves grew. This suggests that the value of Y differed between the treatments, being less than P for control plants, but equal to or more than P for those subjected to root excision or root cooling.

In summary, the data obtained suggests that root excision and root cooling affected the yielding properties of leaf cell walls, and that these effects may have contributed to the reductions in leaf growth rate observed. However, two important reservations concerning these conclusions must be considered. Firstly the timing of the measurements made was unsuitable, particularly the choice of the first harvest for wall extensibility measurements as 20 or 24 hours after treatment. A more useful design would have had harvests at 2 to 3 hour intervals throughout the first 48 hours of treatment. This would have allowed the details of any effects, including their timing and possible correlation with changes in leaf extension rate to be ascertained. The second reservation about any conclusions drawn from these data is that it is inappropriate to predict detailed mechanisms on the basis of measurements of only a few parameters: this is particularly so when knowledge of these parameters and their control and interaction is so poor (Lawlor and Leach, 1985). For instance wall yeild threshold and wall extensibility are known to vary with changes in wall geometry (Hsaio et al, 1985). Thus it is impossible to show whether the changes in these parameters recorded here were responsible for the recorded effects on leaf cell enlargement, or were actually caused by them.

Despite these reservations, effects of root treatments on Instron-measured extensibility of leaf tissue were recorded consistently enough to suggest

that this may be the basis for a mechanism by which leaf growth rate might be altered to suit the condition of the root system; the possible details of such a mechanism are now discussed.

6.3 POSSIBLE MECHANISMS OF CONTROL OF LEAF CELL WALL BIOPHYSICAL PARAMETERS BY ROOTS

That cell wall extensibility is an important variable in the control of leaf cell growth has been confirmed by several published studies (eg Bunce, 1977; Acevedo, Fereres, Hsiao and Henderson, 1979; Van Volkenburgh and Cleland, 1980; Taylor and Davies. 1985). The parameter has been shown to vary with season, light quality and environment, and to limit cell enlargement even in the presence of values of turgor which would otherwise sustain high growth rates (Bunce, 1977; Davies and Van Volkenburgh, 1983). Of particular interest are the findings that drought and salinity frequently reduce the sensitivity of leaf growth to turgor (Matthews, Van Volkenburgh and Boyer, 1984; Termatt, Passioura and Munns, 1985). Although in some studies it was not determined, in others wall extensibility was found to be altered by treatment and when it decreased, was considered to be responsible for the decrease in sensitivity to turgor which reduced growth (Bunce, 1977; Matthews et al, 1984).

One of the principal effects of the root treatments applied in the present study was to reduce water uptake; in this respect root cooling and root excision resemble stress treatments such as drought and salinity. Since wall extensibility also decreased, the mechanism involved could be analagous. Exactly how reduced water supply might affect leaf cell wall extensibility is not clear (Lawlor and Leach, 1985) although the timing of the effect appears not to be critical since both long and short-term drought treatments are effective (Matthews et al, 1984; Hsaio, Silk

and Jing, 1985). One group has suggested that water deficit could alter wall extensibility by altering the rate of wall-loosening reactions or some aspect of cell wall structure (Matthews et al, 1984); there is also evidence that water deficit may interfere with proton extrusion and acid growth (Van Volkenburgh and Boyer, 1985). However an alternative mechanism might involve the growth inhibitor abscisic acid (ABA).

In the present study, ABA began to accumulate in the primary leaves within one day of the beginning of treatment, thus approximately coinciding with the recorded decreases in plastic extensibility and leaf growth rate. Exogenous ABA applied to Phaseolus leaf tissue has been shown to reduce cell growth by reducing plastic extensibility (Van Volkenburgh and Davies, 1983), although the effect may have been mediated by an associated reduction in stomatal aperture. In the present experiments, the increase in ABA content of the primary leaves was also associated with a decrease in stomatal aperture. Therefore, a possible sequence of events could be that root treatments caused the accumulation of ABA in the leaves which induced stomatal closure, and inhibited cell growth by reducing WEx. Such a mechanism fulfills the requirement for a rapid means of communication to the shoot of a change in the state of the root, and also facilitates the shoot response - a reduction in leaf growth.

Indirect evidence that leaf growth in RE and RC plants may have been reduced by the accumulation of some substances which lowered WEX is provided by the observation that floating leaf pieces on polyethylene glycol (for Y determination) for several hours apparently increased WEx and stimulated growth. Such observations are consistent with the hypothesis that an inhibitor of growth was present in the leaf tissue and was leached out following excision and floating on the PEG solution. The time period involved

(5 to 6 hours) would be sufficient for a change in WEx sufficiently large to enhance cell growth to take place (Van Volkenburgh and Cleland, 1981).

Other indirect evidence that wall extensibility may have been reduced by the accumulation of some inhibitor is that regeneration of new root apices in plants subjected to a single root excision treatment was associated with a rapid increase in leaf growth rate. Perhaps the production of new root apices caused the inhibitor to be removed from the leaves, and allowed growth potential accumulated during the period of inhibition to be expressed. Such a process might be analagous to that recorded in plants subjected to mild, transient drought (Acevedo, Hsaio and Henderson, 1971). Shortly after the commencement of soil drying, leaf extension ceases. However following rewatering, leaf extension rate rises to a value frequently greater than that exhibited by untreated plants. This suggests that the leaf cells of treated plants stored the capacity for growth, perhaps as preconstructed but unused cell wall components (Lawlor and Leach, 1985).

Evidence against the hypothesis that increased leaf ABA might limit growth by lowering wall extensibility is that the concentration of the growth inhibitor is frequently highest in young leaves where growth rate and presumably wall extensibility are maximal (Zeevart, 1977; Digby and Firn, 1985). This would suggest that no relationship existed between wall extensibility and ABA concentration. However the sensitivity of the leaf cells to ABA might not be constant but might vary with leaf age (Trewavas, 1982). In young leaves, stomatal sensitivity to ABA is poorly developed so if wall extensibility was regulated via an effect on stomatal aperture, it would be unaffected by ABA concentration (Radin, 1984). Similarly, if the sensitivity of leaf cell WEX to ABA was due to some

aspect of wall structure (for instance, the presence of specific load-bearing bonds), this might be undeveloped in young leaves, and only develop around the time of unfolding. Thus even though the precise mechanism involved in unclear, it is feasible that one of the factors controlling leaf cell wall extensibility and perhaps limiting leaf growth following root excision or root cooling is leaf ABA concentration.

