EFFECTS OF ROOT COOLING ON LEAF GROWTH AND THE SIGNIFICANCE OF ABSCISIC ACID IN MEDIATING THE RESPONSES

by

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CONTENTS

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ABBREVIATIONS

A	area
ABA	abscisic acid
B	specific binding (antibody + antigen)
B_{max}	maximum specific binding
B_{min}	non-specific binding
BSA	bovine serum albumin
^{o}C	degrees centigrade
cm	centimetre
cpm	counts per minute
DW	dry weight
$\bar{E}$	net assimilation rate
EEx	elastic extensibility
Fig	figure
FW	fresh weight
g	gramme
h	hour
Kg	Kilogramme
L	length
LER	leaf elongation rate
ln	natural logarithm
PTFE	Polytetrafluorethylene
m	metre
McAb	monoclonal antibody
mg	milligramme

min	minutem
m	millimetre
MPa	megapascal
μm	micrometre
n	number of replicates
ng	nanogramme
NS	nutrient solution
P	turgor pressure
PAR	photon flux density
PBS	phosphate buffered saline
PEx	plastic extensibility
pg	picogramme
PVP	polyvinylpyrrolidone
$\bar{R}$	relative growth rate
RC	root cooling
RIA	radioimmunoassay
RVDT	rotary variable displacement transducer
s	second
se	standard error
SW	saturated weight
t	time
T	temperature
TEx	total extensibility
v	volume
VPO	Vapor pressure osmometre
Wd	width

ABSTRACT

The effects of root cooling treatments on leaf growth of seedlings of *Phaseolus vulgaris* L. were examined. Plants were germinated in vermiculite and then grown with their root systems cooled to 10°C or 15°C, or kept at the same temperature as the aerial parts (23°C).

Leaf elongation rates, recorded continuously using Rotary Variable Displacement Transducers, were shown to be very sensitive to root cooling treatments, and at 10°C, elongation rates fell to zero growth within 10 min after start of treatment. When root systems were returned to 23°C, leaf elongation rates increased almost immediately to overshoot the control rates within 5 - 10 min, subsequently declining to control values over the next 50 - 60 min.

Leaf turgor pressure fell at the beginning of the root cooling treatment and was believed to cause the instantaneous growth reduction, and to trigger abscisic acid (ABA) synthesis in leaves. Recovery in bulk leaf turgor occurred within 3 - 4 h from the commencement of root cooling and remained virtually constant throughout the experiment. No detectable changes in leaf turgor were observed when root systems were returned to 23°C.

A rise in abscisic acid occurred in rapidly expanding leaves of *Phaseolus vulgaris*, *Hordeum vulgare* L. and

Helianthus annuus L.. Root cooling treatment did not lead to a rise in leaf ABA content in the fully expanded first leaf of *H.vulgare*. In *H. annuus* as well as in *H. vulgare* the levels of abscisic acid declined as leaves aged.

Analysis of abscisic acid present in xylem sap of *P. vulgaris* seedlings showed that the amount of this hormone coming from the roots was insufficient to account for the observed rise in leaf ABA. However, the responses involving high concentration of abscisic acid in the leaves, may be indirectly mediated by ABA coming from the root systems.

The results point to a control system in which a stimulus from roots is sent to the shoot and is expressed as a change in leaf elongation rate. A transient fall in leaf turgor at the beginning of root cooling treatment is the hydraulic signal believed to cause the initial growth reduction. Rise in abscisic acid in the leaves results in reduction in the cell wall plastic extensibility which remains low while root cooling lasts and is the main factor limiting leaf growth.

CHAPTER 1

INTRODUCTION

1.1- Introduction

The importance of leaf production and performance to the yield and stability of most ecosystems justify the great interest of plant physiologists in studying the factors controlling leaf growth. The subject has been extensively examined and discussed in several books (Maksymowych, 1973; Dale and Milthorpe, 1983; Baker, Davies and Ong, 1985) and review articles (Humphries and Wheeler, 1963; Halperin, 1978; Green, 1980; Cosgrove, 1986; Dale, 1988), and in a recent review Dale, (1988) concluded that despite much progress in this area, many gaps remain in an understanding.

It is known that genotype is the ultimate source of control of leaf growth and development. However, environmental conditions exert important effects on growth and development and the final size and shape of the leaves are the result of complicated genotype versus environmental interactions. Whilst the environmental factors to which leaves are exposed, for example, light intensity and quality, wind and air temperature and humidity, may directly affect leaf growth and functioning, there may also be effects of the soil environment mediated via the root system. The inverse

situation is also true. Root growth and development are affected by soil as well as air conditions. Because root growth depends on a supply of carbohydrate, any environmental factor which reduces leaf area, usually reduces root growth (Kramer, 1983).

That root and shoot functioning are interrelated and interdependent is evident from the contributions of inorganic and organic constituents that one organ makes to the other and which are essential to build and maintain the complete plant. However, it has been shown that the interrelations between root and shoot also involve regulatory control that include supply of hormones and other physiologically-active substances whose functions are not simply the direct provision of energy. These interactions are referred to root-to-shoot communication. This thesis covers aspects of this general problem.

Much effort has been made to elucidate the nature of the communication between above and below ground parts of the plant. For the researchers, one of the most attractive aspects of the problem is the role of the root system in regulating leaf growth and development. A recent multiauthor work (Davies and Jeffcoat, 1990) covered a variety of topics relevant to the description and interpretation of the mechanisms involved. Such mechanisms seem to be highly complex and to involve several types of messages or signals which may include both physical (electrical and hydraulic) as well as

chemical ones.

One of the common approaches used in studying root-to-shoot communication is to subject the root system to an experimental stress condition (water, nutrient, salinity, temperature, aeration) and to examine which aspects of leaf function are altered and to identify the factor, or factors, responsible for the observed responses.

This study examines root-to-shoot communication when roots are subjected to cooling treatments, which have been shown to reduce leaf growth (Milligan and Dale, 1988*a*) and to stimulate a rise in abscisic acid (ABA) in leaves (Dale, Stacciarini Seraphin and Sattin, 1990; Smith and Dale, 1988). It is therefore necessary to understand some of the complex responses of plants to temperature.

1.2- The Temperature Relation of Plants

Temperature is one of the most important environmental factors affecting both the natural distribution of plants and their growth. Temperature is not a stable factor and plants can suffer stress either through too high or too low temperatures, or both, to different extents, over short or long time intervals.

Since plants are poikilothermic organisms, i.e. they are unable to maintain their cells and tissues at a constant optimum temperature, they cannot develop

low-temperature avoidance (Levitt, 1980*a*) and therefore growth and metabolism are greatly affected by changes in environmental temperatures. However, it is difficult to establish precise relationships between plant processes and environmental temperature because of the extreme and sometimes rapid variation of soil and air temperatures.

Air and soil temperatures are subjected to wide diurnal and seasonal fluctuations. Soil temperature also varies with depth, and physical properties which control the energy balance at the surface and transfer of heat through the soil (Fitter and Hay, 1987). Temperature gradients are greatest at, and immediately below the soil surface. Since most of the root systems tend to occur at deeper (>0 - 5 cm) soil layers, the greatest temperature fluctuation will be avoided (Kramer, 1983). Nevertheless, in the field, at any one time the different organs of a plant may be exposed to temperatures which vary as much as 10 - 15°C. Depending on the factors controlling the insolation of the soil and hence its heat gain, for instance, leaf area index and time of day, roots in the upper soil layers may experience significant temperature stress. Thus, steep positive and negative temperature gradients can exist along the length of a root system (Lake, 1987; Cooper, 1973; Russell, 1973).

The temperature variation between above and below ground and the fact that different stages of plant development, and different physiological processes, can

have different temperature optima (Fitter and Hay, 1987) make field experiments difficult so that investigations of plant responses to root temperature have been carried out more frequently in the laboratory. Since the responses of a plant to root cooling depend on the previous history and on how the treatments have been imposed (Minorsky, 1989; MacDuff, 1989; Markhart, Fiscus, Naylor and Kramer, 1979) it is important to record the growth conditions before and during treatment, in particular, i) the nature and duration of pretreatment; ii) the alternation of day/night temperature regimes; iii) the shoot temperature and iv) the rate of change in temperature. Experiments under controlled conditions where root and shoot temperatures are varied independently, have provided valuable data on the effects of root temperature on water and ion uptake and shoot growth (Milligan and Dale, 1988*a*, *b*; Milligan, 1986; Moorby and Nye, 1984; Markhart, *et al.*, 1979; Clarkson, 1976; Watts, 1972).

1.3- Root Cooling and Plant Growth

Interpretation of the responses of plants to differences in root temperature has developed from the idea of a functional equilibrium between root and shoot specific activities (Brouwer, 1962). This concept assumes that the rate of photosynthesis (shoot specific activity) is proportional to the rate of nutrient uptake

(root specific activity) and thus, the plant achieves this balance by adjusting the relative size of the shoot and root masses (Davidson, 1969).

Recently, Johnson and Thornley (1987) have proposed a mechanistic model of partitioning between shoot and root. The model considers partitioning as a response to changes in substrate concentrations, for example, carbohydrates and amino acids within the plant, which in turn, are influenced by environmental factors acting on specific activities of shoot and root. Although the model is concerned with plant growth, as a function of carbon and nitrogen, the authors indicate how other nutrients as well as growth factors may be incorporated.

1.3.1- Effects of Root Cooling on Root Growth and Functioning

Low root temperatures markedly reduce root growth and functioning. However, these effects are complex and change with time, probably because the response to temperature depends on the extent to which the plant become acclimatized and on the approaches used (see section 1.2).

Although long-term root cooling treatments have been shown to reduce root extension, root surface area and number and lengths of root hairs (Milligan and Dale 1988*a*; MacDuff, Wild, Hopper and Dhanoa, 1986), Milligan (1986) has shown that root cooling has little effect on

root growth and functioning over short periods when the treatment begins to affect leaf growth. Root tip number increased rapidly over the first 24 h after treatment had started, but was inhibited later, indicating that root cooling did not affect the apices that had already been initiated, but inhibited the emergence and initiation of new laterals (Milligan, 1986).

In long-term experiments, Milligan (1986) has demonstrated a pronounced effect of root cooling on root growth of *Phaseolus* seedlings. The dry weight increase of all growing parts of the plant, in particular of the root system, was reduced; root dry weight was shown to be only 20% of that of the control. Changes in the pattern of relative growth rates of different organs, upon root cooling treatment, have also been shown to occur in *Phaseolus*. For example, in seedlings grown in favourable conditions, the relative growth rate of the root exceeds that of the shoot, so that root/shoot ratio increases with time. However, in root-cooled plants the root and shoot relative growth rates are approximately equal and thus, root/shoot ratio remains constant (Milligan, 1986).

Reduction in root volume and weight would be expected to reduce the amount of tissue contributing to root metabolism, thus reducing both the capacity of root system for synthesis and catabolism, and also its sink size. A reduction in the number of root tips as part of a general reduction in root growth, may also reduce the

ability of the root to perform synthetic and catabolic functions, such as the synthesis and interconversion of certain plant growth regulators such as cytokinin and gibberellin (Atkin, Barton and Robinson, 1973), indole-3-acetic acid (IAA, Phillips, 1964) and abscisic acid (ABA, Jackson and Hall, 1987).

By reducing root surface area and permeability, low root temperature might lower the capacity of the root for the uptake of water and minerals. Water uptake has been shown to be restricted at low root temperature (Brouwer and Hoogland, 1964; Kramer, 1969). The main causes suggested for this restriction are: i) increased viscosity of water in the root medium; ii) decreased permeability of the root membranes; iii) increased viscosity of protoplasm in the roots; iv) reduced root conductance; v) increased suberization of the roots; and vi) poor development of the root system (Kramer, 1969).

The rate of water uptake by roots is often 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 low-temperature threshold was shown to coincide with changes in the molecular state of the root cell membranes (McWilliam, Kramer and Musser, 1982) and to vary between species. It may thus be a factor influencing plant distribution (Teskey, Hinkley and Grier, 1984).

It has been proposed that upon root cooling treatment the total plant demand for mineral nutrients and its ability to utilize them is lowered since growth is usually slowed down (Clarkson, Earnshaw, White and Cooper, 1988; Brouwer, 1964). The complex effects of low root temperatures on ion uptake and its regulation have recently been reviewed by Clarkson *et al.*, (1988). The uptake of phosphorus is particularly affected by root temperature (Atkin *et al.*, 1973) whereas nitrogen and potassium is less affected (Cooper, 1973). Studies on the effects of low root temperature on nitrogen uptake have shown an increased preference for ammonium (NH_4^+) over nitrate (NO_3^-) uptake (MacDuff and Jackson, 1991; MacDuff, Hopper and Wild, 1987; Clarkson, Hopper and Jones, 1986). It was not clear however, whether temperature acted differentially solely on the respective ion transport `capacities' for $NH_4^+$ and $NO_3^-$ or on the intrinsic `activities' of the two transport systems (MacDuff and Jackson, 1991).

Whether or not root temperature limits plant growth via effects on nutrient or water fluxes into the root may depend on the time-scale considered. In short-term experiments a consistent observation is that shoots rarely show visible deficiency symptoms of mineral nutrient (Setter, Greenway, 1988; Milligan, 1986), whereas fall in leaf turgor is often observed (Milligan and Dale, 1988*a*, *b*; Brouwer, 1964). Milligan (1986) has concluded that in *Phaseolus* seedlings shortage of

minerals was not the cause of leaf growth reduction, and no visible symptoms of nutrient deficiency were observed, at least up to day six after root cooling treatment had started; nor were the synthesis of protein and nucleic acids affected by treatment in the short-term. Setter and Greenway (1988) have shown that a low root temperature treatment reduced the percentage of nitrogen and phosphorus in the dry weight of rice plants, however this was not the cause of the observed reduction in leaf extension rates over short-term.

It has been suggested that the reduced rates of ion uptake *per se* does not limit growth at low root temperature, but rather the growth may be reduced by some other temperature-sensitive mechanism, acting on cell expansion, leading to a reduced growth and hence, causing the demand for nutrient uptake to be diminished (MacDuff and Jackson, 1991)

1.3.2- Effects of Root Cooling on Leaf Growth and Functioning

An effect of root cooling to reduce leaf growth has been shown for several species, in many cases associated with parallel decrease in leaf water content (Dale *et al.*, 1990; Milligan and Dale, 1988*b*; Setter and Greenway, 1988, McWilliam *et al.*, 1982; Kleinendorst and Brouwer, 1972, 1970; Watts, 1972; Brouwer, 1964). Changes in leaf water status have been attributed to a

lowering of root permeability to water and reduction in the hydraulic conductance of the root:shoot pathway (Milligan and Dale, 1988*b*, Markhart *et al.*, 1979; Kleinendorst and Brouwer, 1972).

Studies by Milligan and Dale (1988*a*, *b*) showed that growth of *Phaseolus* primary leaves was reduced by root cooling, and that leaf turgor was also reduced. The fall in turgor was short-lived, whereas reduction in growth continued. The reduction in leaf elongation is due to reduced leaf cell enlargement and leaves are generally thinner with smaller cells and fewer intercellular spaces; cell number was not affected (Milligan and Dale, 1988*a*; Brouwer and Hoogland, 1964). These responses resemble the responses to other root stress conditions such as drought (Quarrie and Jones, 1977) and salinity (Waldron, Terry and Nemson, 1985; Wignarajah, Jennings and Handley, 1975), although in some cases leaf cell number was reduced by water deficit (Clough and Milthorpe, 1975).

1.4- Root-to-Shoot Communication

The reported direct evidence for root-to-shoot communication in ordinarily growing, unstressed plants is minimal. However, there is much more information for situations where the root system is under stress (see page 2).

1.4.1- Electrical signals

Electrical signals have been reported to occur in plants and to be responsible for leaf folding movements in *Mimosa* and in *Dionaea* the Venus Fly Trap (Pickard, 1973; Simons, 1981).

Amongst the electrical signals, action potentials are the best documented signalling system in plants. They can be transmitted through plant tissue at about 3 - 5 cm min^{-1}, and are negatively going (to the apoplast) at -25 to -75 mV (Davies, 1987). Action potentials involve ion flux (K^+ and Cl^- efflux and Ca^{2+} influx), current flow, changes in membrane potential, and turgor loss. Davies (1987) has proposed that action potential, as a whole, may act as a major intercellular signal whereas its various components may act independently or cooperatively as intracellular signals.

The role of action potentials in mediating plants responses to wounding have been reviewed by Davies (1987, 1990). Action potentials have also been shown to play a role in response to cold stress (Minorsky, 1985). However, the involvement of action potentials in root-to-shoot communication in response to root stress needs further investigations (Jones, 1990).

1.4.2- Hydraulic Signals

If root functioning is altered by changes in the

specific environment, the effects will be reflected in the leaves. In some cases this results in a restriction of the water supply to the aerial part generating a water deficit. This type of communication is termed a **hydraulic signal** and, to some extent, has been shown to be one of the primary signals that reduces leaf growth upon specific changes in the root environment.

The generation of short-term leaf water deficits in *Phaseolus* seedlings, subjected to root cooling treatment (Milligan and Dale, 1988*b*; Milligan, 1986; Brouwer, 1964) has been suggested to be the primary cause of reduced elongation rates. Neuman and Smit (1991) have shown similar results for *Phaseolus* seedlings subjected to root hypoxia. In their study, a transitory decline in leaf water potential was observed and accounted for the initial reduction in leaf growth rates. These observations are in agreement with those presented by Schildwacht (1989) and Wadman-van Schravendijk and van Andel (1985) also using bean systems subjected to root hypoxia and waterlogging treatments respectively. Therefore, it seems common for bean plants, subjected to root stresses, to develop leaf water deficits, even though for short periods. Although root cooling may be associated with oxygen shortage to the root system, Milligan's experiments have demonstrated that cooling bean roots to 10^{o}C did not lead to root hypoxia (Milligan, 1986).

In soybean plants, a hydraulic signal has been

demonstrated to be very important in controlling leaf growth. Using a guillotine thermocouple psychrometer to make continuous water potential measurements, Boyer and co-workers (Matyssek, Tang and Boyer, 1991; Matyssek, Maruyama and Boyer, 1991; Nonami and Boyer, 1989; 1990*a*, *b*; Boyer, Cavalieri and Schulze, 1985) have demonstrated that a hydraulic signal can affect growth when the external water supply is either diminished or unavailable. In these plants, when the seedlings were transplanted from wet to dry vermiculite, hydraulic signal was the primary cause of reduced leaf growth (Nonami and Boyer 1990*a*) which could be transmitted from the basal stem to the elongating stem tissues as fast as 1 minute (Matyssek *et al.*, 1991). Despite several publications showing that leaf responses to soil environment stress, mainly to soil water deficits, sometimes precede changes in leaf water status (see Davies and Jeffcoat, 1990; Passioura, 1988*a*), the idea of hydraulic signals as the main root:shoot signal in field conditions remains strong (Kramer, 1988).

1.4.3- Chemical signals

The hypothesis that plants have evolved a control system which enables them to cope with soil water deficits by sensing the soil water availability and regulating their gas exchange and growth rate accordingly, thus avoiding excessive water loss, was put

forwards in early 1980's by Jones (1980) and Cowan (1982). Since then, many reports have demonstrated the importance of such mechanisms, not only for drought stress, but nearly for all types of stress. Marked and often rapid changes in the hormonal content of plant tissues are generally observed in response to stress conditions as drought, salinity, flooding, chilling and high temperature (Levitt, 1980*a*, *b*).

This hypothesis suggests that the root system can sense conditions which resulted from a restricted water supply and send a chemical signal to the shoot before any detectable water deficit could be measured in the aerial part (Davies, Mansfield and Hetherington, 1990; Saab and Sharp, 1989; Zhang and Davies, 1989*a*, *b*; Passioura, 1888*b*; Munns, 1987; Jackson and Hall, 1987; Blackman and Davies, 1985; Turner, Schulze and Gollan, 1985), thus allowing a water consuming strategy to be activated.

According to Jackson and Kowalewska (1983) and Jackson (1990), a chemical signal can be manifested as one of the three types: i) negative messages; ii) positive messages; and iii) accumulative messages, or a combination of them.

Perturbations of the root environment may result in reduced supply to the shoot of root-sourced physiologically-active compound (s). This kind of signal, a negative message, is considered to be an important potential element of root-to-shoot

communication. Cytokinin and gibberellin have been identified in xylem exudate and many reports have shown that perturbation in the root environment resulted in reduced cytokinin and gibberellin export from root to shoot, thereby reducing the level of these hormones in the shoot and triggering changes in leaf physiology such as reduced growth and accelerated senescence of the leaves (Atkin *et al.*, 1973; Reid and Crozier, 1971; Carr and Reid, 1968).

The hypothesis that reduced supply of cytokinin can act as a negative message is supported by the fact that i) roots are sites of cytokinin synthesis (van Staden and Davey, 1979; Skene, 1975; Kende and Sitton, 1967); ii) the concentration of cytokinin in xylem sap decreases in response to root stress such as low and high root temperature (Atkin *et al.*, 1973), flooding (Carr and Reid, 1968), root hypoxia (Smit, Neuman and Stachowiak, 1990; Neuman, Rood and Smit, 1990), and water stress (Itai and Vaadia, 1965; 1971); and iii) application of cytokinins has been shown to alleviate the effect of stress on shoot growth and stomatal conductance (Blackman and Davies, 1985, Burrows and Carr, 1969).

However, Neuman *et al.*, (1990) using poplar and bean plants concluded that although root hypoxia reduced the transport of cytokinin through the transpiration stream, there was no reduction of the hormone within the leaves in response to the treatment. The treatment reduced leaf

elongation rates, however, exogenously applied cytokinin did not reverse the growth response observed upon root hypoxia.

A decrease in concentration of gibberellin in xylem sap reported for flooded (Reid and Crozier, 1971) and root cooled (Atkin *et al.*, 1973) plants, suggests that a smaller flux of gibberellin activity circulating from root to shoot contributes to the shoot response, mainly to leaf senescence and reduced shoot elongation (Reid and Crozier, 1971). Reid and Crozier's results showed that initiation of adventitious roots at the base of the stem within 3 days of flooding was accompanied by increased gibberellin activity in the shoot. However, neither increase of gibberellin activity in xylem exudate, nor recovery of shoot extension were observed upon adventitious root initiation. This result suggests that gibberellin may act as a negative message in flooded plants, but its effects may be restricted to the beginning of treatment, and another growth factor becomes increasingly important.

It seems probable that root stress reduces the concentration of cytokinin and gibberellin in xylem. What is less clear is the effect of these negative messages on shoot behaviour (Jackson, 1990).

Positive messages are considered as an increase in the supply, from stressed roots to the shoot, of some physiologically-active compound (s) that would not

normally be supplied or could be supplied in smaller quantities if the roots have not been stressed.

Abscisic acid has been reported as one of the most important positive messages controlling responses to environmental stress. At least in the case of soil drying, it is postulated that shallow roots, which dehydrate independently of the deeper ones, will measure the water available in the soil. Reduced root turgor will trigger abscisic acid synthesis in roots (Walton, Harrison and Cotê, 1976; Walton, 1980) and this hormone will be sent, via the transpiration stream, to the shoot where it will reduce growth and cause stomatal closure. At the same time, the deeper roots will continue to supply sufficient water to keep the leaves turgid (Sharp and Davies, 1989). This kind of response has been demonstrated to occur for sunflower and maize plants in the laboratory (Zhang and Davies, 1989*b*; 1990*a*) and for maize and almond in field experiments (Tardieu, Katerji, Bethenod, Zhang and Davies, 1991; Wartinger, Heilmeier, Hartung and Schulze, 1990).

However, it seems that other compounds can also act as a positive message, not only in the case of soil drying but also in the case of other environmental stress such as salinity. Bean plants, for instance, subjected to drying soil, did not show a substantial rise in abscisic acid in xylem sap (Trejo and Davies, 1991; Hartung and Radin, 1989) and stomatal closure could not be attributed as a consequence of increased

abscisic acid in xylem sap (Trejo and Davies, 1991). Similarly, in wheat plants, Munns and King (1988) have demonstrated that xylem sap must contain a potent inhibitor of transpiration and although this compound is not yet known, it was demonstrated not to be abscisic acid.

The data about abscisic acid in response to waterlogging treatment are still contrasting. Jackson (1985, 1990) and Jackson and Hall (1987) reported that the accumulation of abscisic acid in leaves of flooded pea plants is a result of reduced transport out of leaves, thus constituting an accumulative message. However, Zhang and Davies (1987*b*), also using pea plants, reported that abscisic acid in leaves originates in perturbed roots and is transported to leaves, constituting a positive message. Zhang and Davies do not rule out the possibility that in a later stage of stress, a build-up of abscisic acid can occur in leaves themselves as the total abscisic acid in roots declined.

In addition, Jackson and co-workers and Zhang and Davies have shown that abscisic acid accumulated in leaves of pea plants in the absence of a reduction in leaf turgor whereas Wadman-van Schravendijk and van Andel (1986) reported that in bean plants there was a decrease in water potential and leaf turgor in response to flooding and that these changes were positively correlated with increase in abscisic acid within the leaves.

Redistribution of abscisic acid within leaf compartments and organs is another important mechanism that one must consider when studying plant response to environment stress. It is known that the distribution of abscisic acid between leaf compartments i. e. apoplast, cytosol, chloroplasts and vacuole, is determined by the pH gradient between each compartment (Cowan, Raven, Hartung and Farquhar, 1982). High membrane permeability to the protonated form (ABAH) and low permeability to the dissociated anion (ABA^-), results in the accumulation of ABA in the most alkaline compartment of the cell (Cowan, *et al.*, 1982). Upon illumination, nearly 70% of the total leaf ABA is trapped in the chloroplasts, whereas in darkness this value falls to around 40% (Cowan, *et al.*, 1982). This constitutes one of the mechanisms that leaves utilize to open and close stomata.

Recently, Hartung and Slovik (1991) have suggested that abscisic acid can be redistributed between leaf mesophyll and apoplast in response to water deficit. As the leaf apoplast pH becomes more alkaline upon dehydration (Hartung and Radin, 1989; Hartung, Radin and Hendrix, 1988) abscisic acid is released from the mesophyll to the apoplast in response to the changes in pH gradient which in turn, may be regulated by stress-sensitive ATPase of the mesophyll plasma membranes (Hartung and Slovik, 1991).

Another important positive message links poorly aerated roots with fast production of ethylene and leaf epinasty (Jackson, 1990). It has been demonstrated that anaerobic roots of tomato synthesise 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor of ethylene, and this compound is then transported to shoots via the transpiration stream, where it is converted to ethylene (Bradford and Yang, 1980). In bean leaves, a drastic increase in ethylene was observed within 2 h as a result of waterlogging treatment (Wadman-van Schravendijk and van Andel, 1986).

Whether the enhanced production of ethylene reduces leaf expansion is not well documented. However it has been shown that this hormone inhibits leaf expansion in pea and bean (Kays and Pallas, 1980) and maize (Jackson, Drew and Giffard, 1981). In bean plants it has been shown that ethylene interferes with abscisic acid regulation of stomatal closure (Wadman-van Schravendijk and van Andel, 1986).

1.5- Plan of investigation

It appears that root-to-shoot communication in response to environmental stress is mediated by some kind of signal originating in the roots. What is more difficult to define precisely, is the exact nature of the signal involved. Most published studies refer to leaf responses to water deficit and in this case,

abscisic acid has been demonstrated to be the main chemical signal involved. A limited number of reports are available on the effects of low temperature in general and fewer still on the effects of low root temperature.

Studies using *Phaseolus vulgaris* seedlings have shown that root cooling treatments lead to reduced leaf growth, with resultant changes in leaf anatomy, reduced transpiration rates (Milligan and Dale, 1988*a*, *b*) and increased abscisic acid (ABA) concentration in leaves (Dale *et el.*, 1990; Smith and Dale, 1988). Milligan and Dale (1988*a*, *b*) detected a significant reduction in the elongation rates of the primary leaves 3 - 6 h after the root cooling treatment started, but did not determine the time course of this response, particularly in the short-term. They considered changes in cell wall extensibility to be the major determinant of growth reduction. However, they did not rule out the possibility that the short-lived fall in leaf turgor observed during the first 3 - 4 h of treatment also had some effect on reducing leaf growth. Added to these findings, the increased abscisic acid concentration in leaves, observed by Dale *et al.*, (1990) and Smith and Dale (1988) gives an indication that this hormone may play a role in root-to-shoot communication when *Phaseolus* plants are subjected to root cooling treatment.

In light of these results, it was considered

important to examine shorter-term aspects of root-to-shoot communication associated with root cooling treatment. If the time course of the effects of treatment on leaf elongation and leaf abscisic acid content differ, then the implication that the latter causes the former may prove untenable. Therefore, the aims of this study were to determine: i) the short-term effects of root cooling treatments on leaf elongation rates measured using Rotary Variable Displacement Transducers; ii) the origin of leaf abscisic acid, determining abscisic acid concentrations by radioimmunoassay, and iii) whether or not this hormone is controlling changes in cell wall extensibilities. *Phaseolus vulgaris* was chosen as the main species for these investigations, although some comparisons were also made using *Helianthus annuus* and *Hordeum vulgaris* seedlings.

CHAPTER 2

MATERIAL AND METHODS

2.1- Plant Material

The dicotyledon species used were Dwarf French Bean (*Phaseolus vulgaris* L.) var. Canadian Wonder, and Tall Single Sunflower (*Helianthus annuus* L.). Seeds were purchased from WmK McNair (Edinburgh, U. K.). Only seeds in the weight range 0.35 g to 0.55 g (95% of the bean seeds) and 0.09 g to 0.15 g (85% of the sunflower seeds) were used to ensure uniformity of the seedlings.

Some experiments were also done using Maris Mink Barley (*Hordeum vulgare* L.). The grains were obtained from locally-grown plants. Only grains in the weight range 0.035 g to 0.055 g (90% of the grains) were used.

2.2- Germination of Seedlings

Seeds of *P. vulgaris* and *H. annuus* (50 per tray) were germinated in trays (30 cm x 55 cm x 8 cm) containing dry-vermiculite (Vermiperl, Garden Horticultural Vermiculite, Silvaperl, Limited), in a controlled environment growth room at 23 ± 0.5°C. At planting, the seeds were watered with 2000 cm^3 per tray of nutrient solution (Table 1A) and the trays covered with a polythene sheet to reduce evaporation. The polythene

Table 2.1. Nutrient solution (N.S.) for growing *Phaseolus vulgaris* and *Helianthus annuus* (A) and *Hordeum vulgare* (B) plants.

Nutrient	g/1000 cm^3	
	A	B
Calcium Nitrate	35.500	
Potassium Nitrate	50.500	
Potassium Sulphate		87.13
Potassium Dihydrogen Orthophosphate	13.600	27.20
Magnesium Sulphate	37.000	98.50
Calcium Chloride		109.50
Sodium Nitrate	17.000	84.99
Ferric EDTA	0.367	0.25
Boric Acid	0.057	2.86
Cupric Sulphate	0.004	0.08
Potassium Chloride	0.105	
Manganese Sulphate	0.081	
Manganese Chloride		1.81
Ammonium Molybdate	0.002	
Molibdic Acid		0.09
Zinc Sulphate	0.022	0.22

10 cm^3 of each were used to make up 1000 cm^3 of N.S.

sheet was taken off after 4 days, when the hypocotyls had started to emerge above the vermiculite surface.

Barley grains (4 per pot) were sown in 500 cm^3 plastic pots containing a basal layer, 1 cm deep, of gravel stones and filled up with river-washed sand. Before planting, the sand was moistened with 30 cm^3 of tap water and 4 holes, 2 cm deep, were made in the sand where the grains were laid. After covering the grains with sand, another 20 cm^3 of tap water was added. The pots were kept in a controlled environmental growth room at 23 ± 0.5°C. Every 3 days after planting 40 cm^3 of nutrient solution (Table 1B) was added to each pot.

2.3- Seedlings Growth and Transplantation

The seedlings were grown in a controlled environment growth room at a constant temperature of 23 ± 0.5°C. A light/dark cycle of 12 h day, 12 h night with a Photon Flux Density (PAR) of 250 $\mu mol.m^{-2}s^{-1}$, at leaves levels, were provided by Metal Halide Lamps (Thorn EMI Lighting Ltd). A coefficient of variation between 10 and 15 per cent in light intensity over the growing table was found. The relative humidity fluctuated between 65 and 80 per cent and was not controlled.

At the day of the experiment, uniform seedlings were carefully removed either from vermiculite (bean and sunflower) or sand (barley) and their root systems thoroughly rinsed with tap water. Seedlings were

transferred to black painted Kilner jars (1000 cm^3) which were filled with 950 cm^3 of nutrient solution (Tables 1A, B). Seedling transplantation started at the beginning of the light period.

Bean and sunflower seedlings (one per jar) were kept in position by wrapping the base of the hypocotyl with a foam strip (2.5 cm × 30 cm × 0.5 cm) which was inserted into a polythene cup. A PVC ring held this set on the top of the jar as shown in Figure 2.1A.

For barley seedlings, a foam strip (1.5 cm × 4.0 cm × 0.5 cm) was wrapped around the base of the coleoptile and inserted into a PVC ring which held a set of 4 plants on top of the jar (Fig. 2.1B). As the meristematic zone and the base of the growing leaf are considered to be important sites of temperature perception in grasses (Peacock, 1975), a polystyrene disc (2.5 cm thick) which matched to the PVC ring was placed on top of the nutrient solution to insulate the basal area from the root medium (Fig. 2.1B).

2.4- Root Cooling Treatments

After being transferred to the Kilner jars containing nutrient solution, the seedlings were separated in two uniform groups, one of them was subjected to root cooling treatment while the second was kept as control. The beginning of the root cooling treatment (RC) was taken as time zero (t_o) and started within 2 - 3 h from

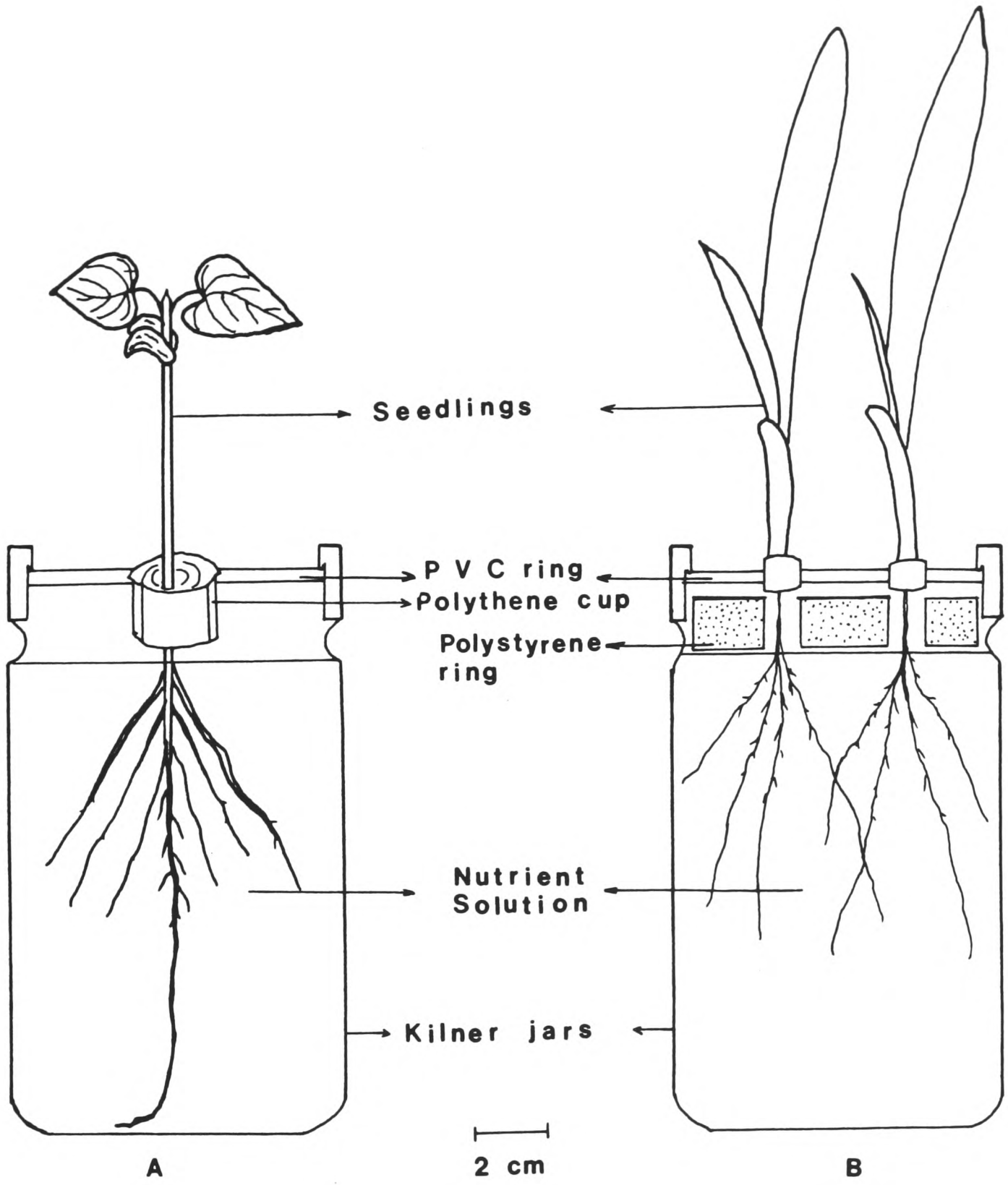


Figure 2.1- Diagram of the Kilner jars used for culturing either *Phaseolus vulgaris* and *Helianthus annuus* (A) or *Hordeum vulgare* (B) plants. Longitudinal section.

the beginning of the light period.

The treatment was given by placing the jars in a PVC tank (35 cm × 55 cm × 30 cm), the water in which was already cooled and maintained at 15°C or 10°C by means of a cooling system (Grant Instrument (Cambridge) Ltd) as shown in Figure 2.2.

The time courses of the absolute changes in temperature and the cooling rates of the nutrient solution in the jars, when placed in the cool water bath, are shown in Table 2.2. The cooling rates averaged 0.26°C min^{-1} and 0.28°C min^{-1} for the 15°C and 10°C treatments respectively. However these rates were not constant. They were higher immediately after the jars were placed in the cool bath as shown in Table 2.2.

The aerial parts of the treated plants were maintained at controlled growth room temperature of 23°C. To avoid the effects of low temperature above the bath on the aerial parts, the bath was covered with a 5 cm thick polystyrene sheet. Two cooling tanks were available at the same time allowing a total of up to 24 jars per experiment. The control set of plants was maintained with their root system and aerial parts at 23°C (Control plants - C).

Phaseolus vulgaris seedlings were subjected to root-cooling (RC) treatment at day 7 after sowing. At this stage the hypocotyl was 6.5 ± 1.0 cm long and the primary leaves had begun to unfold (see Fig. 3.1, Chapter 3). *Helianthus annuus* seedlings were subjected

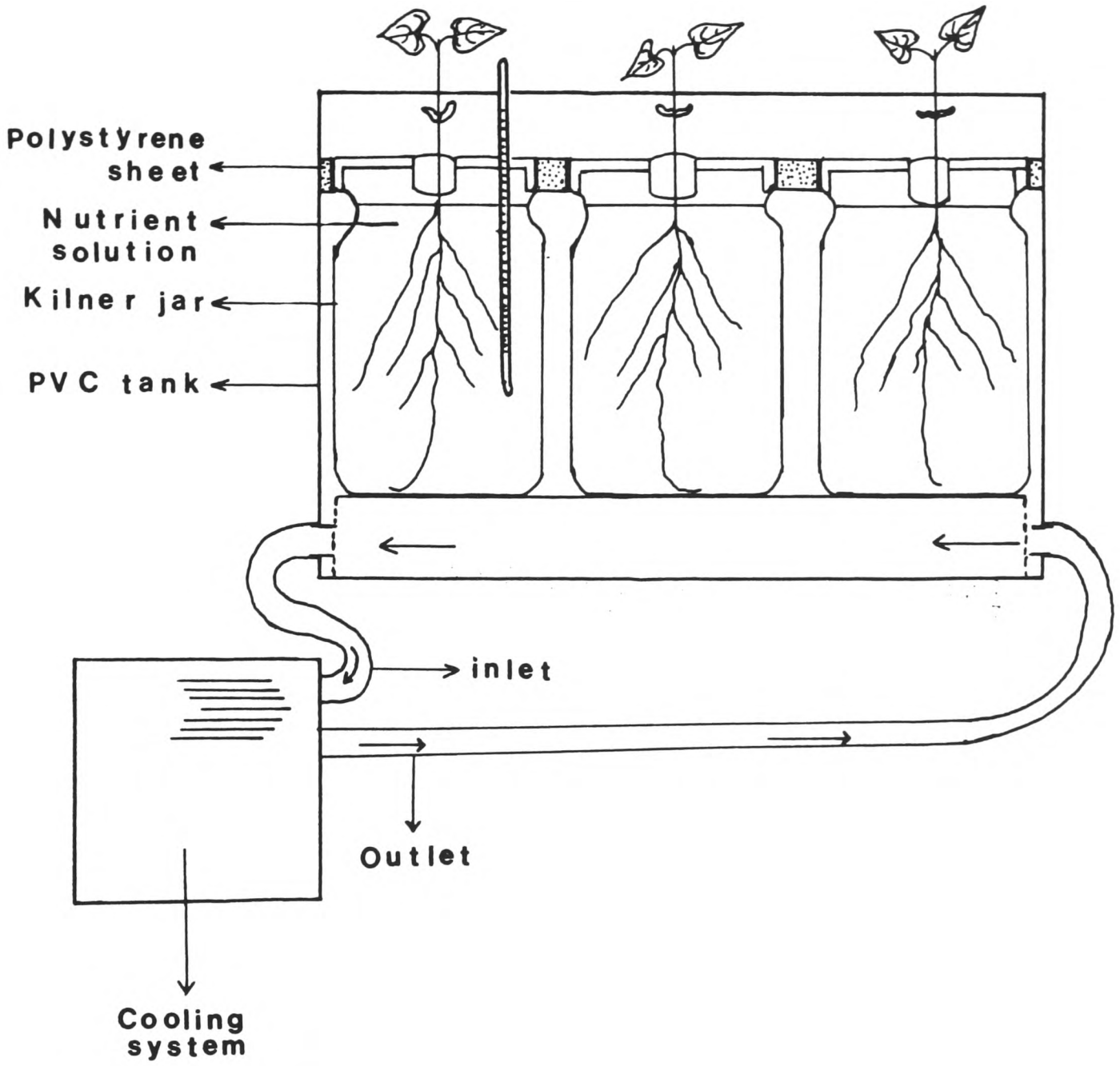


Figure 2.2- Diagram of the cooling system and cooled water bath used to cool the root medium in all experiments. Longitudinal section.

Table 2.2- The time course of absolute change in temperature and cooling rates of the nutrient solution in the Kilner jars after transferring to cooled water bath. The initial temperature was 23°C for both treatments.