The finding that leaf growth in RE plants was greatly enhanced by the appearance of new lateral roots could also be interpreted as showing that the root apices supply the shoot with a factor that promotes cell growth, possible by increasing WEX. The root tips regenerated in these plants contributed little to the total area of the root system and so are unlikely to have substantially increased its uptake of mineral nutrients or water (although the possibility was considered in Chapter 4 that the emergence of new primordia may have increased water uptake by breaking the continuity of the endodermis). However, even unemerged root primordia have been found to be capable of substantial cytokinin production so these very small roots may nevertheless have contributed significantly to the concentrations of root-derived growth substances reaching the shoot (Gollnow and Letham, 1978; Van Staden and Davey, 1979).

The evidence that cytokinins and gibberellins are produced and/or metabolised in plant root apices is now considerable and the implications for plant growth and development well understood (Carr and Reid, 1968; Gollnow and Letham, 1978; Van Staden and Davey, 1979). However remarkably few instances of root-derived growth regulators influencing leaf growth have been described (Digby and Firn, 1985). The work of Carmi and co-workers has provided correlative evidence that root-derived cytokinins may affect the

expansion of Phaseolus primary leaves (Carmi and Koller, 1978; Carmi and Van Staden, 1983). However most of that work was conducted on relatively mature tissue and the situation in the newly expanding leaf is still unclear. Some instances of exogenous cytokinins promoting leaf growth have been found, for instance in Phaseolus (Scott and Liverman, 1956; Hayes, 1978; Yokoyama, Naito and Suzuki, 1981) and Nicotiana (Engelke, Hamzi and Skoog, 1973). However frequently such treatments have no effect or may actually inhibit leaf growth (eg Richards, 1980). The lack of agreement may indicate that no relationship exists between leaf growth and cytokinin content, or may be due to the use of unsuitable concentrations or the incorrect type of cytokinin, or to interactions between applied and endogenous plant growth regulators. Leaf disk experiments do lend some support to the hypothesis that cytokinins promote leaf growth (Powell and Griffith, 1960; Grierson, Chambers and Penniket, 1977).

Nevertheless the inconsistency of these data, and the poor reliability of correlative studies when a process of the complexity of leaf growth is involved, suggest that a simple link between leaf growth and cytokinin concentration does not exist (Goodwin and Erwee, 1983; Digby and Firn, 1985).

In contrast to the case for cytokinins, good correlations have been established between leaf growth rate and gibberellin content (Goodwin, 1978), although most positive results were obtained with cereals (Digby and Firn, 1985). However, few consistent correlations between applied gibberellins and leaf growth have been established (Goodwin and Erwee, 1983). A popular hypothesis is that gibberellins are involved in the modification of leaf growth but probably do not control it directly. Once again however, such conclusions are difficult to draw from such studies since so many factors may be interacting (Digby and Firn, 1985).

In order to avoid some of the complications of whole plant systems, several recent studies have been conducted using excised tissue *in vitro*, and concentrating on the biophysical parameters of the Lockhart equation (Cleland, 1981). Gibberellins have been found to promote or have no effect on the wall extensibility of stem tissue. However no measurements of the effects of these substances on leaves have been attempted (Cleland, 1981). In the case of cytokinins, expansion of excised cotyledons of radish and cucumber has been shown to be promoted by exogenous zeatin (Huff and Ross, 1975); apparently cell enlargement is promoted through a cytokinin-induced increase in wall extensibility (Thomas, Ross, Chastain, Koomanoff, Hendrix and Van Volkenburgh, 1981). Such correlations between cytokinin and/or gibberellin supply and wall extensibility support the hypothesis that these substances play mediating roles in the effects of certain treatments which modify leaf growth. In one system, white light has been shown to promote cell enlargement in red-light-grown *Phaseolus* leaves by increasing leaf cell wall extensibility (Van Volkenburgh and Cleland, 1980). A recent short communication claims that gibberellins and cytokinins can cause the same changes, the latter actually mediating the white light effect (Brock and Cleland, 1983).

The finding that gibberellins and cytokinins may affect the wall extensibility of *Phaseolus* primary leaf tissue is of great significance in the context of the present work since it suggests a further mechanism by which the effects of root excision and root cooling on leaf growth could be mediated. Thus the root treatments used could inhibit leaf growth by limiting the supply of cytokinins and/or gibberellins reaching the shoot and thus reducing the extensibility of the leaf cell walls. Although highly speculative, such a mechanism would have several of the features necessary for it to operate in the living plant. In particular

it would be sufficiently sensitive to allow a response in the leaf cells to relatively minor changes in the state of the root (Bradford and Hsiao, 1982(b); Blackman and Davies, 1984; Termatt, Passioura and Munns, 1985).

That plant roots might control leaf growth via specific positive and negative chemical messengers (here cytokinins, gibberellins and abscisic acid were suggested) has been proposed since the early work of Went (1938) and Chibnall (1954). The attractions of such a mechanism over others based on water supply or the distribution of mineral or organic nutrients are its specificity and sensitivity. However care must be taken not to implicate plant growth regulators unnecessarily (Digby and Firn, 1985). Much of the data obtained in the present experiments point to a control system in which a stimulus received by the roots is communicated to the shoot and expressed, possibly via an effect on wall extensibility, as a change in the rate of leaf cell enlargement. While plant growth regulators might mediate these effects, a great deal of work must be performed before this can be confirmed.

6.4 CONCLUSION

Although its identity is unknown, that a control system exists between the root and shoot is confirmed by numerous published accounts (for review, see Section 1.2); the present study provided the opportunity to inquire why such a system is necessary. Its main function appears to be to ensure the coordination of growth of the different parts of the plant and the response, in terms of growth, to changing environmental conditions which might impose different demands. For instance, since the roots provide the plant with almost all its mineral nutrient and water supplies, any damage or stress imposed on the roots must be reacted to, to prevent disruption of the functional equilibrium between root and shoot. This

reaction might involve the diversion of assimilate and other organic materials to the root for the regeneration or repair of tissue. Consequently shoot demand for those materials, as well as water and minerals, which may be in short supply must fall. By the possession of a control system which responds to damage or stress in the roots by causing the slowing or cessation of leaf growth, this requirement is met.

The principal advantage to the whole plant of a reduction in leaf growth might be the creation of an excess of assimilate and other metabolites for use in regeneration and repair of roots. However another important consequence confirmed by the present work is the conservation of water. Since water loss is related to lamina area, any reduction in the rate of lamina expansion must inevitably slow the increase in demand associated with seedling growth. As further indirect evidence, other responses aiding water conservation or tolerance of water deficit were recorded including partial stomatal closure, changes in leaf anatomy and the formation of small cells with highly elastic cell walls (Tyree and Jarvis, 1982; Kim and Lee-Stadelman, 1984; Stadelman, 1984).

The evidence presented suggests that the effects of root treatments at the level of the leaf cells might be mediated by changes in wall extensibility. Other findings suggest that the communication between root and shoot may involve chemical messengers, possible known plant growth regulators. It is in these two areas that the greatest scope for future work now exists. An early priority must be to establish the precise biophysical mechanisms underlying leaf cell growth. An approach must be chosen which will overcome the problem of spatial heterogeneity encountered in the present work. One method might be to exploit the spatial separation

of developmental events which occurs in certain leaves, using for instance cereal leaf extension zones or isolated epidermal layers of broadleaves. Once a suitable system is selected, the biophysical parameters of the Lockhart model could be measured on single cells. Such a study would also require to measure all the biophysical parameters contributing to the control of growth, since the complex interactions between parameters make assumptions about unknowns potentially misleading; a more thorough understanding of cell wall structure and development would aid the interpretation of such findings. Correct timing of such measurements would also be important since in the present study, inappropriate timing of harvests meant that it was not possible to state categorically whether recorded changes in PEx actually brought about, or were caused by changes in cell growth rate.

With the development of techniques for the accurate measurement of the different growth parameters, it may become necessary to reappraise the Lockhart equations. As shown, this model provides a useful framework for investigations of cell growth and its control. However, growing evidence suggests that it fails to take into account interactions between different parameters and is limited to equilibrium or steady-state growth (Ortega, 1985).

With the establishment of suitable techniques for the measurement of the growth parameters at the cell level, it should be relatively straightforward to begin identifying the mechanisms involved in their control. An in vitro system could be developed for the assessment of the effects of specific substances, including plant growth regulators, on the different parameters. A simultaneous investigation of the effects of different root treatments on the composition of the xylem sap might identify other possible chemical messengers.

Such studies are complicated by numerous technical and theoretical difficulties. However by adhering to the dual approach of seeking both the basis of communication from root to shoot and the mechanism involved in the reception of the stimulus at the leaf cells, the long-term reward should be an understanding of the mechanism by which plant roots influence , and perhaps ultimately control, leaf growth.

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A1 CONSTRUCTION AND OPERATION OF THE EDINBURGH TENSIOMETER

A1.1 INTRODUCTION

The Edinburgh Tensiometer was built to measure the wall extensibility of plant tissue as part of the present investigation. Although designed for use on *Phaseolus* leaf tissue, it has been employed successfully with a variety of material including *Lycopersicon* leaves and *Spirodela* fronds. In operation, it is similar to the Instron-type machines used by Olsen, Bonner and Morre (1965), Cleland (1967) and Van Volkenburgh, Hunt and Davies (1982). Its design is based on that of an instrument built at the University of Lancaster (Van Volkenburgh et al, 1982) except that it incorporates several important modifications which make it cheaper to build and easier to use. According to Cleland (1981) and others, the rheological properties of plant cell walls can be determined from load-extension curves produced for strips of tissue pre-treated to eliminate metabolic activity and remove proteins. In the Instron technique, as the method is known, a strip of plant tissue is stretched at a constant rate (ie subjected to a constant rate of increase of strain) and stress on the tissue recorded. When a pre-defined stress is reached, the tissue is returned to its original length and the procedure repeated. The resultant load-extension curves give respectively, the total and elastic extensibilities of the tissue. The difference between the two gives irreversible or plastic extensibility ($P\dot{E}x$), the parameter thought to represent the wall extensibility ($W\dot{E}x$) of living cells (Section A1.3.4). Although the interpretation of Instron-measured extensibilities, particularly the relationship between $P\dot{E}x$ and $W\dot{E}x$ is subject to debate (Taiz, 1984; Cleland, 1984; see Section A1.4), the Edinburgh Tensiometer provides at least qualitative estimates of plant tissue extensibility with speed, accuracy and repeatability.