Time	15 °C		10 °C	
	T °C	°C min^{-1}	T °C	°C min^{-1}
0	23.0		23.0	
5	21.0	0.4	19.0	0.8
10	19.0	0.4	17.0	0.4
15	17.5	0.3	15.0	0.4
20	16.5	0.2	13.5	0.3
25	15.5	0.2	12.0	0.3
30	15.0	0.1	11.5	0.1
35	15.0	0.0	11.0	0.1
40	15.0	0.0	10.5	0.1
45	15.0	0.0	10.0	0.1

to RC treatment either at day 7 or 11 after sowing. At the former stage, the seedlings had their first pair of leaves beginning to emerge. At day 11, the first pair of leaves was 4.0 ± 0.8 cm long and 2.0 ± 0.3 cm wide and the second pair of leaves was emerging. For *Hordeum vulgare* seedling, the treatment started at day 10 after sowing. At this stage, the first leaf was fully expanded and the emerged portion of the second leaf was about 8.0 ± 1.0 cm long.

2.5- High Relative Humidity Treatment

A fall in turgor of primary leaves of *Phaseolus* seedlings after the commencement of root cooling treatment has been observed by Milligan and Dale (1988*a*). An attempt to minimize this effect was performed in some experiments by subjecting the aerial parts of both control and root-cooled *Phaseolus* plants to high relative humidity.

Seedlings were transferred to Kilner jars with nutrient solution as described in section 2.3, and their aerial parts were enclosed in a transparent polythene bag (21 cm x 19 cm) in which water had been sprayed over the inner surface to raise the atmospheric humidity to approximately 100%. The bag was sealed on top of the Kilner jar by mean of masking tape.

Preliminary experiments showed that changes in stomatal conductance occur when the aerial parts were

enclosed in the bag. Therefore, in these experiments, the stomatal conductance of the abaxial surface of the primary leaves was monitored by means of porometer (see below section 2.11) before and after the plants' tops were bagged. Changes in stomatal conductance after bagging the aerial parts were monitored in separate sets of plants, at 15 min intervals, to detect when conductance became steady. This was after 2 h, and the root-cooling treatment started then. The aerial parts were maintained bagged over the period of root cooling treatment.

2.6- Measurements of Leaf Growth

2.6.1- Leaf Area

The area of the primary leaves of plants of *Phaseolus vulgaris* was estimated using the relationship:

$$\mathbf{A} = 0.97 + (L \times Wd) \times 0.86 \qquad (2.1)$$

and leaf area of plants of *Helianthus annuus* was estimated by:

$$\mathbf{A} = 0.220 + (L \times Wd) \times 0.725 \qquad (2.2)$$

where, for both equations, **A** is the estimated leaf area (cm^2); L is the total length from tip to petiole and Wd is the maximum width of the leaves, both in cm, measured with ruler.

The constant values in (2.1) and (2.2) were established in preliminary experiments using a linear regression procedure, where the actual leaf areas were

obtained by tracing the leaf (n = 50) on to paper, cutting out and weighing the shapes (Dale. 1964). The leaf areas were then plotted against the product of leaf dimensions (L x Wd). For both equations the correlation coefficients (r) were greater than 0.95.

The length of the blade of the second expanding leaf of plants of *Hordeum vulgare* was measured, using a ruler, from its tip to the ligule of the first expanded leaf. The leaf elongation rate (LER) was then determined by taking the difference in leaf lengths at two times and dividing by the time interval.

2.6.2- Leaf Elongation Rates

Short-term elongation rates of the leaves were determined by auxanometric techniques. Lamina extension rates of the primary leaves of plants of *Phaseolus vulgaris*, first pair of leaves of *Helianthus annuus* and the second expanding leaf of *Hordeum vulgare* were measured using Rotary-Variable Displacement Transducers (RVDTs; model 3810/60, Penny & Giles, Bournemouth, Dorset U. K.). This technique has been used for short-term elongation measurements of *Phaseolus* leaves (Sattin, Stacciarini Seraphin and Dale, 1990; Van Volkenburgh and Cleland, 1986) and other species (Waldron *et al.*, 1985, in *Beta*; Taylor and Davies, 1985, in *Betula* and *Acer*).

A schematic diagram of the components of the RVDT is

shown in figure 2.3. A polytetrafluorethylene (PTFE, 0.26 mm) thread (a) was attached to the tip of the leaf by means of a small spring (b), turned thrice round the transducer (c) and tensioned using a 2.0 g weight (d). Any change in the length of the leaf registered a voltage output change in the transducer. The output was monitored on a Data Logger (e) (MADC 12 Oasis Products, Norwich U. K.) and calibrated such that changes of 1 μm could be registered. The output from each RVDT was scanned every minute, or at longer intervals if desired, on a microcomputer (f) from which data were accessed and passed to a mainframe computer for processing. Each set of data came from at least two runs yielding qualitatively similar results and each was duplicated with two RVDTs for each treatment.

The measurements of leaf elongation were routinely scanned at 5 min intervals, except when root cooling started or was reversed and during the light-dark, dark-light transitions, the measurements were scanned every minute.

Growth movements of the leaf over the experimental period, were prevented by clamping its petiole to a steel rod (g in Fig. 2.3), to the transducer frame. Before being clamped, the petiole was wrapped in a plastic tubing to avoid damage.

Preliminary work showed that when the RVDTs are connected to a non-growing object there was a very slight oscillation (shrinkage or stretching) by about

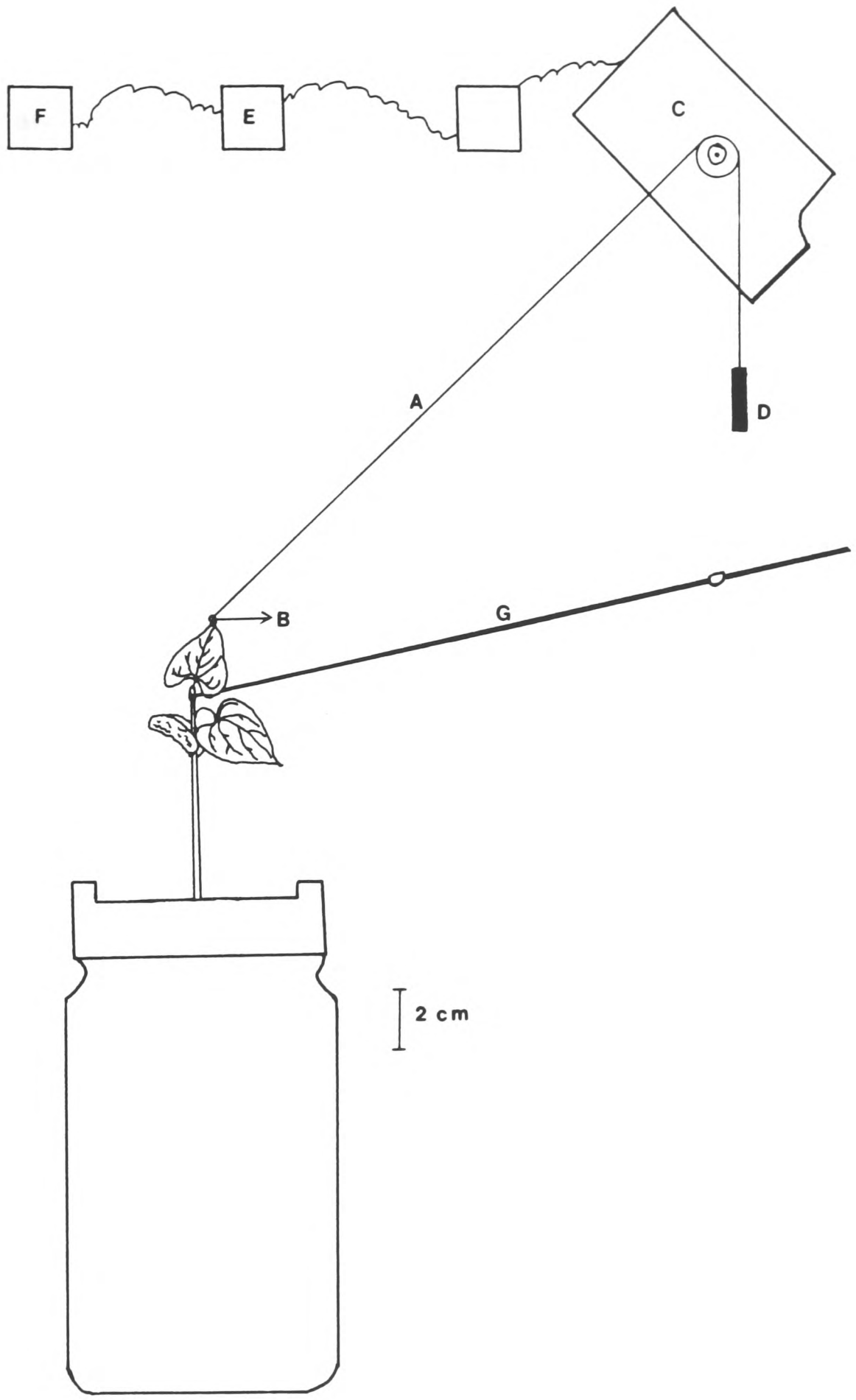


Figure 2.3- Schematic diagram of RVDT assembly. a- PTFE; b- spring for attachment of the leaf; c- Transducer and frame; d- tensioning weight; e- MADC 12 Data Logger; f- microcomputer; g- steel rod for fixing the petiole.

1 μm of the PTFE thread at the time when the lights went on and off (Figure 2.4). For the first reading after lights on and off an arbitrary correction of ± 1 μm was made to all data. Apart from these very small responses, there was no detectable change or drift in readings obtained over a 24 h period.

2.7- Leaf Cell Number Counting

The total number of cells per leaf was determined using the technique described by Brown and Rickless (1949).

Following harvest, the leaves were weighed and immersed in 5% aqueous solution of chromic acid (chromium trioxide) at 23°C for 24 h. After this period, the excess solution was carefully poured off and the leaf tissue macerated by stirring it in the solution, using the tip of a Pasteur pipette to give a turbid suspension.

The suspension was taken up six times into a Pasteur pipette and six times into a 10 cm^3 syringe fitted with an 0.7 mm bore stainless steel needle and squeezed out to form a suspension with no clumps visible to the naked eye. The pipette and syringe were rinsed in a known volume of distilled water and the suspension diluted to known volume. The cells were counted by drawing an aliquot of the suspension and placing a drop of it under the cover-slip of a haematocytometer slide (Hawksley

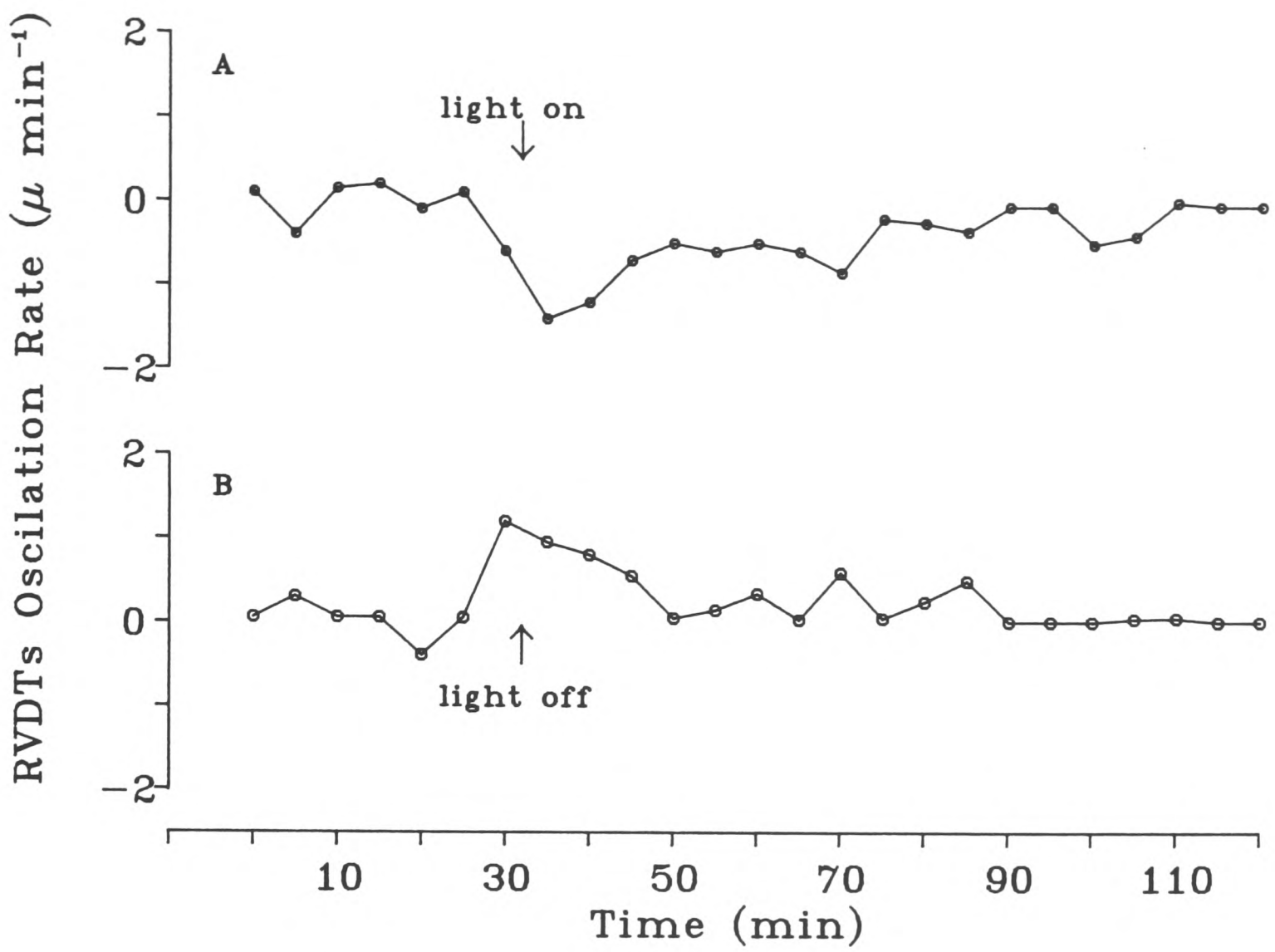


Figure 2.4- Apparent extension of PTFE thread when attached to a non-growing object at the beginning (A) or at the end (B) of the photoperiod.

Crystallite, mod-Fuch Rosenthal; 0.2 mm depth).

For each sample, six 3 mm x 3 mm fields were counted to give cell number per 1.8 mm^3. The number of cells per leaf was calculated knowing the volume of the suspension from which the aliquots were drawn.

2.8- Measurements of Fresh, Saturated and Dry Weights

Fresh weight (FW) saturated weight (SW) and dry weight (DW) were determined separately for roots, leaves, shoot and cotyledons as required.

For barley plants, the fresh and dry weights of the root system included the remains, if any, of the grain.

To obtain the saturated weight of the leaves, each leaf was individually weighed fresh and hydrated overnight by immersing its petiole in freshly boiled and cooled water and then weighed.

Dry weights of each organ were measured after material had been weighed fresh, and dried to a constant weight at 80°C.

The Relative Growth Rate (R) and Net Assimilation Rate (E) were calculated according to equations (2.3) and (2.4) respectively (Hunt, 1982 and 1990)

$$\bar{R} = (\ln W_2 - \ln W_1) / (t_2 - t_1) \qquad (2.3)$$

and

$$\bar{E} = (W_2 - W_1)/(t_2 - t_1) \times (\ln A_2 - \ln A_1)/(A_2 - A_1) \qquad (2.4)$$

where (t_2 - t_1) is the time interval between successive harvests; W_1 and W_2 the dry weights at times t_1 and t_2 respectively and A_1 and A_2 the leaf area at these times.

2.9- Leaf Water Relations Parameters

2.9.1- Leaf Water Potential (ψ)

Leaf water potential (ψ) was measured using the pressure chamber technique (Scholander, Hammel, Bradstreet and Hemmingsen, 1965).

The inside surface of the chamber was lined with wet filter paper, the leaf was excised from the plant and its petiole inserted through a silicone rubber disk which was fitted in the lid of the chamber. The lid was then sealed with a rubber O-ring and screwed into place.

The whole section of the lid contained an aperture through which the cut end of the petiole protruded and could be observed with a binocular microscope (Vickers, eyepiece 10x and objective 3x). Light was provided by fiber-optics (Nachet, E.F. 50s).

The gas-flow regulator was then opened increasing the pressure into the chamber at a rate of 0.02 MPa min^{-1}. When the sap began to exude, the gas supply was switched off. The pressure at which the sap exudation first occurred was read on the gauge of the pressure chamber device and was considered to be a measure of the xylem water potential and equivalent to bulk leaf water potential.

2.9.2- Leaf Osmotic Potential (π)

The osmotic potential (π) was measured using the same leaf sampled for the measurement of the leaf water potential. The leaf was removed from the pressure chamber and placed in an eppendorf vial (1.5 cm^3) and quickly frozen by immersing the vial in liquid nitrogen. The sample was then stored frozen (-20°C) until measured.

At the time of measurement, the samples were allowed to thaw at 23°C and placed in a disposable syringe (1 cm^3). The sap was extracted by squeezing the sample into an eppendorf vial (0.5 cm^3); 8 μl of sap was placed on a filter paper disc (0.5 cm diameter) on the sample carrier of a vapour pressure osmometer (VPO, Wescor, model 5100C, Wescor, Inc.). The osmotic potential of the leaf was measured in dew-point mode. The value obtained was converted to MPa assuming that 410 mmol Kg^{-1} (read on VPO) was equivalent to 1 MPa.

The VPO was calibrated before each set of samples were measured, using osmolality reference standard solutions of osmotic potential 290 and 1000 mmol Kg^{-1}.

2.9.3- Leaf Turgor Potential (P)

Turgor potential (P) was estimated from the leaf water (ψ) and osmotic (π) potentials by difference.

2.10- Leaf Cell Wall Parameters

The mechanical properties of leaf tissue were determined by the Instron technique (Cleland, 1967, Van Volkenburgh, Hunt and Davies, 1983), using the extensiometer device (Fig. 2.5) described by Milligan (1986).

2.10.1- Preparation of Leaf Tissue for Extensibility Measurements

Strips (5 mm wide × 12 mm long) were prepared from freshly excised primary leaves of *Phaseolus*, and cut parallel to midrib, avoiding the prominent veins. Uniform strips were cut using a disposable pair of scalpel blades taped together to give the chosen strip width. The strips were immediately killed in boiling methanol and stored in cold methanol until used.

2.10.2- Measurements of Leaf Tissue Extensibilities

For measurement, the strips were taken from methanol, trimmed to around 10 mm long and placed between the extensiometer clamps (Fig. 2.5) which initially, were 5 mm apart. The clamps were screwed tight to hold the strip in place. The tissue was then rehydrated by directing a small jet of distilled water on to it.

The total (TE_x) and elastic (EE_x) extensibilities of

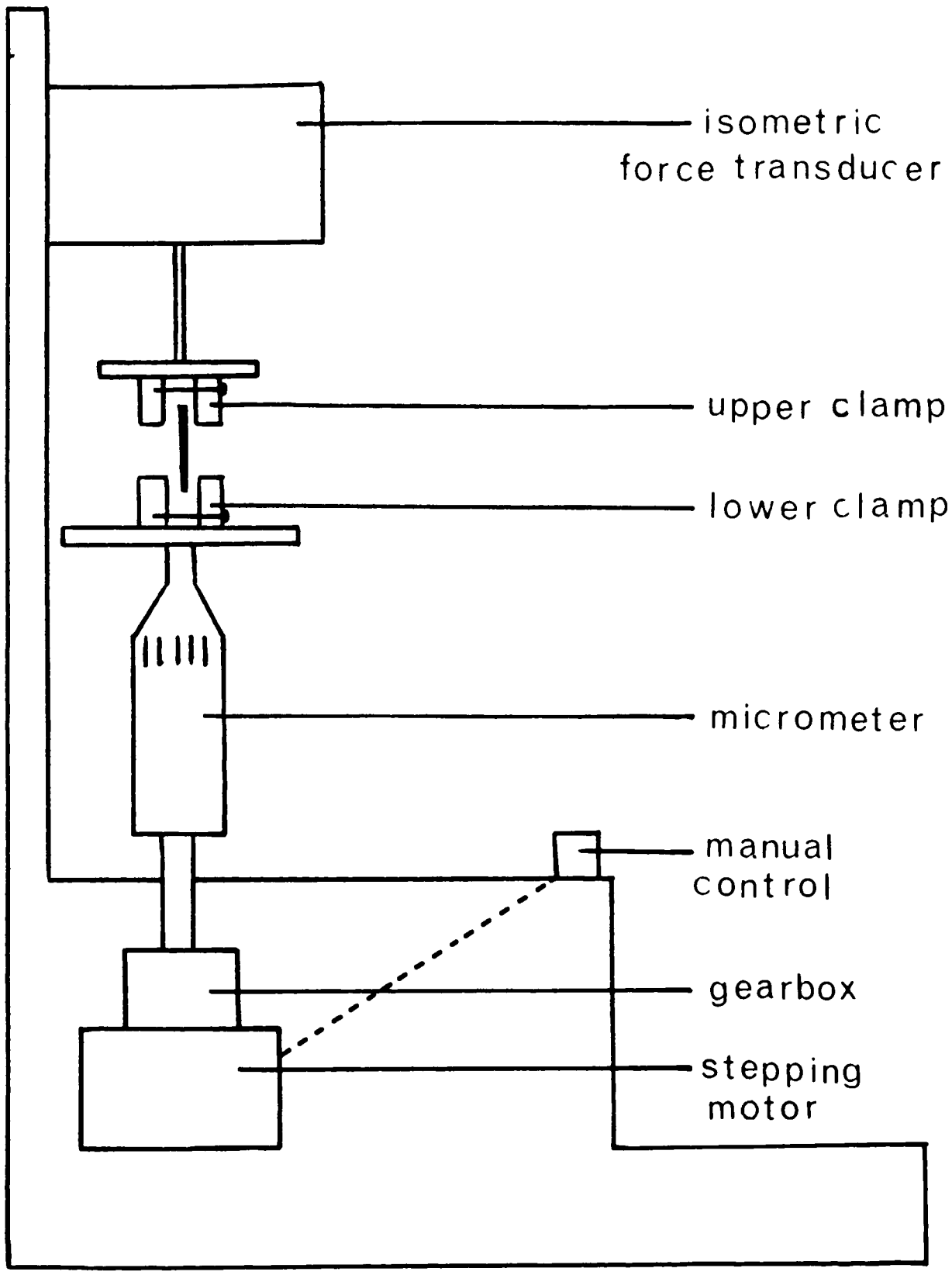


Figure 2.5- Diagram of the Edinburgh Tensiometer used to measure cell wall extensibility.

the leaf tissue were measured extending the strip by moving the lower clamp downwards (90 mm h^{-1}), to 20 g load. The clamp was returned to its initial position and a second extension was performed to the same 20 g load.

The resulting load-extension curves (Fig. 2.6) were recorded against time on an XY-axis chart recorder. The output of the load transducer plotted against time gives a curve which can be treated as a load-extension relationship (Milligan, 1986). The calibration of the time axis, in units of displacement, was calculated by dividing Vc/Vp, where Vc is the velocity of the lower clamp (90 mm h^{-1}) and Vp is the velocity of the chart paper (600 divisions h^{-1}). Thus, one chart division represented (Vc/Vp =) 0.15 mm of tissue extension. This conversion factor was used to calculate tissue extensibilities (section 2.10.3).

2.10.3- Interpretation of Load-Extension Curves

The two load-extension curves are shown in Figure 2.6. From the point where the total 20 g load was reached, for each extension, two strait lines where traced back to the X-axis as shown in Figure 2.6. One tangent to the linear portion of the load curve, and one perpendicular to the X-axis. The points where these two lines touched the X-axis, give the displacement (number of chart divisions) of the clamps necessary to initiate tissue extension (d) and to extend the tissue to 20 g

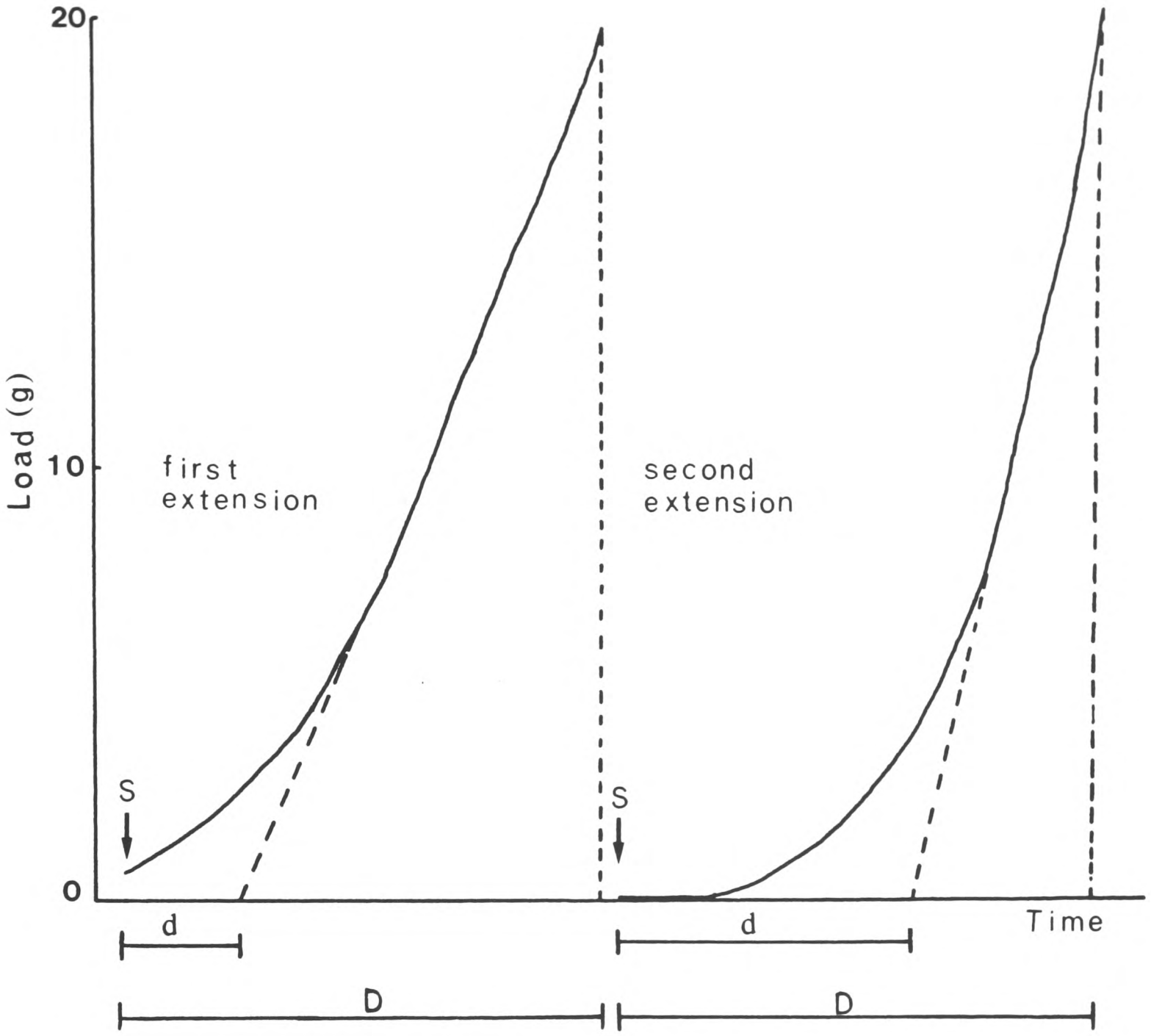


Figure 2.6- Typical pair of load-displacement curves for a strip of primary leaf tissue of *Phaseolus vulgaris*. (S) is the starting distance between clamps (5 mm); (d) the displacement of clamps necessary to commence tissue extension; and (D) displacement of clamps necessary to extend tissue to a load of 20 g.

load (D). The initial (L_i) and final (L_f) lengths of the tissue, at these loads, were then calculated by equations (2.5) and (2.6) respectively:

$$L_i = (d \times 0.15 \text{ mm}) + 5 \text{ mm} \qquad (2.5)$$

and

$$L_f = (D \times 0.15 \text{ mm}) + 5 \text{ mm} \qquad (2.6)$$

where: d is the displacement necessary to initiate tissue extension; D is the displacement necessary to extend the tissue to a total of 20 g load; 0.15 mm is the X-axis calibration value obtained from Vc/Vp (see section 2.10.2); and 5 mm is the initial length of the tissue. Once the values of L_i and L_f were known for both curves, the TEx and EEx extensibilities of the tissue were calculated from the first and second extension curve respectively, using the equation:

$$[(L_f \times L_i) / L_i] \times (100 / 2) \qquad (2.7)$$

The plastic (PEx) extensibility was then estimated by the difference: PEx = TEx - EEx.

2.11- Leaf Conductance and Transpiration Rates

Leaf conductance and transpiration rates, both expressed in mmol $m^{-2}s^{-1}$, and leaf temperature, expressed in ^{o}C, were measured with a steady-state

porometer, model LI-1600 (Li-Cor, inc. Lincoln, Nebraska, USA).

Before any measurements were made, the cuvette was allowed to equilibrate to growth room conditions, and the null point humidity carefully monitored. The measurements were performed on the abaxial surface of the leaf inserting and clamping this over the aperture in the cuvette wall. The broadleaf aperture cap used for all measurements was a circular 2 cm^2 opening. Simultaneous measurements of photon flux density was made in the same plane of the leaf. The values obtained for each measurement were read directly on the display of the porometer.

2.12- Xylem Sap Collection

To analyse the concentrations of abscisic acid in xylem sap and to investigate the effects of xylem sap on seedlings of *Phaseolus vulgaris*, root exudates were collected from root-cooled and control plants without pressurizing the root system.

When sap collection was about to begin the aerial parts of the two sets of 24 plants each (root-cooled and control) were removed by cutting off the shoot, firstly at a higher position (2 cm below the cotyledons) to release xylem tension and then at a lower position (0.5 cm). The remaining stump (3 cm) was immediately covered with a foil-wrapped plastic tube to prevent evaporation.

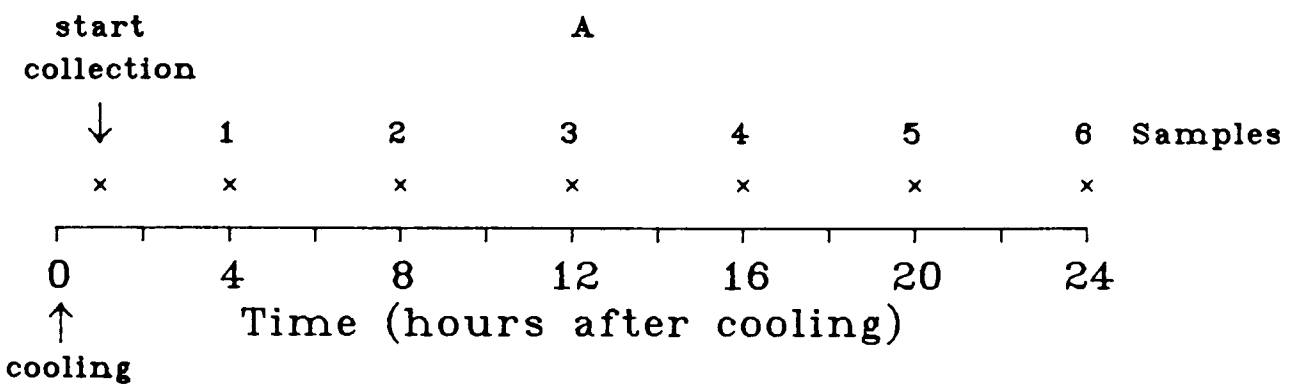
The sap drops which accumulated on the cut surface of the stumps were collected using a microcap pipette (0.05 cm^3) and pooled together in foil-wrapped eppendorf vials. After each collection the sap was immediately frozen until used.

Two kinds of experiments were performed for sap collection. A diagram of the time of collection is shown in Figure 2.7. Sap collection started either 1 h after the commencement of root-cooling treatment (Fig.2.7A) or after 24 hours (Fig.2.7B). For both, root cooling started at day 7.

When sap collection was performed following root cooling for 1 h the sap was collected over four hour periods, during a total period of 24 h (Fig. 2.7A). For each interval of collection, sap was pooled to give the samples 1 to 6.

In the second experiment (Fig.2.7B), plants were 8-day old and the root cooling treatment was started 24 h before (day 7 from sowing). Sap was collected every hour within the first 8 hours when the interval of collection was then extended to 4 hours. The sap collected over each interval was pooled, giving the samples 1 to 12 (Fig. 2.7B).

Because of the small volume of sap produced by root-cooled plants, and because of the limitation of available space imposed by the cooling system (no more than 24 root-cooled plants could be sampled at the same



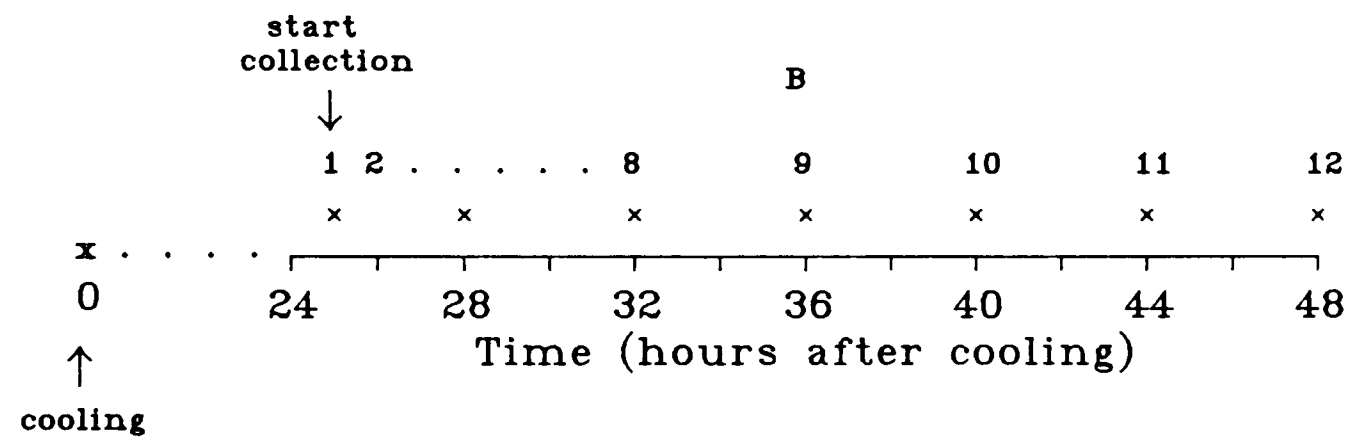


Figure 2.7- Diagram of the time of xylem sap collection.

time), several runs were performed to collect enough xylem sap to be used in further experiments.

2.13- Procedure for Feeding Experiments

Feeding experiments were used to investigate the relationship between ABA concentration in xylem sap with leaf growth, cell wall properties, leaf water relations and leaf conductance of seedlings of *Phaseolus vulgaris*. The procedure for these experiments was as follow:

i) Uniform seedlings of *Phaseolus vulgaris* grown as described in section 2.3 were removed from vermiculite on day 7. Their root systems were immersed in distilled water and the tops were excised, 5 cm below the cotyledons. The excisions were performed under water to avoid air bubbles.

ii) Derooted seedlings were then transferred to plastic vials (3.0 cm^3) containing distilled water. Leaf conductance was monitored using a Li-Cor porometer. Seedlings which showed comparable stomatal conductance were selected for experimental treatment.

iii) Seedlings were then transferred to similar plastic vials containing 0.5 cm^3 of distilled water (control), or xylem sap from root cooled and control plants (section 2.12) or (+)-ABA solutions at concentrations 60, 170 and 1000 $\mu mol\ m^{-3}$.

iv) When the solutions were taken up (around 3 h from the beginning of the supply) 1.0 cm^3 of distilled water

was added to all vials.

v) The amount of ABA taken up per leaf from 0.5 cm^3 of xylem sap or ABA solution was estimated having the fresh weight of one leaf, taken to be 0.15 g (see Appendix A.1), and the ABA concentration in xylem sap from control and root cooled plants equal 20 and 60 $\mu mol\ m^{-3}$ respectively. (1 $\mu mol\ m^{-3}$ ABA = 0.264 pg/μl ABA).

2.14- Abscisic Acid (ABA) Analysis

The quantification of ABA in leaf and cotyledon tissues and xylem sap were performed using a radioimmunoassay (RIA) technique described by Quarrie, Whitford, Appleford, Wang, Cook, Henson and Loveys (1988) and Quarrie (1988).

The immunoassay is based on the competition of a known amount of labelled antigen (^{3}H-ABA) and an unknown amount of sample antigen ((+)-ABA) for a limited number of high-affinity monoclonal antibody (McAb) binding sites. The unknown concentration of the antigen in the samples is determined from a reference dose response curve constructed with known concentration of standard antigen.

2.14.1 - Validation of the Radioimmunoassay (RIA)

Abscisic acid, as other plant growth regulators, is present within plants at very low levels. The full range

of measurements for ABA was quoted to be 3 - 20,000 $ng.g^{-1}$ (Addicott and Carns, 1983). The concentration quoted by Neil, Horgan and Walton, (1984) is much lower (20 - 1000 $ng.g^{-1}$). Quantification of ABA from plant tissue has been done mainly by use of either bioassays (Tillberg, 1975; Ogunkammi, Tuckey and Mansfield, 1973; Tuckey and Mansfield, 1971) or physical chemical techniques (Quarrie, 1978; Ciha, Brenner and Brun, 1977; Sweetser and Vatvars, 1976). The main disadvantage of using these techniques are that they lack specificity and require great amounts of plant material, with extensive and laborious methods of extraction and purification (Weiler, Eberle, Mertens, Atzorn, Feyerabend, Jourdan, Arnscheidt and Weiczorek, 1986; Weiler, 1979).

Those disadvantages are avoided by the application of immunological techniques for plant growth regulator analysis, which had its beginning in the early 70's (Fuchs, Mayak and Fuchs, 1972; Fuchs, Haimovich and Fuchs, 1971).

Since then, there has been an increasing interest in this approach, and several radioimmunoassays (RIA) and enzymeimmunoassays (EIA) have been reported using either polyclonal (Le Page-Degivri, Duval, Bulard and Delaage, 1984; Weiler, 1982; 1979; Walton, Dashek and Galson, 1979) or monoclonal antibodies (Quarrie *et al.*, 1988; Quarrie and Galfre, 1985; Mertens, Deus-Neumann and Weiler, 1983).

The monoclonal antibody used in this study (MAC 62) is specific for (+)-ABA (Quarrie *et al.*, 1988). Crude aqueous extracts of primary leaves of *Phaseolus vulgaris* (Trejo and Davies, 1991; Quarrie *et al.*, 1988; Smith and Dale, 1988), green leaves of *Hordeum vulgare* (Quarrie, 1988) and unpurified xylem sap of *Phaseolus vulgaris* (Trejo and Davies, 1991) and *Helianthus annuus* (Zhang and Davies, 1990*b*) have been assayed using MAC 62, showing that the extent of immunoreactive contaminants was no greater than 10 - 15%, an acceptable range of contamination (Quarrie, 1988).

However, since interfering compounds can vary within extracts of tissue from different species (Crozier, Sandberg, Monteiro and Sundberg, 1986) and within different experimental treatments of the same species (Pengelly, Bundurski and Schulze, 1981) it is advisable to check the interferences of competitive and non-competitive compounds which could give counterfeit results (Pengelly, 1986; Crozier, *et al.*, 1986; Pengelly and Meins, 1977).

The accuracy and precision of the RIA, for use with crude sample extracts of bean, sunflower and barley leaves and xylem sap of bean plants, were confirmed by the method of parallelism (Pence and Caruso, 1988 and 1986; Jones, 1987; Pengelly, 1986). Crude sample extracts not diluted or diluted with distilled water (v/v; 1/2 and 1/4) were assayed in the presence of different amounts of added internal (+)-abscisic acid

standard in a range of twofold dilutions from 4 - 0 ng/vial. The regression lines (Fig. 2.8) were parallel when compared to the control line (standard only), showing that there was not significant non-specific interference and that the use of these unpurified samples is reliable.

2.14.2- Reagents and Instrumentation for RIA

Reagents:

The monoclonal Antibody (AFRC MAC 62) was kindly provided by Dr. Quarrie and has been produced in his laboratory in response to immunisation of rats with (*S*)-ABA conjugated to a carrier protein through the ring ketone group (Quarrie and Galfre, 1985). Therefore, MAC 62 discriminates between (*R*)- and (*S*)-ABA and between the free and ester derivatives of the ABA carboxyl group and is specific for (*S*)-ABA (Quarrie, *et al.*, 1988).

^{3}H-Abscisic acid (DL - *cis,trans* - [G-^{3}H] Abscisic acid) specific radioactivity 107.6 MBq mg^{-1} was purchased from Amesham International.

(±) *cis-trans*-Abscisic acid (No A-1012) , Bovine γ-Globulin (No G-5009), Bovine Serum Albumin (No A-7030), Polyvinylpyrrolidone PVP-40 (No 45F-0372) and Ammonium Sulphate (No A-5132) from Sigma.

Sodium dihydrogen orthophosphate (Prod. 10245), *di*Sodium hydrogen orthophosphate (Prod. 10249) and Sodium chloride (Prod. 10241) from BDH laboratories.

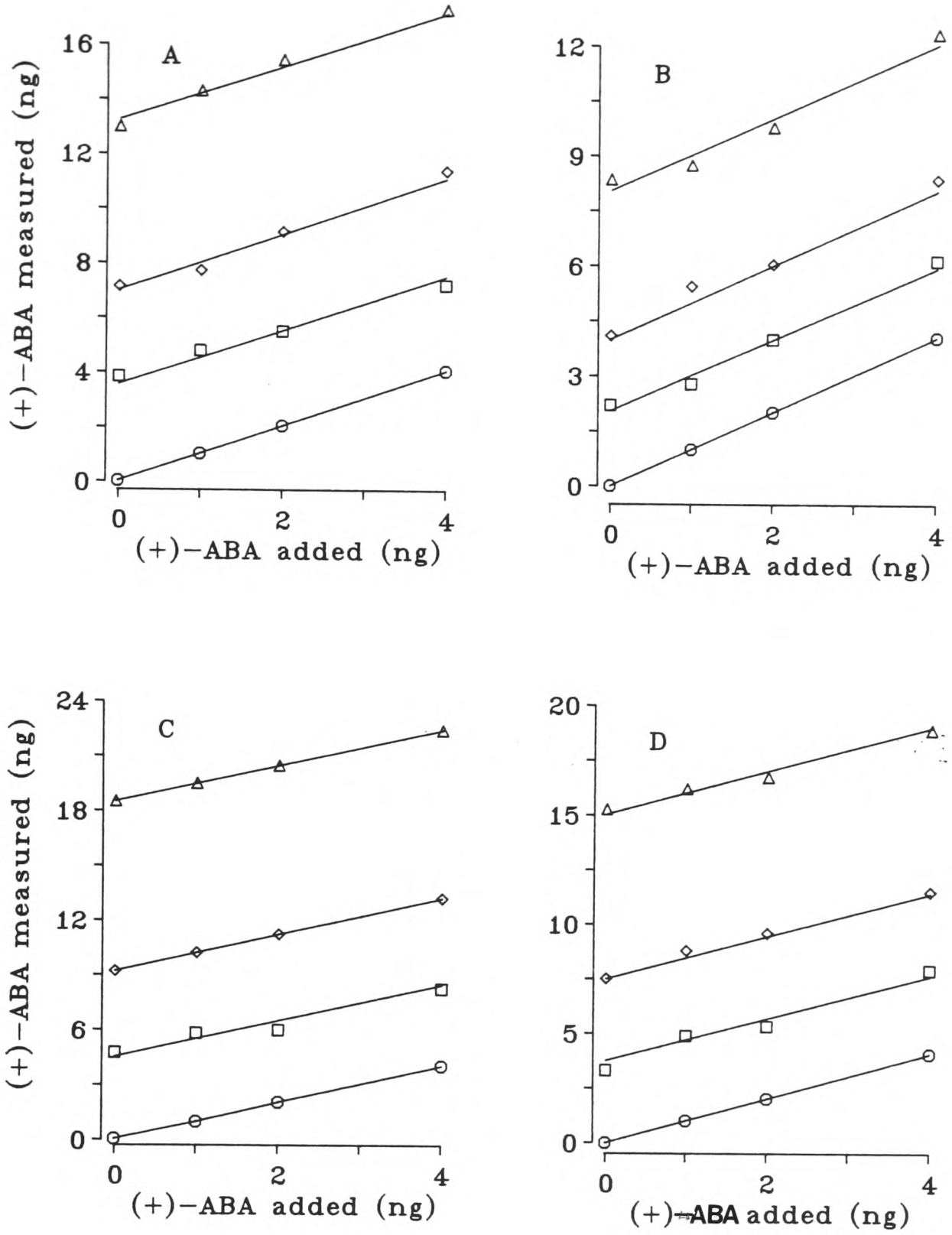


Figure 2.8- Dilution tests for non-specific interference in the ABA raidioimmunoassay in the presence of internal standard as a control (circles), or added sample not diluted (triangles), and diluted 1/2 (diamonds), and 1/4 (squares). (A) *Phaseolus* primary leaves; (B) *Phaseolus* xylem sap; (C) *Helianthus* leaves; and (D) *Hordeum* leaves.

scintillation cocktail (supersolve-X) from Koch-light Laboratories.

Instrumentation:

The assay was performed in eppendorf vials (1.5 cm^3) supported in 3 cm deep foam racks (Alpha Laboratories: Cat. No AW 2625) with a capacity to hold 50 vials. The reagents were added to eppendorfs using a repetitive pipette (BCL 8000), except the abscisic acid standard solutions and the samples which were added with a microcap pipette (0.05 cm^3 Pipetman, Gilson) and scintillation cocktail which was added using a bottle dispenser. Spinning was performed using micro-centrifuge (Eppendorf centrifuge model 5413) at 8800 g. Radioactivities were counted in a liquid scintillation counter (model SL-3000, Intertechnique).

2.14.3- Solutions for RIA

Stock solutions:

Phosphate Buffered Saline (PBS): 50 mol m^{-3} solutions of sodium dihydrogen orthophosphate (NaH_2PO_4) and *di*sodium hydrogen orthophosphate (Na_2HPO_4) were prepared in distilled water and the Na_2HPO_4 solution slowly added to the NaH_2PO_4 to pH 6.0. Sodium chloride (100 mol m^{-3}) was then added to the buffer.

50% Buffer Solution: PBS was diluted 1:1 with distilled water to make 50% PBS.

Working [^{3}H]-Abscisic acid solution (^{3}H-ABA): Stock solution from Amesham International was diluted tenfold with sterile water and frozen in aliquots of 0.1 cm^3.

Monoclonal Antibody (McAb): the lyophilised sample (0.5 cm^3) of monoclonal antibody (MAC 62) from AFRC was rehydrated by adding 0.475 cm^3 sterile water. Aliquots (0.1 cm^3) of this stock was stored frozen.

γ-globulin for [^{3}H]-ABA : γ-globulin was dissolved in PBS at 5.0 mg cm^{-3} and stored frozen in 10 cm^3 aliquots.

Bovine Serum Albumin (BSA) for MAC 62: BSA (5.0 mg cm^{-3}) and PVP (4.0 mg cm^{-3}) were dissolved in PBS. Aliquots of 10 cm^3 were stored frozen.

(±)*cis-trans* Abscisic acid standard solutions ((±)-ABA): (±)-ABA were prepared in a range of twofold dilutions from 250 to 4000 pg per vial.

Freshly prepared solutions:

[^{3}H]-ABA solution: the working ^{3}H-ABA solution was diluted in γ-globulin solution to give 5.0 μl cm^{-3}.This solution should give 8000-8500 cpm.

MAC 62 solution: The antibody solution (McAb) was diluted in BSA + PVP solution to give 2.5 - 5.0 μl cm^{-3}, depending on the batch of monoclonal antibody.

Ammonium Sulphate Saturated Solution: Ammonium sulphate (50 g) was dissolved in water (60 cm^3) giving a solution with solid residue; Part of this solution was diluted in water 1:1 to give 50% ammonium sulphate saturated solution.

2.14.4- Sampling Material for RIA

The material used in the RAI for ABA were primary leaves of seedlings of *Phaseolus vulgaris*; the first pair of leaves and the cotyledons of *Helianthus annuus* and the first full expanded and the second expanding leaves of *Hordeum vulgaris* seedlings. For *Phaseolus* and *Helianthus*, the leaves or cotyledons were harvested as a whole, excluding the petiole. For *Hordeum*, the analysis was performed for the whole blade of the first full expanded leaf, whereas the second expanding leaf was divided in emerged and unemerged portion, with a further distinction being made between the proximal and distal portions. ABA analysis was performed separately for each portion of the second leaf of barley.

Leaves, leaf portions and cotyledons were weighed immediately after severing from the plant, then quickly placed into a plastic tube and frozen in liquid nitrogen. The tissues were then ground into powder using a glass rod and kept frozen until used.

Xylem sap from seedlings of *P. vulgaris* were sampled for ABA analysis as described above (section 2.12). Xylem sap was added to the assay without purification.

2.14.5- Extraction Method for RIA

For leaf and cotyledon tissues, distilled deionised water was used as a solvent. The solvent was added to

the sample and the tubes were placed on a Coulter mixer (Coulter Electronics Limited) shaken overnight at 2°C in darkness. The extraction ratio for leaf tissue was 8:1, solvent volume (cm^3):leaf FW (g). The sample was then centrifuged at 500 g for 2 min. The supernatant was assayed without purification.

2.14.6- RIA Procedure

Solutions were added to assay vials in the following order:
0.2 cm^3 of 50% PBS; 0.05 cm^3 of (±)-ABA standards in a range of twofold dilutions from 4000 - 250 pg/vial for dose response curve or 0.05 cm^3 sample; 0.1 cm^3 of [^{3}H]-ABA solution in γ-globulin; and 0.1 cm^3 of MAC 62 solution in BSA + PVP. Non-specific binding (B_{min}) was determined by omitting the MAC 62 and adding an equal volume of the lowest concentration (250 pg) of the (±)-ABA standard. Maximum specific binding (B_{max}) was determined by omitting the (±)-ABA standard or sample and adding an equal volume of distilled, dionized water.

The contents were mixed, by shaking briefly, and then incubated at 2°C for 45 min. After incubation, the vials were spun briefly (1 min) to remove any drop adhered on the cap. 0.5 cm^3 of saturated ammonium sulphate solution were added and the contents mixed thoroughly by shaking the rack. Vials were left at room temperature for 30 min to separate the free and bound antigen. The precipitated

antibodies were then pelleted by centrifugation for 6 min at 8800 g. The caps were removed, placed face down on several layers of paper towelling and pressed to remove any moisture. The rack was turned upside down and gently tapped on to paper towelling to remove all residual supernatant. The pellet was then washed by resuspending it in 1.0 cm^3 of 50% saturated ammonium solution, centrifuging again (8 min) and discarding the supernatant. The pellet was dissolved in 0.1 cm^3 of sterile water. 1.4 cm^3 of scintillation cocktail were added to all vials. They were then capped and placed inside scintillation vials (20 cm^3) and the radioactivity counted once for 10 min.

2.14.7- Calculation of ABA Concentrations

A standard curve and its logit transformation is shown in Figure 2.9. For each assay run one standard curve, with triplicates was constructed.

The values obtained for bound radioactivity (B), in counts per minute (cpm), for each standard concentration, were corrected by subtracting them from the non-specific binding (B_{min}) value i.e. ($B - B_{min}$). Corrected B values were divided by the corrected maximum specific binding ($B_{max} - B_{min}$) value. The logit B were then calculated from the equation (2.8):

$$\text{Logit } B = \ln (p / q) \qquad (2.8)$$

where:

$p = [(B - B_{min})/(B_{max} - B_{min})]$

and

$q = (1 - p)$

Estimated ABA concentrations either in the standard solutions or in the samples were then calculated from the equation (2.9):

$$[(+)\text{-ABA}] = \left\{2^{[(\text{logit } B - a)/b]}\right\} \times 125 \qquad (2.9)$$

where a = constant and b = slope, both obtained from linear regression; and 125 = the smallest (+)-ABA concentration (pg/vial) from the standard solutions.

To obtain the constant (a) and the slope (b), the values of logit B (Y values) for each concentration of ABA standard were fitted in a linear regression against X values (0, 1, 2, 3 and 4). The X values were calculated on a logarithmic scale base 2 of the concentrations of the doses of ABA standard (125, 250, 500, 1000 and 2000 pg) according to equation (2.10).

$$X = \log_2((+)\text{-ABA concentration} / 125) \qquad (2.10)$$

An example of the logit calculation for each standard concentration and the values for the construction of the standard curve (showed in Figure 2.9) is presented in Table 2.3. The values of estimated (+)-ABA for each

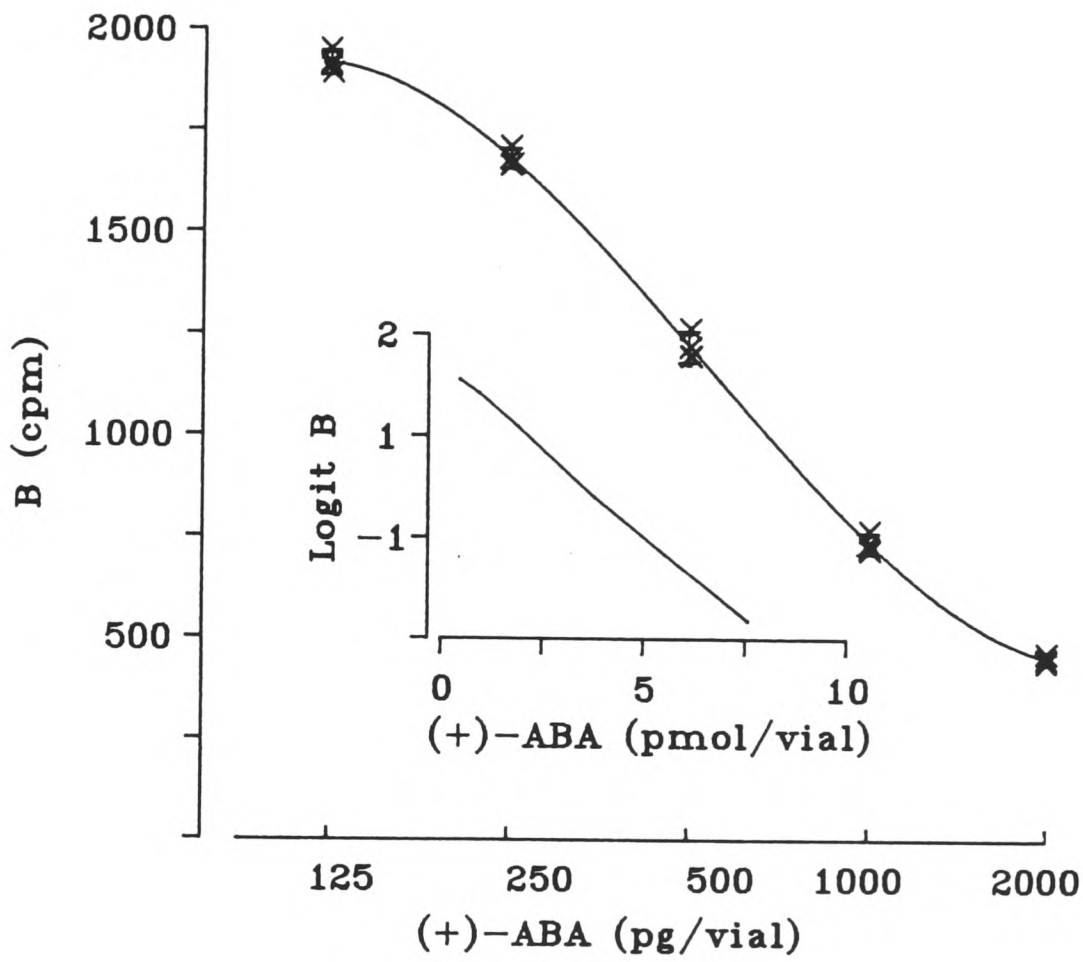


Figure 2.9- Standard curve for the RIA and its linear regression, showing the results for triplicate RIA vials of each (+)-ABA concentration.

Table 2.3- Value of radioactive binding B (cpm) and logitB ($\ln(p/q)$) for a triplicate RIA vials of each (+)-ABA concentration.

(+)-ABA (pg)	B_{mean} (cpm)	$(B-B_{mim})$	p	q	Logit B $\ln(p/q)$
125	1916	1723	0.8817	0.1183	2.0086
250	1682	1489	0.7620	0.2380	1.1636
500	1220	1027	0.5255	0.4745	0.1020
1000	739	546	0.2794	0.7206	-0.9474
2000	454	261	0.1335	0.8665	-1.8703

B_{max} (cpm)= 2147;

B_{min} (cpm)= 193; $B_{max} - B_{min} = 1954$

50% ($B_{max} - B_{min}$) = 977pg (+)-ABA/vial

Logit-transformed regression coefficients:

const. (a) = 2.0650 slope (b) = -0.9868

Logit-transformed correlation coefficient (r) = -0.9993

Table 2.4 - Specific binding (cpm) and estimated values of (+)-ABA (pg/vial).

Radioactivity (cpm)	ABA added (pg/vial)				
	125	250	500	1000	2000
cpm 1	1891	1670	1198	721	445
cpm 2	1908	1666	1265	769	468
cpm 3	1950	1712	1199	727	450
Mn cpm	1916	1683	1221	739	454
se mean	± 17	± 14	± 22	± 15	± 6
Calc. ABA (pg/vial)	130	235	496	1037	1983

concentration was then calculated by fitting the values of logit B in equation (2.9) and the results are shown in Table 2.4.

2.14.8- Concentration of ABA in Samples

The concentration of ABA in the samples were calculated from equation (2.9) by fitting the logit B value obtained for each sample. The results were presented either in ng g^{-1}F.W. or in μmol m^{-3}.

2.15- Statistical Analysis

The numerical data presented in this study were statistically analysed using the one way analysis of variance (Mead and Curnow, 1983).

Most of the results presented are 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 figures, plus and minus standard errors are shown by a vertical bar through each point.

Significant differences between treatment means were tested using 95% limit of confidence.

CHAPTER 3 - RESULTS

LEAF ELONGATION

3.1- Introduction

This chapter deals with the short-term pattern of primary leaf elongation of seedlings of *Phaseolus vulgaris* grown under controlled conditions; it also examines the short-term effects of root-cooling treatments and reversing root cooling on leaf elongation rates.

Results for leaf elongation of seedlings of *Helianthus annuus* and *Hordeum vulgare* in response to root cooling treatments are also reported.

3.2- Development of the Seedling of *Phaseolus vulgaris*

The successive stages of development of seedlings of *P. vulgaris*, from planting (day 0) to day 7, are shown in Figure 3.1. Germination occurred at day 2 and lateral root primordia were present by day 3. At day 4, the hypocotyl appeared above the vermiculite surface, the leaves were expanding within the cotyledons, and the testa had ruptured; the length of the main root was about 7 - 9 cm. Between days 5 and 6 the length of the hypocotyl was 3 - 5 cm and it had straightened by day 6. By this time the leaves had expanded beyond the

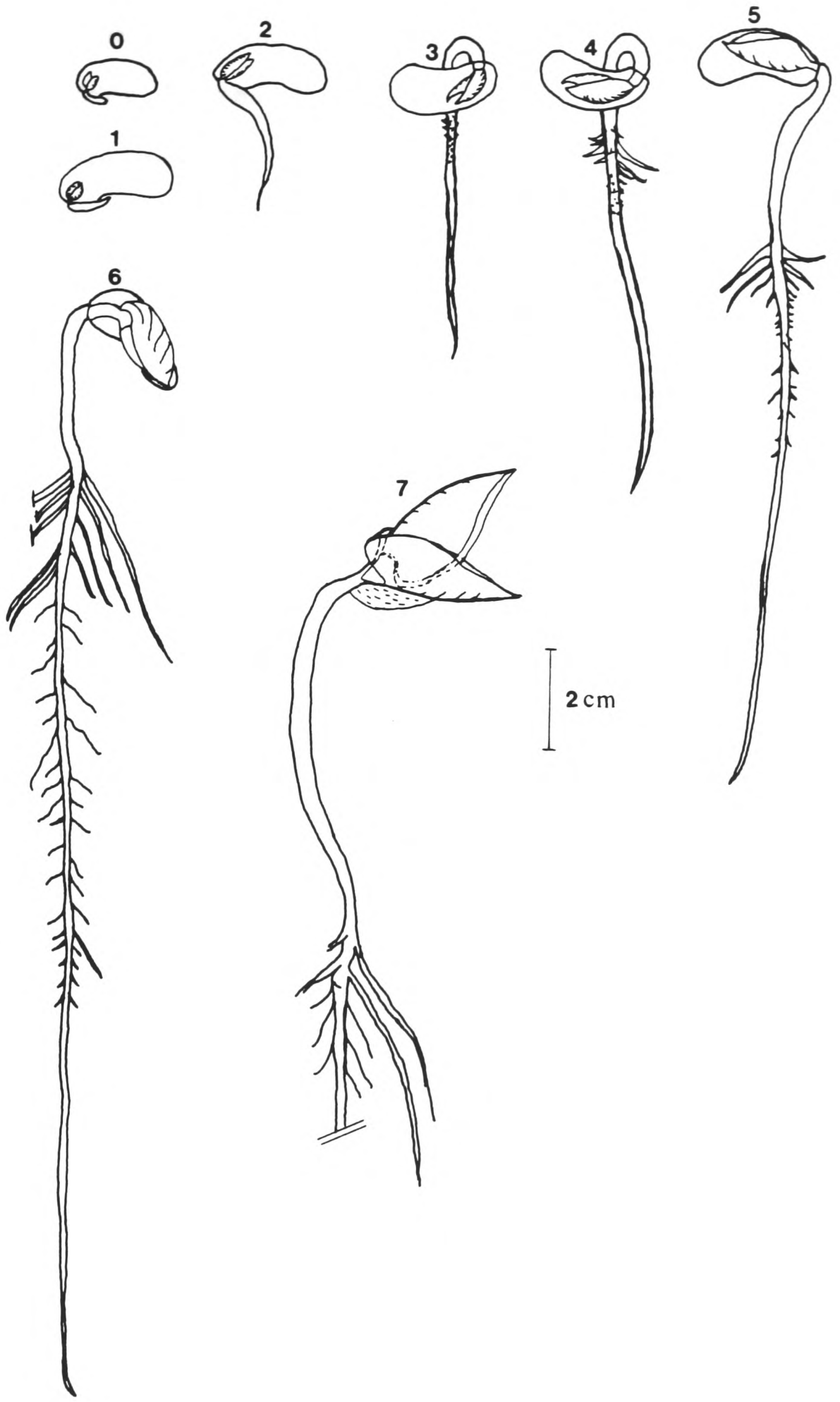


Figure 3.1- Diagram of the successive stages of development of *Phaseolus vulgaris* seedling from the dry seed, day 0, to day 7.

cotyledons and the main root had reached 9 - 15 cm, with lateral roots formed 4 - 10 cm from the tip. By day 7, the primary leaves were unfolding; the midrib was 3 - 5 cm and the area of the primary leaves about 15 - 20 cm^2 per pair. The length of the main root was 18 - 22 cm.

3.3- Changes in Leaf Elongation and Water Relations of *Phaseolus vulgaris* Grown in Controlled Conditions (i.e. Not Subjected to Root Cooling)

The measurements of growth of primary leaves of *Phaseolus vulgaris* started on day 7 after sowing when seedlings had straightened (Fig. 3.1). At this stage the seedlings were transferred to solution medium and one of the primary leaves was attached to the transducer (see Figure 2.4, Chapter 2). The experiments began 2 - 3 h after the start of the light period and 0.5 - 1 h were allowed before a steady-state rate of elongation was achieved and measurements started.

The cumulative growth of the primary leaves for plants transferred or not from vermiculite medium to nutrient solution is shown in Figure 3.2. Transference of the seedling to nutrient solution had no effect on growth. The absolute growth rate averaged over 15 h was 0.6 mm h^{-1} for both sets of plants.

Changes in elongation rate after transferring the seedlings to nutrient solution, is shown in Figure 3.3A, B and Figure 3.4 over the alternating cycle of 12 h

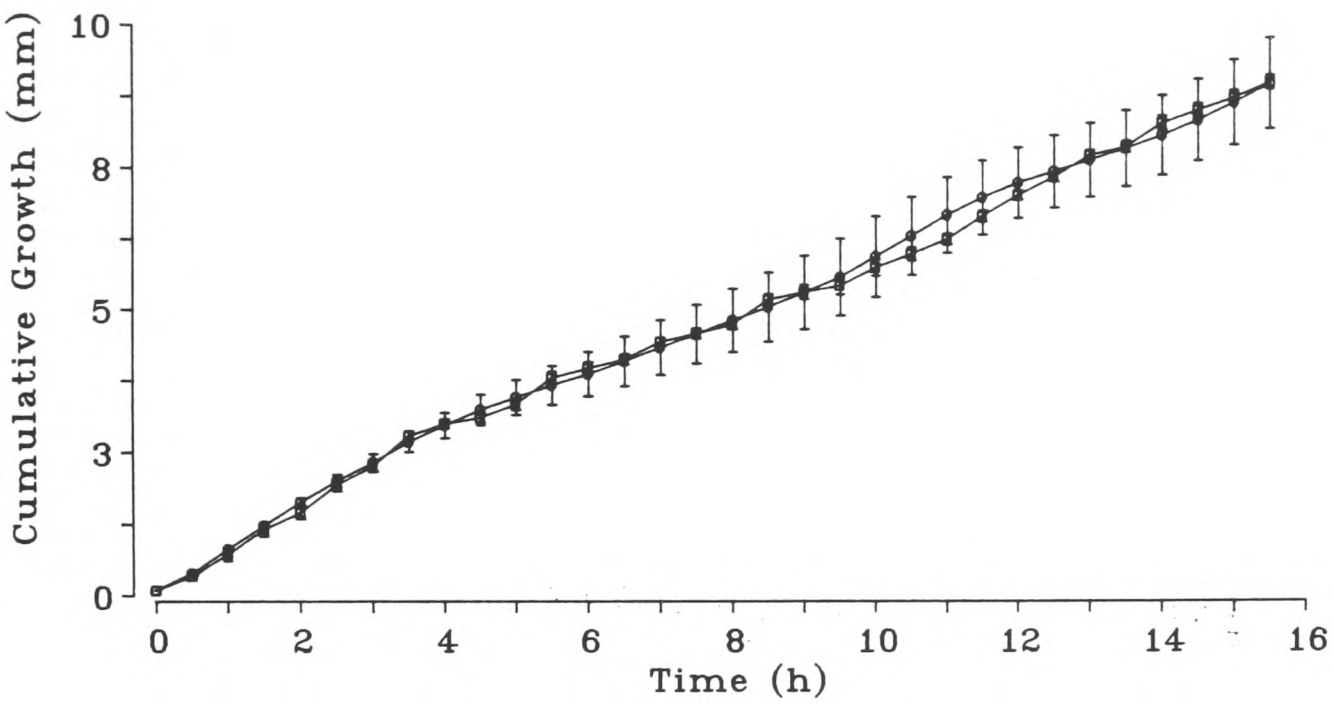
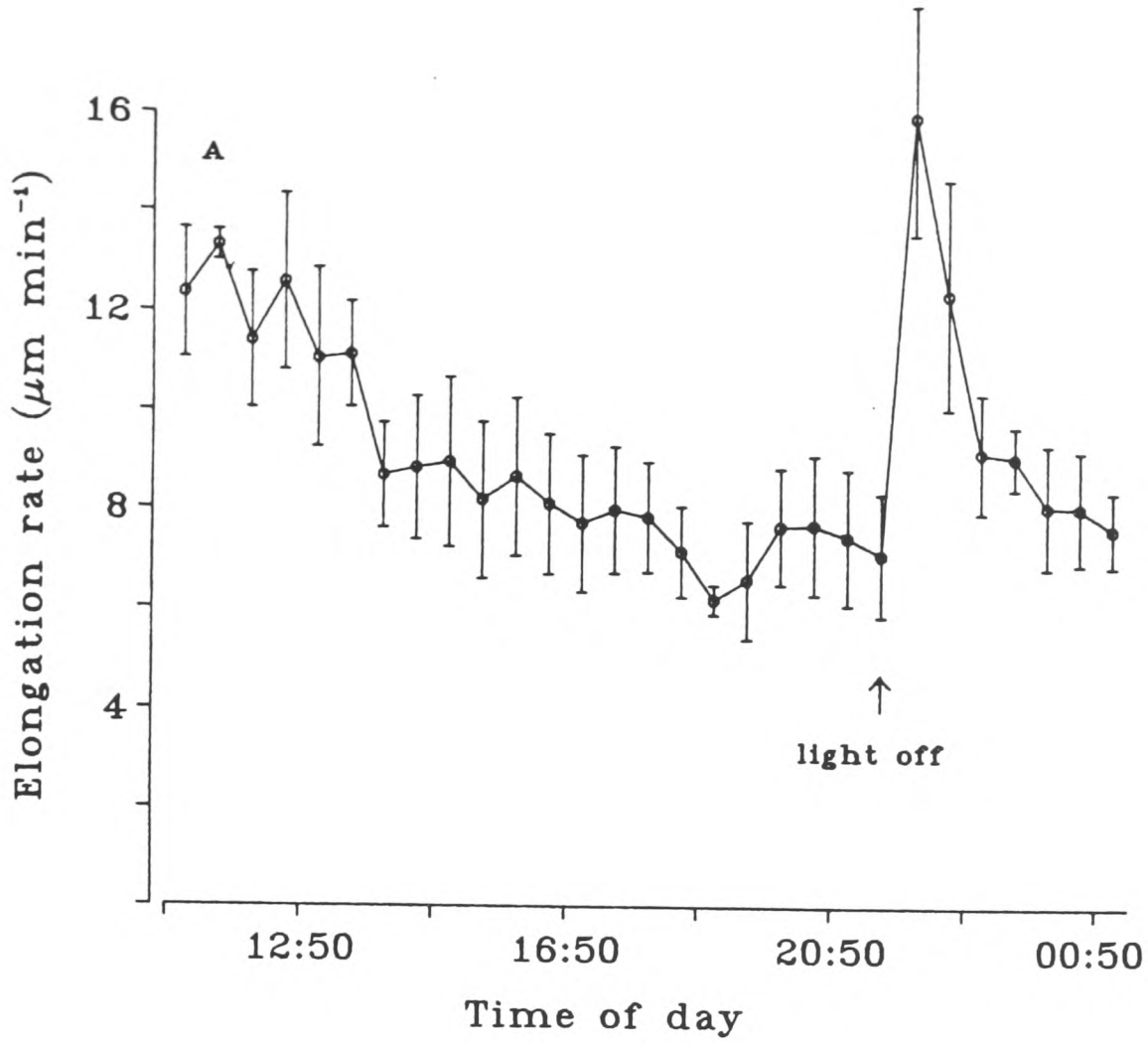


Figure 3.2- The time courses of the cumulative growth of the lamina of primary leaves of *P. vulgaris* in vermiculite (squares) and after transfer to nutrient solution (circles), measured by RVDT. Standard errors are shown for n = 4.

light and 12 h dark. The leaf elongation rate was steady averaging 10 μm min^{-1} before the lights went off, and a large increase was found at the commencement of the dark period (Fig. 3.3A). The response to lights-off was rapid and over the short-term leaf elongation rates increased within 1 - 2 minutes to values considerably higher than those in the light period (Fig. 3.3B). For example, the leaf elongation rate just prior to the end of the light period was about 10 μm min^{-1} whereas 6 min into the dark period the rate had risen to 34 μm min^{-1} (Fig 3.3B). This high rate did not persist and fell over 2 - 3 h (Fig. 3.3A) to values comparable to those found in the preceding light period.

At the beginning of the light period, leaf elongation rate slowed (Fig. 3.4) but this fall was not as immediate as the rise at the end of the light period. The fall began 5 min of the start the light period and the minimum values, which were negative and imply shrinkage, were found 15 min after lights were switched on. It was followed by a rapid and progressive increase to reach the previous values within 1 h (Fig. 3.4).

Since the plants were grown in alternating cycles of 12 h light and 12 h dark from sowing, leaf growth rate could fluctuate on a rhythmic basis. To differentiate between effects due to endogenous rhythms and effects due to light-dark transition, leaf elongation rates were measured for plants in which the light period was extended by 2 h or curtailed by 3 h (Fig. 3.5A, B).



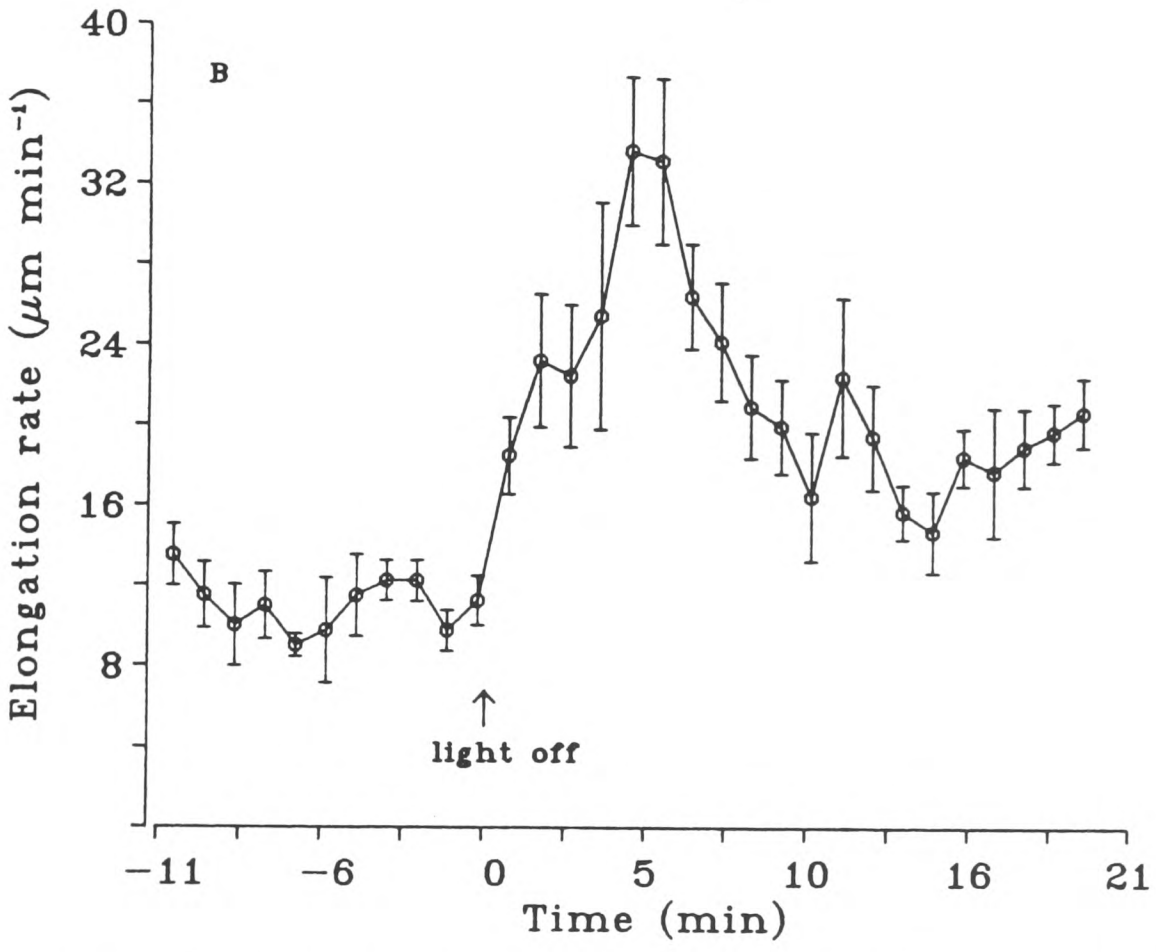


Figure 3.3- Long (A) and short-term (B) changes in the elongation rate of the lamina of the primary leaves of *P. vulgaris*. The negative time in B indicates the time before to the end of light period. Standard errors are shown for n = 4.

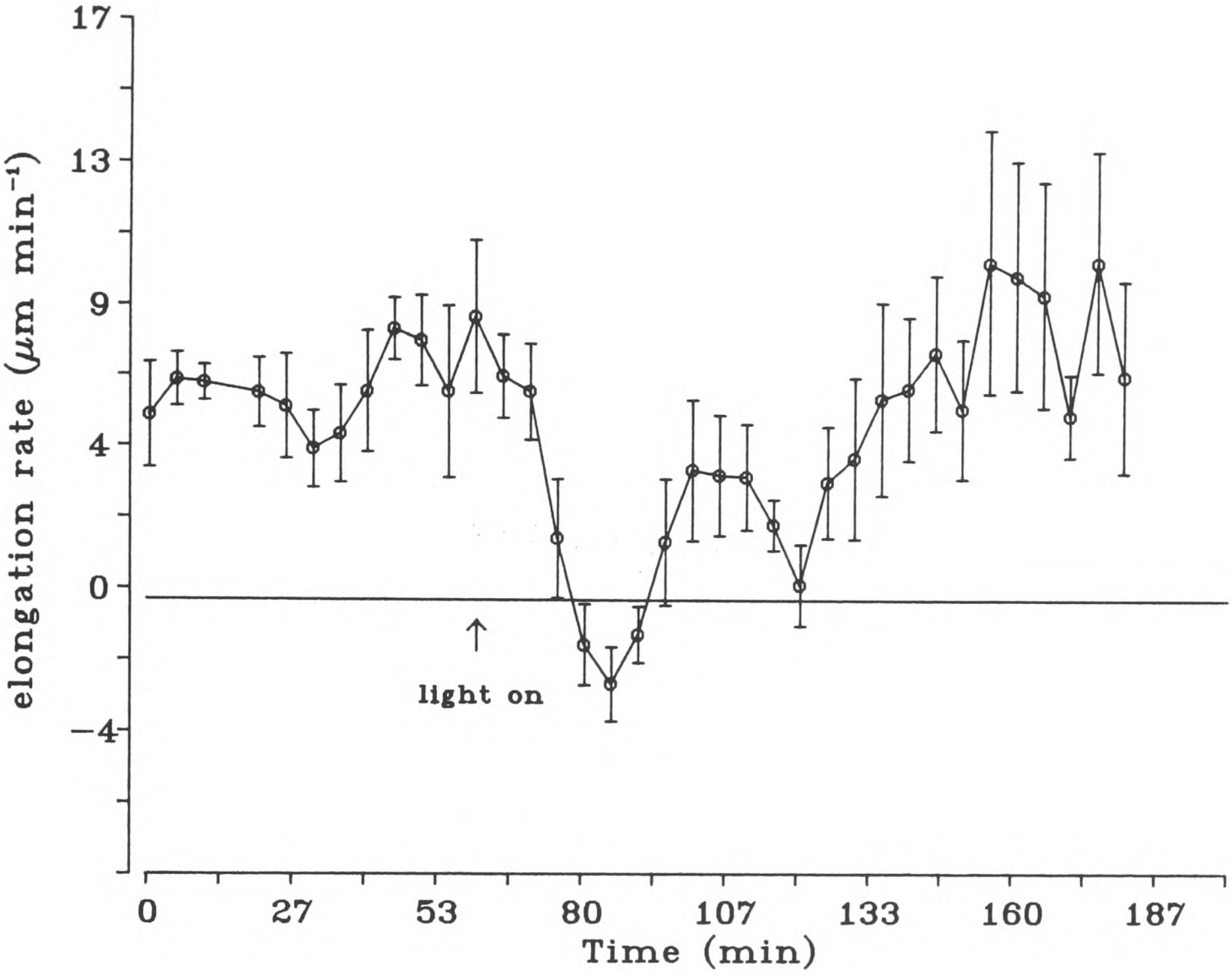


Figure 3.4- Short-term changes in the elongation rate of primary leaves of *P. vulgaris* at the beginning of the light period. Standard errors are shown for n = 4.

When the light period was extended by 2 h (Fig. 3.5A), leaf elongation rate began to increase at the normal lights-off time. This rise was significant but less pronounced than that found at the end of the extended light period when there was a further increase in leaf elongation rate. This was followed, as usual, by the slow decline in rate of elongation (Fig. 3.5A).

When the light period was ended 3 h early (Fig. 3.5B), the increase associated with the end of the light period was found, although less pronounced, followed by a fall and then a further rise at the time when the light period would normally have ended.

As the photoperiod normally ended or began by the automatic and abrupt switching of lights on or off, experiments were run in which the end of the light period was achieved by dimming the lights gradually and determining the effects on leaf elongation. In these gradual light-dark transition experiments the response observed was less extreme, the peak of leaf elongation rate was found some 5 min after the beginning of complete darkness and declined within 10 min (Fig. 3.6).

It may be that in controlled conditions, where the lights are normally switched on and off abruptly, such an abnormal situation may lead to a disruption of the mechanisms which control growth and normally prevent overshoot.

Milligan and Dale (1988*a*) have reported higher leaf elongation rates during dark period for primary leaves

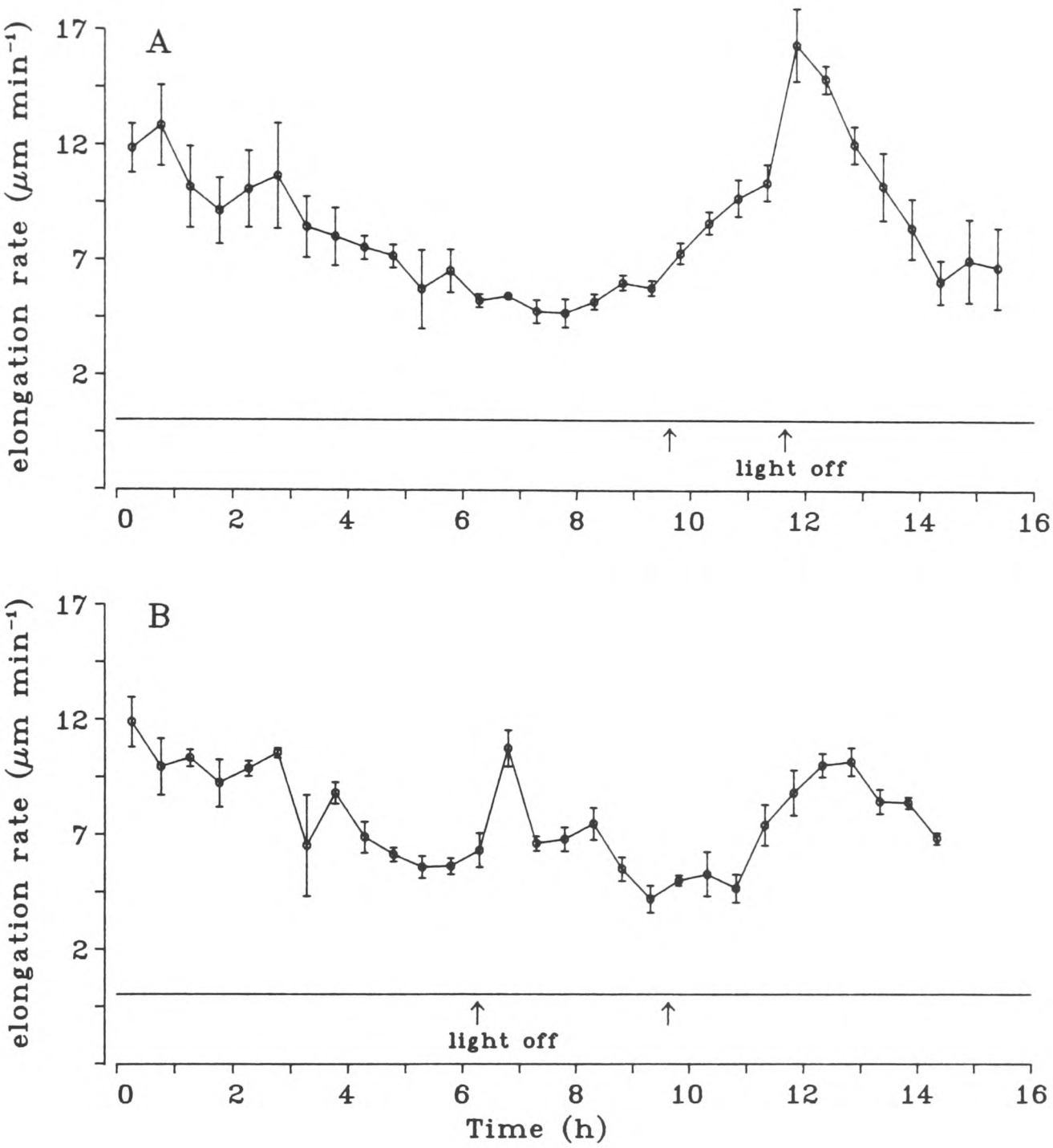


Figure 3.5- Long-term changes in the elongation rate of the lamina of primary leaves of *P. vulgaris* subjected to a 2 h extension (A) or a 3 h reduction (B) in the light period. Labelled arrows indicate when the lights went off, unlabeled arrows, the normal end of the light period. Standard errors are shown for n = 4.