A1.2 DESCRIPTION

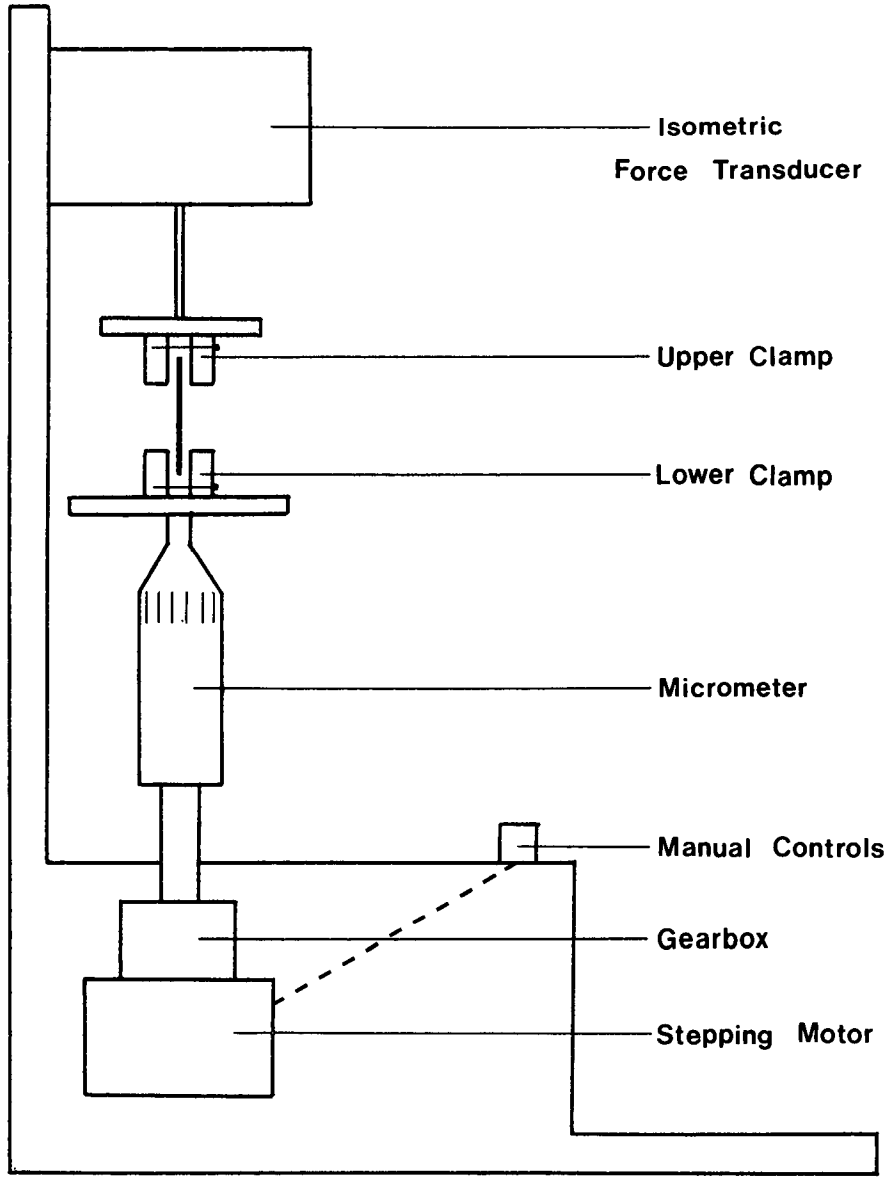
The instrument (Fig A1.1, p 272) consists of a pair of clamps, between which the plant tissue is held, a motor which drives the extension of the tissue, a screw micrometer which indicates the distance between the clamps and a force transducer which records the load applied.

The clamps which hold the tissue are made of aluminium and have neoprene jaws to ensure a firm grip without damage. The upper clamp is attached to the armature of an isometric force transducer and is stationary. The lower clamp is moved by a stepping motor which is driven by a custom-designed integrated-circuit and associated logic circuitry. Control of the motor may be manual, in which case speed and direction are adjustable, or automatic when both are preset. Manual control, which is necessary for correct positioning of the lower clamp, is facilitated by inter-locking push-buttons, one for selection of motor SPEED (fast or slow), one for DIRECTION (forward or reverse) and a third which is depressed to SHIFT the motor. The automatic mode, which is used to control the extension of the tissue, is selected by pressing a fourth button, RUN. In this mode, the lower clamp is moved downwards at a rate of 90mm h^{-1}.

The motor is attached to the lower clamp via a reduction gearbox and a sliding coupling. Incorporated into the vertical assembly is a screw micrometer which indicates the distance between the clamps with an accuracy of 0.01mm.

While the tissue is being extended, the output from the force transducer is amplified and plotted against time on a chart recorder. Since the motor speed in the automatic mode is constant and known, displacement of the lower clamp and extension of the tissue are proportional to time. Therefore, the load/time curve obtained can be regarded as a load/extension

Figure A1.1


Isometric
Force Transducer
Upper Clamp
Lower Clamp
Micrometer
Manual Controls
Gearbox
Stepping Motor

relationship (see Section A1.3.2).

The main advantages of this instrument over similar models are firstly, that the use of a variable-speed motor means that the lower clamp can be returned to its original position, following the first extension of the tissue, at a particularly high speed. This lessens the likelihood that the tissue will undergo any additional deformation once the extension has been stopped. Secondly, the fact that the extension of the tissue is constant with time, and that the length of the tissue at any time can be determined from the micrometer reading at the beginning of the extension means that tissue length need not be monitored during extension. In other models, linearly variable displacement transducers were used to monitor tissue length. However as well as being expensive and often difficult to use, these components require continuous recording (as does the force transducer) so either two y - t recorders or an x - y recorder must be used. The instrument described here requires only one y - t recorder. (For further technical details, see Section A1.5).

A1.3 OPERATION

A1.3.1 Preparation of plant material

In choosing and harvesting plant material for extensibility measurements, several points were considered. Firstly, tissue strips had to be large enough to manipulate between the clamps, but not so large that they became tangled. For Phaseolus leaves, strips 5mm wide and 12mm long (which included 3mm - 4mm at each end for the clamps to grip) were found to be most satisfactory. Secondly, the tissue strips to be extended had to be homogenous in structure, so in excising material, prominent veins and marginal regions were avoided. Tissue strips had also to be cut cleanly and not damaged in any way. This was achieved by using only freshly excised leaves, in which turgor was high, and cutting with disposable scalpel blades.

Tissue strips were obtained by placing the leaf, adaxial surface downwards, on a wad of paper towels and cutting with a pair of scalpel blades taped together to give the chosen strip width. As soon as they were excised, the strips were immersed in boiling methanol for three minutes. Tissue prepared in this way could be stored in cold methanol for up to six months without changes in extensibility.

A1.3.2 Calibration

An important feature of the operation of this instrument is that the output of the load transducer plotted against time gives a line which can be treated as a load-displacement or load-extension relationship. To calibrate the time axis in units of displacement, two constants were required:-

Vc the velocity of movement of the lower clamps (90mm h^{-1}).

Vp the velocity of movement of the chart paper (1200 divisions h^{-1}).

Then one chart division represented (Vc/Vp =) 0.075mm of tissue extension.