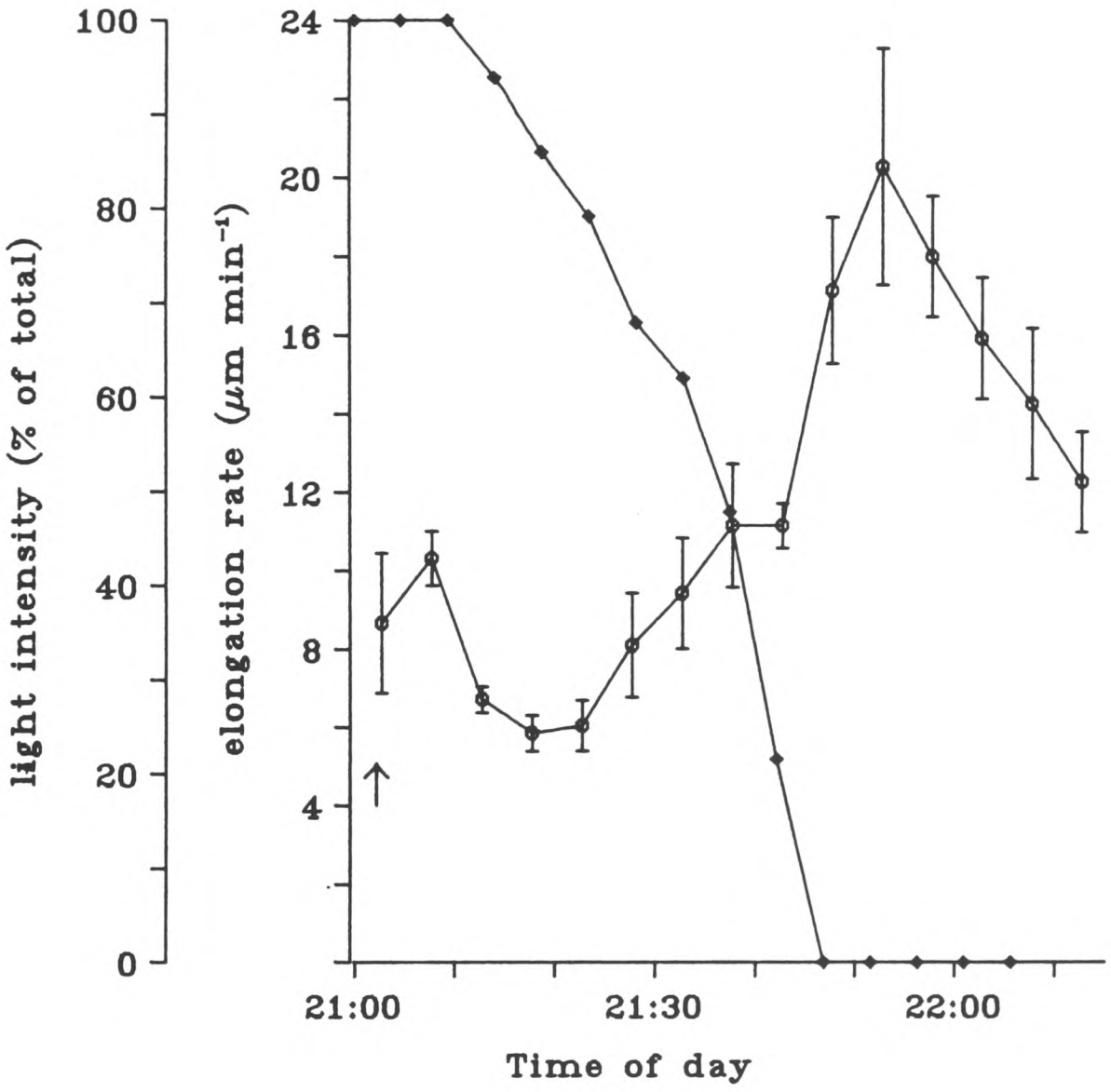


Figure 3.6- Short-term changes in the elongation rate (circles) of the primary leaves of *P. vulgaris* following dimming of the lights (diamonds) from an initial intensity of 280 μmol $m^{-2}s^{-1}$. The arrow indicates the normal end of the light period. Standard errors are shown for n = 4.

of *Phaseolus*. The results in Figures 3.3 - 3.6 support this findings and give evidence for leaf growth being regulated by an endogenous rhythm as well as by light-dark transition and that these effects are independent.

As for bean plants, increases in leaf elongation rates associated with the beginning of the dark period were also found for *Helianthus annuus*. The elongation rates of the first leaf of 7-day old seedlings increased from a steady state rate, over the light period, of about 9 - 11 $\mu m\ min^{-1}$ to about 17 $\mu m\ min^{-1}$ within 1 h after the lights went off. This was followed by a decline to the previous values (see Fig.3.13 , Chapter 3).

The rapid changes in leaf elongation rates at light-dark, dark-light transitions showed here for both bean and sunflower plants, are consistent with most studies performed in controlled environments (Waldron, *et al*, 1985; Taylor and Davies, 1985; Matthews *et al.*, 1984; Parish and Wolf, 1983; Christ, 1978*a*, *b*; Boyer, 1968). These studies have shown that leaf elongation rates are often different in light and dark, and change rapidly in light-dark transition. These abrupt changes in growth are normally attributed to an alleviation of cumulative water stress built up over the light period (Waldron *et al*, 1985; Parrish and Wolf, 1983). However, it seems likely that the mechanisms by which lights, or its absence, control leaf growth differ between species,

and changes in leaf turgor as well as in cell wall extensibility may be involved (Taylor and Davies, 1985).

To examine whether the rapid changes in leaf elongation rate of *Phaseolus* plants, at light transitions, were associated with changes in leaf turgor, bulk water potential was measured by pressure chamber; osmotic potential was determined on the same samples and turgor estimated by differences.

The results for short-term successive measurements performed in individual leaves are shown in Figure 3.7A, B. The values obtained 15 min before and 5 min after the light-dark and dark-light transitions showed little detectable changes in water potential; osmotic potential was more variable, but the resulting changes in turgor were small. However, there was a trend of increase or reduction in turgor with time, as the photoperiod ended or began, respectively.

These trends are shown in Tables 3.1A, B, where the values presented are from longer intervals (40 min) with repetitions. For control plants there was an increase in leaf water potential of 0.10 MP_a, 40 min after the end of the light period (Table 3.1A) and a decrease of 0.12 MP_a, for the same elapsed time, when the light period began (Table 3.1B).

A small detectable change occurred in osmotic potential, and consequently leaf turgor actually varies during light-dark, dark-light transitions.

When data for changes in leaf turgor (Fig. 3.7 and

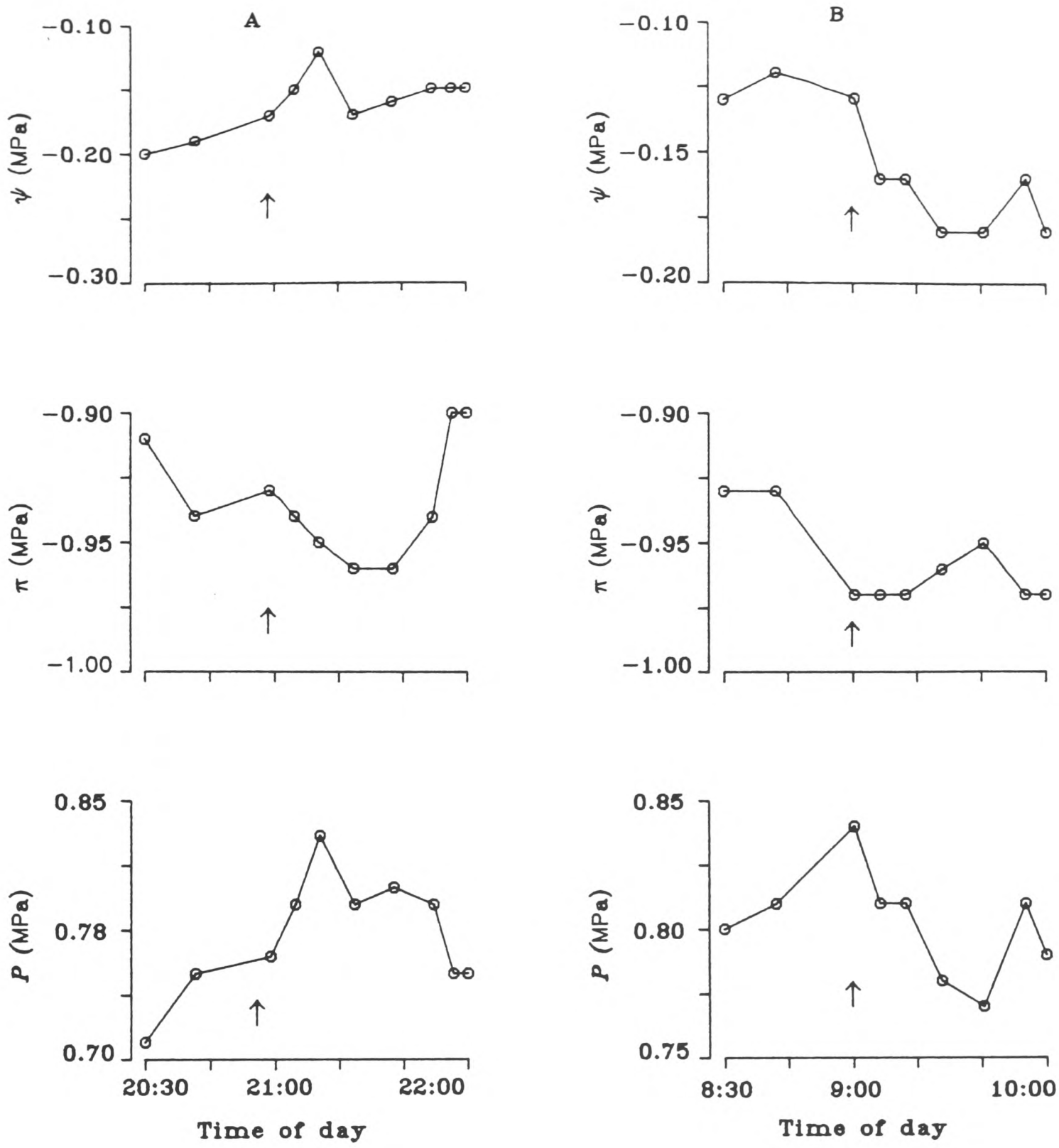


Figure 3.7- Effects of light-dark (A, left) and dark-light (B, right) transitions on leaf water relations (MPa) of seedlings of *P. vulgaris*. Top, ψ; middle, π; and bottom, P. Unlabelled arrows show the end or beginning of light period.

Table 3.1- Effects of light-dark (A) and dark-light (B) transitions on leaf water relations (MPa) of seedlings of *P. vulgaris*. Standard errors are shown for n = 4.

A

Water Relations (MPa)	Time of day		
	20:20-21:00*	21:00-21:40	21:40-22:20
ψ	-0.25 (±0.01)	-0.15 (±0.03)	-0.14 (±0.04)
π	-0.94 (±0.02)	-0.94 (±0.05)	-0.93 (±0.06)
P	0.69 (±0.02)	0.79 (±0.04)	0.79 (±0.03)

* End of the light period

B

Water Relations (MPa)	Time of day		
	8:20-9:00*	9:00-9:40	9:40-10:20
ψ	-0.13 (±0.01)	-0.25 (±0.04)	-0.27 (±0.04)
π	-0.90 (±0.04)	-0.86 (±0.06)	-0.85 (±0.04)
P	0.77 (±0.03)	0.61 (±0.02)	0.58 (±0.03)

* Beginning of the light period

Table 3.1A, B) are compared with the changes in leaf elongation rates at light transition (Fig. 3.3A and Fig. 3.4), they do not show any consistent correlation between these two variables. It seems that changes in leaf turgor follow, rather than lead the changes in elongation rate, since the response of elongation is instantaneous while leaf turgor took around 40 min to give a significant response.

If any instantaneous change occurred in leaf turgor at the light-dark transition it could not be detected by the pressure chamber. The pressure probe could not be used to achieve shorter-term turgor measurements in these experiments, since this technique required lighting for the measurements.

Lack of change in bulk leaf turgor may obscure more important local turgor effects. If leaf elongation is constrained by particular cells, such as the epidermis, a rise in turgor of these cells, not necessarily reflected in bulk turgor values, might allow their expansion and hence remove the constraint on others. This assumes that turgor of the epidermis is suboptimal for cell expansion during the light period.

3.4- Effects of Root Cooling Treatments on Leaf Elongation Rates

3.4.1- Experiments with *Phaseolus vulgaris* Seedlings

To examine the effects of root cooling treatments on

leaf elongation rate of *P. vulgaris*, the seedlings were transferred to Kilner jars with nutrient solution (see section 3.3) and the treatments started 2 - 3 h into the light period by placing the jars in the cooled water bath (see section 2.4, Chapter 2).

Figure 3.8A, B shows the effects of cooling the root system to 10°C (A) and 15°C (B) on the cumulative growth of the primary leaves. The effects were greater at 10°C where the growth was completely stopped over the first 6 h after the start of the cooling. It only started to increase again at the end of the light period (Fig. 3.8A). Although the effect was less accentuated at 15°C (Fig. 3.8B) it was significant and showed about the same general pattern as at 10°C, i.e., with a rapid reduction after cooling and an increase when the light period ended.

The rapidity of the response of elongation rates to root cooling at 10°C and 15°C is shown in Figure 3.9A, B and Figure 3.10. For both treatments the response was almost instantaneous (Fig. 3.9A, B) and the reduction in leaf elongation rate took only about 30 min to fall to zero. This reduction was sharper at the lower temperature. The rapid and extreme effect at 10°C led to negative values of the leaf elongation rate implying shrinkage (Fig. 3.9A and Fig. 3.10).

For both sets of temperature the low leaf elongation rate lasted for about 6 h, when the light period ends. A rise in elongation rate then occurred in response to

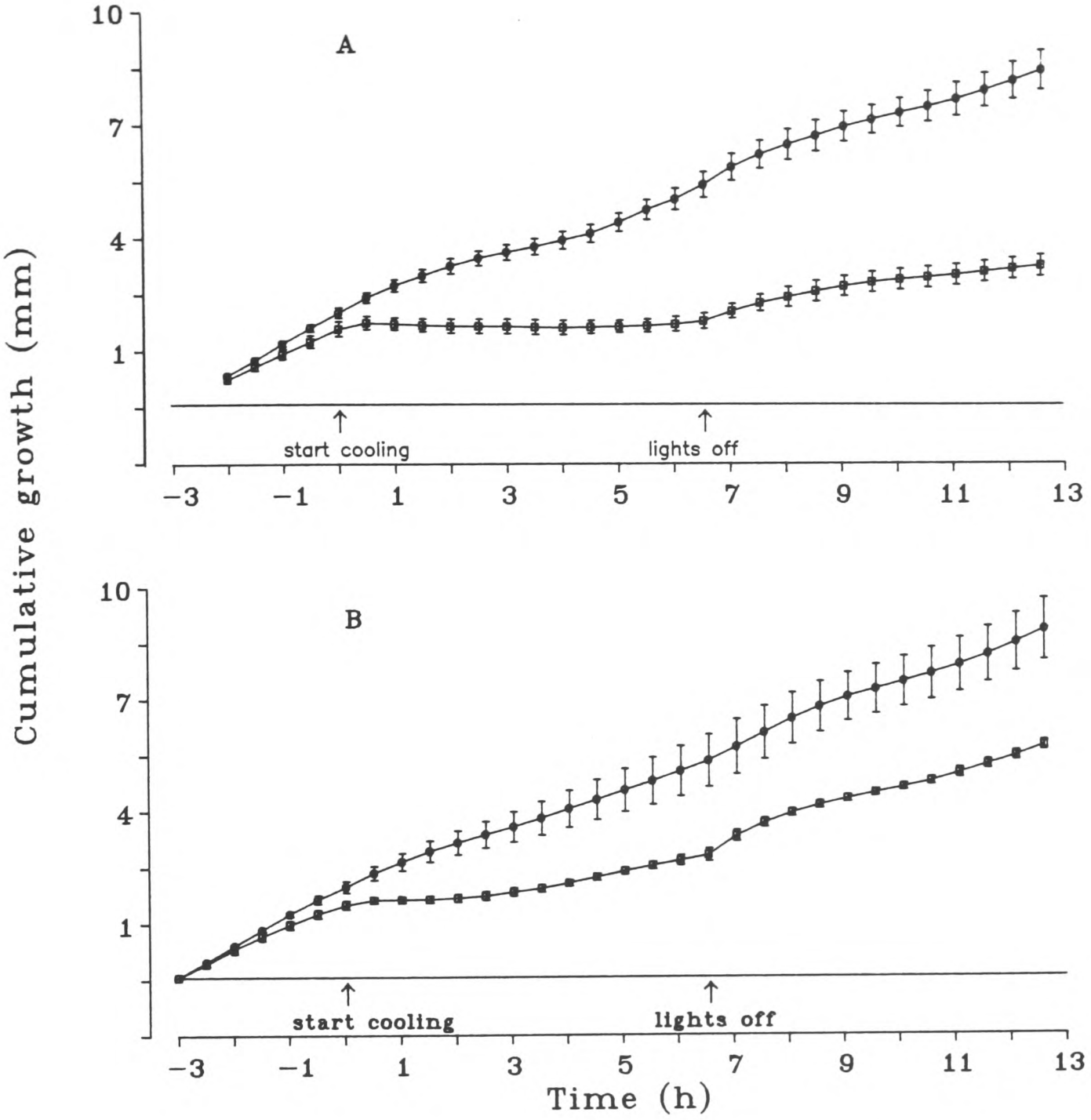


Figure 3.8- The time courses of the cumulative growth of the lamina of primary leaves of *P. vulgaris* subjected to root cooling (squares) at 10°C (A) or 15°C (B); for control plants (circles) root temperature was 23°C. The onset of root cooling and the time of lights-off are indicated by arrows. Standard errors are shown for n = 4

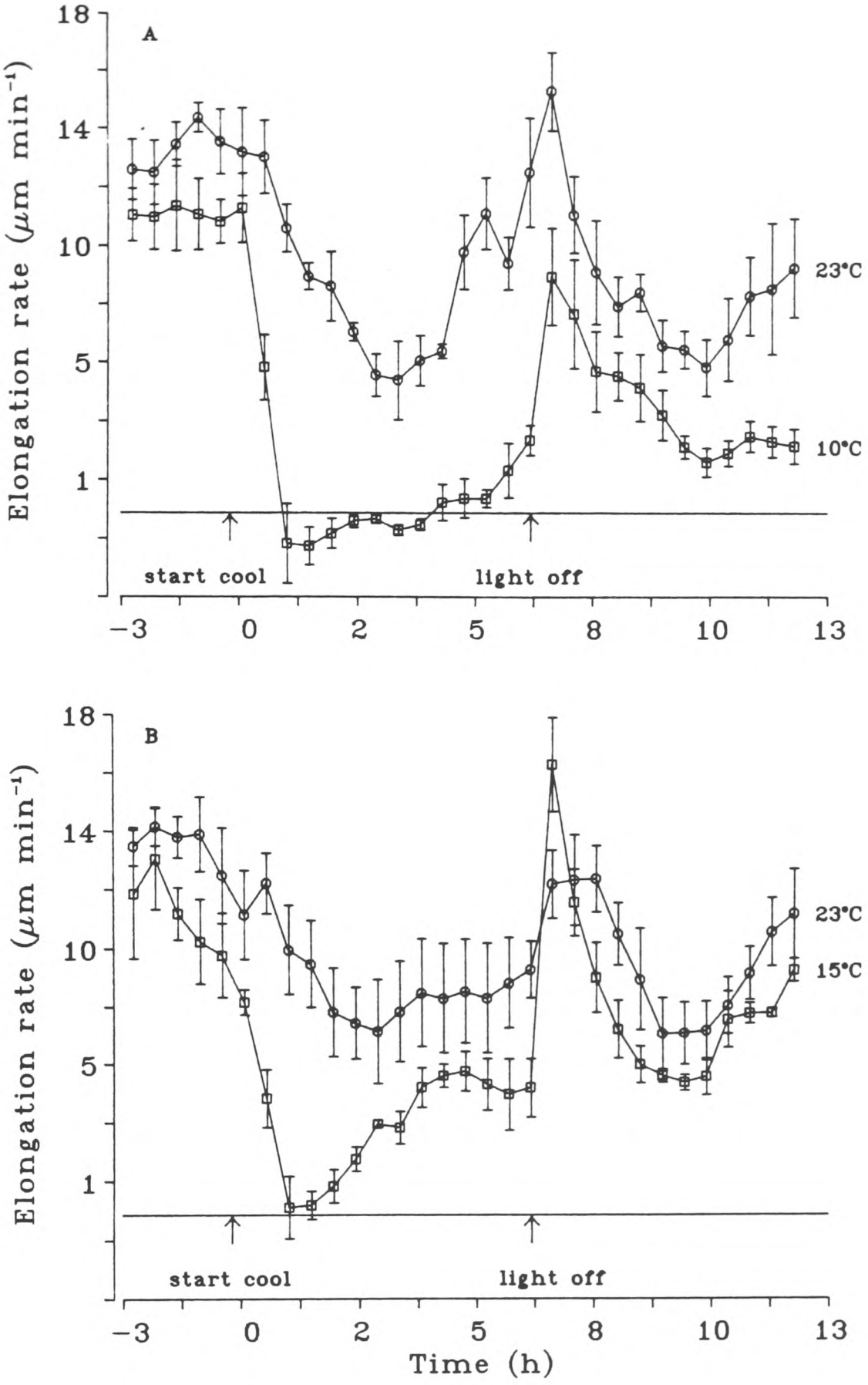


Figure 3.9- Long-term changes in the elongation rate of the lamina of primary leaves of *P. vulgaris* subjected to root cooling treatment (squares) at 10°C (A) or at 15°C (B); for control plants (circles) root temperature was 23°C. The onset of root cooling and the time of lights-off are indicated by arrows. Standard errors are shown for n = 4.

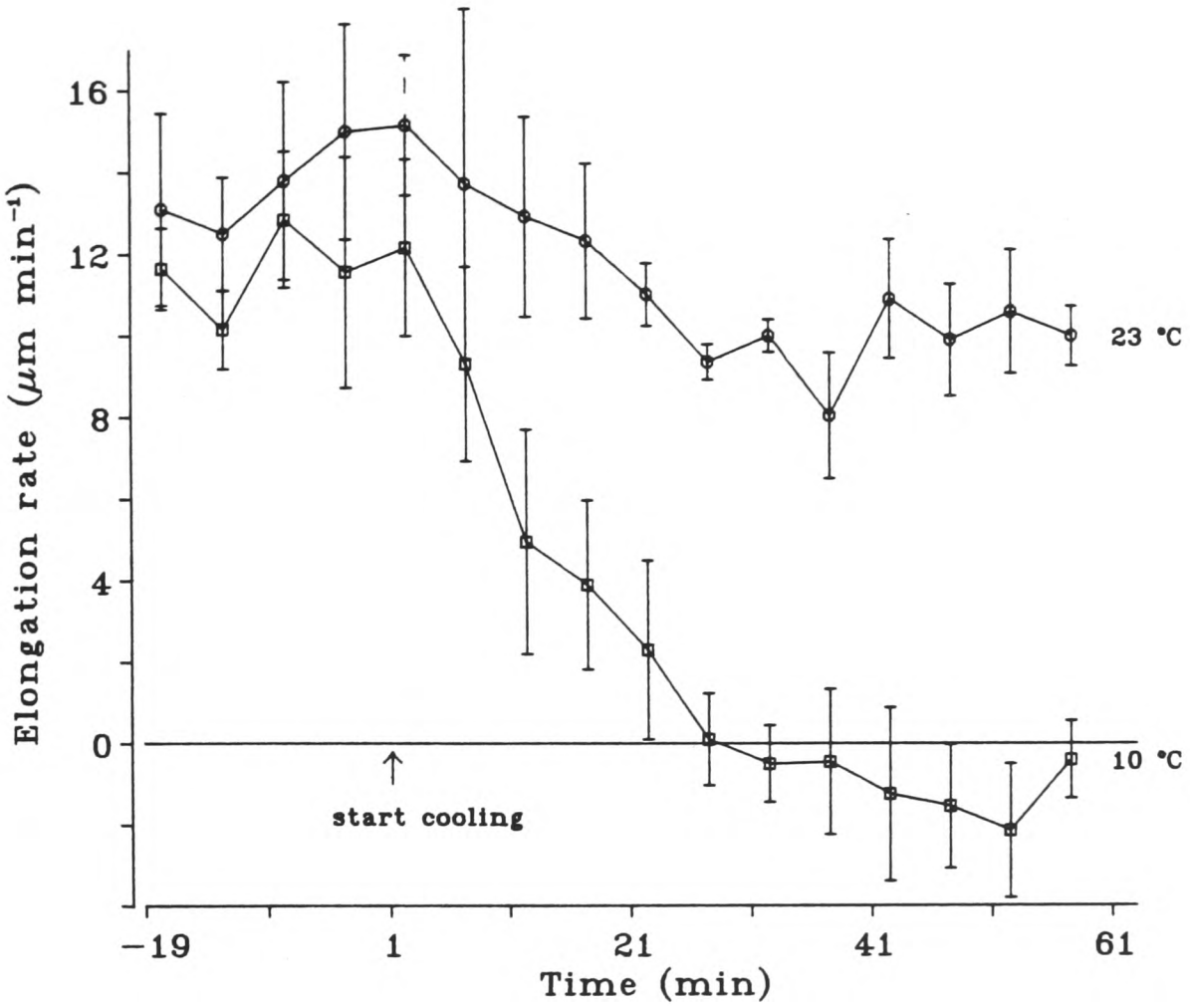


Figure 3.10- Short-term effects of root cooling treatment at 10°C (squares) on the elongation rate of the lamina of primary leaves of *P. vulgaris*; for control plants (circles) the root temperature was 23°C. The onset of cooling is indicated by the arrow. Standard errors are shown for n = 4.

light-dark transition and it was followed by a decline to low values again, about 1 - 2 μm min^{-1} (Fig. 3.9A, B). Thereafter, for the 10°C set, leaf elongation was kept at these low value, but it did not fall back to zero until the beginning of the next light period. At this time, the elongation rate fell quickly, soon after lights went on, to values below zero, but it was followed by a rapid and progressive increase to reach the previous values of about 1 - 2 μm min^{-1} (Fig 3.11).

Experiments were performed to investigate the short-term effects of reversing the root-cooling treatment on leaf elongation rate. In these experiments the cooling treatment (10°C) was performed as before (see above). 3 - 4 h after the start of cooling the solution around the root system was rapidly replaced by solution at 23°C.

The effect of root cooling on LER was rapidly reversed, and within 5 min the elongation rate rose from about 0 - 1 μm min^{-1} to 13 μm min^{-1} (Fig. 3.12B). This rate was even greater than the control values which were about 8 - 10 μm min^{-1}. These high values remained for up 1 - 2 h, gradually declining thereafter to values below the control level (Fig. 3.12A).

3.4.2- Experiments with *Helianthus annuus* and *Hordeum vulgare* Seedlings

The effects of root cooling on leaf growth were also

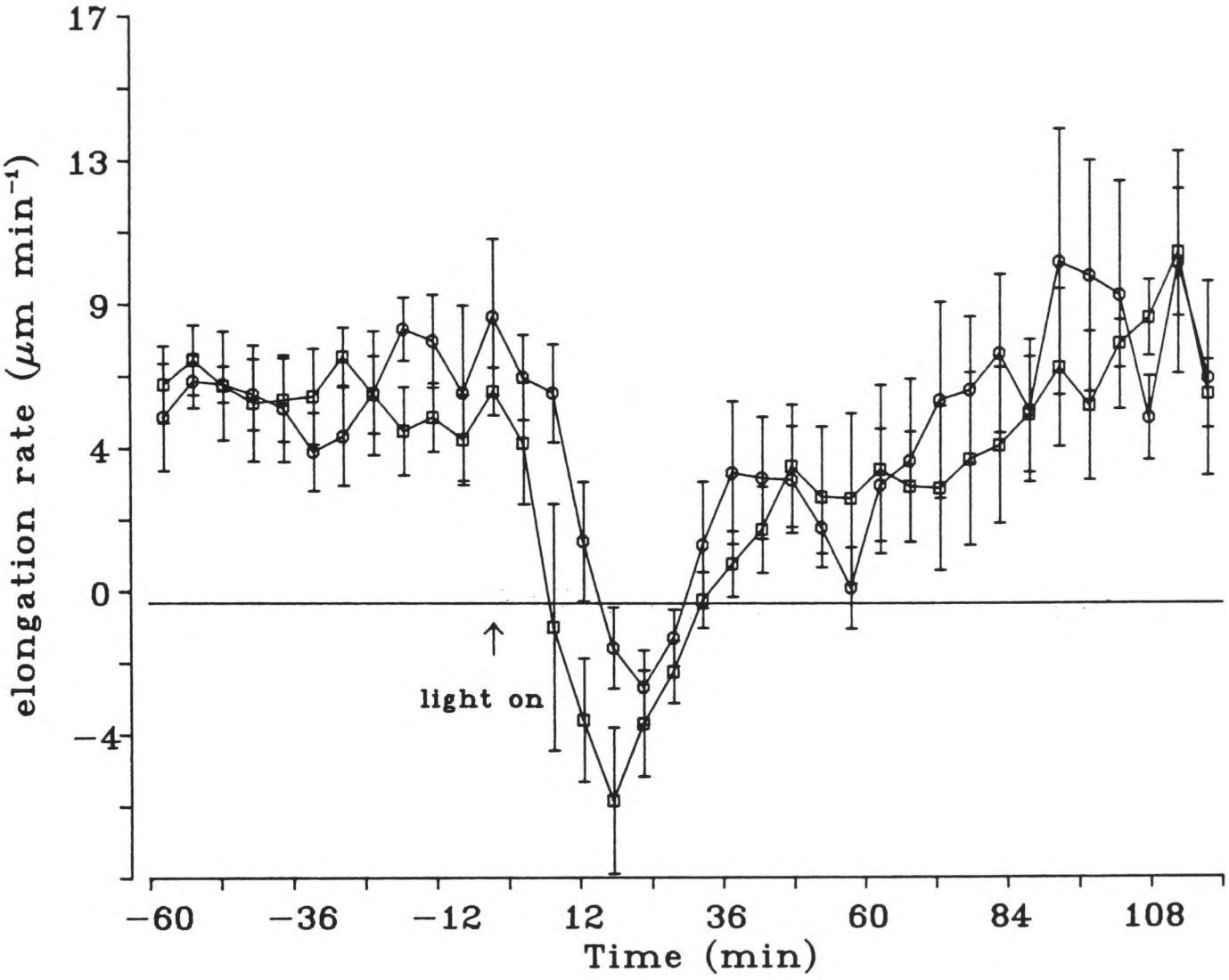
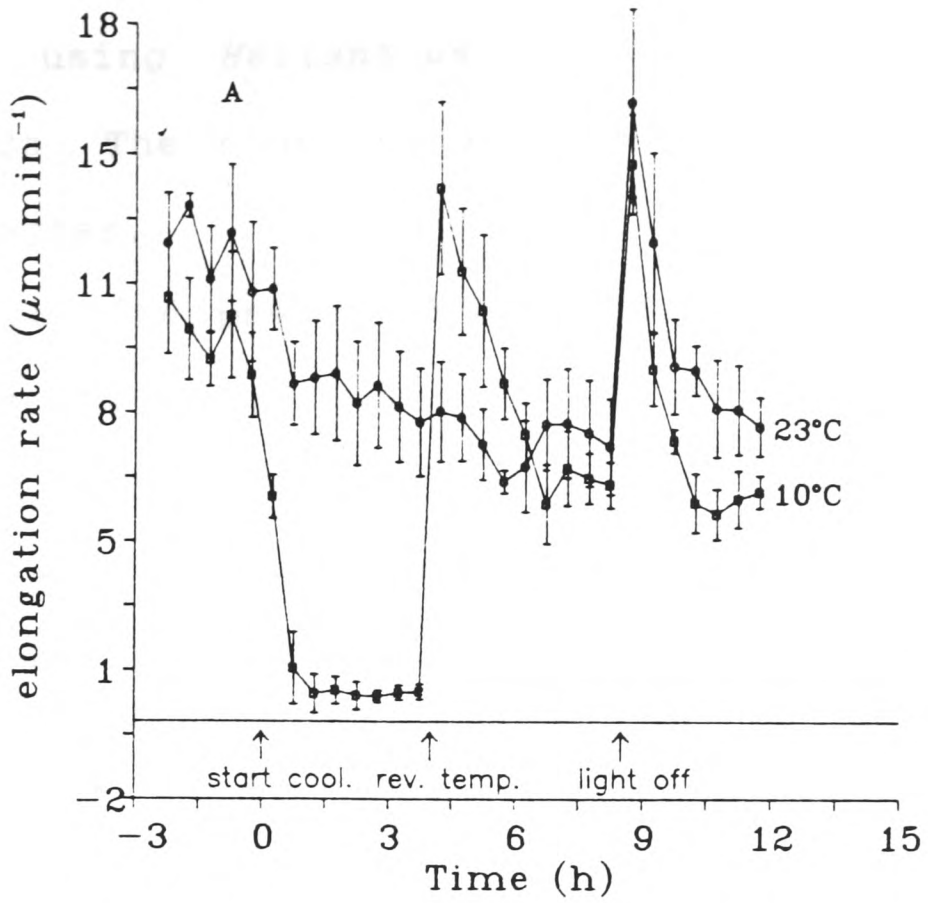


Figure 3.11- Short-term changes in the elongation rate of the lamina of primary leaves of *P. vulgaris* subjected to root temperature at 10°C (squares) or 23°C (circles) following transition from dark to light (arrow). Standard errors are shown for n = 4.



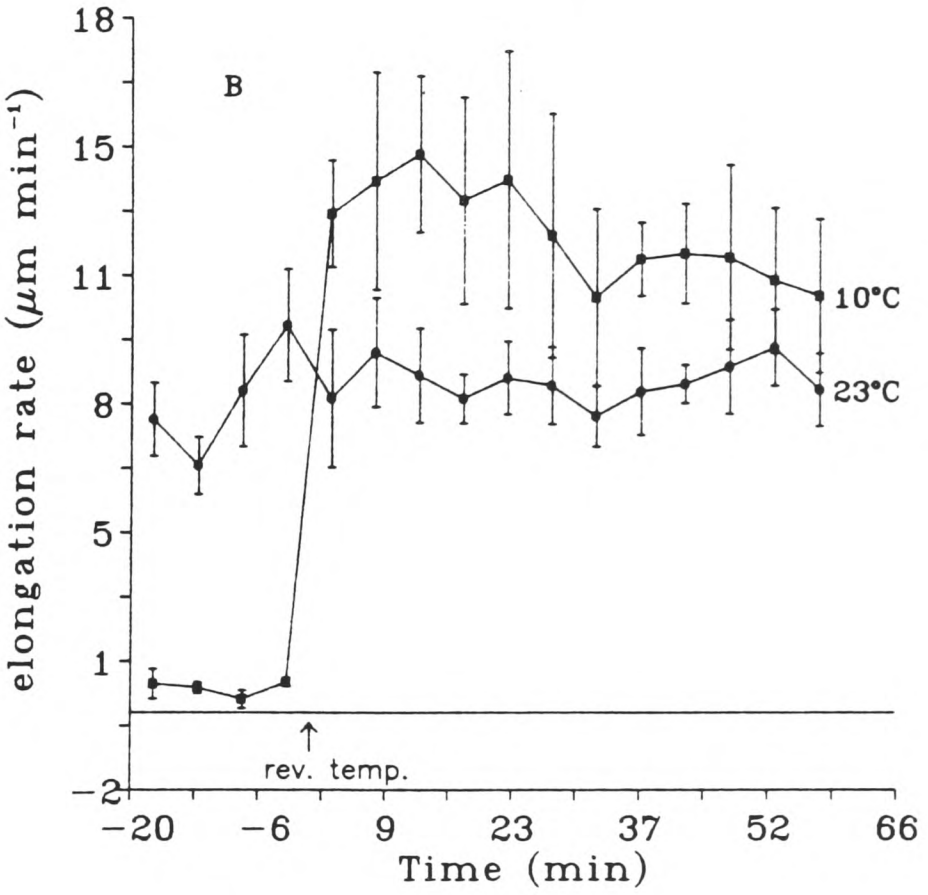


Figure 3.12- Long (A) and short-term (B) effects of reversing the treatment of root-cooling (rev. temp.) from 10°C (squares) to 23°C on the elongation rate of the lamina of primary leaves of *P. vulgaris*; for control plants (circles) the temperature was 23°C. Standard errors are shown for n = 4.

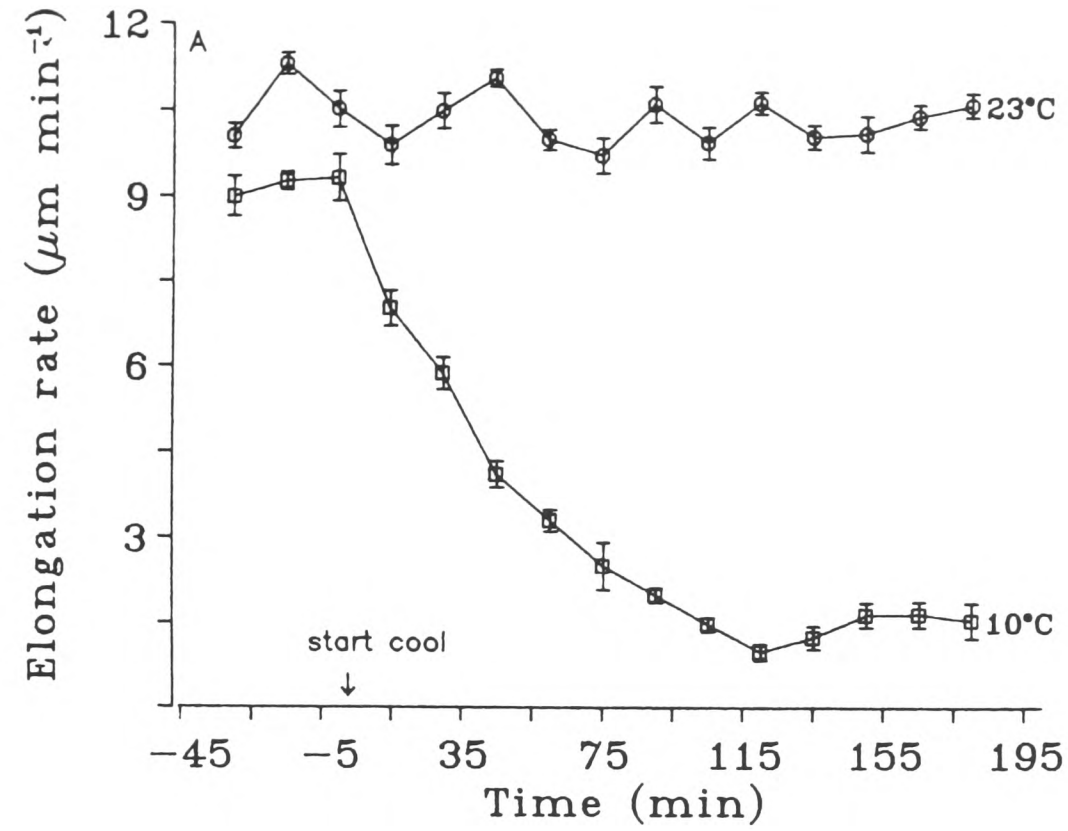
studied using *Helianthus annuus* and *Hordeum vulgare* seedlings. The root temperature treatment was 10°C for both species.

The results presented here for long- and short-term measurements of leaf elongation rates and leaf area were achieved using RVDT or ruler respectively.

Root cooling treatment also reduced leaf growth of *Helianthus annuus* and *Hordeum vulgare* as showed for bean plants.

The kinetics of leaf elongation rate measured for the first leaf of 7 day old plants is shown in Figure 3.13A, B. Leaf elongation rate fell from about 9 - 11 μm min^{-1} to about 1 μm min^{-1} within 2 h after cooling the root system whereas the control remained at a steady value, around 10μ m min^{-1} (Fig. 3.13A). This reduction in LER was not as rapid as observed for bean plants subjected to the same root temperature (10°C) but was significant from the beginning and similar to that of bean plants subjected to 15°C.

A significant reduction in the area of the first leaf of seedlings of *H. annuus* occurred when the cooling treatment started either at day 7 or at day 11 after sowing (Figure 3.14A, B). When treatment started at day 7 the leaf area was reduced by 40% at the end of the experiment (day 4 after cooling) in relation to the control (Fig. 3.14A). The reduction was less pronounced (25%) for the corresponding leaf when the treatment started at day 11 and, in this case, a larger effect was



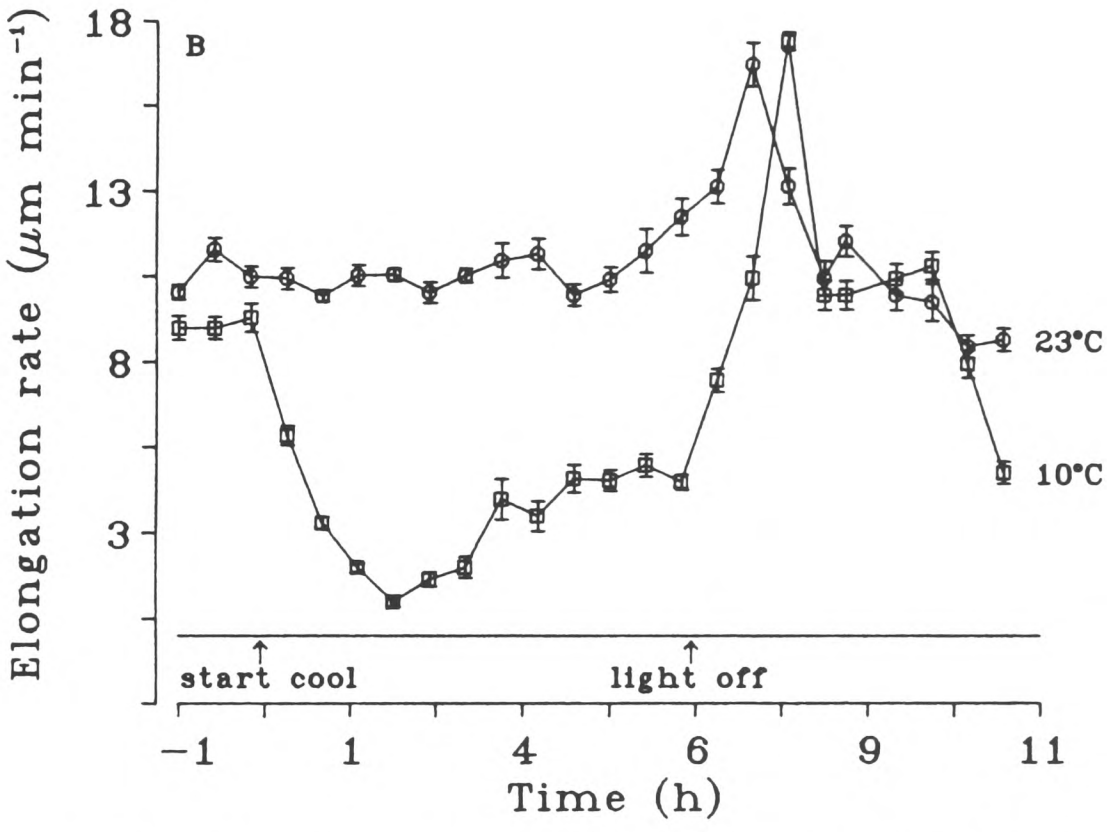


Figure 3.13- Short- (A) and long-term (B) changes in the elongation rate of the first leaf of *H. annuus* subjected to root cooling (squares); for control plants (circles) root temperature was 23°C. The onset of root cooling and the end of the light period are indicated by arrows. Standard errors are shown for n = 4.