A1.3.3 Measurement of extensibilities

The two load-extension curves required for the calculation of total, elastic and plastic extensibilities were obtained as follows. Using the manual controls and reading from the micrometer scale, the position of the lower clamp was adjusted to give the required starting distance (5mm). Then the tissue strip was removed from the methanol and inserted between the clamps, and the clamp screws tightened. Rehydration of the tissue was achieved by suspending a drop of distilled water between the clamps. Then the chart recorder was switched on and extension of the tissue commenced when the pen reached a pre-determined and marked point. When a load of 20g was indicated, the extension was stopped and the lower clamp quickly returned to its starting position using the manual controls. Then the extension was repeated and the strip removed and clamps dried before insertion of the next sample.

A1.3.4 Interpretation of load-extension curves (Fig. A1.2, p 277)

For each curve, the marked point corresponding to the beginning of the extension indicated the starting distance between the clamps (5mm) and was used as a reference point on the extension axis. Beginning with the curve of the first extension, a straight line was drawn through the linear portion and the points at which it crossed the lines corresponding to zero and 20g load marked. Tissue lengths at these loads were calculated by counting the number of chart divisions fromthe starting point, multiplying by a conversion factor (=0.075; see Section A1.3.2) to give the extension in mm and adding to the starting length (5mm). Total extensibility (TEx) was then obtained from:

$$\% \text{ extensibility} = \left(\frac{Lf - Li}{Li} \right) \times \frac{100}{2} \qquad \text{Equation A1.1}$$

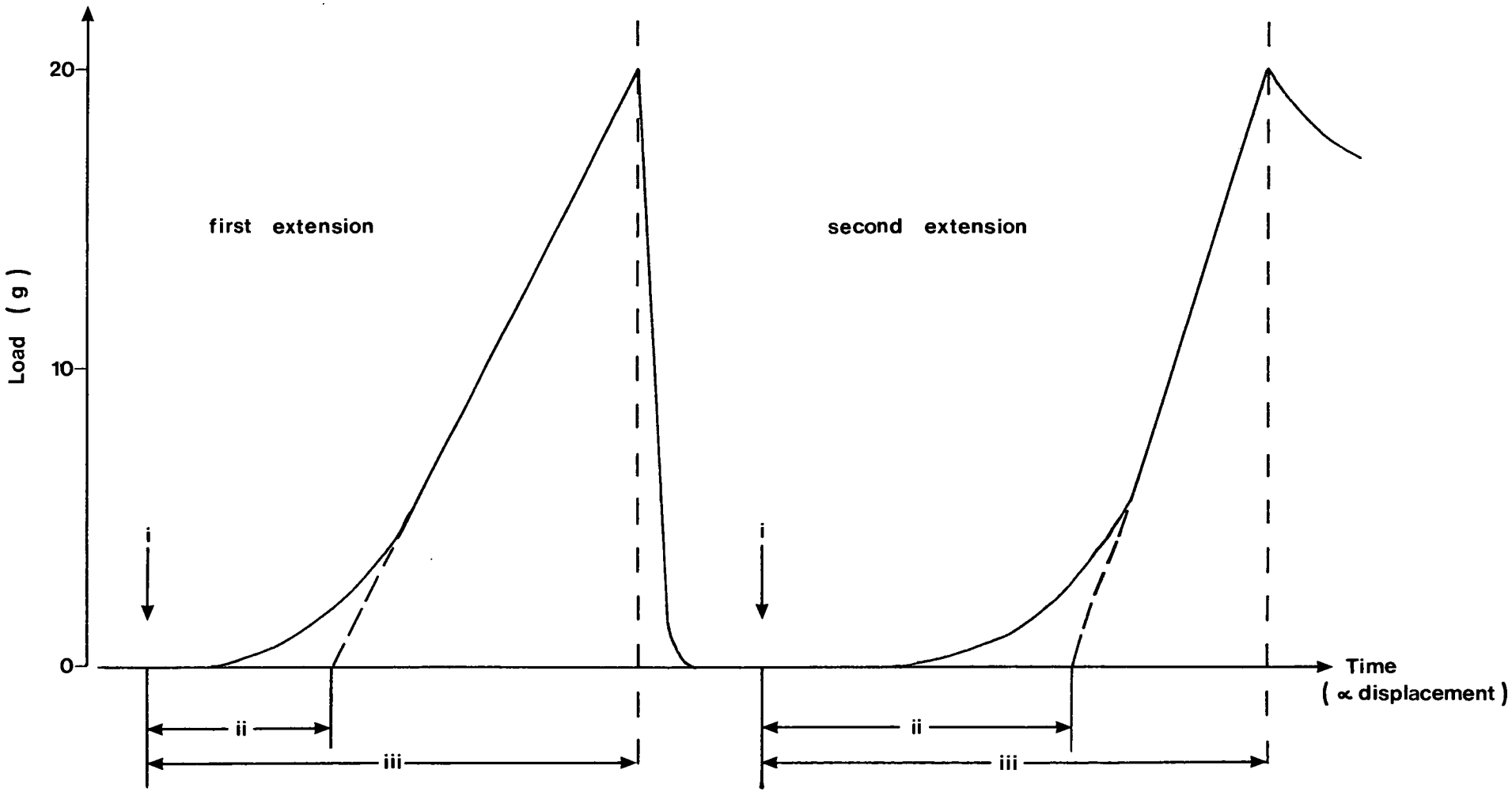
Figure A1.2

Fig. A1.2. Typical pair of load extension or load-displacement curves for a strip of Phaseolus primary leaf tissue.

i. → starting distance between clamps (5mm)

ii. → displacement of clamps necessary to commence tissue extension

iii → displacement of clamps necessary to extend tissue to a load of 20g.


20
10
0
Load (g)
first extension
second extension
i
i
ii
iii
ii
iii
Time
(∝ displacement)

where Li was the tissue length at zero load and Lf the length at 20g load. (Note that the result was divided by two to allow expression of extensibility in the conventional form of % extensibility per 10g load).