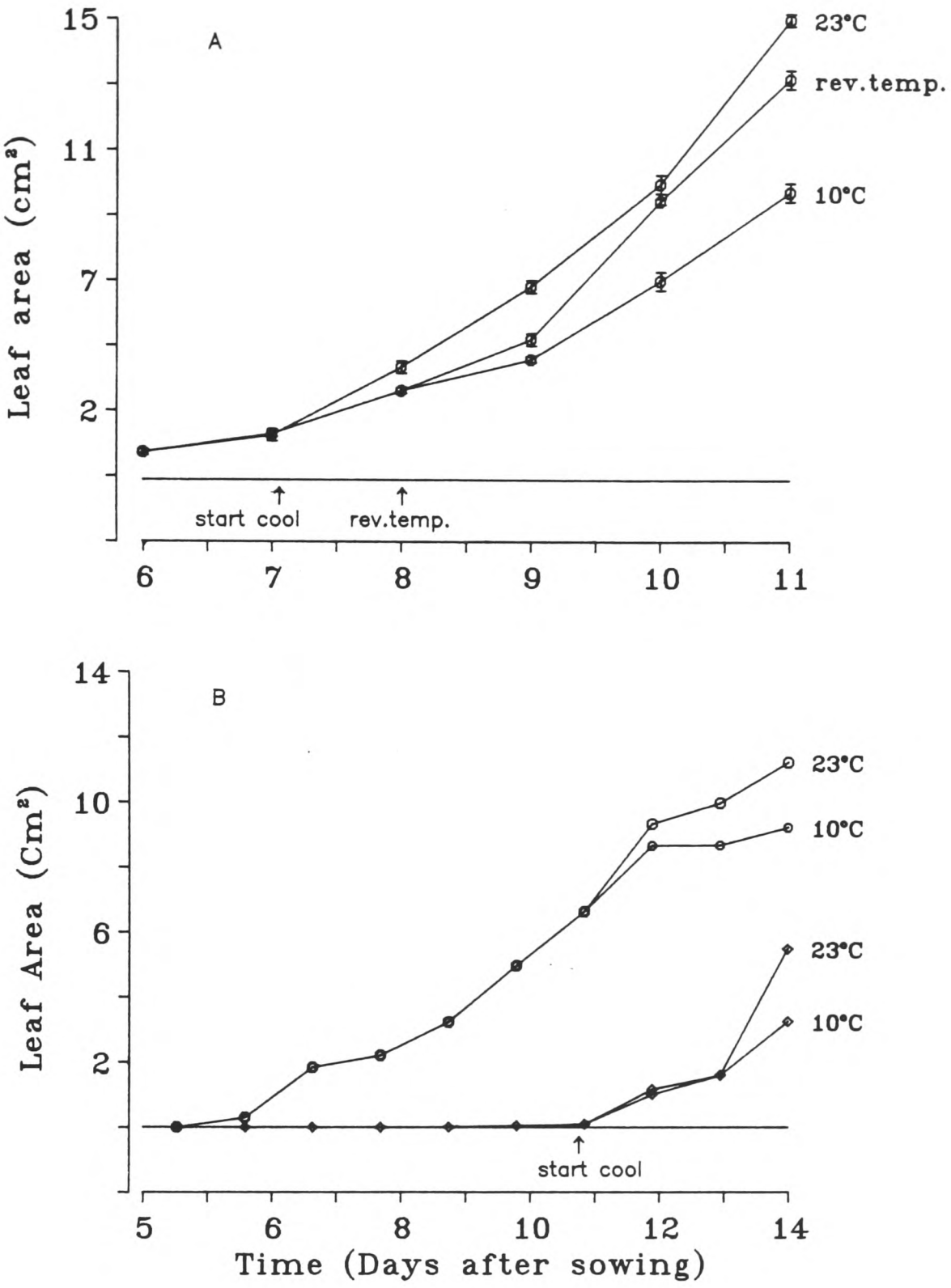


Figure 3.14- Time course of the effects of root cooling at 10°C on the area of the first leaf (circles in A and B) and on the area of the second pair of leaves(diamonds in B) of *H. annuus*. The onset and reversing of root cooling treatment are indicated by arrows.

found for the second pair of leaves which had their area reduced by 40% (Fig.3.14B).

There was no significant difference in cell number of the first leaf of *Helianthus* up to day 9. By this time, cell number per leaf (x 10^5) were 65.5 (se = ± 12, n = 7) and 68.6 (se = ± 14, n = 7), for control and root cooling set respectively. Treatment did not affect the final leaf cell number, suggesting that the reduced leaf area in response to root cooling is mainly due to reduced cell enlargement as reported for bean plants (Milligan and Dale, 1988*a*).

When 7-day old *H. annuus* seedlings subjected to root cooling treatment for 24h, were returned to root temperature of 23°C the relative elongation rate, averaged between 24 and 48 h after the treatment had been discontinued (days 9 and 10 in Fig. 3.14A), was much higher (2.84×10^{-2} cm $cm^{-1}h^{-1}$) than the control (1.76×10^{-2} cm $cm^{-1}h^{-1}$) over the same period. In this case the, leaf area was reduced by only 13% in relation to the control by the end of the experiment.

The results of experiments performed using *Hordeum vulgare* seedlings showed that root cooling treatment at 10°C also decreases growth rate. The absolute growth rate of the second expanding leaf of seedling of *Hordeum vulgare* was reduced from about 1.6 mm h^{-1}, found in control plants, to about 0.95 mm h^{-1} for root-cooled plants, both averaged over 24 h.

Continuous measurements of elongation rate of the second leaf of *H. vulgare* was recorded over 24 h from the start of root cooling.

As soon as the solution was cooled, the growth rate decreased from an average of 27.5 μm min^{-1} found in the control plants to a minimum value of about 11.2 μm min^{-1} (Fig. 3.15). This value was reached approximately 30 min from the start of cooling. The low elongation rate last for about 2 h when it started to increase and reached values of 15.5 μm min^{-1}.

The smaller effect of root cooling treatment on leaf elongation rates of *Hordeum* plants compared to *Phaseolus* and *Helianthus* may be due to the difference between these species in cooling tolerance. It is known that roots of chilling-resistant species such as barley tend to have a higher capacity to maintain their physiological processes at low root temperatures than have chilling-sensitive species such as bean (Chapin, 1974; Duke *et al.*, 1979; Levitt 1980*a*; Markhart, 1986).

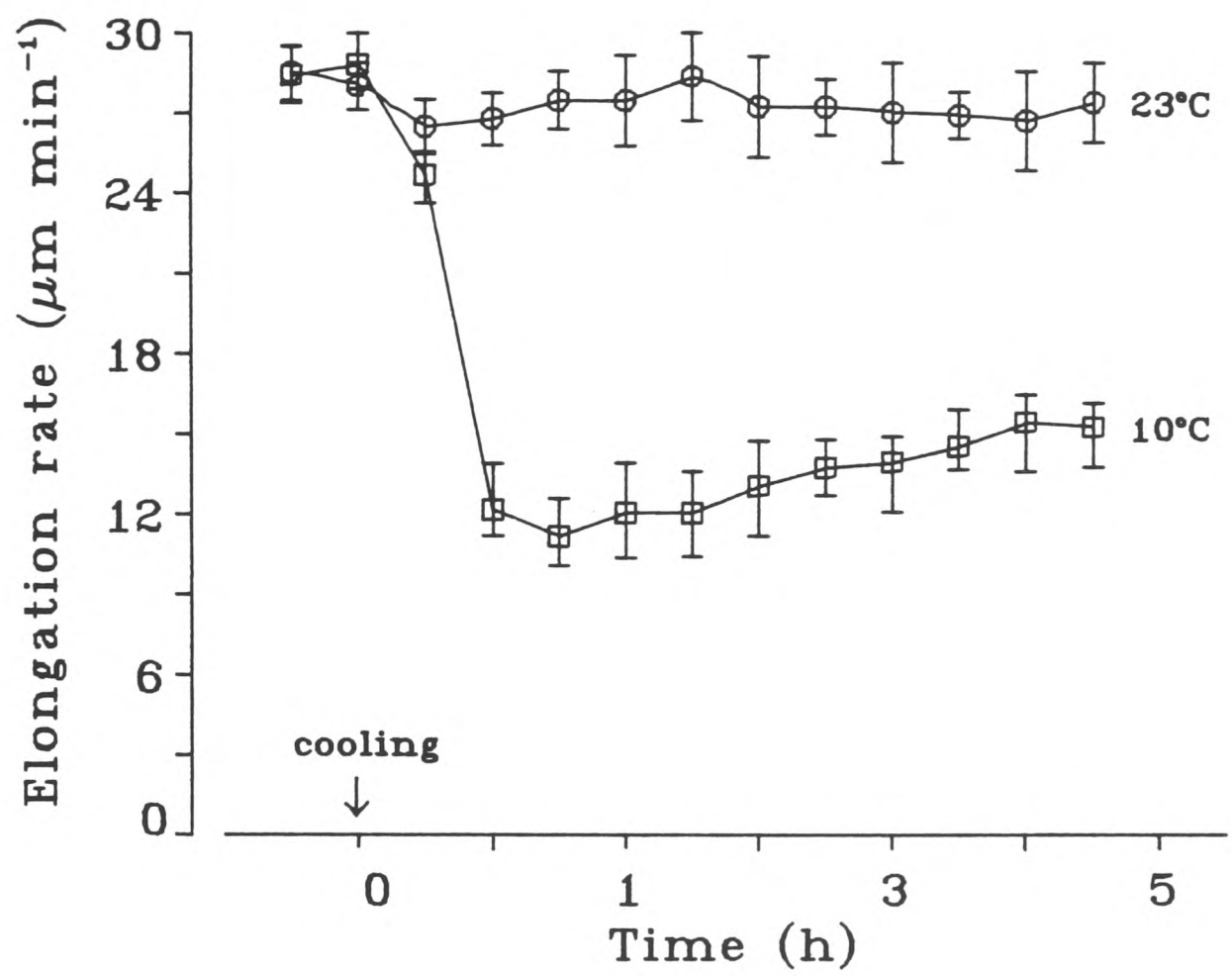


Figure 3.15- Changes in the elongation rate of the second leaf of *Hordeum vulgare* subjected to root cooling (10°C); for control plants root temperature was 23°C. The onset of root-cooling is indicated by arrows. Standard errors are shown for n = 4.

CHAPTER 4 - RESULTS

LEAF WATER RELATIONS AND CELL WALL PROPERTIES

4.1- Introduction

In chapter 3, the effects of root-cooling treatments on early growth of leaves were studied and the speed of the response of leaf elongation rate to root-cooling was established.

This chapter examines the possible physical and physiological mechanisms, such as rapid changes in bulk leaf water and cell wall properties, which could be linked with the short-term response of leaf elongation rate to root cooling.

4.2- Effects of Root Cooling on Leaf Water Relations

Short- and long-term changes in bulk leaf water potential were measured in seedlings of *Phaseolus vulgaris* and *Helianthus annuus* routinely using a pressure chamber. The immediate effect of root cooling on leaf turgor of *P. vulgaris* was also measured in one experiment only using a pressure probe (Murphy and Smith, 1989).

4.2.1- Experiments with *Phaseolus vulgaris*

Cooling the root system from 23°C to 10°C or 15°C led to a fall in bulk leaf water potential measured by pressure chamber (Fig. 4.1A, B). This fall occurred within 30 min and the values persisted low over 3-4 h after the onset of the root cooling treatments. Since there was no detectable changes in leaf osmotic potential, over the same period, leaf turgor for the root-cooled plants, determined by difference, was also lower than the control values.

Thereafter a recovery of water potential occurred, but the leaf osmotic potential fell gradually in root-cooled plants, consequently the turgor reached higher values than the control for both sets of temperature.

To investigate the immediate effect of cooling the root system from 23°C to 10°C using the pressure probe technique, 8 day old intact seedlings of *P. vulgaris* were used, since the cell size was too small for use in seedlings on day 7.

The results presented in Figure 4.2 are from measurements performed on the abaxial epidermal cells (6 cells) of the main vein of the same primary leaf before and after cooling the root system. The values are not replicated since they are from successive measurements over a short period.

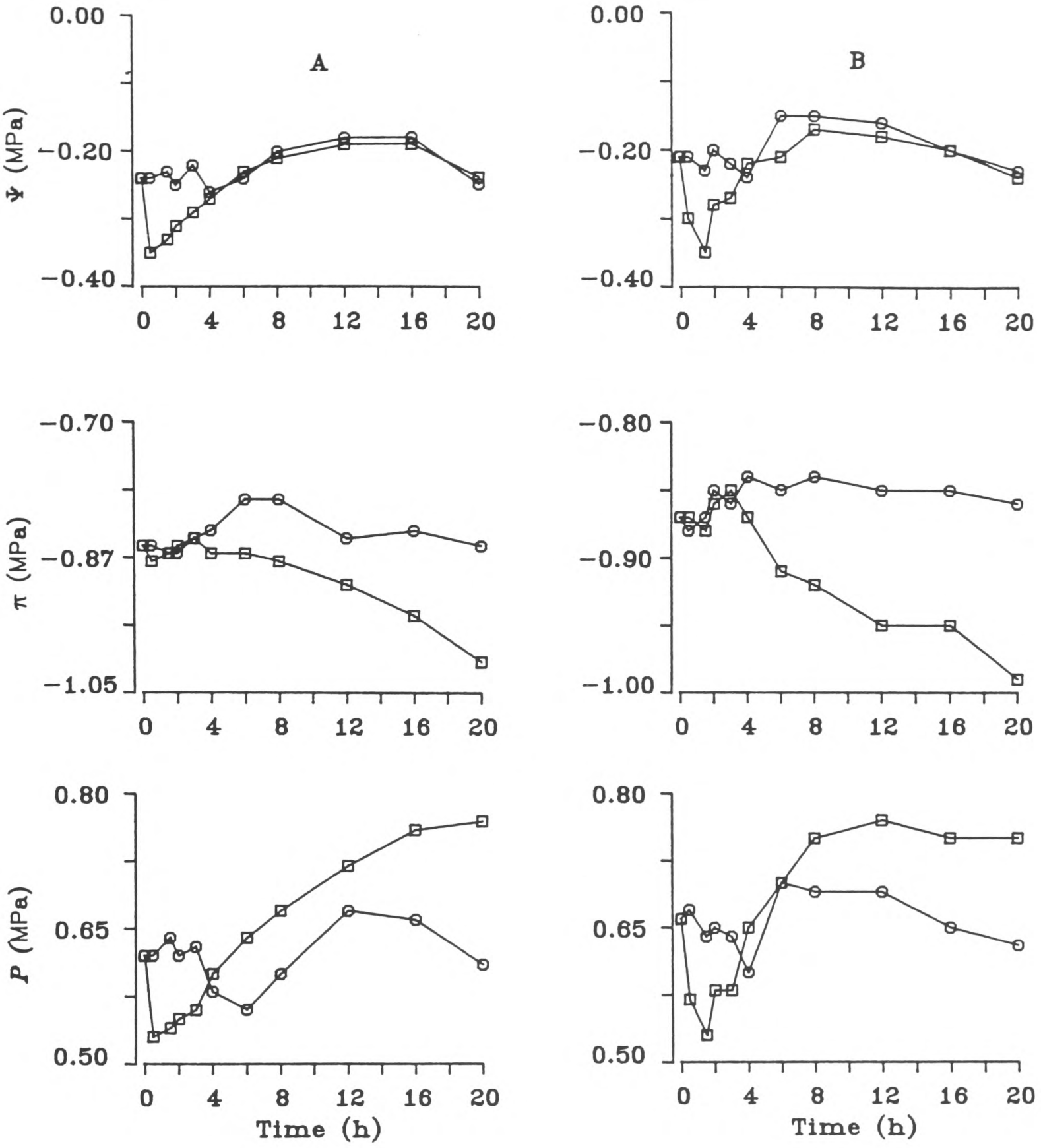


Figure 4.1- Effects of root cooling treatments (squares) at 10°C (A, left) and 15°C (B, right) on water relations (MPa) of primary leaves of *P. vulgaris*; for control plants (circles) root temperature was 23°C. Top (water potential, ψ), middle (osmotic potential, π) and bottom (turgor, P).

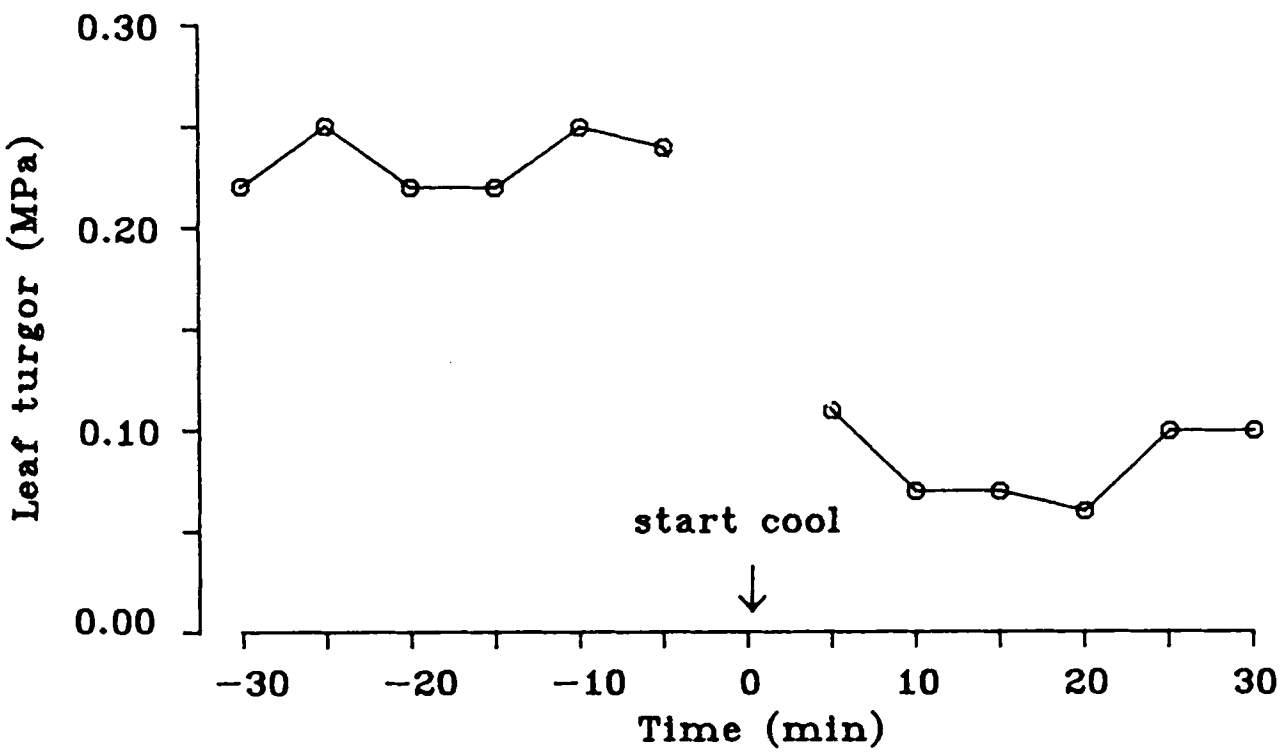


Figure 4.2- Short-term effects of root cooling (10°C) on turgor potential (MPa) of abaxial epidermal of the primary leaves of *P. vulgaris*. Results are not replicated since they are from successive measurements, using the pressure probe, before and after cooling the root system.

Leaf turgor fell immediately after the temperature of the solution was changed (Fig. 4.2). The mean value of leaf turgor (0.085 ± 0.021 MP_a) of root-cooled plants, averaged over 30 min after treatment, showed a statistically significant reduction compared to leaf turgor averaged 30 min before cooling (0.233 ± 0.015). The lower values of leaf turgor found for control plants, compared with the values presented in Figure 4.1, might be because of the leaf consists of a heterogeneous population of cells with different turgor (Tyree and Jarvis, 1982). However, it is also possible that these difference were due to the artificial conditions that plants were set during measurements. Light intensity as well as the temperature surrounding the aerial parts of the plant were higher than those of the controlled growth room.

4.2.2- Experiments with *Helianthus annuus*

The effects of root cooling 10°C on water relation of the first leaf of *H. annuus* seedlings showed the same pattern of *P. vulgaris*, that is, leaf turgor was reduced after root cooling treatment due to a reduction in leaf water potential. This fall in turgor was short-lived and the values rose to control levels within 3 - 4 h (Fig. 4.3). 6 h after the onset of treatment, osmotic potential had fallen and leaf turgor assumed values higher than the control.

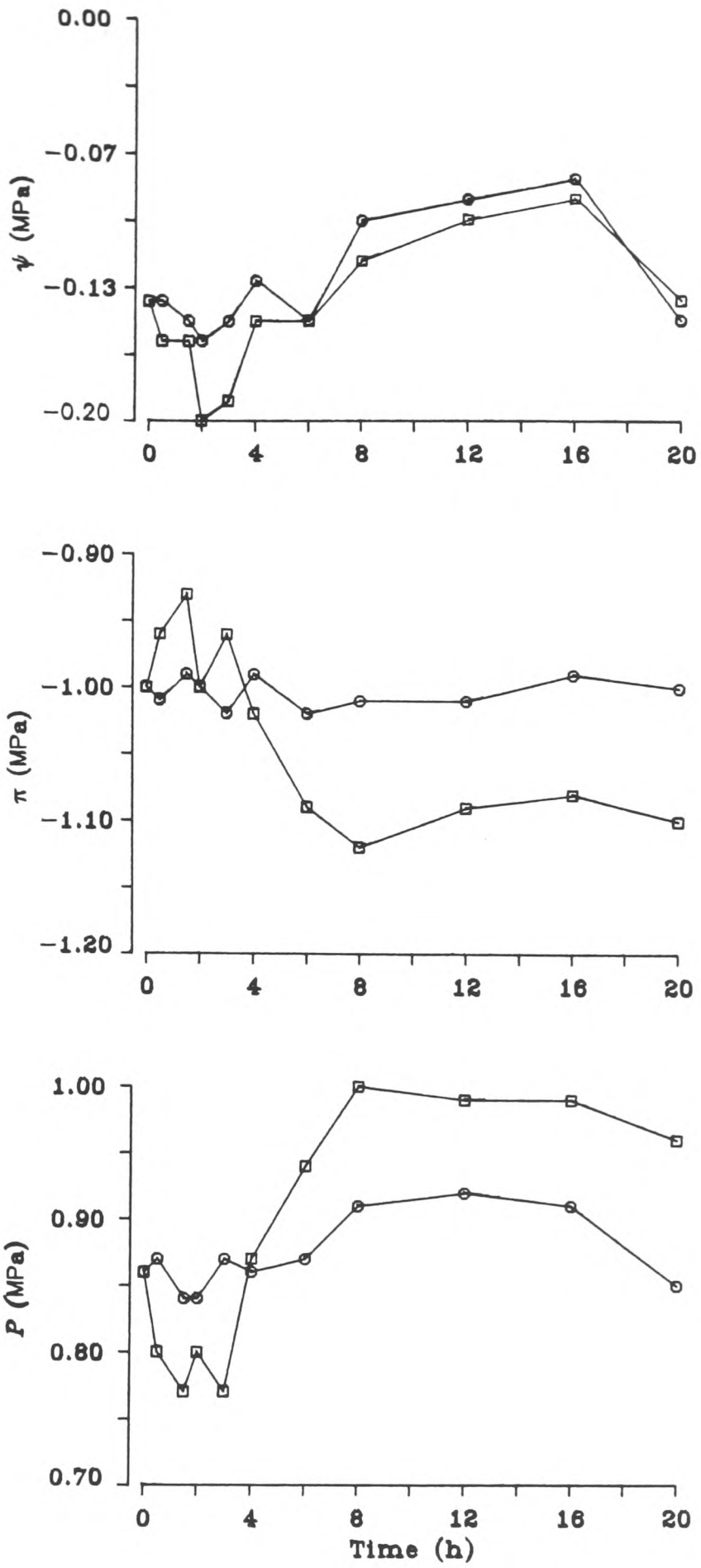


Figure 4.3- Effects of root-cooling treatment at 10°C (squares) on water relation (MPa) of the first leaf of *H. annuus*; for control plants (circles) the root temperature was 23°C. Top (water potential, ψ), middle (osmotic potential, π) and bottom (turgor potential, P).

The results presented for bean and sunflower give evidence that the primary cause of the reduction in leaf elongation rate in response to root cooling may be the fall in leaf turgor associated with the treatment. However, the recovery in leaf turgor which occurred within a few hours and the lack of a relationship between turgor and leaf expansion, when leaf regains its turgor, indicates the possibility that cell wall extensibility is the parameter that keeps elongation rates low.

4.3- Leaf Conductance and Transpiration Rates

Cooling the root system from 23°C to 10°C caused a substantial restriction of leaf stomatal conductance of the primary leaves of *P. vulgaris*, which was apparent 10 - 20 minutes after cooling (Fig. 4.4). Leaf conductance was around 140 mmol $m^{-2}s^{-1}$ at the beginning of the treatment and fell to around 50 mmol $m^{-2}s^{-1}$, 1 h after the onset of cooling, that is, stomatal conductance was reduced by 64% compared to control values; 2 h after the start of root cooling stomatal conductance was reduced to a lower steady value around 20 - 30 mmol $m^{-2}s^{-1}$.

Measurements of stomatal conductance to determine diurnal variation in stomatal behavior of control and root cooled plants (Fig 4.5A, B) showed that the restriction of stomatal opening in response to root-cooling treatment was maintained over the

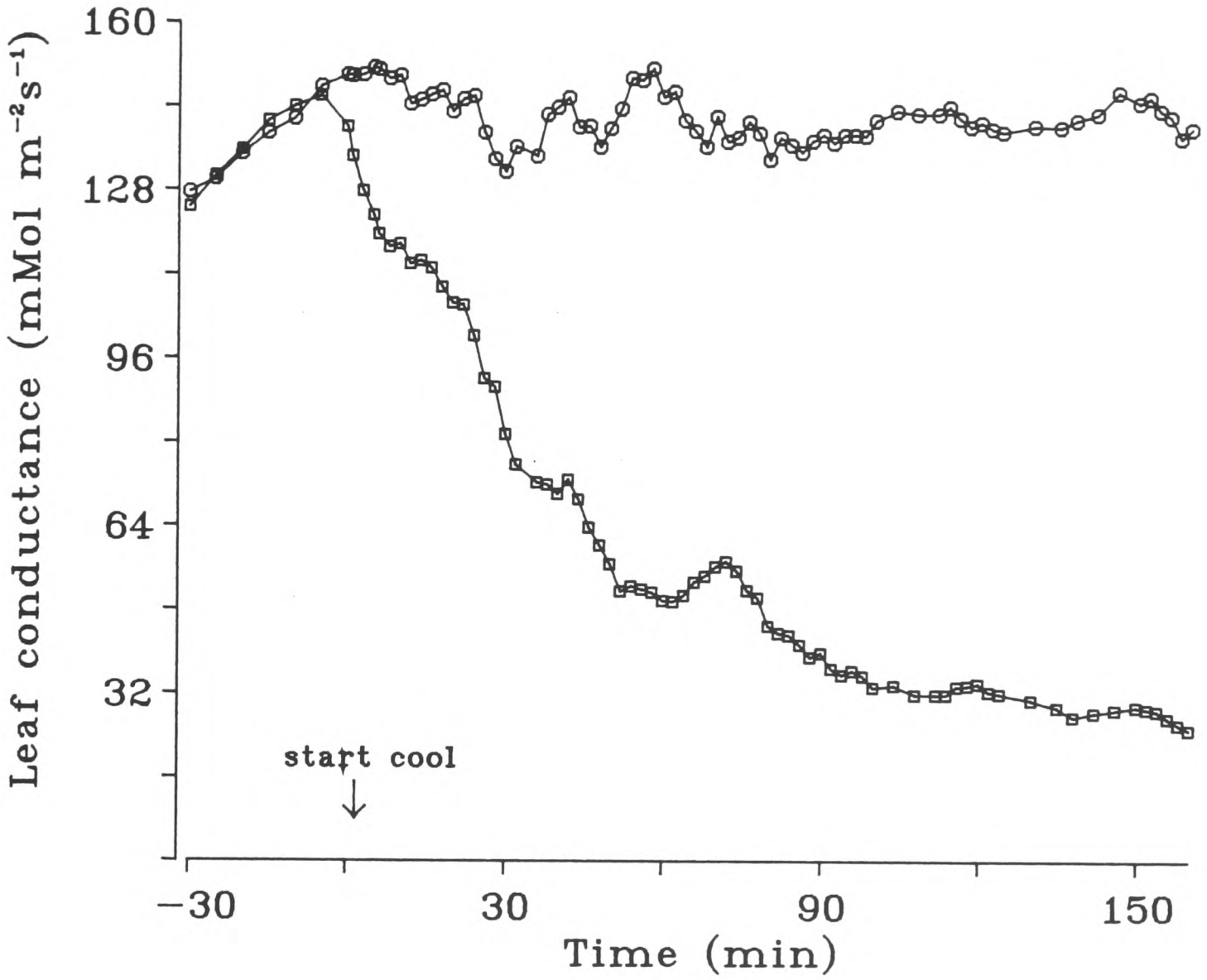


Figure 4.4- Short-term effect of root cooling treatment (10°C, squares) on stomatal conductance of the abaxial surface of the primary leaves of *P. vulgaris*; for control plants the root temperature was 23°C.

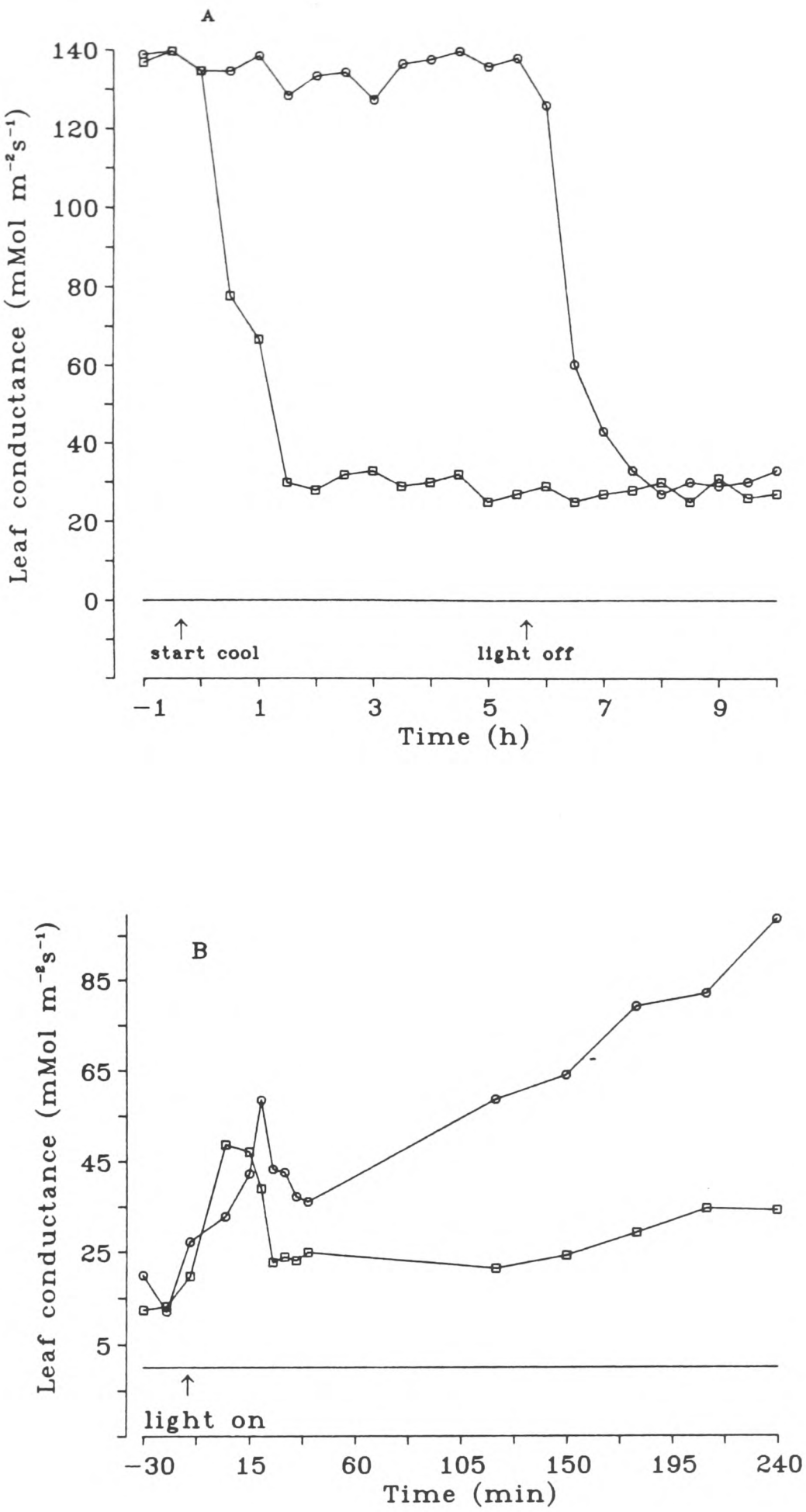


Figure 4.5- Diurnal variation in stomatal behavior measured on the abaxial surface of the primary leaves of *P. vulgaris* plants. Control (circles) and root-cooled at 10°C (squares).

experimental period. Ending the light period had no effect on stomatal conductance of treated plants, since it was already restricted as a result of root-cooling treatment (Fig. 4.5A). At the beginning of the light period, however, stomatal conductance of these plants increased and assumed values slightly higher than the control over a short period. Thereafter it fell to previous values and remained low (Fig. 4.5B).

The diurnal variation of stomatal conductance of control plants showed a typical response to light, with closure in darkness and opening over the light period (Fig. 4.5A, B).

Transpiration rate for the abaxial surface of primary leaves of *P. vulgaris* slowed in the short-term after the onset of root cooling (Table 4.1). After a lag of only 30 min transpiration had fallen to half that of control values. Within 5 h of the onset of the treatment, transpiration rate was reduced by 90% compared to values at time zero for the same set of plants and to control values at the same time.

4.4- Simultaneous Effects of Root Cooling and Atmospheric Humidity on Leaf Elongation and Water Relations

If the sudden reduction in leaf elongation rate in response to root-cooling treatment is caused by the

Table 4.1- The effects of root cooling treatment at 10°C (RC) on transpiration rate (mmol $m^{-2}s^{-1}$) of primary leaves of *P. vulgaris*; for control plants root temperature was 23°C. Standard errors are shown for n = 6.

Time after cooling (h)	Transpiration rate (mmol $m^{-2}s^{-1}$)	
	Control	RC
0	3.49 ± 0.45	3.61 ± 0.63
1/2	3.67 ± 0.81	2.34 ± 0.31
1	3.91 ± 0.55	0.98 ± 0.21
3	2.89 ± 0.48	0.71 ± 0.25
5	3.01 ± 0.51	0.33 ± 0.13
20	3.68 ± 0.64	0.35 ± 0.15

initial fall in leaf turgor, then if turgor is maintained one would not expect an effect on leaf growth.

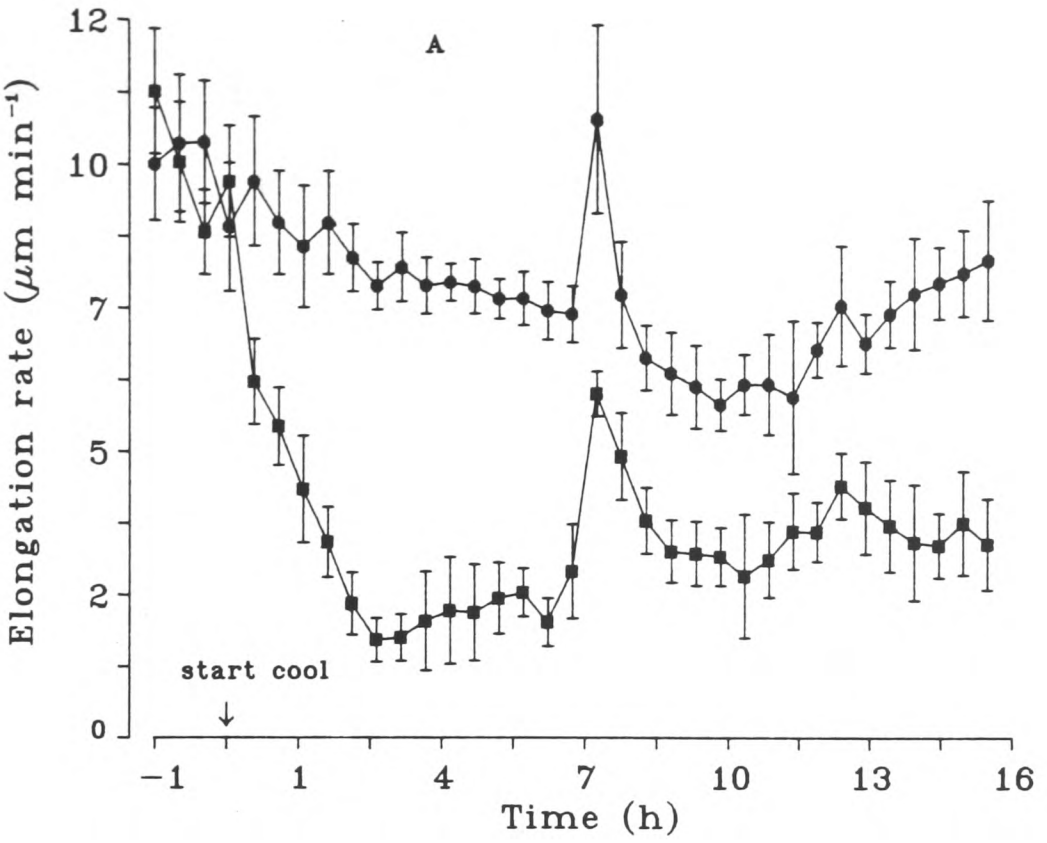
This hypothesis was tested in experiments where the aerial parts of 7 day old seedlings of *P. vulgaris* were subjected to high atmospheric humidity (RH 100%) over the period of root cooling treatment to 10°C (see section 2.5, Chapter 2). Due to the small number of RVDTs available, it was not possible to measure leaf elongation rates of non-bagged control plants.

Placing the tops of the plants in polythene bags prevented the sudden fall in leaf elongation rate when root cooling commenced (Fig. 4.6A, B). There was some reduction in leaf growth rate but this was much smaller than previously observed (Fig.3.9; Chapter 3), and the lowest values of elongation rate (1 - 2 $\mu m\ min^{-1}$) were found after a lag of 2 - 3 h, much later than when tops were not bagged (Fig. 3.9).

Although, the sudden fall in leaf elongation rate was avoided, the final cumulative growth (Fig. 4.7A) was reduced by root cooling treatment to the same extent, as previously observed, regardless of the atmospheric humidity.

Leaf area (Fig. 4.7B) was also markedly reduced by root cooling despite bagging.

No drop in leaf turgor was observed over 4 h after the onset of the treatments (Fig. 4.7D). Instead, turgor tended to higher values between 2 - 4 h after treatment



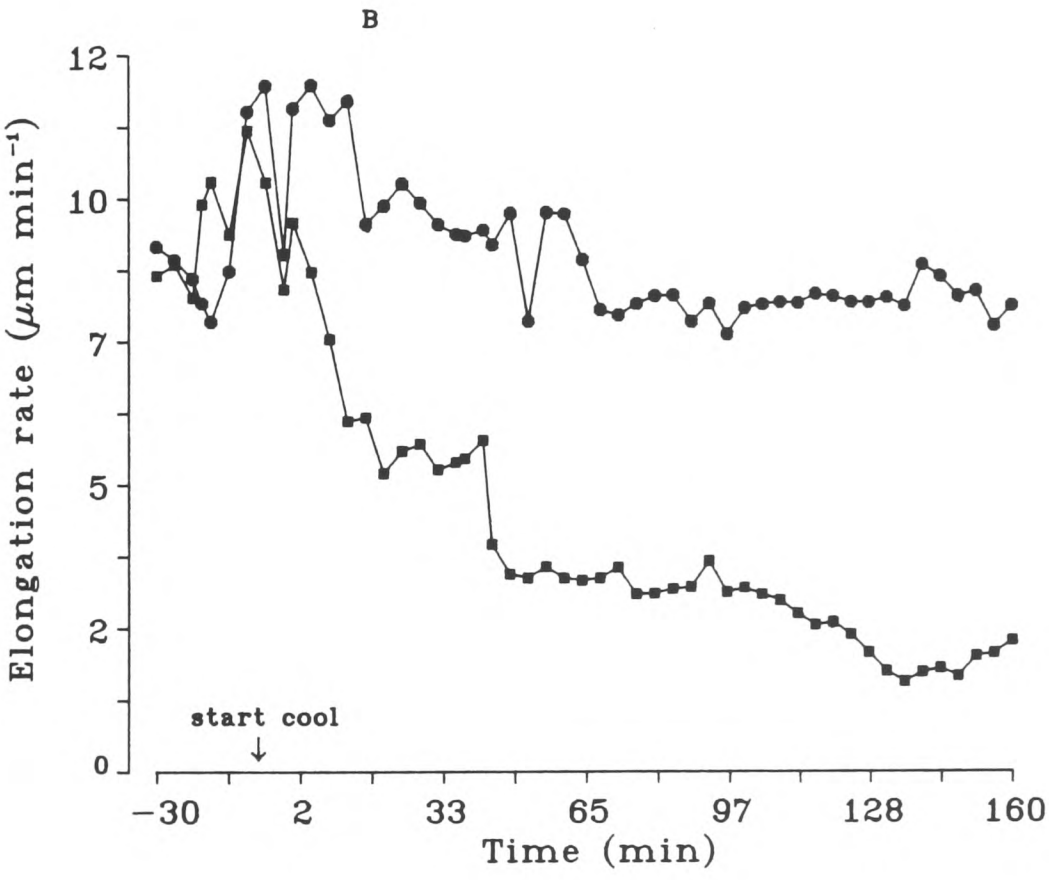


Figure 4.6- Long (A) and short-term (B) effects of root temperature at 10°C (squares) or at 23°C (circles) on elongation rate of primary leaves of *P. vulgaris* subjected to high atmospheric humidity.

started, due to the slight decrease in osmotic potential; water potential did not change over the experimental period. This result also suggests that the short-term reduction in leaf elongation upon root cooling, is due to reduction in leaf turgor.

One striking point when plants were subjected to high atmospheric humidity and root-cooling treatments, was the reduction in *g* (Fig. 4.7C). Stomatal conductance was reduced by 50 - 60% after a lag of 2 h, just whilst the turgor was about to increase, suggesting that conductance is reduced by the root cooling treatment regardless of leaf turgor and another factor must be controlling the stomatal response to root cooling.

4.5- Effects of Root Cooling on Cell Wall Properties of Primary Leaves of *P. vulgaris*

One possible cause of the reduction in leaf elongation rate after root cooling treatment was found to be a decrease in leaf turgor brought about by a decrease in the water potential soon after treatment (0 - 4 h).

During the subsequent experimental period (4 - 20 h) as the turgor rises, due to changes in leaf water and osmotic potential, another mechanism, rather than turgor must be operating to maintain the low elongation rate.