Elastic extensibility (EEx) was obtained by repeating the above procedure for the curve of the second extension, and plastic extensibility (PEx) obtained by subtraction, since:

$$TEx = PEx + EEx \qquad \text{Equation A1.2}$$

A1.3.5 Correction of measured extensibilities for differences in tissue thickness or cross-sectional area

Since the measured extensibility of any material is dependant upon the area over which force is applied, an index of cross-sectional area is required when comparing samples which may differ in this parameter. In plant material, such correction may be important since tissue thickness and internal structure may vary, particularly between plants receiving different treatments (Cleland, 1967; Van Volkenburgh, Hunt and Davies, 1982). One way of approximating tissue area subjected to force is to measure mass per unit length (assuming tissue width to be constant). Then corrected extensibility is given by:

$$\text{measured extensibility} \cdot \left(\frac{\text{strip mass}}{\text{strip length}}\right) \qquad \text{Equation A1.3}$$

and expressed as a percentage extensibility per 10g load per unit mass. For samples of plant tissue, a correction based on unit mass rather than cross sectional area may be most appropriate because it is not affected by tissue internal structure. However whether such corrections are accurate or necessary is contentious (see Section 5.4).

A1.4 THE RELATIONSHIP BETWEEN INSTRON-MEASURED PLASTIC EXTENSIBILITY (PEx) AND THE GROWTH PARAMETER WALL EXTENSIBILITY (WEx)

According to Lockhart (1965), the rate of expansion of a plant cell can be defined by a simple model incorporating several biophysical parameters (For discussion, see Chapter 1). One of these, wall extensibility, is defined as the rate at which the cell wall undergoes irreversible extension under constant strain (Cleland, 1981). The Instron technique and two others, the Creep and Stress-relaxation tests, were developed to provide estimates of this parameter; the Instron technique in particular because of its ease of use, has been employed extensively, particularly in the study of the effects of plant growth regulators on cell growth (see Cleland (1981) and references therein). However it is clear that the parameter actually measured (PEx) cannot be exactly equivalent to the growth parameter (WEx). One major criticism of the Instron technique is that the stress applied is uniaxial while that which drives cell expansion (ie turgor) is multiaxial. Another major fault of the technique is that because it uses tissue in which metabolism has been eliminated, the extensibility it measures is not present (instantaneous) but probably past (Cleland, 1984). This is because present or steady-state expansion of the cell wall probably requires a number of metabolic processes including wall loosening and the synthesis and/or release of a wall-loosening factor (Taiz, 1984). What the Instron technique may measure is the extent of wall loosening which has occurred and which has not been eliminated by additional wall synthesis. Thus PEx values may be regarded as measurements of the average WEx of the tissue over the one to two hours prior to harvest (Cleland, 1984; Tomos, 1985).

Despite these problems of interpretation, satisfactory correlations have been shown between results obtained by the Instron technique and the

other methods (Tomos, 1985), proving it to be a satisfactory qualitative method of studying the extensibility of plant cell walls. Its particular advantages over the Creep and Stress-relaxation techniques are that it is rapid and easily repeated, and that it provides estimates of both reversible (elastic) and irreversible (plastic) extensibilities.

A1.5 ADDITIONAL TECHNICAL DETAILS

A general circuit diagram of the Edinburgh Tensiometer is shown in Fig A1.3 (p 284). The principal components used in its construction are as follows:

a) Stepping motor and gearbox

Stepping motor - Crouzet 82750 (12V, 3.4W, 20 steps rev^{-1})
(Crouzet Ltd)

Gearbox - RS 336-444 (Ratio 25:1)
(RS Components)

A stepping motor was chosen to give positional accuracy and flexibility in selection of speed and direction. When used in conjunction with the gearbox, it gives one revolution of the micrometer screw per 500 steps, or a displacement of the clamp of 1 μm per clock pulse received.

b) Motor drive circuit and logic circuitry

Motor drive circuit - Signetics SA1027 (For 12V stepping motor)
(Farnell Electronics Components Ltd)

The motor drive circuit (Fig A1.4, p 284) has three control lines for speed (T), direction (R) and stop/start (S). Four switches, operated manually by interlocking push buttons, and several Cmos logic gates are used to control these lines as follows:

NAND gates E and F form slow and fast oscillators respectively. These are adjustable, allowing manual setting of slow and fast drive rates. The oscillator output required is selected by gates A, B, H and C. Closing SPEED switch S1 opens gate A and closes gate B, while opening S1 closes A and opens B. The selected oscillator output is passed by NOR gate C to drive line T, driving the motor at the required rate. Direction is controlled by NAND gate G. Closing the DIRECTION switch S2 takes line R 'low' and sets the motor to run forward. Opening S2

sets the motor to run in reverse.

Speed and direction now set, the motor is run manually by closing SHIFT switch S3, which controls gate D and line S. Safety microswitch S5 also controls gate D, so that if the drive runs to its limit, S5 opens and stops the motor.

The RUN switch, which is used to enter the automatic mode S4(A and B) overrides S1, 2 and 3. Closing S4A selects the slow oscillator. At the same time, S4B selects forward direction and runs the motor by taking pin 13 out of gate D 'low' via the diode.

The above circuit requires a stabilised +12V supply of approximately 400mA.

c) Force transducer and amplifier.

Isometric force transducer - Bioscience BIO-50020-7 UFI (+ 200g) (Bioscience)

Amplifier - CIL Electronics SGA 302 (Strain Gauge amplifier) (CIL Electronics Ltd)

An isometric force transducer was used because the armature to which the upper clamp is attached must not be displaced by the movement of the lower clamp.

Figures A1.3 and A1.4

Fig. A1.3. (opposite) General circuit diagram of the Edinburgh Tensiometer.

Fig. A1.4. (overleaf) Diagram of the motor drive circuit used in the Edinburgh Tensiometer. (Drawn from a sketch by R Hart).