As cell wall extensibility is one of the factors controlling growth (Tomos, 1985), it is possible that

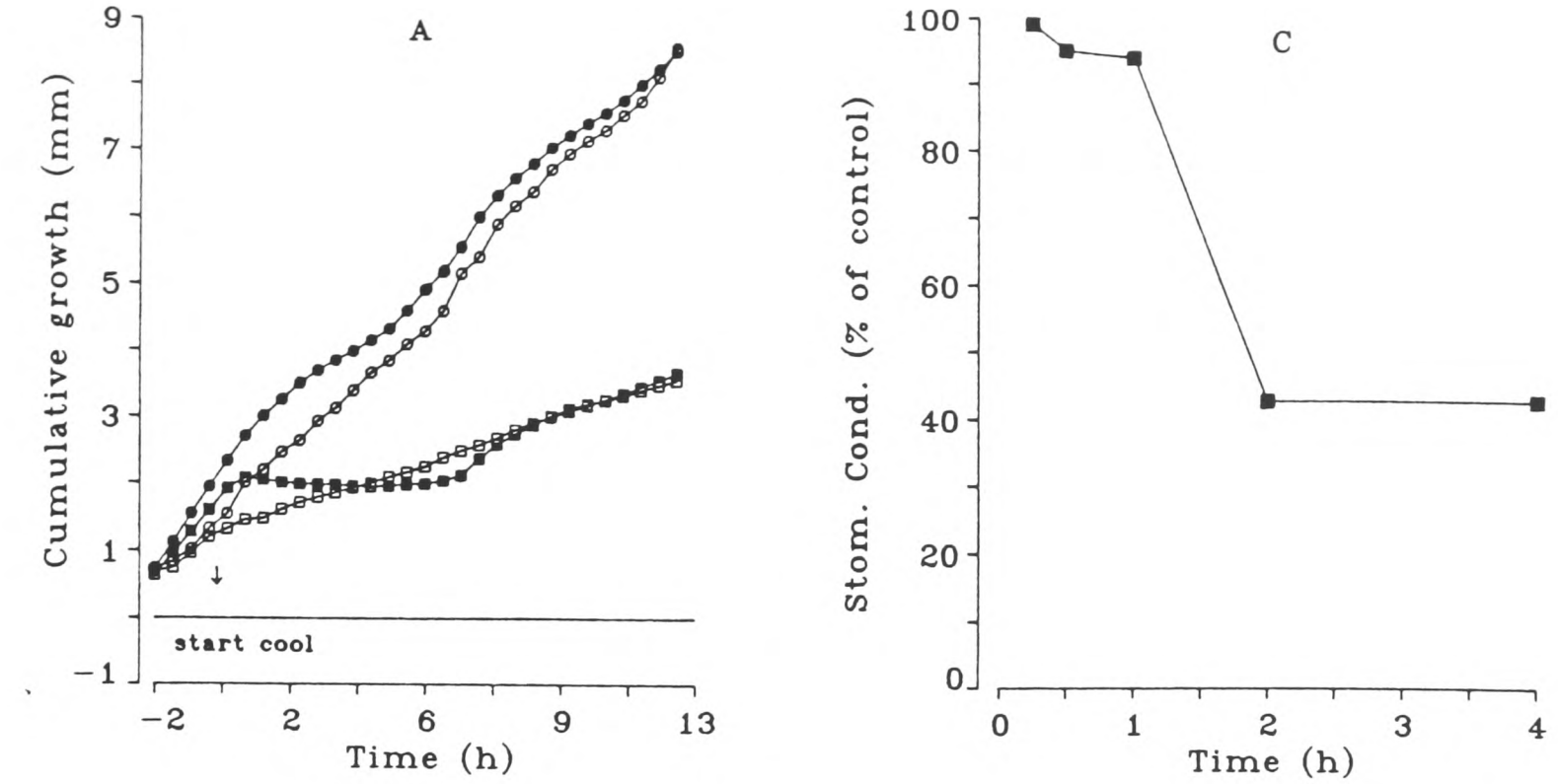


Figure 4.7- Effects of root temperature at 10°C (squares) or 23°C (circles) on cumulative growth (A), area (B), stomatal conductance (C) and on water relations (D), of primary leaves of *P. vulgaris* subjected (close symbols) or not (open symbols) to high atmospheric humidity. Standard errors are shown for n=4.

changes in this could affect cell and leaf elongation rates.

Experiments were performed to investigate the short and long-term effects of root-cooling and reversing treatment on cell wall extensibility of primary leaves of *Phaseolus vulgaris*.

Cell wall properties were affected by root cooling treatment as shown by Milligan and Dale (1988*b*). A reduction in total (TEx) and plastic (PEx) extensibilities was observed after a lag of only 15 min, the earliest time sampled (Fig. 4.8B). These low values continued through the experimental period (20 h, Fig 4.8B). The lowest values for total and plastic extensibilities were observed after a lag of 6 h after the onset of the root-cooling treatment (TEx = 5.38% 10 g load; PEx = 1.60% 10 g load). At this time the cell turgor had already recovered from the initial fall (Fig. 4.1), indicating that cell wall extensibility could be the factor keeping the elongation rates low.

The total and plastic extensibilities for control plants increased at 15 min and thereafter they showed only slight variation (Fig. 4.8B). The values of TEx and PEx 6 h after the onset of the treatment were 7.09% 10. g load and 3.00% 10 load respectively. These values were significantly higher than those for treated plants at the same period.

Reversing the temperature from 10°C to 23°C, at 20 h after the onset of root-cooling, slightly increased TEx

and PEx after a lag of 1 - 2 h, but this increase was not statistically significant (Fig. 4.8C). The control plants also showed a slight increase in total and plastic extensibilities, and the wall extensibilities remained significant lower for the treated plants, compared to those of the control.

When these results are compared with Figure 3.12 (Chapter 3) they suggest that reduction in leaf extensibility is indeed one of the factors which maintain the low leaf elongation rate in response to root-cooling treatment. When root-cooling is reversed there was an immediately increase, even greater than the control, in the elongation rate. However, it was short-lived and fell back to values lower than the control, possibly due to the low values of cell wall extensibilities, even when the treatment is discontinued.

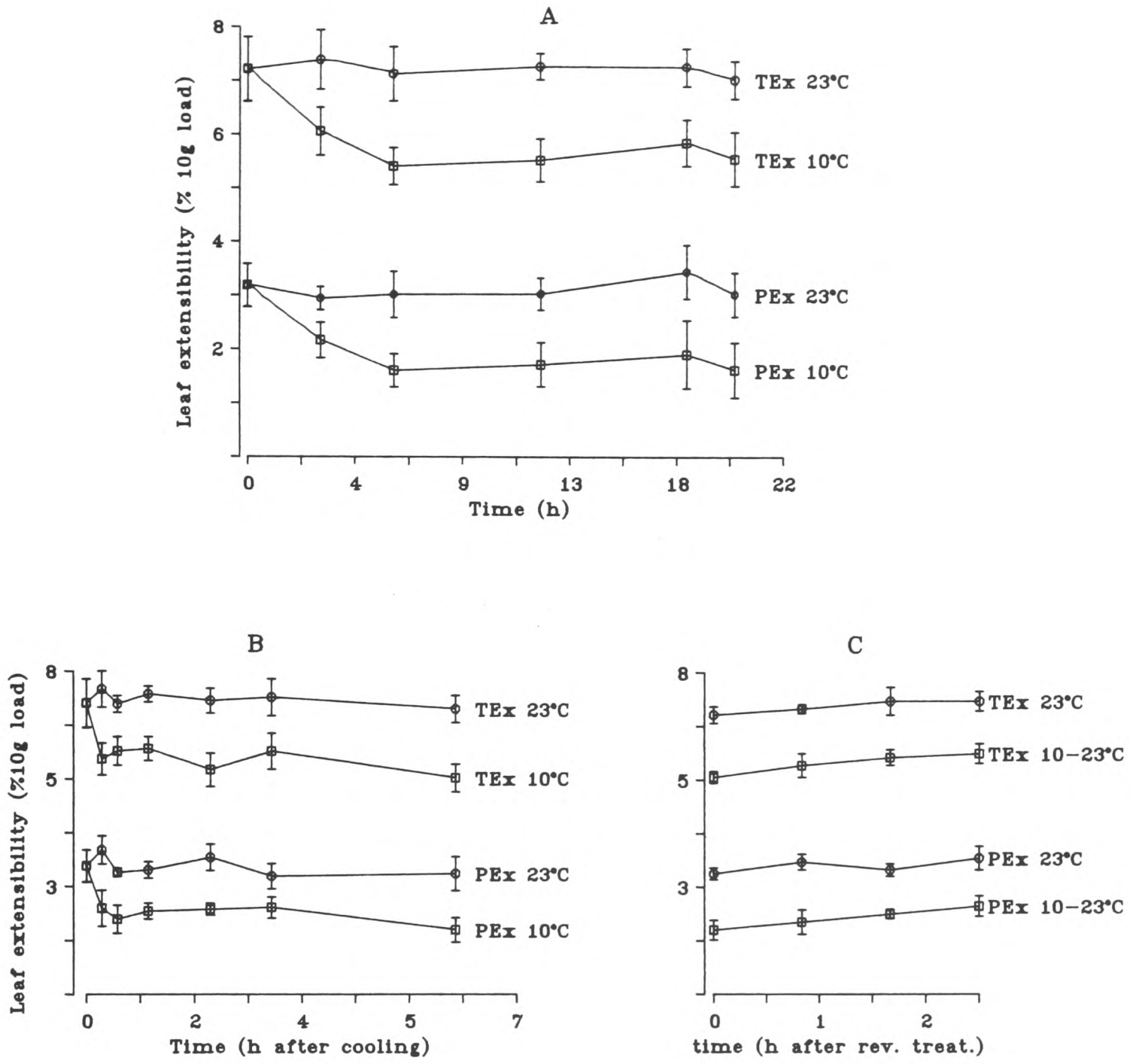


Figure 4.8- Effects of root-cooling at 10°C and reversing treatment (squares) on total (TEx) and plastic (PEx) extensibilities of primary leaves of *P. vulgaris*; for control plants root temperature was 23°C. A- long-term; B- short-term, and C- Reversing root-cooling. Standard errors are shown for n = 10.

CHAPTER 5 - RESULTS

EFFECTS OF ROOT COOLING ON ABSCISIC ACID CONTENT

5.1- Introduction

So far, the results presented in this study, have given evidence that: i) in response to root cooling treatment, the reduction in leaf elongation rate is very rapid and can be reversed; ii) the fall in leaf turgor after root cooling may be the first signal which triggers the reduction in leaf elongation, at least when transpiration is not inhibited; and iii) the cell wall extensibility, one of the primary factors which determines cell enlargement (Lockhart, 1965), is also reduced by the treatment.

Since leaf turgor recovers 3 - 4 h after the onset of treatment, a continuing effect of leaf turgor reducing leaf growth can be ruled out, although effects on wall extensibility could continue.

There is abundant evidence that the plant hormone abscisic acid (ABA) is an important chemical signal mediating plants responses to environmental stress (for reference see section 1.4.3).

Smith and Dale (1988) showed a significant rise in free ABA content of expanding primary leaves on root-cooled seedlings of *Phaseolus vulgaris* 2 h after the start of cooling. They were also able to show that

leaf ABA content were reversed when the low root temperature treatment was discontinued.

ABA is also known to be involved in growth inhibition in plants of wheat (Quarrie and Jones, 1977), bean (Van Volkenburgh and Davies, 1983) and maize (Kutschera and Schopfer, 1986) by affecting growth parameters such as cell expansion.

These findings and those of Smith and Dale (1988) coupled with those of the present study, suggest that abscisic acid could possibly be one of the chemical signals mediating the leaf response to root cooling treatment.

This chapter investigates this hypothesis. The content of free ABA in the leaf blade, cotyledons and xylem sap of control and root-cooled plants were analysed. The method chosen for ABA analysis was the radioimmunoassay described by Quarrie *et al.*, (1988), (see section 2.14).

5.2- Effects of Root Cooling on Abscisic Acid Contents in Leaves

5.2.1- Analysis of ABA in Primary Leaves of *Phaseolus vulgaris*

A rise in free ABA concentration in expanding primary leaves of bean in response to root cooling was shown to occur within 2 h of the onset of the treatment (Smith and Dale, 1988).

Experiments were now performed to investigate whether the rise in ABA occurred before 2 h, i.e. closer to the time when treatment began to affect leaf elongation.

Seedlings of *P. vulgaris* were grown as described in section 2.3 and the root cooling treatment was imposed at day 7 after sowing (section 2.4). Primary leaves were sampled 15 and 30 minutes after the onset of root cooling and ABA content was determined.

A significant increase was found in leaf ABA within only 15 - 30 min after the commencement of root cooling to 10°C (Fig. 5.1A). The ABA content rose rapidly, from 50 ng g^{-1}FW found in control plants, to around 200 ng g^{-1}FW, which represented a 4-fold increase.

A less marked (2-fold), but still significant rise occurred over the same period when the root system was cooled to 15°C (Fig. 5.1B).

From Figure 5.1A and B, it can be seen that ABA accumulated rapidly after cooling the root system and no further significant changes were observed over the next 6 h of treatment. After 24 h of root cooling the ABA content of leaves of treated plants showed similar values to those measured in shorter-terms.

Loss of turgor has been shown to be the critical parameter of cell water relations that initiates abscisic acid accumulation in leaves of bean, cocklebur and cotton (Pierce and Raschke, 1980) and spinach (Creelman and Zeevaart, 1985).

Milligan and Dale (1988*b*) showed that leaf turgor of

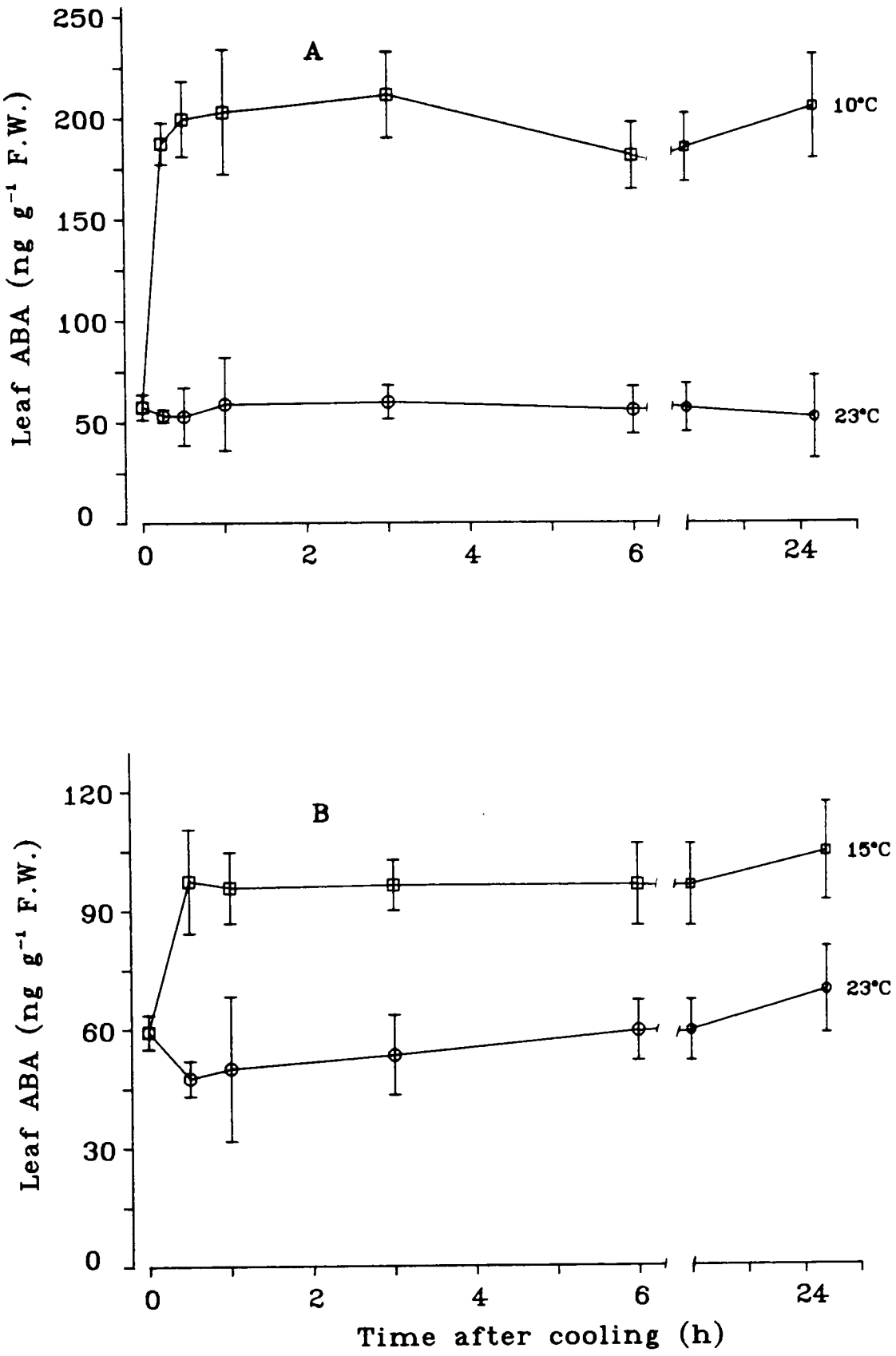


Figure 5.1- Effects of root cooling treatments (squares) to 10°C (A) and 15°C (B) on ABA contents of primary leaves of *P. vulgaris*; for control plants (circles) the root temperature was 23°C. Standard errors are shown for n = 4.

bean plants is lowered for a short period at the beginning of root cooling treatment to 10°C. In this study, similar treatment showed similar results for bean and sunflower plants and root cooling treatment to 15°C also lowered leaf turgor of bean plants.

When the initial fall in leaf turgor showed in Figure 4.1 (Chapter 4) is compared to the rise in leaf ABA content showed in 5.1 (this Chapter) it seems reasonable to assume that any effect of root cooling treatment on leaf ABA content was through its effect on lowering the leaf turgor potential.

This assumption was investigated by analysing the contents of abscisic acid in primary leaves of seedlings of *P. vulgaris* subjected to high atmospheric humidity (RH 100%), over the period of root cooling treatment to 10°C, since this treatment was shown to prevent fall in leaf turgor after root cooling (Chapter 4).

The rise in leaf ABA observed in this experiment (Fig.5.2) took longer than previously observed (see Fig. 5.1). ABA levels started to rise 1 h after the onset of treatment and by 2 h the leaf ABA content of root cooled plants was 4-fold higher than that of the control (Fig. 5.2). By the end of 4 h, this level had risen to values 7-fold higher than the control.

In these experiments there was no relationship between leaf turgor and leaf ABA content (see Figs. 4.8D and 5.2). However, the rise in leaf ABA observed 2 h after the onset of root cooling is closely related with

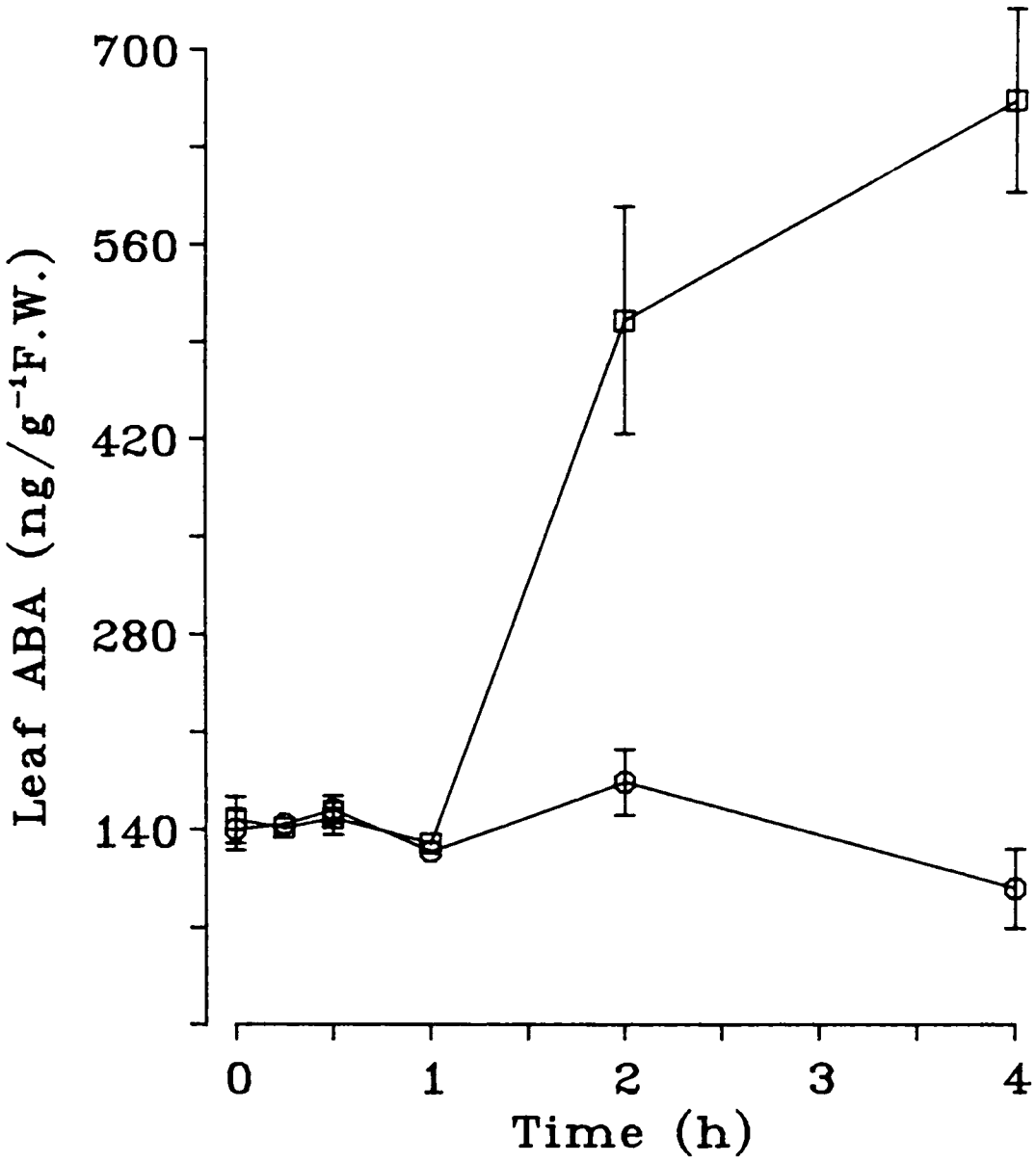


Figure 5.2- Effects of root cooling at 10°C (squares) or at 23°C on ABA content of primary leaves of *Phaseolus vulgaris* subjected to high atmospheric humidity. Standard errors are shown for n = 4.

the decline in stomatal conductance, which is reduced by around 50% over this period (see Figs 4.8C and 5.2). It suggests that leaf ABA content rises in response to root cooling treatment, and that this increase will affect stomatal opening , even though leaf turgor is maintained at control values.

A significant reduction, around 25%, in leaf growth, measured as leaf area, was observed within 12 h. Significant reduction in leaf growth, measured as elongation rate using RVDT, was observed earlier. Within 30 min leaf elongation rate on root-cooled plants was reduced by around 40% in relation to that of the control values. Elongation rates continued to fall and showed values around 1 - 2 $\mu m\ min^{-1}$ within 2 - 3 h. At this time, leaf elongation rate for control plants was around 9 $\mu m\ min^{-1}$.

5.2.2- Analysis of ABA in Leaves of *Helianthus annuus* and *Hordeum vulgare*

Since the effects of root-cooling treatment upon growth and water relations of leaves were also found for *Helianthus and Hordeum* (see Chapters 3 and 4), experiments were performed to investigate whether the treatment causes increased ABA levels in leaves of such plants, and whether the effect is a general feature for all leaves regardless of whether or not they are actively expanding.

5.2.2.1- Experiments with *Helianthus annuus*

Experiments using seedlings of *H. annuus* were performed to allow the analysis of ABA content for actively expanding and half expanded aerial organs.

Seedlings grown as described in section 2.3 were subjected to root cooling treatment and harvested for ABA assay at 2 different stages of development.

In an earlier stage, root cooling treatment was imposed on 7-day old seedlings and ABA analysis performed for the cotyledons and the first pair of leaves, which were in their exponential growth phase.

In a later stage, 11-day old seedlings were treated. The analysis of ABA were performed for the cotyledons and the first pair of leaves, which were completing expansion and for the second pair of leaves, which had emerged between days 8 - 9 and was actively expanding.

The results in Table 5.1A show the effects of root cooling on ABA content of 7-day old seedlings of *H. annuus*. There was a rise in ABA of the first pair of leaves from 250 ng g^{-1}FW, found in the control, to 465 ng g^{-1}FW found in root-cooled plants by 1 h after the start of the treatment. An increased content of free ABA was also found for the cotyledons at the same time. By the end of 24 h, the ABA contents for both leaves and cotyledons had fallen to around 360 ng g^{-1}FW for the treated plants i. e. 25% lower than the previous values. A significant fall in ABA was also observed in the

leaves and cotyledons of control plants.

When *H. annuus* seedlings were treated at day 11 after sowing (Table 5.1B), there was only a slightly increase in ABA content of the first pair of leaves with a much larger effect on the cotyledons and the largest effect of all on the second pair of leaves, whose ABA content was enhanced by 2.5-fold 1 h after the commencement of the treatment. This high level was maintained over 24 h.

5.2.2.2- Experiments with *Hordeum vulgare*

Root cooling treatment of seedlings of *H. vulgare* commenced at day 10 by which stage the first leaf (leaf 1) was fully expanded and the second leaf (leaf 2) was about one quarter expanded. ABA content was measured for the blade of the leaf 1 and for the emerged and unemerged parts of the leaf 2, with a further distinction being made between the distal and proximal unemerged portion of the leaf 2 (see section 2.14.3).

Table 5.2 shows the results for ABA contents of leaves 1 and 2 of seedlings of *H. vulgare*. Root cooling did not cause any rise in ABA of the fully expanded leaf 1, at 6 h after the onset of the treatment. The ABA content actually fell by the end of 24 h, but a similar fall occurred in the control plants.

There was an effect of root cooling treatment on ABA content of the growing leaf 2 (Table 5.2). Here total ABA in leaf 2 increased from 133ng g^{-1}FW to 345ng g^{-1}FW

Table 5.1- The time curse of the effects of root cooling treatment at 10°C (RC) starting at day 7 (A) and day 11 (B) after sowing on ABA contents (ng g^{-1}FW) of the first and second pair of leaves and cotyledons of seedlings of *Helianthus annuus*; for control plants the root temperature was 23°C. Standard errors are shown for n=6.

A Root cooling starting at day 7

Time after cooling (h)		ABA (ng g^{-1} FW)	
		1st leaf	Cotyledons
0	Control	250 ± 20	265 ± 15
	RC	-	-
1	Control	245 ± 15	300 ± 20
	RC	465 ± 15	475 ± 25
24	Control	190 ± 30	220 ± 30
	RC	370 ± 20	350 ± 20

B Root cooling starting at day 11

Time after cooling (h)		ABA (ng g^{-1} FW)		
		2st leaf	1st leaf	Cotyledons
0	Control	210 ± 15	105 ± 10	150 ± 15
	RC	-	-	-
1	Control	195 ± 10	120 ± 11	121 ± 21
	RC	485 ± 21	150 ± 21	260 ± 17
24	Control	200 ± 18	110 ± 22	114 ± 20
	RC	480 ± 16	155 ± 16	240 ± 20

Table 5.2- Time course of the effects of root cooling treatment at 10°C starting at day 10 (RC) on ABA contents (ng g^{-1}FW) of the whole blade of leaf 1 and of the different portions of leaf 2 of seedlings of *Hordeum vulgare*; for control plants the root temperature was 23°C. Standard errors are shown for n = 4.

Leaf	6 h cooling		24 h cooling	
	Control	RC	Control	RC
1	60 ± 3	57 ± 5	27 ± 5	21 ± 1
2				
Emerged	30 ± 9	97 ± 18	11 ± 2	46 ± 2
Unemerged distal	42 ± 18	81 ± 8	17 ± 4	50 ± 1
Unemerged proximal	62 ± 9	167 ± 16	48 ± 5	83 ± 3
Total leaf 2	134	345	76	179

i.e. 2.5-fold higher than that of the control by 6 h after the onset of treatment. By the end of 24 h, the total ABA in leaf 2 of root-cooled plants fell to 179 ng g^{-1}FW. This value was still 2.5-fold greater than the control, where ABA content also fell over this period.

The results in Figures 5.1 and Tables 5.1A, B and 5.3 showed that: i) the rise in leaf ABA in response to root cooling was not unique for *P. vulgaris*; ii) although the amount of abscisic acid accumulated by the leaves of the three species (*P. vulgaris, H. annuus and Hordeum vulgare)* were somewhat variable from species to species, on average, leaves of treated plants showed an increase in ABA content around 2.5 to 4-fold higher than their control at 23°C, and iii) in seedlings of sunflower and barley, leaf age is an important factor in determining ABA levels since young, rapidly expanding leaves had a much higher ABA content than mature leaves.

5.3- Analysis of Abscisic Acid in Xylem Sap of Seedlings of *Phaseolus vulgaris*

Considerable evidence has been obtained recently supporting the hypothesis of ABA redistribution in leaves according to the anion-trap mechanism for weak acids. Any environmental factor which causes a shift in compartment pH, especially between cytosol and apoplast, will affect the compartmentation of ABA in the leaf (Cowan *et al.*, 1982; Hartung *et al.*, 1983; Cornish and

Zeevaart, 1985*a*; Radin and Hendrix, 1988; Hartung *et al.*, 1988; Hartung and Slovik, 1991).

Such a mechanism of redistribution may lead to a substantial amount of ABA, trapped in the cytosol of the leaf, becoming available at sites of action at, for example, the guard cell.

Furthermore, there is evidence that ABA produced by roots in drying soil moves in the transpiration stream and accumulates at or near the guard cell and thereby promotes stomatal closure (for review see Hartung and Davies, 1991)

Faced with this evidence, it was considered worthwhile to investigate whether root cooling treatment exerts similar effects on ABA redistribution and/or synthesis in the leaf itself and synthesis in the roots of bean plants.

To achieve this, the ABA concentrations in xylem sap of control and root-cooled plants of seedlings of *P. vulgaris* were analysed in samples collected as described in sections 2.12.

5.3.1- Root Exudate Experiments

When shoots are removed the rate of exudation of xylem sap from roots of control plants is relatively high, but rate of exudation from root systems subjected to cooling is much lower.

In experiments where plants were detopped following

root cooling treatment the rate of exudation of sap from roots of treated plants, averaged over 24 h, was 1.6 μl plant^{-1} h^{-1}. For control plants, over the same period, the rate was 7.1 μl plant^{-1} h^{-1}. Typical data showing this differences are presented in Table 5.3A.

When shoots were removed 24 h after the onset of root cooling (Table 5.3B) the rate of exudation remained much higher in the controls. The rates of sap exudation from control and treated plants were 11.7 μl plant^{-1} h^{-1} and 3.0 μl plant^{-1} h^{-1} respectively.

The highest volume of sap exudation from control plants, in both experiments, was found at the first collection, it was them reduced and maintained stable in subsequent collections (Table 5.3). The sap volume coming from root-cooled plants did not vary over the collection period in experiments were plants were detopped following root cooling (Table 5.3A). When root-cooled plants were detopped 24 h after treatment the volume of sap exudation from these plants increased over the collection period (Table 5.3B).

5.3.2- Abscisic Acid Analysis

For ABA analysis a minimal volume of exudate of 0.04 cm^3 was necessary and this imposed restriction on how experiments could be done, since many plants were needed to contribute small amounts of exudate which were then bulked for analysis.

Table 5.3- Rates of xylem sap exudation from the root system of *Phaseolus vulgaris* seedlings subjected to root cooling at 10°C (RC) or 23°C (control). Plants were topped immediately following root cooling treatment (A) or 24 h later (B). Data were calculated for 24 plants.

A

Time after cooling (h)	Xylem sap exudation (μl plant^{-1}h^{-1})	
	Control	RC
1 - 4	13.75	1.50
5 - 8	5.89	1.61
9 - 12	5.83	1.35
13 - 16	6.40	1.60
17 - 20	5.20	1.68
21 - 24	5.41	1.77

B

Time after cooling (h)	Xylem sap exudation (μl plant^{-1}h^{-1})	
	Control	RC
25	28.75	1.66
26	7.08	2.50
27	6.66	2.08
28	6.25	2.50
29	11.66	5.00
30	8.75	3.30
31	12.91	4.16

This was particularly difficult in the case of plants sampled immediately after the start of root cooling, since it was sometimes found that some plants failed to exude at all. However, a number of experiments were performed and results of two such runs are now reported.

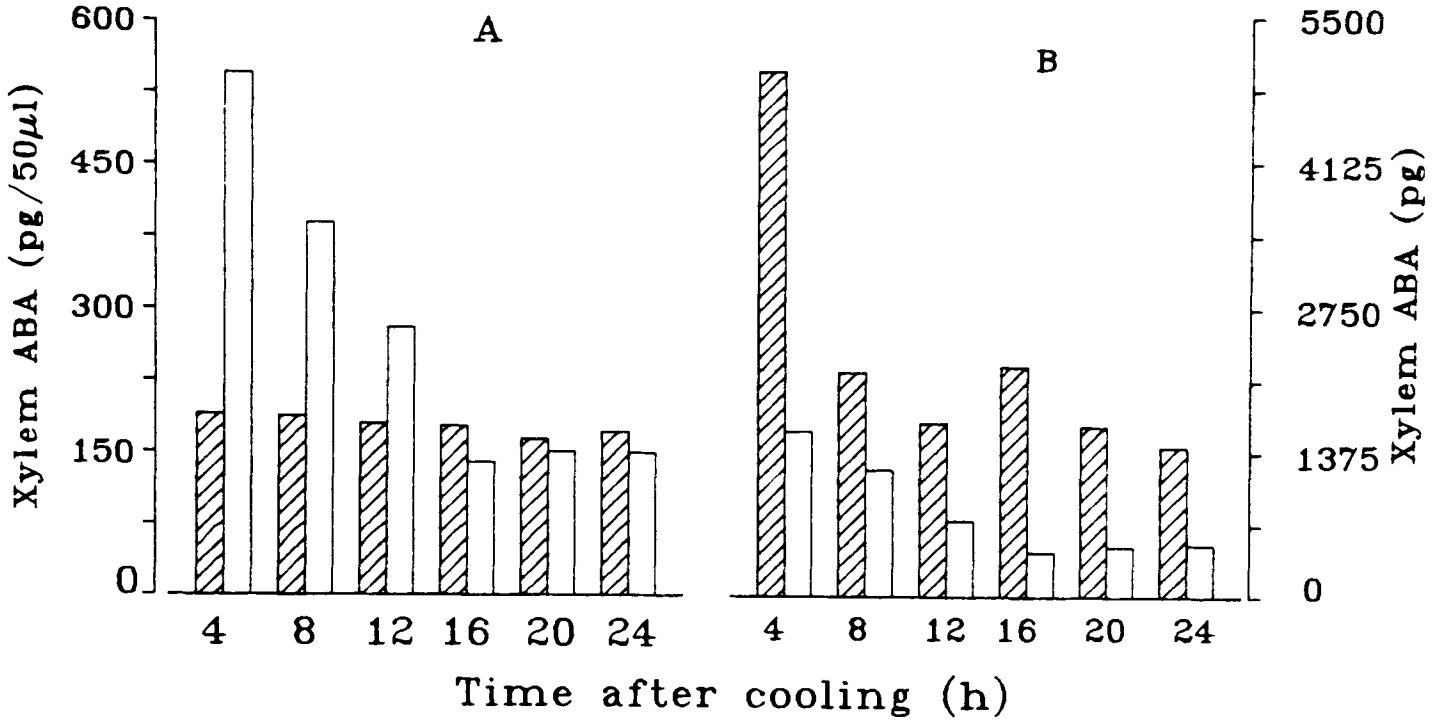
In the first, root cooling was commenced in the usual way and plants were topped after 20 min from the start, and sap collected for analysis of ABA. Data are given in Figure 5.3A, B.

In the second experiment, root-cooling was continued for 24 h before detopping, sap then being collected at hourly intervals for analysis (Figure 5.3C, D).

In both experiments the concentrations of ABA in xylem sap of control plants were very stable and averaged around 150 pg/50μl over the experimental period regardless of the volume of sap which was exudating (Fig. 3.5A, C). For root-cooled plants ABA concentrations in xylem sap were initially high (excluding the first analysis in Figure 5.3C) and then decreased with collection time (Fig. 5.3 A and C).

Comparing ABA concentrations, they were lower in xylem sap of control than root-cooled plants (Fig. 5.3A and C), which showed concentration as high as 800 pg/50μl (Fig. 5.3C). However, the total ABA contents (Fig. 5.3B, D) in xylem sap exuded over the experimental period was much higher for control than for root-cooled plants.

Assuming that exuded rates of xylem sap in detopped plants are similar to that in intact ones, it may be



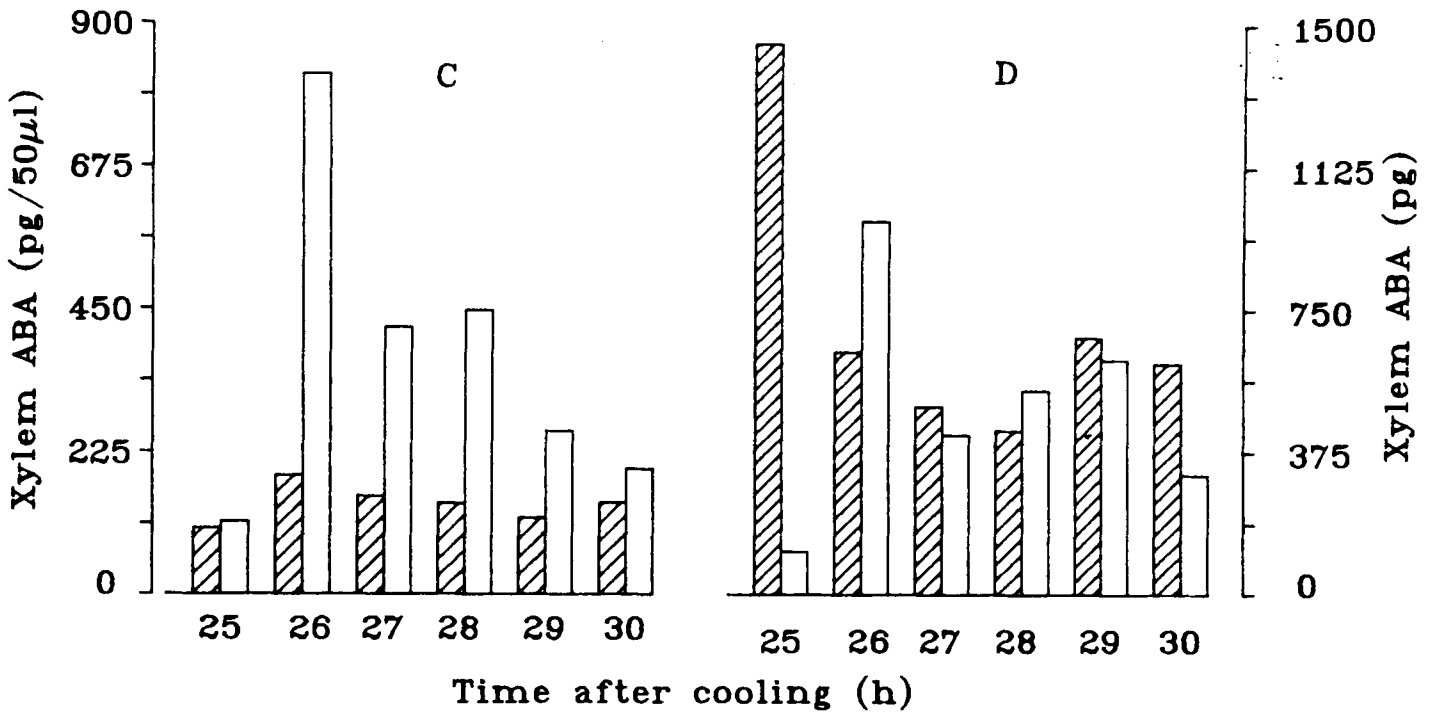


Figure 5.3- ABA concentration (A, C) and content (B, D) in xylem sap of roots of *Phaseolus vulgaris* seedlings subjected to root cooling at 10°C (open histograms) or 23°C (shaded histograms). Plants were detopped for xylem sap collection immediately following root cooling treatment (A, B) or 24 h later (C, D).

concluded that the amounts of ABA that could be going to the leaves on root-cooled plants could well be much smaller than that in the control plants and also that the volume of sap must be taken in account for estimations of ABA moving from roots to the shoots. From the results in Figure 5.3A, C it can also be concluded that reduced sap volume did not result in higher ABA concentration either in control or in root-cooled xylem sap.

CHAPTER 6 - RESULTS

EFFECTS OF EXOGENOUS ABA AND XYLEM SAP

6.1- Introduction

The hypothesis that a message from roots controls the shoot responses to environmental stress, and that the plant hormone abscisic acid is linked with these responses, has been extensively tested by means of feeding experiments. These approaches are based on feeding derooted plants, or parts of the plant, grown in standard conditions, with xylem sap collected from stressed and non stressed roots and/or exogenous ABA (Munns and King, 1988; Henson *et al.*, 1989; Zhang and Davies, 1990*a*, *b*; Zhang *et al.*, 1990; Munns, 1990; Neuman and Smit, 1991; Zhang and Davies, 1991).

In this chapter, feeding with sap and exogenous abscisic acid has been used to investigate the relationship between ABA concentration in xylem sap and leaf growth, cell wall properties, leaf water relation and leaf conductance.

Xylem sap, used for feeding experiments, was collected as described in section 2.12 and only sap collected within 2 h from the start was used. Since the volume of sap exuded from treated plants was very small, several runs were necessary to obtain a reasonable volume of sap. The procedure of feeding experiments is

detailed in section 2.13.

There was no difference in osmotic potential of sap collected from control and root cooled plants (control -0.32 ± 0.02 MPa; root cooling -0.34 ± 0.02 MPa n = 6).

Synthetic ABA solutions were supplied at a range of concentrations equal and higher (60, 170 and 1000 μmol m^{-3}) to that found in xylem sap. The racemate (±)-ABA was used and it was assumed to have equal amounts of (+) and (-) enantiomers; the concentrations stated are the (+)enantiomer.

Seven-day-old seedlings of *Phaseolus vulgaris*, grown as described in section 2.3 were used in these experiments. Seedlings were removed from vermiculite and their root systems were cut under distilled water. Stomatal conductance was monitored using a Li-Cor porometer. Those derooted plants which showed comparable stomatal conductance were selected for experimental treatment. Leaf strips were also used.

In experiments using excised seedlings, the solutions were fed through the transpiration stream by placing the shoot in plastic vials (3.0 cm^3), containing 0.5 cm^3 of xylem sap, synthetic ABA solutions or distilled water.

When leaf strips were used, they were removed from the leaves, parallel to the midrib, and incubated in Petri dish containing 1.0 cm^3 of xylem sap, synthetic ABA or distilled water. The strip dimensions were 5 mm

wide x 10 mm long.

6.2- Effects of Feeding Treatments on Leaf Growth

Increase in leaf area was inhibited by both, exogenous ABA and xylem sap from root cooled plants (Table 6.1). The leaf area measured 24 h from the beginning of feeding treatments was 30% less compared with distilled water (control) with the highest concentration of ABA (1000 μmol m^{-3}). Smaller, but still significant, effects were found when lower ABA concentrations (60 μmol m^{-3} and 170 μmol m^{-3}) were supplied. These concentrations reduced leaf area by around 20% compared with that of the control. When excised seedlings were fed with xylem sap from root-cooled plants leaf area was reduced by 26%. There was no effect on leaf area of xylem sap from control plants compare treatments 1 and 3 in Table 6.1.

6.3- Effects of Feeding Treatments on Cell Wall Extensibility

6.3.1- Experiments using Leaf Strips

Cell wall extensibility of primary leaves of *P. vulgaris* was measured in experiments where leaf strips were incubated in ABA solutions at different concentrations, or in xylem sap from both control and root-cooled plants, or in distilled water. The effects

Table 6.1- Effects of synthetic (+)-ABA at different concentrations and xylem sap from control (Sap-C) and root cooled (Sap-RC) plants on primary leaf area of excised seedlings of *Phaseolus vulgaris* , 24 h after treatment. ABA solutions, saps and distilled water were fed through the transpiration stream. The initial leaf area averaged for 24 plants was 6.8 ± 0.71 cm^2. Standard errors shown are shown for n = 4.

Incubation medium	Leaf Area (cm^2)
1- Water	10.8 ± 1.01
2- ABA ($\mu mol\ m^{-3}$)	
60	8.6 ± 0.60
170	8.5 ± 0.60
1000	7.5 ± 0.81
3- Sap-C	10.7 ± 0.73
4- Sap-RC	8.0 ± 1.02

of treatments on total and plastic extensibilities were determined 24 h after the onset of the incubation period and more regularly, for the control in distilled water.

The results in Table 6.2 show that plastic extensibility was reduced by ABA treatments. The total extensibility was less affected, but for both total and plastic extensibilities, the degree of reduction was dependent upon the ABA concentration. Total extensibility was reduced by 12, 21 and 26% with increased ABA concentration. The reduction were much greater for plastic extensibility (24, 44 and 61%).

Xylem sap from root-cooled plants fed to leaf strips also reduced cell wall extensibility (Table 6.2). As with exogenous ABA, the effects were larger on plastic extensibility. The reductions in total (21%) and plastic (42%) extensibility were comparable to the values recorded for intact plants subjected to root cooling treatment which had total and plastic extensibilities reduced by 24% and 46% respectively (see section 4.6 Chapter 4).

Xylem sap from control plants had no effect on extensibility of leaf strips.

6.3.2- Experiments using Excised Seedlings

The effects of exogenous ABA (0, 60, 170 and 1000 μmol m^{-3}) on cell wall extensibilities of primary leaves of excised seedlings of *Phaseolus vulgaris* were also

Table 6.2- Effects of synthetic (+)-ABA at different concentrations and xylem sap from control (Sap-C) and root cooled (Sap-RC) plants on cell wall extensibility of primary leaf strips of seedlings of *P. vulgaris*, 24 h after treatment. Measurements for the control in distilled water were made either at the beginning of the incubation period (time 0) or 8 h (time 1) and 24 h (time 2) later. Standard errors shown are shown for n = 12.

Incubation medium	Extensibilities (% 10g load)	
	Total	Plastic
1- Water		
time 0	7.36 ± 0.84	3.80 ± 1.01
time 1	7.38 ± 0.91	3.20 ± 0.78
time 2	7.68 ± 0.70	3.60 ± 0.88
2- ABA (μmol m^{-3})		
60	6.72 ± 0.65	2.73 ± 0.60
170	6.02 ± 0.80	2.00 ± 1.00
1000	5.63 ± 1.05	1.38 ± 0.90
3- Sap-C	7.56 ± 1.00	4.07 ± 0.90
4- Sap-RC	6.05 ± 0.85	2.06 ± 0.80

Table 6.3- Effects of exogenous ABA at 0, 60, 170 and 1000μmol m^{-3} fed to excised seedlings of *Phaseolus vulgaris* on total (A) and plastic (B) extensibilities of primary leaves. Standard errors are shown for n = 4.

A

ABA Concentration (μmol m^{-3})	Total Extensibility (% 10g load)		
	Time after starting treatment (h)		
	8	16	24
0	7.3 ± 0.5	7.4 ± 0.7	7.3 ± 0.6
60	6.7 ± 0.4	6.5 ± 0.6	6.6 ± 0.5
170	6.1 ± 0.5	6.0 ± 0.5	6.0 ± 0.7
1000	5.4 ± 0.5	5.5 ± 0.7	5.3 ± 0.5

B

ABA Concentration (μmol m^{-3})	Plastic Extensibility (% 10g load)		
	Time after starting treatment (h)		
	8	16	24
0	3.5 ± 0.8	3.4 ± 0.6	3.5 ± 0.4
60	2.8 ± 0.3	2.7 ± 0.6	2.7 ± 0.5
170	2.1 ± 0.5	1.9 ± 0.4	2.2 ± 0.6
1000	1.4 ± 0.4	1.5 + 0.7	1.4 ± 0.7

investigated. Measurements were made at different intervals after the onset of feeding experiment.

Eight hours after the start of feeding all ABA-treated plants had reduced leaf extensibility (Table 6.3). The values recorded 8 h after the commencement of the experiment remained stable over the 24 h of the experimental period. On average, the percentages of reduction caused by each concentration tested were similar to those recorded for leaf strips (Table 6.2), where, the degree of reduction was also dependent on ABA concentration.

6.4- Effects of Feeding Treatments on Water Relations

The effects of synthetic ABA and xylem saps on bulk leaf water potential were determined in excised seedlings of *P. vulgaris* fed with ABA solutions or xylem saps. The measurements were carried out 8 h after the commencement of feeding.

Leaf water potential did not differ between treatments (Table 6.4). The osmotic potential was reduced by 0.08 to 0.1 MPa when excised plants were fed either ABA or xylem sap from root-cooled plants. This reduction reflected an increase, although not significant, in leaf turgor of up to 0.07 MPa.

Table 6.4- Water potential (ψ), osmotic potential (π) and turgor (P) of primary leaves of excised plants of *Phaseolus vulgaris* fed with xylem sap from control (Sap-C) and root cooled (Sap-RC) plants or synthetic ABA through the transpiration stream. Measurements were made 8 h after the start of feeding experiment. Standard errors are shown for n = 4.

Incubation medium	Water Relations (MPa)		
	ψ	π	P
1- Water	-0.20 ± 0.05	-0.81 ± 0.02	0.61 ± 0.07
2- ABA (μmol m^{-3})*			
60	-0.24 ± 0.04	-0.89 ± 0.03	0.65 ± 0.04
170	-0.22 ± 0.02	-0.90 ± 0.03	0.68 ± 0.08
1000	-0.23 ± 0.05	-0.91 ± 0.04	0.68 ± 0.05
3- Sap-C	-0.20 ± 0.04	-0.83 ± 0.03	0.63 ± 0.04
4- Sap-RC	-0.22 ± 0.04	-0.90 ± 0.06	0.68 ± 0.08

* Expressed as (+) enantiomer of the (±) racemate.

6.5- Effects of Feeding Treatment on Leaf Conductance

The stomatal conductance of leaves of excised seedlings fed with ABA or xylem sap was measured 3, 6 and 9 h after the start of the feeding.

For all excised plants fed ABA leaf conductance (Table 6.5) was reduced progressively with increased ABA concentration. Significant reduction occurred within 3 h of the beginning of feeding treatment and no further reduction was observed. On average, conductance was reduced by 21%, 40% and 55% with ABA concentrations of 60, 170 and 1000 μmol m^{-3} respectively.

Xylem sap from root-cooled plants reduced leaf conductance by 42%, a value comparable with the 170 μmol m^{-3} ABA solution. No significant reduction was observed for the treatment with sap from control plants.

6.6- Effects of Feeding Treatment on ABA Contents of Leaves

The effects of feeding xylem sap from control and root-cooled plants, and ABA solutions, on leaf ABA content were investigated in excised seedlings of *Phaseolus vulgaris*.

Xylem sap and ABA solutions at different concentrations were fed (0.5 cm^3) through the transpiration stream and leaves were harvested for ABA analysis 6 h from the beginning of the experiment. The

Table 6.5- Leaf conductance (mmol $m^{-2}s^{-2}$) of primary leaves (abaxial epidermis) of excised plants of *Phaseolus vulgaris* fed with xylem sap from control (Sap-C) and root cooled (Sap-RC) plants or synthetic ABA through the transpiration stream. Measures were made 3, 6 and 9 h after the start of feeding experiment. Standard errors are shown for n = 4.

Incubation medium	Stomatal Conductance (mmol $m^{-2}s^{-1}$)		
	Time after starting treatment (h)		
	3	6	9
1- Water	101 ± 18	86 ± 10	92 ± 14
2- ABA(μmolm^{-3})			
60	81 ± 12	68 ± 12	72 ± 12
170	64 ± 18	50 ± 10	51 ± 15
1000	45 ± 13	40 ± 13	37 ± 20
3- Sap-C	90 ± 17	80 ± 18	81 ± 18
4- Sap-RC	59 ± 13	51 ± 11	51 ± 12

amount of ABA fed per leaf showed in Table 6.6 was calculated on the assumption that the mean leaf fresh weight was = 0.15 g (see Appendix A.1).

Feeding experiments enhanced the ABA content in leaves in two ways. First, in all treatments the content of ABA was greater than the amount fed (Table 6.6), and second, the ABA levels in leaves of excised seedlings were higher than those measured previously for intact seedlings.

The ABA content reported previously (Chapter 5, see Fig. 5.1) in leaves on intact control seedlings of *Phaseolus vulgaris* averaged 50 ng $g^{-1}FW$ or 7.5 ng $leaf^{-1}$. For the leaves on root-cooled plants the ABA content was around 200 ng $g^{-1}FW$ or 30 ng $leaf^{-1}$ (considering in both cases a mean leaf fresh weight of 0.15 g).

Leaf ABA content measured for excised control plants (no ABA fed) was around 130 ng $g^{-1}FW$ i.e. 2.5-fold higher than the value for control intact plants. In experiments fed xylem sap from root-cooled plants (ABA fed 26.6 ng $g^{-1}FW$) and for those fed ABA solution at the same concentration (60 μmol m^{-3}), the amount of ABA measured was around 350 ng $g^{-1}FW$ (Table 6.6). These two treatments did not show any significant difference. Feeding ABA solution at higher concentration (170 and 1000 μmol m^{-3}) raised the leaf ABA to around 470 and 800 ng $g^{-1}FW$ respectively.

The results presented in this Chapter showed that

Table 6.6- Leaf ABA content of excised plants of *P. vulgaris* fed with xylem sap from control (Sap-C) and root cooled (Sap-RC) plants or synthetic ABA through the transpiration stream. Measurements were made 9 h from start of feeding experiment. Standard errors are shown for n = 4.

Incubation medium	ABA Fed	ABA Measured
	ng $g^{-1}FW^{-1}$	ng $g^{-1}FW^{-1}$
1- Water	0.0	133 ± 50
2- ABA fed (μmol m^{-3})		
60	26.6	372 ± 51
170	74.6	478 ± 73
1000	440.0	842 ± 85
3- Sap-C	8.8	176 ± 53
4- Sap-RC	26.6	401 ± 60

there was an inverse correlation between exogenously applied abscisic acid and xylem sap from root-cooled plants with leaf growth. Leaf area as well as leaf tissue extensibility was reduced by these treatments and both were dependent on the concentrations of abscisic acid that was fed. Exogenous ABA and xylem sap from root-cooled plants stimulated abscisic acid synthesis in leaves of excised plants. Since the plants were excised, some of the abscisic acid measured (Table 6.6) may have resulted from accumulation in leaves due to blockage of export. However, it was not the only cause, as the abscisic acid concentration in leaves increased with concentration of ABA that was fed.

CHAPTER 7

DISCUSSION

7.1- Objectives

The main aims of the work presented here were to examine the role of the root system on leaf growth and the significance of root:shoot signalling when root are subjected to adverse environmental conditions.

The approach chosen was to submit the root system to low root temperature treatment, known to affect leaf growth and several other parameters of leaf functioning, while the aerial parts were held at optimal constant temperature. By recording the short-term changes in leaf elongation rate, leaf conductance, water potential, turgor and levels of abscisic acid in leaves and xylem sap, it was possible to examine the inter-relations of these parameters and the order of response to root cooling.

7.2- Short-Term Responses of Leaf Elongation to Root Cooling

The reduction in leaf growth rate following root cooling is very rapid in all three species studied (*Phaseolus vulgaris, Helianthus annuus* and *Hordeum vulgare)* and is reversible on stopping the treatment.

Early studies by Milligan and Dale (1988*a*) using 7-day seedlings of *P. vulgaris* recognized that cooling the root system from 23°C to 10°C reduced the elongation rate (LER) of the primary leaves; measuring the elongation of the midrib with a ruler they were able to detect a significant inhibitory effect of root cooling on leaf growth within 3 - 6 h.

When the growth of primary leaves of *P. vulgaris* was continuously recorded using Rotary Variable Displacement Transducers, the response of leaf elongation to root cooling could be detected within minutes from the start of the treatments (Fig. 3.9), i.e. much earlier then previously thought.

The most striking effects of root cooling observed here, were the rapidity of the growth inhibition (lag time of around 2 min, Fig 3.9) and the completeness of the inhibition at 10°C, with smaller effect at 15°C (Fig.3.9). It suggests that the plant can sense the adverse conditions soon after the treatment starts and that a very rapid signal may be moving from root to shoot upon start of the root cooling treatment.

It has been shown that restricted water supply to shoots from the roots may be responsible for a transient change in leaf water status in response to root cooling (Milligan and Dale, 1988*a*, *b*; Dale *et al.*, 1990) and to other stress treatment such as root hypoxia (Schildwacht, 1989). It was concluded that during the hour immediately following treatment leaf elongation is

limited by leaf water status. Evidence for leaf water deficit immediately after root cooling was found in this study (see Chapter 4) and therefore, it can be suggested that the same mechanism may be operating from the onset of treatment.

7.3- Reversibility of the Response

The other equally rapid response observed in this study was the increase in leaf elongation rate when root cooling is stopped (Fig. 3.11) but the mechanism controlling this response is not well established. Milligan and Dale (1988*b*) found that 4 h after the start of root cooling estimated leaf turgor was slightly greater than for the controls whose roots were at 23°C and these findings are now confirmed (Figs. 4.1, 4.2). Yet here the leaves grow very much more slowly (Fig. 3.12) making difficult the interpretation that, on reversing root temperature, increasing bulk turgor, leads to the fast growth response observed unless the wall yield threshold (Y) is greater in treated plants.

Measurements of Y and effective turgor for growth (P-Y) can now be achieved rapidly (Cosgrove, 1987; Matyssek *et al.*, 1988) and such investigations, though not attempted here, should aid the explanation of such a rapid change in growth on stopping root cooling. The only available evidence for bean leaves on root-cooled plants (Milligan and Dale, 1988*b*), suggests that Y is

lower at least after 24 h treatment.

The possibility that leaf cells undergo wall loosening during the period of low turgor and "store growth" (Acevedo, Hsiao and Henderson, 1971) may provide the mechanism by which *Phaseolus*, and *Helianthus* plants resume growth after reversing the root cooling treatment. Since turgor affects the rate of H^+ efflux (Cleland, 1967), any factors that affect turgor may affect wall extensibility (Cleland, 1986).

It is also possible that, on reversing root cooling, changes in the gradients of water potential occur, extending from the xylem to the growing tissue causing water entry for cell enlargement, as was reported for soybean (Nonami and Boyer, 1990*a*; 1989; Boyer and Nonami, 1990) and maize (Westgate and Boyer, 1884; Michelena and Boyer, 1882).

7.4- Sequence of Events after Root Cooling

There may be several stages involved in the reduction of leaf elongation rate in response to root cooling. In view of the rapidity of the response, it is reasonable to suggest that firstly, the initial cause of leaf growth reduction is due to water deficit caused by the reduction in water flux. This will cause a transient reduction in turgor and consequently growth will decrease. Secondly, a rapid mechanism must operate to close stomata and reduce transpiration, avoiding water

loss and allowing turgor to be regained. At the same time, changes in cell wall characteristics may be elicited so that a low leaf elongation rate can be maintained even when leaf turgor is regained.

The interpretation follows that a signal originating in roots has immediate but potentially short-lived effects on leaf growth while at the same time initiating a local control mechanism which can maintain a low rate of leaf growth for as long as the adverse conditions remain, but which is flexible enough to be reversed when more favourable conditions return.

Are these above mechanisms explained by the rise in leaf ABA observed here? Abscisic acid is known to regulate stomatal closure (Zeevaart and Creelman, 1988) and has been shown to reduce the rate of leaf growth of *Phaseolus* (Van Volkenburgh and Davies, 1983) by reducing cell wall extensibility, although the effect may have been mediated by an associated reduction in stomatal aperture.

In bean leaves, both stomatal conductance and cell wall extensibility fall rapidly after root cooling. The rise in leaf ABA was also rapid supporting the view that ABA could be mediating these effects (Dale *et al.*, 1990). Therefore, a possible sequence of events could be that root cooling caused the accumulation of ABA in leaves which induced stomatal closure, and inhibited cell growth by reducing cell wall extensibility.

Turgor loss is the critical parameter of water

relations that stimulates ABA synthesis in the mesophyll cells of the leaf (Creelman and Zeevaart, 1985; Ackerson and Radin, 1983, Pierce and Raschke, 1980). Although the values for leaf turgor estimated here after the onset of root cooling did not fall to zero, it is plausible to expect that ABA accumulates in leaves as they consist of different cells which differ from each other in their osmotic potential.

7.5- Nature of the Root-to-Shoot Signal

7.5.1- Evidence for Hydraulic Signal

The mechanisms discussed above support the view (Dale *et al.*, 1990 and Milligan and Dale 1988*a*) that the prime cause of reduced leaf elongation is a fall in turgor. Leaf water potential, and turgor, fall quickly, but temporarily, on root cooling (Dale *et al.*, 1990; Milligan and Dale, 1988*b*). Confirmation of loss of leaf turgor in primary leaves of *P. vulgaris*, immediately after root cooling, was obtained in this study by direct but very preliminary, measurements using the pressure probe (Fig.4.2), indicating that leaf water deficit can precede growth reduction.

Although in *H. annuus* the growth reduction occurred slower than the almost instantaneous effect observed in bean, the response was also rapid (15 - 30 min, Fig. 3.13A) and similar to the response observed in bean plants subjected to root cooling at 15°C (Fig. 3.9B). In

both cases it was also possible to detect a transient turgor fall, which gives support for the hydraulic signal hypothesis.

There is also evidence that root cooling reduces the hydraulic conductance of the root:shoot pathway in *Phaseolus* (Milligan and Dale, 1988*b*; Davies and Van Volkenburgh, 1983; McWilliam *et al.*, 1982); sunflower (Kramer, 1969) and other species (McWilliam *et al.*, 1982; Markhart *et al.*, 1979; Kramer, 1969); root cooling also reduced water permeability of the root tissue in *Phaseolus* (Brouwer, 1964) and thus the water supply to the leaves may also be reduced.

A rapid leaf growth response to root cooling was also observed in *Hordeum vulgare*. Although in this study turgor pressure was not determined in the growing zone of barley leaves, changes in water relations may determine the growth response to root cooling in barley plants, as suggested by MacDuff and Jackson, (1991) and in other monocotyledons species (Setter and Greenway, 1988 (in rice); Kleinendorst and Brouwer, 1972 (in maize)).

The evidence that the hydraulic signal mediates the transient leaf water deficit and the initial growth reduction observed in this study is in agreement with results reported for *Phaseolus* plants subjected to other forms of root stress such as root hypoxia (Neuman and Smit, 1991), and increased salinity (Neuman, Van Volkenburgh and Cleland, 1988). However, these data

conflict with reports that attribute changes in leaf growth and stomatal conductance of pea plants with oxygen-deficient roots entirely to a metabolically-produced root signal (Zhang and Davies, 1987; Jackson and Kowalewska, 1983).

It should be noted that in the case of root cooling treatment imposed here and that reported by Milligan and Dale (1988*a*, *b*) and that of root hypoxia (Neuman and Smit, 1991) the period of water deficit was brief, implying that if measurements of leaf water status had been made at longer intervals no changes in water relations would have been recorded.

7.5.2- Evidence for a Chemical Signal Moving From Roots

In the case of the root cooling treatment imposed here, and in the field where plants can experience conditions of low soil temperature (see Chapter 1), the roots may play a central role in regulating how the plant adjusts to these conditions, as seems to be the case for other environmental stresses.

It has been proposed that for plants subjected to soil drying, leaf elongation rate and stomatal conductance are more closely associated to water contents of roots and soil than leaves. Stomatal behaviour and leaf growth may respond to soil drying before changes in leaf water status occurs (see Davies and Zhang, 1991 for review).

Supporting this hypothesis, Zhang, Gowing and Davies (1990); Zhang and Davies (1990*a*, *b*, 1989*a*, *b*); Zhang, Schurr and Davies (1987) showed that, at least for maize and sunflower plants, roots are the primary sensor of soil drying and that ABA from the root system, produced in response to reduced root turgor, is transported to the leaves via the transpiration stream, to cause reduced leaf conductance before any sign of leaf water deficit is detected. By this mean the plant is able to cope with the adverse environmental conditions by determining the availability of water in the soil and regulating growth rate and gas exchange accordingly.

The possibility that the rise in ABA in leaves of root-cooled bean plants is the result of import of ABA passing from roots to shoots has been examined in this study and the evidence fails to support this hypothesis. The total amount of ABA in xylem sap exudate of root-cooled bean plants did not differ from that of control plants at optimal root temperature (Fig 5.3B, D), although concentrations differ (Fig. 5.3A,C).

Interpretations of ABA contents in xylem sap collected from detopped plants are complicated by doubts about the actual volume flux. One of the criticism is that the volume flux of water would be many times lower from the roots after detopping than when the plants were transpiring and so ABA would be concentrated (Munns, 1990; Zhao, Munns and King, 1991). However, ABA assay of samples of xylem sap collected sequentially from both

control and root-cooled plants did not show that it was the case. In fact the first few hundred microlitres of sap, which should be the sap which was in the xylem vessels before the plants were detopped showed the highest ABA concentration.

The use of the balancing pressure approach described by Passioura and Munns (1984) was not appropriate for here the plants were hydroponically grown and the pressure could not be imposed to the cooling bath. In addition, comparison of three methods of sap collection (centrifuging, collection of exudate and pressurizing) using sunflower and maize plants showed that collection of sap exudate from stem stumps is a reliable method of sampling for ABA assay (Zhang and Davies, 1990*b*). Similar conclusion was drawn by Neales and McLeod (1991) with sunflower plants.

Considering the mean transpiration rate of primary leaves is 3.5 mmol $m^{-2}s^{-1}$ (Table 4.1) and their area around 10 cm^2 (see Appendix A.1), bulk flow to the leaves should be equal 226 μl $leaf^{-1}h^{-1}$. If the ABA content in xylem sap of cooled root was 0.016 ng μl^{-1} (the highest value from Figure 5.3A, C) then only 3.6 ng of ABA would go to the leaves over 1 h. This is much lower than the ABA measured in bean leaves on root-cooled intact plants (see section 6.6, Chapter 6). This estimated amount is very close to that presented by Smith and Dale, (1989). It is important to note that the calculations presented here, and those presented by

Smith and Dale, take no account of the fact that transpiration rate in leaves of root-cooled plants was reduced by about 30% (Table 4.1) over the controls as a result of stomatal closure (Fig. 4.5), nor of the reduced flux due to changes in root hydraulic conductivity on cooling (Dale *et al.*, 1990). Therefore, the actual amount of abscisic acid in xylem sap of root-cooled plants could be much lower than the 3.6 ng calculated above.

In addition, if the rise in leaf ABA is due to an increased concentration in the transpiration stream one might expect it to occur for non-expanding, mature leaves as well since these are freely transpiring. This was not the case for barley where the first leaf showed no change in ABA following root cooling; on the contrary, the older leaf seems to export ABA to the younger (see below).

These findings and the fact that the rise in leaf ABA occurs within 15 min of the commencement of treatment (Fig. 5.1) strengthen the view that the rise in leaf ABA in root cooled plants is mainly due to local synthesis in the leaves themselves (Dale *et al.*, 1990; Smith and Dale, 1988). However, the possibility that the stimulus provoking this comes from roots cannot be ruled out.

It has also been proposed that the increased leaf abscisic acid observed in pea plants subjected to root hypoxia is due to restricted transport from leaves to hypoxic roots (Jackson and Hall, 1987). In the present

study, *Phaseolus* primary leaves on detopped plants showed a rise in ABA concentration (133 ng g^{-1}FW) compared to the value of 50 ng g^{-1}FW for leaves on control intact plants (Fig. 5.1), suggesting that blockage of ABA export from leaves may have occurred.

Abscisic acid has been shown to occur in both xylem and phloem. Wolf, Jeschke and Hartung (1990) showed that in NaCl-treated *Lupinus* plants, the transport of ABA in xylem and phloem increased 10- and 5-fold respectively and that the ABA present in xylem is originated part in roots and part in the leaves. This ABA originated in leaves was transported in the phloem to the roots and recirculated back to the aerial parts via xylem (Wolf *et al.*, 1990). In osmotically stressed sunflower plants, the leaves were the main source of abscisic acid synthesis present in xylem sap (Hoad, 1975).

Abscisic acid metabolites have also been demonstrated to occur in xylem as well as in phloem (Hoad and Gaskin, 1980; Zeevaart, 1977) therefore, it does not necessarily follow that the occurrence of ABA and its metabolites in a particular organ can be interpreted as evidence of synthesis and metabolism in that organ (Loveys and Milborrow, 1984).

In *Phaseolus* plants subjected to root cooling it is possible that xylem sap contains a compound which is effective in mediating the leaf growth and stomatal conductance responses and/or to stimulate ABA synthesis in leaves. Xylem sap from root-cooled plants was as

effective in reducing cell wall extensibility (Table 6.3) and stomatal conductance in derooted plants as was the root cooling treatment (Figs. 4.8 and 4.4) in intact plants. It is particularly important to note that, in addition to these effects, xylem sap from root-cooled plants induced ABA synthesis in leaves (Table 6.6). This effect was also observed using exogenous applied ABA. Equivalent concentrations of ABA in xylem sap and ABA solution gave similar responses. These results suggest that ABA present in xylem sap may stimulate ABA synthesis in leaves, and that the responses involving high concentrations of ABA in the leaf may be indirectly mediated by ABA coming from the roots.

Trejo and Davies (1991) have shown that the concentration of ABA in xylem sap did not rise in water-stressed *Phaseolus* plants up to day 5 after the onset of treatment. After this period, ABA in xylem sap rises only slightly compared to that of the control. They concluded that the amount of ABA measured in xylem sap of bean plants was not enough to induce stomatal closure in maize and sunflower. They also postulated that *Phaseolus* plants are either very sensitive to small amounts of ABA or there must be an additional factor in the sap acting synergistically with ABA. This view is shared by others (Zhao *et al.*, 1991; Munns, 1990; Munns and King, 1988).

Munns and King (1988) found that although xylem sap from wheat plants in drying soil was effective in

inhibiting transpiration, the amount of ABA present in the sap was not enough to account for this effect and removal of ABA from xylem sap using a immunoaffinity column did not eliminate the inhibitory effect. They concluded that xylem sap from wheat plants in drying soil contains an inhibitor of transpiration, suggesting that this unknown inhibitor could be a precursor or regulator of ABA synthesis.

ABA content also rose in expanding leaves of *Helianthus annuus* and *Hordeum vulgare* plants subjected to root cooling treatment at 10°C (Tables 5.1A, B and 5.2). It was not possible to analyse the amounts of ABA present in xylem sap for these species in this study, but such data might help to explain the source of ABA in leaves of plants subjected to root cooling.

For sunflower, the evidence available from other studies suggests that the absolute concentration of ABA in xylem sap of root-cooled (5°C) plants was low and did not differ from that in control plants (Durka, Gollan and Schurr, 1990). For osmotically stressed sunflower plants, Neales and McLeod, (1991) found that roots as well as leaves were contributing to the ABA present in xylem sap. For barley plants, Zhao *et al.*, (1991) showed that the rise in leaf ABA of NaCl-treated plants was due to the response of roots to the salinity and not triggered by leaf water deficit.

It is also possible that in sunflower and barley plants, older leaves which showed a reduction in ABA

contents over 24 h, act as a source and export ABA to younger actively expanding leaves, as reported for *Xanthium* (Cornish and Zeevaart, 1984). However, for bean plants it seems unlikely that older organs e.g. cotyledons act as a source for ABA to the primary leaves (Smith and Dale, 1988).

If ABA present in xylem sap stimulates ABA synthesis in leaves, it could explain the rise in leaf ABA when root cooling treatment was carried out with plants in polythene bags to give high relative humidity around the aerial parts and to prevent water loss (Figure 7.1A). This treatment prevented leaf dehydration and there was no reduction in turgor compared with control plants grown at standard conditions (Fig 7.1B). In this circumstance leaf elongation rate was reduced on root cooling but more slowly than in conditions allowing turgor loss. Reduction in stomatal conductance was observed concomitant with the rise in leaf ABA.

7.6- Role of Endogenous Abscisic Acid

The speed of the growth response to root cooling appears to rule out an effect on extension involving, for example, the synthesis of plant growth regulators. However, the fall in leaf turgor that occurs at the onset of the treatment may trigger hormone synthesis in leaves and the longer-term reduction in extension rate, after gross water stress is relieved (Dale *et al.*, 1990;

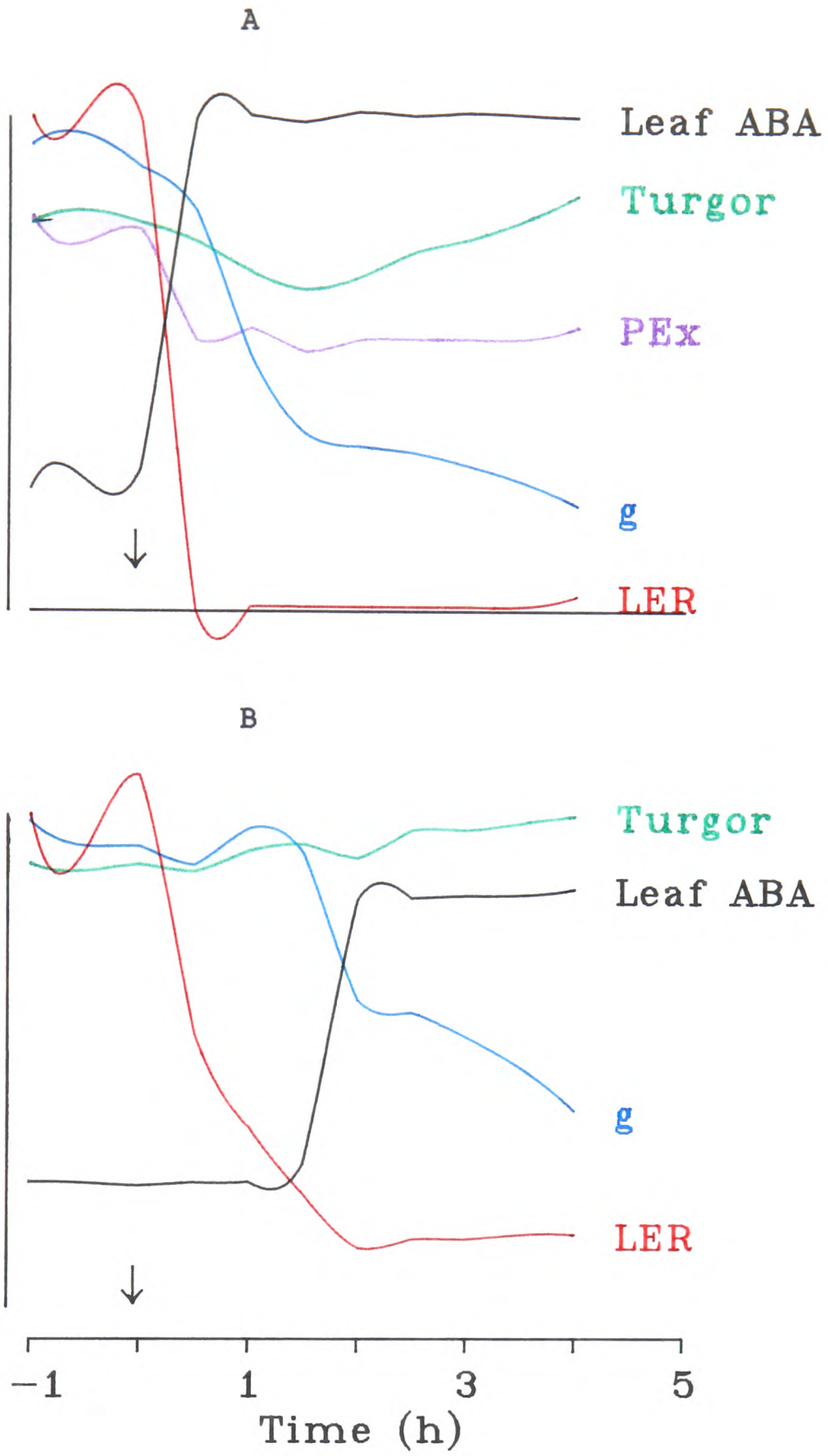


Figure 7.1- Time course of responses to root cooling. Aerial parts at high (A) or low (B) vapour pressure. The arrows indicate the start of root cooling.

Milligan and Dale, 1988).

In this is the case, abscisic acid is a candidate since it accumulates in bean leaves in response to root cooling treatment (Dale *et al.*, 1990; Smith and Dale, 1988) and the 4-fold increase in leaf ABA found in this study confirms earlier findings. ABA also increased in leaves of several species in response to other environment stress such drought (Henson, Jensen and Turner, 1989), flooding and oxygen depletion (Smit *et al.*, 1990; Jackson and Hall, 1987; Jackson, 1985; Wadman-van Schravendijk and van Andel, 1985) and salinity (Zhao *et al.*,1991).

Dale *et al.*, (1990) showed that when bean leaves on root-cooled plants regained turgor within 4 h from the start treatment the levels of ABA had already risen; they suggested that the endogenous ABA might be reducing wall plastic extensibility thus keeping the growth rate low whilst the root cooling treatment lasts.

The short-term analysis of the effects of root cooling on leaf ABA (Fig. 5.1) and cell wall extensibility (Fig. 4.8) of intact bean seedlings showed an inverse correlation between ABA concentration and leaf extensibility. This finding confirms the view that there might be a link between endogenous ABA and reduced leaf growth through an effect on wall plastic extensibility.

This idea is reinforced by experiments where exogenous ABA solutions were fed to excised seedlings

and leaf strips. In these experiments both leaf area (Table 6.1) and wall plastic extensibility (Table 6.2, 6.3) were reduced by ABA. Wall plastic extensibility was significantly reduced by ABA concentration of 60 μmol m^{-3}. It was somewhat surprising that this low concentration was effective in reducing extensibility but this treatment stimulated a 2-fold increase in leaf ABA, compared with the initial value. The amount of ABA measured in this treatment (370 ng g^{-1} FW) is slightly greater than that observed in leaves of intact plants subjected to root cooling (Fig. 5.1).

Exogenous ABA has been shown to reduce growth of *Phaseolus* leaves (Van Volkenburgh and Davies, 1983) and maize coleoptiles (Kutschera and Schopfer, 1986). In both cases, the effect of ABA was attributed to its effect on reduction of cell wall extensibility.

Considering the fact that there was an inverse correlation between endogenous leaf ABA and wall extensibility on root-cooled plants, and that applied ABA mimicked the effects of root cooling on leaf extensibility, it seems reasonable to suggest that ABA might be mediating the effects of root cooling treatment.

Changes in cell wall extensibility may result from metabolic loosening which involve breakage of load bearing bonds and deposition of new material in the wall. Cell wall extension may also depend on supply of wall loosening factor (Rayle and Cleland, 1972; Cleland,

1981). According to the acid-growth hypothesis (Cleland, 1981) any agent which causes the cell to excrete H^+ into the wall space may stimulate growth by increasing cell wall extensibility. The acidification of the cell wall, possibly involving activation of hydrolytic enzymes, catalyses the hydrolysis of polysaccharide bonds allowing wall components to move apart or slide past each other, inducing wall loosening. Treatment with growth inhibitor may retard cell growth by the peroxidase-catalysed crosslinking of phenolic side chains on wall polysaccharides and glycoproteins (Fry, 1986; 1988).

The very rapid, almost instantaneous reversibility of the root cooling response suggest that the cell wall rheological properties can readily be altered. The fact that, on returning roots to the higher temperature, rates of leaf elongation are initially higher than the controls suggest that the potential for expansion already exist or is acquired very quickly. Here, two possibilities must be considered: i) if wall extensibility restricts expansion it must change rapidly, within minutes and ii) if leaf ABA is causing the reduced wall extensibility the levels of this hormone in leaves must change equally rapidly.

Results from Smith and Dale (1988) showed that bulk leaf ABA in bean plants decreased slowly on stopping root cooling treatment and took about 2 - 4 h to be reduced to half of the previous value. Measurements of

wall plastic extensibility on reversing root cooling treatment (Fig. 4.8) showed that this parameter increased only slightly during growth recovery i.e. coinciding with the decrease in ABA.

Acevedo *et al.*, (1971) have suggested that the rapid recovery in leaf extension rate when water stressed maize plants were rewatered was due to the ability of plants to "store growth". As stress develops, wall material may be synthesised and stored in the cytosol or incorporated into the wall. Upon relief of stress, rapid extension may occur by expanding the preformed wall or by drawing on preformed materials (Lawlor and Leach, 1985).

The results showed in this study suggest that a causal relationship exist between ABA and the inhibition of leaf growth through its effect on wall plastic extensibility. However the mechanisms by which ABA reduces extensibility is not clear yet. It could be either a prevention of wall loosening or a positive stiffening.

In bean leaves it was suggested that the mechanism by which ABA reduces wall extensibility is apparently by reduction in the capacity of the cell to respond to acidity (Van Volkenburgh and Davies, 1983). There is also increased evidence for the role of abscisic acid in ameliorating chilling resistance in plants (Pan, 1990). During cold hardening, plants showed a transient increase in ABA content (Chen, Li and Brenner, 1983),

suggesting that ABA may play a role in the adaptation to cold stress. ABA was found to stabilize microtubules in cold-hardened cotton cotyledons and this might aid the flow of structural elements to the wall (Rikin, Atsmon and Gitler, 1983). In germinating seeds of chick-pea, ABA was shown to affect the cell wall composition by a pH-induced inhibition of α-galactosidase activity (Labrador, Rodriguez and Nicolas, 1987). Since peroxidases are thought to be involved in wall stiffening by supporting cross-linking reactions (Goldberg, Liberman, Mathieu, Pierron and Catesson, 1987) it could be of value to investigate whether ABA can induce wall peroxidases during root cooling.

7.7- Conclusion

From the results presented in this study, a possible mechanism by which root cooling leads to reduced leaf growth is proposed in Figure 7.2. The most direct short-term effect of root cooling is to reduce hydraulic conductance of the root shoot pathway and water permeability, consequently lowering the water supply to the leaves. A transient fall in leaf turgor (P) will be generated, thus reducing leaf elongation rate (LER) almost instantaneously. At the same time, low turgor will reduce cell wall plastic extensibility (PEx) and stomatal conductance (*g*) to maintain elongation rates low and to prevent excessive water loss. Concurrently,

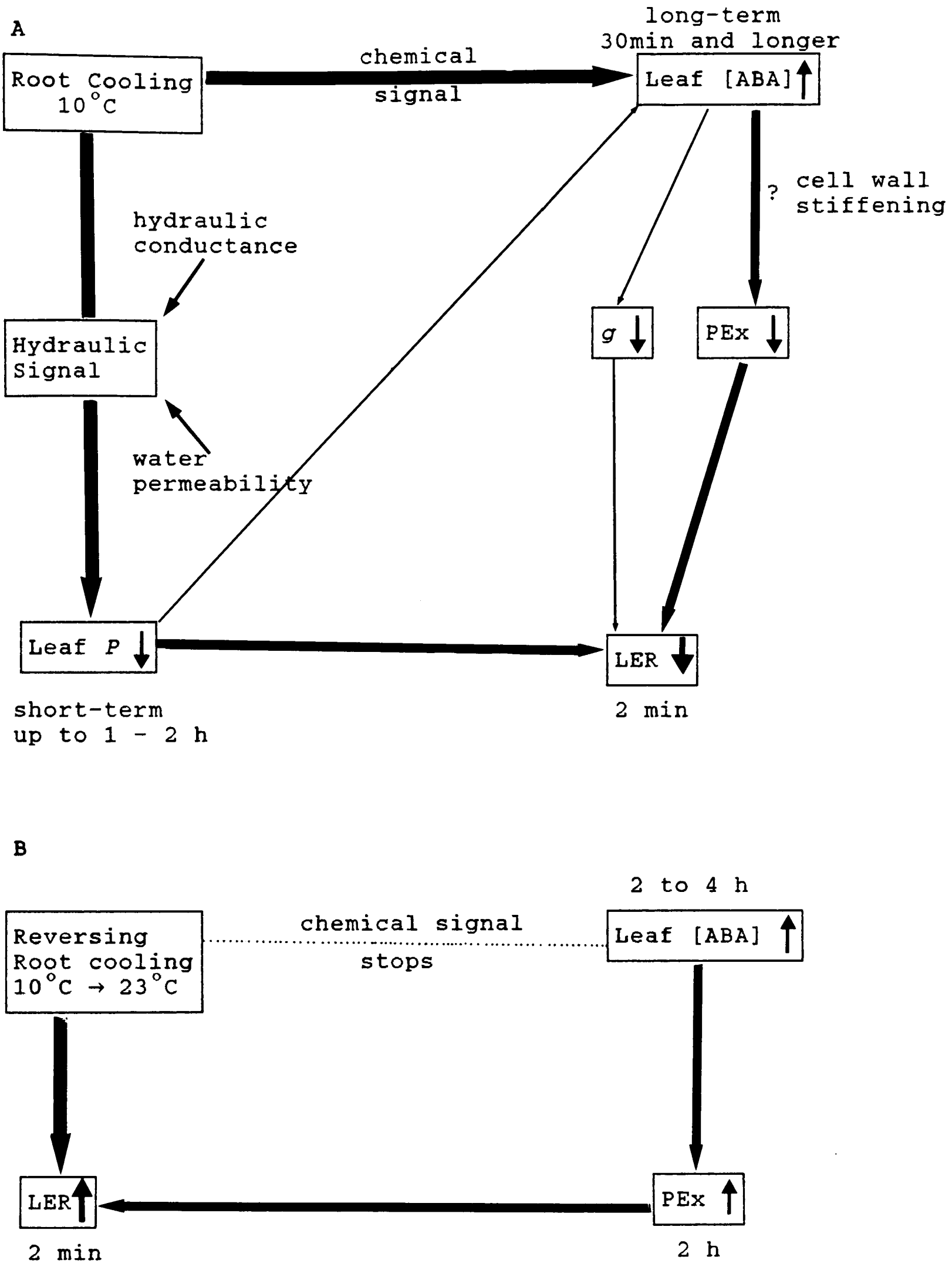


Figure 7.2- Diagram of the leaf responses to root cooling (A) and reversal of treatment (B).

another regulatory mechanism is generated in roots operating to send a chemical signal (s) to stimulate ABA synthesis in leaves and maintaining the levels high.

It is suggested that the main chemical signal is ABA which stimulates its own synthesis while unfavourable conditions last. During the period of root cooling the cells can adjust osmotically and recover their initial turgor.

On reversing root cooling, the rapidity of the growth recovery suggests that again a very rapid and sensitive mechanism is operating. It is possible that rapid changes in water potential gradients extending from the growing tissue through the xylem favour water entry to the enlarging tissue so that growth can rapidly overshoot the control value. At the same time the root signal no longer operates so that ABA synthesis in the leaves is reduced while the rates of catabolism and compartmentation may increase, and ABA effects on cell wall plastic extensibility cease.

Although the evidence presented suggests that the effects of root cooling treatments at the level of the leaf cell might be mediated by abscisic acid affecting cell wall extensibility, more studies are required to unravel the effects of this hormone on leaf growth. In this area, it is valuable to investigate whether abscisic acid induces the activity of cell wall peroxidases which control the wall stiffening process, and whether the effect, if any, can be rapidly reversed.

REFERENCES

ACEVEDO, E., HSIAO, T.C. & HENDERSON, D.W., 1971. Immediate and subsequent growth responses of maize leaves to changes in water status. *Plant Physiol.*, **48**, 631-636.

ACKERSON, R.C. & RADIN, J.W., 1983. Abscisic acid accumulation in cotton leaves in response to dehydration at high pressure. *Plant Physiol.*, 71, 432-433.