The switches in the diagram correspond to the following:

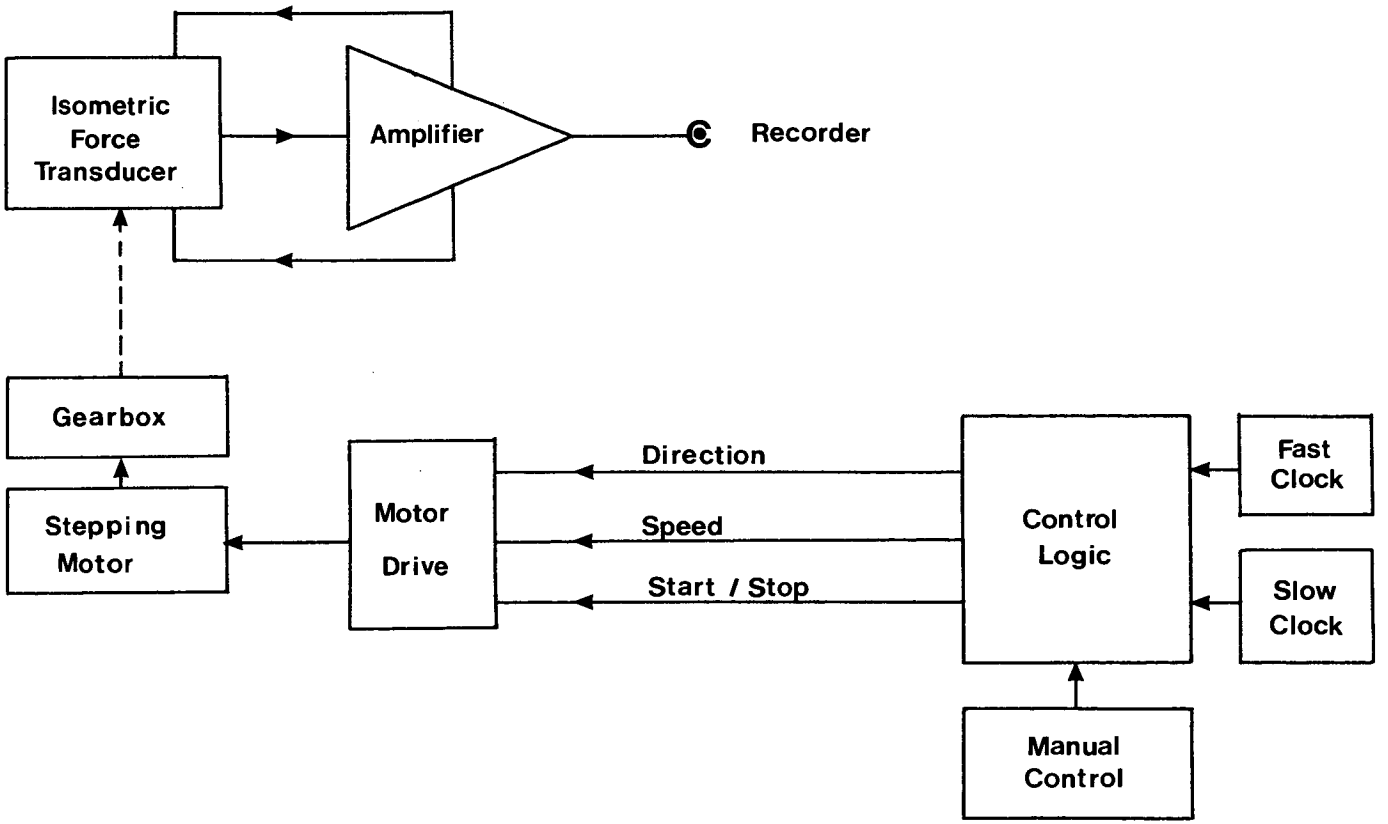
S1 speed

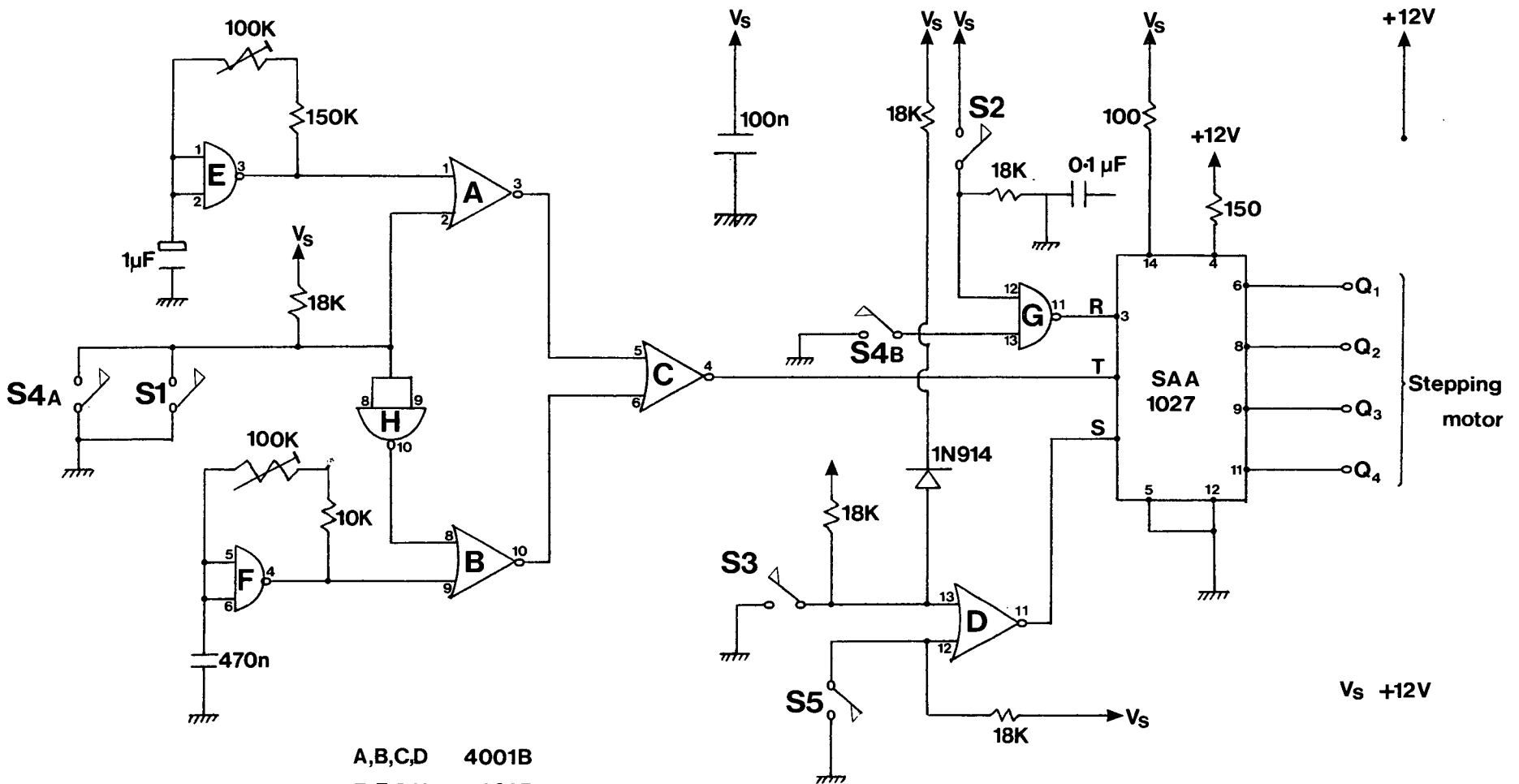
S2 direction

S3 shift

S4 run

S5 safety microswitch.


Isometric
Force
Transducer
Amplifier
Recorder
Gearbox
Stepping
Motor
Motor
Drive
Direction
Speed
Start / Stop
Control
Logic
Fast
Clock
Slow
Clock
Manual
Control


100K
150K
E
A
1µF
Vs
18K
S4A
S1
H
100K
10K
F
B
470n
C
Vs
100n
Vs
Vs
18K
S2
18K
0·1 µF
Vs
100
+12V
150
+12V
G
S4B
R
T
S
SAA
1027
Q1
Q2
Q3
Q4
Stepping
motor
1N914
18K
S3
D
S5
18K
Vs
Vs +12V
A,B,C,D 4001B
E,F,G,H 4093B
both pin 7 ground
pin 14 +ve

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A2 EFFECTS OF ROOT EXCISION AND ROOT COOLING ON THE CONCENTRATION OF THE MAJOR MINERAL NUTRIENTS IN WHOLE PLANTS AND PRIMARY LEAVES.

Table A2.1 (p 289) Effects of root excision (RE), root cooling (RC) or no treatment (CONTROL) on the total content (g) and concentration (g per unit dry weight) of nitrogen (N), phosphorus (P) and potassium (K) in whole seedlings harvested on days 7 (the first day of treatment), 11 and 13.

Table A2.2 (p 290) Effects of root excision (RE), root cooling (RC) or no treatment (CONTROL) on the total content (g) and concentration (g per unit dry weight) of nitrogen (N), phosphorus (P) and potassium (K) in primary leaves harvested on days 7 (the first day of treatment), 11 and 13.

In both tables, the results represent means of 6 values with standard errors in brackets.

Treatment	Day	Content of element in whole plant (g)		
		N	P	K
CONTROL	7	14.01 (0.95)	2.29 (0.10)	7.67 (0.41)
	11	25.65 (0.42)	3.63 (0.12)	29.47 (0.75)