ADDICOTT, F.T. & CARNS, H.R., 1983. History and Introduction. In: *Abscisic Acid*. F.T. Addicott (ed.). New York, Praeger. pp. 1-21.

ATKIN, R.K., BARTON, G.E. & ROBINSON, D.K., 1973. Effect of root-growing temperature on growth substances in xylem exudate of *Zea mays*. *J. Exp. Bot.*, **24**, 475-487.

BAKER, N.R., DAVIES, W.J. & ONG, C.K., 1985. *Control of Leaf Growth*. Soc. Exp. Biol. Seminar Serie **27**. Cambridge: Cambridge University Press. 350 pp.

BLACKMAN, P.G. & DAVIES, W.J., 1985. Root to shoot communication in maize plants of the effect of soil drying. *J. Exp. Bot.*, **36**, 39-48.

BOYER, J. S., 1968. Relationship of water potential to growth of leaves. *Plant Physiol.*, **43**, 1056-1062.

BOYER, J. S., CAVALIERI, A.J. & SCHULZE, E.-D., 1985. Control of the rate of cell enlargement: excision, wall relations, and growth-induced water potential.

Planta, **163**, 527-543.

BOYER, J. S. & NONAMI, H., 1990. Xylem hydraulics, turgor and wall properties during growth. In: *Importance of Root to Shoot Communication in the Responses to Environmental stress*. W.J. Davies & B. Jeffcoat (eds). British Society for Plant Growth Regulation, Monograph 21. pp 45-52.

BRADFORD, K.J. & YANG, S.F., 1980. Xylem transport of 1-aminocyclopropane-1-carboxylic acid, an ethylene precursor, on waterlogged tomato plants. *Plant Physiol.*, **65**, 322-326.

BROUWER, R., 1962. Distribution of dry matter in the plant. *Neth. J. Agric. Sci.*, **10**, 361-376

BROUWER, R., 1964. Response of bean plants to root temperatures. I. Root temperature and growth in the vegetative stage. *Jahrbuch Instituut voor Biologisch Onderzoek van Landbouwgewassen, Wageningen*, 11- 22.

BROUWER, R.& HOOGLAND, A., 1964. Responses of bean plants to root temperature. II. Anatomical aspects. *Jahrbuch Instituut voor Biologisch Onderzoek van Landbouwgewassen, Wageningen*, 23-31.

BROWN, R.B. & RICKLESS, P., 1949. A new method for the study of cell division and cell extension with some preliminary observations of the effects of temperature and nutrients. *Proc. R. Soc.*, Series B. **136**, 110-125.

BURROWS, W.J. & CARR, D.J., 1969. Effects of flooding the root system of sunflower plants on the cytokinin

content of the xylem sap. *Physiol. Plant.*, 22, 1105-1112.

CARR, D.J. & REID, D.M., 1968. The physiological significance of the synthesis of hormones in roots and their export to the shoot system. In: *Biochemistry and Physiology of Plant Growth Substances*. F. Wightman and G. Setterfield (eds). Runge Press, Ottawa. pp 1169-1185.

CHRIST, R.A.,1978*a*. The elongation rate of wheat leaves. I- Elongation rates during day and night. *J. Exp. Bot.*, 29, 603-610.

CHRIST, R.A., 1978*b*. The elongation rate in wheat leaves. II- Effects of sudden light change on the elongation rate. *J. Exp. Bot.*, 29, 611-618.

CIHA, A.J., BRENNER, M.L. & BRUN, W. A., 1977. Rapid separation and quantification of abscisic acid from plant tissues using High Performance Liquid Chromatography. *Plant Physiol.*, 59, 821-826.

CLARKSON, D.T., 1976. The influence of temperature on the exudation of xylem sap from detached root system of rye (*Secale cereale*) and barley (*Hordeum vulgare*). *Planta*, 132, 297-304.

CLARKSON, D.T., EARNSHAW, M.J., WHITE, P.J. & COOPER, H.D., 1988. Temperature dependent factors influencing nutrient uptake: An analysis of responses of different levels of organization. In: *Plant and Temperature*. S.P. Long & F.I. Woodward (eds). Symposia of the Soc. Exp. Biol. pp. 281-309.

CLARKSON, D.T., HOPPER, M.J. & JONES, L.H.P., 1986. The effect of root temperature on the uptake of nitrogen and the relative size of the root system in *Lolium perenne*. I- Solution containing both NH_4 and NO_3. *Plant, Cell Environ.*, **9**, 535-545.

CLELAND, R.E., 1967. Extensibility of isolated cell walls; measurements and changes during cell elongation. *Planta*, **74**, 196-209.

CLELAND, R.E., 1981. Wall extensibility: hormones and wall extension. **In:** Plant Carbohydrates, Vol. II., Extracellular carbohydrates. Encycl. Plant Physiol., N.S. **13B**, W. Tanner, F.A. Loewus (eds). Springer, Heidelberg. pp 255-273.

CLELAND, R.E., 1986. The role of hormone in wall loosening and plant growth. *Aust. J. Plant Physiol.*, **13**, 93-103.

CLOUGH, B.F. & MILTHORPE, F.L., 1975. Effects of water deficit on leaf development in tobacco. *Aust. J. Plant Physiol.*, **2**, 291-300.

COOPER, A.J., 1973. *Root Temperature and Plant Growth*. Commonwealth Bureau of Horticulture and Plantation Crops, Research Review no 4. East Malling, Maidstone, Kent. 73 pp.

CORNISH, K. & ZEEVAART, J.A.D., 1984. ABA metabolism in relation to water stress and leaf age in *Xanthium strumarium*. *Plant Physiol.*, **76**, 1029-1035.

CORNISH, K. & ZEEVAART, J.A.D., 1985*a*. Movement of abscisic acid into the apoplast in response to water

stress in *Xanthium strumarium* L. *Plant Physiol.*, 78, 623-626.

CORNISH, K. & ZEEVAART, J.A.D., 1985*b*. Abscisic acid accumulation by roots of *Xanthium strumarium* L. and *Lycopersicon esculentum* Mill. in relation to water stress. *Plant Physiol.*, 79, 635-658.

COSGROVE, D.J., 1986. Biophysical control of plant cell growth. *Ann. Rev. Plant Physiol.*, 37, 377-405.

COSGROVE, D.J., 1987. Wall relaxation in growing stems: comparison of four species and assessment of measurement techniques. *Planta*, 171, 266-278.

COWAN, I.R., 1982. Regulation of water use in relation to carbon gain in higher plants. In: *Physiological Plant Ecology II*. O.L. Lange, P.S. Nobel, C.B. Osmond & H. Ziegler (eds). Berlin: Springer-Verlag. pp 589-614.

COWAN, I.R., RAVEN, J.A., HARTUNG, W. & FARQUHAR, G.D., 1982. A possible role for abscisic acid in coupling stomatal conductance and photosynthetic carbon metabolism in leaves. *Aust. J. Plant Physiol.*, 9, 489-498.

CREELMAN, R.A. & ZEEVAART, J.A.D., 1985. Abscisic acid accumulation in spinach leaf slices in the presence of penetrating and nonpenetrating solutes. *Plant Physiol.*, 77, 25-28.

CROZIER, A., SANDBERG, G., MONTEIRO, A.M. & SUNDBERG, B., 1986. The use of immunological techniques in plant hormone analysis. In: *Plant Growth Substances*

1985. M. Bopp (ed). Spring -Verlag. pp 13-21.

DALE, J.E., 1964. Some effects of alternating temperature on growth of French bean plants. *Ann. Bot.*, **28**, 127-135.

DALE, J.E., 1988. The control of leaf expansion. *Ann. Rev. Plant Physiol.*, **39**, 267-295.

DALE, J.E. & MILTHORPE, 1983. *The Growth and Functioning of Leaves*. Cambridge: Cambridge University Press. 540 pp.

DALE, J.E., STACCIARINI SERAPHIN, E. & SATTIN, M., 1990. The effects of root cooling on leaf growth. **In:** *Importance of Root to Shoot Communication in the Responses to Environmental stress*. W.J. Davies & B. Jeffcoat (eds). British Society for Plant Growth Regulation, Monograph 21. pp. 149-161.

DAVIDSON, R.L., 1969. Effect of root/leaf temperature differential on root/shoot ratios in some pasture grasses and clover. *Ann. Bot.*, 33, 561-569.

DAVIES, E., 1987. Action potentials as multifunctional signals in plants: a unifying hypothesis to explain apparently disparate wound responses. *Plant, Cell Environ*, **10**, 623-631.

DAVIES, E., 1990. Plant wound signal and translation. **In:** *Plant Growth Substances 1988*. R.P. Pharis & S.B. Rood (eds). Springer-Verlag Berlin Heidelberg. pp 519-530.

DAVIES, W. J. & JEFFCOAT, B., 1990. *Importance of Root to Shoot Communication in Response to Environmental*

Stress. British Society for Plant Growth Regulation, Monograph 21. 398 pp.

DAVIES, W. J., MANSFIELD, T.A. & HETHERINGTON, A.M., 1990. Sensing of soil water status and the regulation of plant growth and development. *Plant, Cell and Environ.*, 13, 709-719.

DAVIES, W.J. & Van VOLKENBURGH, E., 1983. The influence of water deficit on the factors controlling the daily pattern of growth of *Phaseolus* trifoliates. *J. Exp. Bot.*, **34**, 987-999.

DAVIES, W. J. & ZHANG, J., 1991. Root signal and the regulation of growth and development of plants in drying soil. *Ann. Rev. Plant Physiol. Mol. Biol.*, **42**, 55-76.

DURKA, W., GOLLAN, T. & SCHURR, U., 1990. The effects of root-cooling on xylem sap composition and leaf gas exchange in *Helianthus annuus*. **In**: *Importance of Root to Shoot Communication in the Responses to Environmental stress*. W.J. Davies & B. Jeffcoat (eds). British Society for Plant Growth Regulation, Monograph 21. pp 313-314.

FITTER, A.H. & HAY, R.K.M., 1987. *Environmental Physiology of Plants*. 2nd ed. London: Academic Press. 423 pp.

FRY, S.C., 1986. Cross-linking of matrix polymers in the growing cell walls of Angiosperms. *Ann. Rev. Plant Physiol.*, 37, 165-186.

FRY, S.C., 1988. *The Growing Cell Wall: Chemical and*

Metabolic Analysis. Logman, London & Wiley, New York.

FUCHS, S., HAIMOVICH, J. & FUCHS, Y., 1971. Immunological studies of plant hormones. Detection and estimation by immunological assay. *Eur. J. Biochem.*, **18**, 384-390.

FUCHS, Y., MAYAK, S, & FUCHS, S., 1972. Detection and quantitative determination of abscisic acid by immunological assay. *Planta*, **103**, 117-125.

GOLDBERG, R., LIBERMAN, A.M., MATHIEU, C. PIERRON, M. & CATESSON, A.M., 1987. Development of epidermal cell wall peroxidase along the mung bean hypocotyl: possible involvement in the cell wall stiffening process. *J. Exp. Bot.*, **38**, 1378-1390.

GREEN, P. B.,1980. Organogenesis - a biophysical viewpoint. *Ann. Rev, Plant Physiol.*, **31**, 51-82.

HALPERIN, W., 1978. Organogenesis at the shoot apex. *Ann. Rev. Plant Physiol.*, **29**, 239-262.

HARTUNG, W. & DAVIES, W.J., 1991. Drought-induced changes in physiology and ABA. In: *Abscisic Acid Physiology and Biochemistry*. W.J. Davies & H.G. Jones (eds). Published in the Environmental Plant Biology series. Bios Scientific Publishers. pp 63-79.

HARTUNG, W. & RADIN, J.W., 1989. Abscisic acid in the mesophyll apoplast and the root system sap of water-stressed plants: the significance of pH gradients. *Current Topics in Plant Biochemistry*, **8**, 110-124.

HARTUNG, W., RADIN, J.W. & HENDRIX, D.L., 1988. Abscisic acid movement into the apoplast solution of water

stressed cotton leaves. *Plant Physiol.*, **86**, 908-913.

HARTUNG, W. & SLOVIK, S., 1991. Physicochemical properties of plant growth regulators and plant tissues determine their distribution and redistribution: stomatal regulation by abscisic acid in leaves. *New Phytol.*, **119**, 361-382.

HENSON, I.E., JENSEN, C.R. & TURNER, N.C., 1989. Leaf gas exchange and water relations of lupins and wheat. III. Abscisic acid and drought-induced stomatal closure. *Aust. J. Plant Physiol.*, **16**, 429-442.

HOAD, G.V., 1975. Effects of osmotic stress on abscisic acid levels in xylem sap of sunflower (*Helianthus annuus*). *Planta*, **124**, 25-29.

HOAD, G.V. & GASKIN, P., 1980. Abscisic acid and related compounds in phloem exudate of *Yucca flaccida* Haw and coconut (*Cocos nucifera* L). *Planta*, **150**. 347-348.

HSIAO, T.C., ACEVEDO, E., FERERES, E. & HENDERSON, D.W., 1976. Stress metabolism: water stress, growth, and osmotic adjustment. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **273**, 479-500.

HUMPHRIES, E.C. & WHEELER, A.W., 1963. The physiology of leaf growth. *Ann. Rev, Plant Physiol.*, **14**, 385-410.

HUNT, R., 1982. *Plant Growth Curves. The Functional Approach to Plant Growth Analysis.* London, Edward Arnold. 248 pp.

HUNT, R., 1990. *Basic Growth Analysis.* London, Unwin Hyman. 112 pp.

ITAI, C. & VAADIA, Y., 1965. Kinetin-like activity in

root exudate of water stressed sunflower plants. *Physiol. Plant.*, 18, 441-444.

ITAI, C. & VAADIA, Y., 1971. Cytokinin activity in water-stressed shoots. *Plant Physiol.*, **47**, 87-90.

JACKSON, M.B., 1985. Response of leafed and leafless peas to soil waterlogging. **In:** *The Pea Crops. A Basis for Improvement.* P.B. Hebblethwaite, M.C. Heath & T.C.K. Dawkins (eds). Butterworths, London, pp 163-172.

JACKSON, M.B., 1990. Communication between the roots and shoots of flooded plants. **In:** *Importance of Root to Shoot Communication in the Responses to Environmental Stress*. W. J. Davies & B. Jeffcoat (eds). British Society for Plant Growth Regulation, Monograph 21. pp. 115-133.

JACKSON, M.B., DREW, M.C. & GIFFARD, S.C., 1978. Effects of applying ethylene to the root system of *Zea mays* on growth and nutrient concentration in relation to flooding tolerance. *Physiol. Plant.*, **52**, 23-28.

JACKSON, M.B. & HALL, K.C., 1987. Early stomatal closure in waterlogged pea plants is mediated by abscisic acid in the absence of foliar water deficits. *Plant, Cell and Environ.*, **10**, 121-130.

JACKSON, M.B. & KOWALEWSKA, A.K.B., 1983. Positive and negative messages from roots induce foliar desiccation and stomatal closure in flooded pea plants. *J. Exp. Bot.*, **34**, 493-506.

JOHNSON, I.R. & THORNLEY, J.H.M., 1987. A model of

shoot:root partitioning with optimal growth. *Ann. Bot.*, **60**, 133-142.

JONES, H.G., 1980. Interaction and integration of adaptive responses to water stress: the application of an unpredictable environment. In: *Adaptation of Plant to Water and High Temperature Stress*. N.C. Turner & P.J. Kramer (eds). New York: Wiley. pp 353-365.

JONES, H.G., 1987. Correction for non-specific interference in competitive immunoassays. *Physiol. Plant.*, **70**, 146-154.

JONES, H.G., 1990. Control of growth and stomatal beraviour at the whole plant level: effects of soil drying. In: *Importance of Root to Shoot Communication in the Responses to Environmental Stress*. W. J. Davies & B. Jeffcoat (eds). British Society for Plant Growth Regulation, Monograph 21. pp. 81-93.

KAYS, S.J. & PALLAS, J. E., 1980. Inhibition of photosynthesis by ethylene. *Nature*, **285**, 51-52.

KENDE, H. & SITTON, D., 1967. The physiological significance of kinetin and gibberellin-like root hormones. *Ann. New York Academy Science*, **144**, 235-243.

KLEINENDORST, A. & BROUWER, R., 1970. The effect of temperature of the root medium and of the growing point of the shoot on growth, water content and sugar content of maize leaves. *Neth. J. Agric. Sci.*, **18**,

140-148.

KLEINENDORST, A. & BROUWER, R., 1972. The effect of local cooling on growth and water content of plants. *Neth. J. Agric. Sci.*, 20, 203-217.

KRAMER, P.J., 1969. *Plant and Soil Water Relationships: A Modern Synthesis*. New York, McGraw-Hill.

KRAMER, P.J., 1983. *Water Relations of Plants*. San Diego: Academic Press, Inc. 489 pp.

KRAMER, P.J., 1988. Changing concepts regarding plant water relations. *Plant, Cell and Environ.*, **11**, 565-568.

KUTSCHERA, U. & SCHOPFER, P., 1986. Effects of auxin and abscisic acid on cell wall extensibility in maize coleoptiles. *Planta*, **167**, 527-535.

LABRADOR, E., RODRIGUEZ, D. & NICOLAS, G., 1987. Change in cell wall composition of embryonic axes of germinating *Cicer arietinum* L. seeds. Effects of abscisic acid and temperature. *Plant Sci.*, **48**, 23-30.

LAKE, J.V., 1987. Interaction in the physical environment of plant roots. In: *Root Development and Function*. P.J. Gregory, J.V. Lake & D.A. Rose (eds). Soc. Exp. Biol. Seminar Series 30. Cambridge: Cambridge University Press. pp ix-xiii.

LAWLOR, D.W., & LEACH, J.E., 1985. Leaf growth and water deficits: biochemistry in relation to biophysics. In: *Control of Leaf Growth*. N.R. Baker, W.J. Davies & C.R. Ong (eds). Society for Experimental Biology. Seminar Series 27. Cambridge: Cambridge University

Press. pp 267-294.

LE PAGE-DEGIVRI, M.Th., DUVAL. D., BULARD. C. & DELAAGE, M., 1984. A radioimmunoassay for abscisic acid. *J. Immunl. Methods*, **67**, 119-128.

LEVITT, J., 1980*a*. *Responses of Plants to Environmental Stress*. Vol. I. *Chilling, Freezing and High temperature stress*. New York: Academic Press. 497 pp.

LEVITT, J., 1980*b*. *Responses of Plants to Environmental Stress*. Vol. II. New York: Academic Press.

LOCKHART, J.A., 1965. An analysis of irreversible plant cell elongation. *J. Theor. Biol.*, **8**, 264-275.

LOVEYS, B.R. & MILBORROW, B.V., 1984. Metabolism of abscisic acid. **In**: *The Biosynthesis and Metabolism of Plant Hormones*. A. Crozier and J.R. Hillman (eds). *Soc. Exp Biol.*, Seminar Series **23**. Cambridge, Cambridge University Press. pp 71-104.

MacDUFF, J. H., 1989. Growth and nutrient uptake in response to root temperature. **In**: *Roots and the Soil Environment*. Aspects of Applied Biology 22, pp. 23-32.

MacDUFF, J.H., HOPPER, M.J. & WILD, A., 1987. The effects of root temperature on growth and uptake of ammonium and nitrate by *Brassica napus* L. cv. Bien Venu in flowing solution culture. II. Uptake from solution containing NH_4NO_3. *J. Exp. Bot.*, **38**, 53-66.

MacDUFF, J.H. & JACKSON, S.B., 1991. Growth and preferences for ammonium or nitrate uptake by barley in relation to root temperature. *J. Exp. Bot.*, **42**,

521-530.

MacDUFF, J.H., WILD, A., HOPPER, M.J. & DHANOA, M.S., 1986. Effects of temperature on parameters of root growth relevant to nutrient uptake: measurements of oilseed rape and barley grown in flowing nutrient solution. *Plant and Soil*, **94**, 321-332.

MAKSYMOWYCH, R., 1973. *Analysis of Leaf Development.* Cambridge: Cambridge University Press.

MARKHART, A.H., FISCUS, E.L., NAYLOR, A.W. & KRAMER, P.J., 1979. Effects of temperature on water and ion transport in soybean and broccoli systems. *Plant Physiol.*, **64**, 83-87.

MATTHEWS, M.A., Van VOLKENBURGH, E. & BOYER, J.S., 1984. Acclimation of leaf growth to low water potentials in sunflower. *Plant, Cell Environ.*, 7, 199-206.

MATYSSEK, R., MARUYAMA, S. & BOYER, J.S., 1988. Rapid wall relaxation in elongating tissue. *Plant Physiol.*, **86**, 1163-1167.

MATYSSEK, R., MARUYAMA, S. & BOYER, J.S., 1991. Growth-induced water potential may mobilize water for growth. *Plant, Cell and Environ.*, **14**, 917-923.

MATYSSEK, R., TANG, A.-C. & BOYER, J.S., 1991. Plant can growth on internal water. *Plant, Cell and Environ.*, **14**, 925-930.

McWILLIAM, J.R., KRAMER, P.J. & MUSSER, R.L., 1982. Temperature-induced water stress in chilling-sensitive plants. *Aust. J. Plant Physiol.*, **9**, 343-352.

MEAD, R. & CURNOW, R.N., 1983. *Statistical Methods in Agriculture and Experimental Biology.* London: Champman and Hall. 335 pp.

MERTENS, R., DEUS-NEUMANN, B. & WEILER, E.W., 1983. Monoclonal antibodies for the detection and quantification of the endogenous plant growth regulator, abscisic acid. *FEBS Lett.*, **160**, 269-272.

MICHELENA, V. A. & BOYER, J. S., 1982. Complete turgor maintenance at low water potentials in the elongation region of maize leaves. *Plant Physiol.*, **69**, 1145-1149.

MILLIGAN, S.P., 1986. Effects of root excision and root cooling on primary leaf expansion in *Phaseolus vulgaris* L. PhD Thesis. Department of Botany, University of Edinburgh, U.K.

MILLIGAN, S.P. & DALE. J.E., 1988*a*. The effects of root treatments on growth of the primary leaves of *Phaseolus vulgaris* L.: General Feature. *New Phytol.*, **108**, 27-35.

MILLIGAN, S.P.& DALE, J.E., 1988*b*. The effects of root treatments on growth of primary leaves of *Phaseolus vulgaris* L.: Biophysical analysis. *New Phytol.*, **109**, 35-40.

MINORSKY, P.V., 1985. An heuristic hypothesis of chilling injury in plants: A role for calcium as a primary physiological transducer of injury. *Plant, Cell and Environ.*, **8**, 75-94.

MINORSKY, P.V., 1989. Temperature sensing by plants: a

review and hypothesis. *Plant, Cell and Environ.*, 12, 119-135.

MOORBY, H. & NYE, P.H., 1984. The effect of temperature variation over the root system on root extension and phosphate uptake by rape. *Plant and Soil*, 78, 283-293.

MUNNS, R., 1987. Does leaf turgor determine leaf expansion rates in dry or saline soil? In: *Physiology of Cell Expansion During Plant Growth*. D.J. Cosgrove & D.P. Knievel (eds). American Society of Plant Physiologists, Rockville, Md. pp 241-242.

MUNNS, R., 1990. Chemical signal moving from roots to shoots: The case against ABA. In: *Importance of Root to Shoot Communication in the Response of Environmental Stress*. W. J. Davies & B. Jeffcoat (eds). British Society for Plant Growth Regulation. Monograph 21. pp. 175-183.

MUNNS, R. & KING, R.W., 1988. Abscisic acid is not the only stomatal inhibitor in the transpiration stream of wheat plants. *Plant Physiol.*, 88, 703-708.

MURPHY, R. & SMITH, A.C., 1989. Pressure-clamp experiments on plant cells using a manually operated probe. In: *Plant Membrane Transport*. J. Dainty *et al.*, (eds). Elsivier Science Publishers B.V. (Biomedical Division). pp 577-578.

NEALES, T.F. & McLEOD, A.L., 1991. Do leaves contribute to the abscisic acid present in the xylem sap of "droughted" sunflower plants? *Plant, Cell Environ.*,

14, 979-986.

NEIL, S.J., HORGAN, R. & WALTON, D.C., 1984. Biosynthesis of abscisic acid. In: *Biosynthesis and Metabolism of Plant Hormones*. A. Crozier & J.R. Hillman (eds). Society for Experimental Biology Seminar Series 23. Cambridge: Cambridge University Press. pp 43-70.

NEUMAN, D.S., ROOD, S.B. & SMIT, B.A., 1990. Does cytokinin transport from root-to-shoot in the xylem sap regulate leaf responses to root hypoxia? *J. Exp. Bot.*, **41**, 1325-1333.

NEUMAN, D.S. & SMIT, B.A., 1991. The influence of leaf water status and ABA on leaf growth and stomata of *Phaseolus* seedlings with hypoxic roots. *J. Exp. Bot.*, **42**, 1499-1506.

NEUMANN, P.M., VAN VOLKENBURGH, E. and CLELAND, R. E., 1988. Salinity stress inhibits bean leaf expansion by reducing turgor, not wall extensibility. *Plant. Physiol.*, **88**, 233-237.

NONAMI, H. & BOYER, J. S., 1989. Turgor and growth at low water potential. *Plant Physiol.*, **89**, 798-804.

NONAMI, H. & BOYER, J. S., 1990*a*. Primary events regulating stem growth at low water potentials. *Plant Physiol.*, **93**, 1601-1609.

NONAMI, H. & BOYER, J. S., 1990*b*. Wall extensibility and hydraulic conductivity decrease in enlarging tissues at low water potentials. *Plant Physiol.*, **93**, 1610-1619.

OGUNKAMI, A.B., TUCKER, D.J. & MANSFIELD, T.A., 1973. An improved bio-assay for abscisic acid and other antitranspirants. *New Phytol.*, **72**, 277-282.

PAN, R-C., 1990. The role of abscisic acid in chilling resistance. **In:** *Plant Growth Substances 1988.* R.P. Pharis and S.B. Rood (eds). Berlin, Spring-Verlag. pp 391-399.

PARRISH, D.J. & WOLF, D.D., 1983. Kinetics of tall fescue leaf elongation: Response to changes in illumination and vapor pressure. *Crop Science*, **23**, 659-663.

PASSIOURA, J.B., 1988*a*. Response to Dr P. J. Kramer's article, 'Changing concepts regarding plant water relations', Volume 11, Number 7, pp. 565-568. *Plant, Cell and Environ.*, **11**, 569-571.

PASSIOURA, J.B., 1988*b*. Root signal control leaf expansion in wheat seedling in drying soil. *Aust. J. Plant Physiol.*, **15**, 687-693.

PASSIOURA, J.B. & MUNNS, R., 1984. Hydraulic resistance of plants. II. Effects of rooting medium, and time of day, in barley and lupin. *Aust. J. Plant Physiol.*, **11**, 341-350.

PEACOCK, J.M., 1975. Temperature and leaf growth in *Lolium perenne*. II. The site of temperature perception. *J. Apply. Eco.*, **12**, 115-123.

PENCE, V.C. & CARUSO, J.L., 1986. Auxin and cytokinin levels in selected and temperature-induced morphologically distinct tissue lines of tobacco

crown gall tumors. *Plant Sci.*, **46**, 233-237.

PENCE, V.C. & CARUSO, J.L., 1988. Immunoassay methods of plant hormone analysis. **In:** *Plants Hormones and Their Role in Plant Growth and Development.* P.J. Davies (ed.). Dordrecht: Kluwer Academic Publishers. pp 240-256.

PENGELLY, W.L., 1986. Validation of immunoassay. **In:** *Plant Growth Substances 1985.* M. Bopp (ed). Berlin: Spring -Verlag pp 35-43.

PENGELLY, W.L., BANDURSKI, R.S. & SCHULZE. A., 1981. Validation of a radioimmunoassay for indole-3-acetic acid using gas chromatography-selected ion monitoring-mass spectrometry. *Plant Physiol.*, **68**, 96-98.

PENGELLY, W.L. & MEINS Jr., F., 1977. A specific radioimmunoassay for nanogram quantities of the auxin, Indol- 3 - Acetic Acid. *Planta*, **136**, 173-180.

PHILLIPS, I.D.J., 1964. Root-shoot hormone relation I. The importance of an aerated root system in the regulation of growth hormone levels in the shoot of *Helianthus annuus*. *Ann. Bot.*, **28**, 17-35.

PICKARD, B.G., 1973. Action potentials in higher plants. *The Botanical Review*, **39**, 172-201.

PIERCE, M. & RASCHKE. K., 1980. Correlation between loss of turgor and accumulation of abscisic acid in detached leaves. *Planta*, **148**, 174-182.

PIERCE, M. & RASCHKE, K., 1981. Synthesis and metabolism of abscisic acid in detached leaves of *Phaseolus*

vulgaris L. after loss and recovery of turgor. *Planta*, **153**, 156-165.

QUARRIE, S.A., 1978. A rapid and sensitive assay for abscisic acid using ethyl abscisate as an internal standard. *Anal. Biochem.*, **87**, 148-156.

QUARRIE, S.A., 1988. RAI protocol for ABA measurements. Plant Breeding Institute. Cambridge, U.K.

QUARRIE, S.A. & GALFRE, G., 1985. Use of different Hapten-protein conjugates immobilized on nitrocellulose to screen monoclonal antibodies to abscisic acid. *Anal. Biochem.*, **151**, 389-399.

QUARRIE, S.A. & JONES, H.G., 1977. Effects of abscisic acid and water stress on development and morphology on wheat. *J. Exp. Bot.*, **28**, 192-203.

QUARRIE, S.A., WHITFORD,P.N., APPLEFORD, N.E.J., WANG, T.L., COOK, S.K., HENSON, I.E. & LOVEYS. B.R., 1988. A monoclonal antibody to (S)-abscisic acid: its characterisation and use in a radioimmunoassay for measuring abscisic acid in crude extracts of cereal and lupin leaves. *Planta*, **173**, 330-339.

RADIN, J.W. & HENDRIX, D.L., 1988. The apoplastic pool of abscisic acid in cotton leaves in relation to stomatal closure. *Planta*, **174**, 180-186.

RAYLE, D.L. & CLELAND, R.E., 1972. The in-vitro acid-growth response: relation to in-vivo growth response and auxin action. *Planta*, **104**, 282-296.

REID, D.M. & CROZIER, A., 1971. Effects of waterlogging on the gibberellin content and growth of tomato

plants. *J. Exp. Bot.*, 22, 39-48.

RIKIN, A., ATSMON D. & GITLER, C., 1983. Quantitation of chill-induced release of a tubulin-like factor and its prevention by abscisic acid in *Gossypium hirsutum* L. *Plant Physiol.*, **71**, 474-478.

RUSSELL, E.W., 1973. *Soil Conditions and Plant Growth.* 10th edn. London: Longman. 635 pp.

SAAB, I.N. & SHARP, R.E., 1989. Non-hydraulic signals from maize roots in drying soil: inhibition of leaf elongation but not stomatal conductance. *Planta*, **179**, 466-474.

SATTIN, M., STACCIARINI SERAPHIN, E. & DALE, J.E., 1990. The effects of root cooling and light-dark transition on growth of primary leaves of *Phaseolus vulgaris* L., *J. Exp. Bot.*, **41**, 1319-1324.

SCHILDWACHT, P.M., 1989. Is a decreased water potential after withholding oxygen to roots the cause of decline of leaf elongation rates in *Zea mays* L. and *Phaseolus vulgaris* L.? *Planta*, **177**, 178-184.

SCHOLANDER, P.F., HAMMEL, H.T., BRADSTREET, E.D. & HEMMINGSEN, E.A., 1965. Sap pressure in vascular plants. *Science*, **148**, 339-346.

SETTER, T.L. & GREENWAY, H., 1988. Growth reduction of rice at low root temperature: decreases in nutrient uptake and development of chlorosis. *J. Exp. Bot.*, **39**, 811-829.

SHARP, R.E., 1990. Comparative Sensitivity of root and shoot growth and physiology to low water potentials.

In: *Importance of Root to Shoot Communication in the Responses to Environmental Stress*. W.J. Davies & B. Jeffcoat (eds). British Society for Plant Growth Regulation, Monograph 21. pp. 29-44.

SHARP, R.E. & DAVIES, W.J., 1989. Regulation of growth and development of plants growing with a restricted supply of water. **In:** *Plant under Stress*. H.G. Jones, T.J. Flowers & M.B. Jones (eds). Soc. Exp. Biol. seminar Series **39**. Cambridge: Cambridge University Press. pp 71-93.

SIMONS, P.J., 1981. The role of electricity in plant movements. *New Phytol.*, **87**, 11-37.

SKENE, K.G.M., 1975. Cytokinin production by roots as a factor in the control of plant growth. **In:** *The Development and Function of Roots*. J.G. Torrey & D.T. Clarkson (eds). London: Academic Press. pp 365-396.

SMIT, B.A., NEUMAN, D.S. & STACHOWIAK, M.L., 1990. Root hypoxia reduces leaf growth. Role of factors in the transpiration stream. *Plant Physiol.*, **92**, 1021-1028.

SMITH, P. G. & DALE, J. E., 1988. The effects of root cooling and excision treatments on the growth of primary leaves of *Phaseolus vulgaris* L. Rapid and reversible increases in abscisic acid. *New Phytol.*, **110**, 293-300.

SWEETSER, P.B. & VATVARS, A., 1976. High-performance liquid chromatographic analysis of abscisic acid in plants extracts. *Anal. Biochem.*, **71**, 68-78.

TARDIEU. F., KATERJI, N. BETHENOD, O., ZHANG, J. &

DAVIES, W.J., 1991. Maize stomatal conductance in the field: its relationship with soil and plant water potential, mechanical constraints and ABA concentration in the xylem sap. *Plant Cell and Environ.*, **14**, 121-126.

TAYLOR, G. & DAVIES, W.J., 1985. The control of Leaf growth of *Betula* and *Acer* by photoenvironment. *New Phytol.*, **101**, 259-268.

TESKEY, R.O., HINKLEY, T.M. & GRIER, C.C., 1984. Temperature induced changes in water relations of *Abies amabilis* (Douge) Forbes. *Plant Physiol.*, **74**, 77-80.

TILLBERG, E., 1975. An abscisic acid-like substance in dry and soaked *Phaseolus vulgaris* seeds determined by the *Lemna* growth bioassay. *Physiol. Plant.*, **34**, 129-159.

TREJO, C.L. & DAVIES, W.J., 1991. Drought-induced closure of *Phaseolus vulgaris* L. stomata precedes leaf water deficit and any increase in xylem ABA concentration. *J. Exp. Bot.*, **42**, 1507-1515.

TUCKER, D.J. & MANSFIELD, T.A., 1971. A simple bio-assay for detecting "antitranspirant" activity of naturally occurring compounds such as abscisic acid. *Planta*, **98**: 157-163.

TURNER, N.C., SCHULZE, E-D. & GOLLAN, T., 1985. The response of stomata and leaf gas exchange to vapour pressure deficit and soil water content II. In the mesophytic herbaceous species *Helianthus annuus.*

Oecologia, **65**, 348-355.

VAN STADEN, J. & DAVEY, J.E., 1979. The synthesis, transport and metabolism of endogenous cytokinins. *Plant, Cell and Environ.*, 2, 92-106.

VAN VOLKENBURGH E. & CLELAND, R. E., 1986. Wall yield threshold and effective turgor in growing bean leaves. *Planta*, **167**, 37-43.

VAN VOLKENBURGH, E., & DAVIES, W.J., 1983. Inhibition of light-stimulated leaf extension by abscisic acid. *J. Exp. Bot.*, **144**, 835-845.

VAN VOLKENBURGH, E., HUNT, S. & DAVIES, W.J., 1983. A simple instrument for measuring cell-wall extensibility. *Ann. Bot.*, **51**, 669-627.

WADMAN-VAN SCHRAVENDJK. H. & VAN ANDEL, O.M.,1985. Interdependence of growth, water relations and abscisic acid levels in *Phaseolus vulgaris* during waterlogging. *Physiol. Plant.*, 63,215-220.

WADMAN-VAN SCHRAVENDJK. H. & VAN ANDEL, O.M.,1986. The role of ethylene during flooding of *Phaseolus vulgaris*. *Physiol. Plant.*, **66**, 257-264.

WALDRON, L.J., TERRY, N. & NEMSON, J.A., 1985. Diurnal cycles of leaf extension in unsalinised and salinised *Beta vulgaris*. *Plant, Cell and Environ.*, **8**, 207-211.

WALTON, D.C., 1980. Biochemistry and physiology of abscisic acid. *Ann. Rev. Plant Physiol.*, **31**, 453-489.

WALTON, D.C., DASHEK, W. & GALSON, E., 1979. A radioimmunoassay for abscisic acid. *Planta*, **146**, 139-145.

WALTON, D.C., HARRISON, A. & COTÊ, P., 1976. The effects of water stress on abscisic acid levels in roots of *Phaseolus vulgaris* L. and other plants. *Planta*, 131, 141-144.

WARTINGER, A., HEILMEIER, H., HARTUNG, W. & SCHULZE, E-D., 1990. Daily and seasonal courses of leaf conductance and abscisic acid in the xylem sap of almond trees [*Prunus dulcis* (Miller) D.A. Webb] under desert conditions. *New Phytol.*, **116**, 581-587.

WATTS, W.R., 1972. Leaf extension in *Zea mays* I. Leaf extension and water potential in relation to root-zone and air temperatures. *J. Exp. Bot.*, 23, 704-712.

WEILER, E.W., 1979. Radioimmunoassay for the determination of free and conjugated abscisic acid. *Planta*, **144**, 255-263.

WEILER, E.W., 1982. An enzyme-immunoassy for *cis*- (+)-abscisic acid. *Physiol. Plant.*, **54**, 510-514.

WEILER, E.W., 1984. Immunoassay of plant growth regulators. *Ann. Rev. Plant Physiol.*, **35**, 85-95.

WEILER, E.W., EBERLE, J. MERTENS, R., ATZORN, R., FEYERABEND, M., JOURDAN, P.S., ARNSCHEIDT, A. & WIECZOREK, U., 1986. Antisera- and monoclonal antibody-based immunoassay of plants hormones. **In:** *Immunology in Plants*. Society for Experimental Biology. Seminar Series **29**. T.V. Wang (ed). Cambridge: Cambridge University Press. pp. 27-58.

WESTGATE, M.E. & BOYER, J.S., 1984. Inhibitory effects of water deficit on maize leaf elongation. *Plant*

Physiol., **77**, 190-194.

WESTGATE, M.E. & BOYER, J.S., 1985. Osmotic adjustment and the sensitivity of leaf, root, stem, and silk growth to low water potentials in maize. *Planta*, **164**, 540-549.

WIGNARAJAH, K., JENNINGS, D.H. & HANDLEY, J.F., 1975. The effect of salinity on growth of *phaseolus vulgaris* L. I. Anatomical changes in the first trifoliate leaf. *Ann. Bot.*, **39**, 1029-1038.

WOLF, O., JESCHKE, W.J. and HARTUNG, W., 1990. Long distance transport of abscisic acid in NaCl-treated intact plants of *Lupinus albus*. *J. Exp. Bot.*, **41**, 593-600.

ZEEVAART, J.A.D., 1977. Sites of abscisic acid synthesis and metabolism in *Ricinus communis* L. *Plant Physiol.*, **59**, 788-791.

ZEEVAART, J.A.D. & CREELMAN, R.A., 1988. Metabolism and physiology of abscisic acid. *Ann. Rev. Plant Physiol. Mol. Biol.*, **39**, 439-473.

ZHANG, J. & DAVIES, W.J., 1987*a*. Increased synthesis of ABA in partially dehydrated root tips and ABA transport from roots to leaves. *J. Exp. Bot.*, **38**, 2015-2023.

ZHANG, J. & DAVIES, W.J., 1987*b*. ABA in roots and leaves of flooded pea plants. *J. Exp. Bot.*, **38**, 649-659.

ZHANG, J. & DAVIES, W.J., 1989*a*. Abscisic acid produced in dehydrating roots enable the plant to measure the water status of the soil. *Plant, Cell Environ.*, **12**,

73-81.

ZHANG, J. & DAVIES, W.J., 1989*b*. Sequential response of whole plant water relations to prolonged soil drying and the involvement of xylem sap ABA in the regulation of stomatal behaviour of sunflower plants. *New Phytol.*, **113**, 167-174.

ZHANG, J. & DAVIES, W.J., 1990*a*. Changes in the concentration of ABA in xylem sap as a function of changing soil water status can account for changes in leaf conductance and growth. *Plant, Cell and Environ.*, **13**, 277-285.

ZHANG, J. & DAVIES, W.J., 1990*b*. Does ABA in xylem sap control the rate of leaf growth in soil-dried maize and sunflower plants? *J. Exp. Bot.*, **41**, 1125-1132.

ZHANG, J. & DAVIES, W.J., 1991. Antitranspirant activity in the xylem sap of maize plants. *J. Exp. Bot.*, **42**, 317-321.

ZHANG, J., GOWING, D.J. & DAVIES, W.J.,1990. ABA as a signal in root to shoot communication of soil drying. In: *Importance of Root to Shoot Communication in the Responses to Environmental Stress*. W.J. Davies & B. Jeffcoat (eds). British Society for Plant Growth Regulation, Monograph 21. pp. 163-174.

ZHANG, J. SCHURR, U. & DAVIES, W.J., 1987. Control of stomatal behaviour by abscisic acid which apparently originates in the roots. *J. Exp. Bot.*, **192**, 1175-1181.

ZHAO, K., MUNNS, R. & KING, W., 1991. Abscisic acid

levels in NaCl-treated barley, cotton and saltbush. *Aust. J. Plant Physiol.*, 18, 17-24.

APPENDIX

A.1- General Effects of Root Cooling on Growth Parameters of Seedlings of *Phaseolus vulgaris*

Table A.1- The effects of root cooling (RC) over 24 h on growth parameters of *Phaseolus vulgaris* seedlings

Parameters	Units	Time (h)		
		0	24	
		control	control	RC
Leaf area	cm^2	6 ± 0.2	14 ± 1.2	11 ± 0.80
Midrib L	cm	3 ± 0.1	4 ± 0.1	4 ± 0.05
Leaf FW	mg	155 ± 10.0	515 ± 20.0	230 ± 13.00
Leaf DW	mg	18 ± 2.0	65 ± 5.0	46 ± 6.00
Cotys. FW	mg	486 ± 20.0	365 ± 39.0	400 ± 42.00
Cotys. DW	mg	141 ± 8.0	85 ± 11.0	103 ± 15.00
Root FW	mg	600 ± 26.0	725 ± 30.0	634 ± 28.00
Root DW	mg	30 ± 1.2	36 ± 1.5	34 ± 2.00
DW_r / DW_s		0.14	0.14	0.14
$\bar{R}_{leaf}$			1.20	0.93
$\bar{R}_{aerial\ parts}$			0.21	0.14
$\bar{R}_{root}$			0.18	0.12
$\bar{E}$	mg $cm^{-2}d^{-1}$		4.90	3.36

PUBLICATIONS

DALE, J.E., STACCIARINI SERAPHIN, E. & SATTIN, M., 1990. The effects of root cooling on leaf growth. In: *Importance of Root to Shoot Communication in the Responses to Environmental Stress.* W.J. Davies & B. Jeffcoat (eds). British Society for Plant Growth Regulation, Monograph 21. pp 149-161.

SATTIN, M., STACCIARINI SERAPHIN, E. & DALE, J.E., 1990. The effects of root cooling and light-dark transition on growth of primary leaves of *Phaseolus vulgaris* L., *J. Exp. Bot.*, 41, 1319-1324.