	13	44.14 (2.74)	6.86 (0.43)	55.23 (3.57)
RE	7	13.24 (0.92)	1.90 (0.09)	6.36 (0.09)
	11	15.01 (0.66)	2.50 (0.09)	9.81 (0.23)
	13	15.87 (0.72)	2.59 (0.10)	10.74 (0.33)
RC	7	14.01 (0.95)	2.29 (0.10)	7.67 (0.41)
	11	17.02 (1.19)	2.87 (0.18)	12.70 (1.05)
	13	19.58 (0.71)	2.79 (0.18)	15.17 (1.31)

Treatment	Day	Content of element in whole plant (g $gdwt^{-1}$)		
		N	P	K
CONTROL	7	36.67 (2.15)	5.98 (0.20)	20.11 (1.03)
	11	46.36 (0.86)	6.55 (0.14)	53.20 (0.82)
	13	49.70 (0.74)	7.73 (0.21)	61.65 (1.09)
RE	7	37.84 (3.29)	5.40 (0.31)	18.10 (0.48)
	11	35.53 (1.17)	5.93 (0.22)	23.23 (0.20)
	13	35.93 (0.62)	5.89 (0.24)	24.35 (0.23)
RC	7	36.67 (2.15)	5.98 (0.20)	20.11 (1.03)
	11	36.82 (1.41)	6.24 (0.25)	27.46 (1.56)
	13	35.48 (0.64)	5.04 (0.23)	27.12 (1.10)

Treatment	Day	Content of element in primary leaves (g)		
		N	P	K
CONTROL	7	3.01 (0.44)	0.40 (0.03)	1.25 (0.13)
	11	13.19 (0.38)	1.34 (0.05)	12.39 (0.29)
	13	22.78 (1.48)	2.67 (0.21)	23.37 (1.98)
RE	7	2.63 (0.14)	0.37 (0.01)	1.14 (0.05)
	11	6.00 (0.36)	0.67 (0.02)	3.09 (0.15)
	13	6.23 (0.35)	0.67 (0.02)	3.27 (0.16)
RC	7	3.01 (0.44)	0.40 (0.03)	1.25 (0.13)
	11	6.88 (0.69)	0.70 (0.05)	3.53 (0.52)
	13	9.23 (0.53)	0.86 (0.05)	5.32 (0.64)

Treatment	Day	Content of element in primary leaves (g $gdwt^{-1}$)		
		N	P	K
CONTROL	7	68.3 (1.13)	9.31 (0.33)	28.6 (0.61)
	11	57.5 (1.32)	5.83 (0.11)	53.9 (0.73)
	13	56.3 (0.45)	6.56 (0.13)	57.3 (1.04)
RE	7	67.0 (2.87)	9.44 (0.32)	28.8 (0.58)
	11	48.6 (0.95)	5.47 (0.07)	25.1 (0.33)
	13	51.4 (0.98)	5.48 (0.07)	26.6 (0.41)
RC	7	68.3 (1.13)	9.31 (0.33)	28.6 (0.61)
	11	46.4 (0.57)	4.76 (0.13)	23.4 (0.86)
	13	46.4 (1.24)	4.31 (0.09)	26.1 (0.72)