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Influence of temperature on root water and ion transport and the subsequent effect on shoot water status and growth of barley and sorghum seedlings

BassiriRad, Hormoz, Ph.D.

The University of Arizona, 1990

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INFLUENCE OF TEMPERATURE ON ROOT WATER AND ION TRANSPORT
AND THE SUBSEQUENT EFFECT ON SHOOT WATER STATUS
AND GROWTH OF BARLEY AND SORGHUM SEEDLINGS

by
Hormoz BassiriRad

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A Dissertation Submitted to the Faculty of the
DEPARTMENT OF PLANT SCIENCES
In Partial Fulfillment of the Requirements
For the Degree of
DOCTOR OF PHILOSOPHY
WITH A MAJOR IN AGRONOMY AND PLANT GENETICS
In the Graduate College
THE UNIVERSITY OF ARIZONA

1990
As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Hormoz BassiriRad entitled *INFLUENCE OF TEMPERATURE ON ROOT WATER AND ION TRANSPORT AND THE SUBSEQUENT EFFECT ON SHOOT WATER STATUS AND GROWTH OF BARLEY AND SORGHUM SEEDLINGS* and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of DOCTOR OF PHILOSOPHY WITH A MAJOR IN AGRONOMY AND PLANT GENETICS.

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ABSTRACT

Short term root temperature treatments between 15 to 40°C at a constant air temperature produced a differential response in shoot growth of barley and sorghum seedlings. Maximum growth rate occurred at 25°C in barley and 35°C in sorghum. The stimulation of growth in barley in the suboptimal temperature ranges (15 to 25°C) was associated with both enhanced $L_r$ and $J_l$ whereas growth inhibition at elevated temperatures (>25°C) was associated with no changes in $L_r$, but was accompanied by a severe inhibition of solute fluxes suggesting that supraoptimal temperature inhibition of growth in barley is caused by limited ion and not water supply to the leaves. In sorghum, the enhanced shoot growth in the 15 to 25°C range coincided with stimulated $L_r$ and $J_l$. Between 25 to 35°C temperature induced enhancement of growth was mainly caused by enhanced $L_r$. In both plants even when root $L_r$ appeared to cause temperature induced changes in growth, the bulk leaf water, osmotic and turgor potential remained unaffected. It is suggested that when reduced water supply limits growth, undetectable changes in xylem water potential may mediate temperature response in root $L_r$ and growth. Temperature effects on ion transport across the root were found to be regulated at the site of ion release into the xylem ($\Phi_{x}$) rather than the site of ion entry into the root ($\Phi_{r}$). When ABA was added to the external solution, $L_r$ was enhanced but qualitative responses of $J_l$ and $L_r$ to changes in root temperature remained unchanged in barley. However, addition of ABA to the medium with sorghum roots caused a severe inhibition of solute fluxes at temperatures above 25°C which happened only when the temperature was raised above 35°C in the absence of ABA. The ABA study suggested that temperature induced changes in root transport properties of both plants were not mediated by ABA.
INTRODUCTION

Shoot growth in many crop species responds very strongly to changes in the temperature of the root environment (Cooper 1973, Nielsen 1974). Variation in shoot growth in response to changes in root temperature has been reported to be accompanied by changes in rate of water (Kramer 1940, Brouwer and Hoagland 1964, Radin 1990) and ion uptake (Grobbelaar 1963, Clarkson et al. 1986, Rufty et al. 1981). In many parts of the world, including the Southwestern United States, topsoil temperature commonly exceeds 30°C during the cropping season, particularly during the early stages of development (Cooper 1973). However, only very rarely has the effect of high root temperature on growth and development been studied.

This study is intended to characterize the growth response of barley and sorghum, cool and warm-season species respectively, to a wide range of root temperatures. An attempt has been made to quantify the potential changes in root hydraulic conductance, $L_p$, and fluxes of ions such as $\text{NO}_3^-$, $\text{PO}_4^{3-}$, and $\text{K}^+$ in excised roots, for two purposes, 1) To infer a mechanistic interpretation for temperature induced changes in root pressure exudation and 2) To see if the changes in either hydraulic or osmotic components of transport across the root can potentially act as a signal for the ensuing changes in shoot growth. For the ion transport studies, an attempt was also made to infer the relative importance of "uptake" to the root versus the "release" into the xylem. Permeability to water was assessed by determining $L_p$. Root hydraulic conductance can be estimated using various methods. A comparative study of some of these methods was also carried out. Leaf water status and total water flow were also determined at various root temperatures in intact seedlings of both sorghum and barley. Finally, effects of applied abscisic acid (ABA) on the
root water and ion transport properties were characterized at various root temperatures in both species.
LITERATURE REVIEW

Physiological Responses to Changes in Root Temperature

Effects of root temperature on plant growth. Root temperature has major effects on physiological processes in the shoot. It is one of the most important factors affecting growth at various developmental stages and as suggested by Neilsen (1974) root temperature is even more critical than foliage temperature for vegetative growth. At constant ambient temperatures, most plants exhibit what is often referred to as "Optimum Root Temperature" (ORT) for maximum shoot growth. This temperature optimum varies between species as well as cultivars within the same species. For example in barley, Power (1970) found that ORT was around 20°C. He also found that at 27°C, short term growth was hastened but higher than optimum temperature led to a significant reduction in the yield potential. Therefore, it was concluded that the higher temperature inhibits the plant factor necessary for maximum yield.

Other plants which exhibit relatively lower ORT include oats at 15 to 20°C (Fulton and Findly 1968), wheat at around 20°C (Whitfield and Smika 1971), and many species of native grasses also at around 20°C (Neilson 1974). On the other hand, the ORT for corn, cotton, rice and many vegetable crops such as tomato tends to be at or higher than 30°C (Walker 1969, Pearson et al. 1970, Fujishige and Sugiyam 1968). Because changes in photosynthesis and net assimilation rate often lead to correlative changes in yield, in the following section, a brief summary of the literature with respect to the effects of root temperature on photosynthesis is presented.

The influence of root temperature on photosynthesis. Several studies have shown that photosynthetic rate exhibits a strong dependence on root temperature (Johnson and Ingram 1984, Hurewitz and Janes 1983, Gosselin and Trudel 1986). This effect may result
from changes in the root (sink) metabolic activities, and various workers have suggested that 
modifications in sink strength may mediate the root temperature induced changes in 
photosynthesis (Osmond et al. 1980, Cooper and Thornley 1976). Others have found that 
high rates of photosynthesis at high root temperatures are correlated with enhanced stomatal 
(low stomatal resistance) and non-stomatal (high PEP and RuBP carboxylase) functions 
(Duke et al. 1979). An important implication of such a regulatory role of optimum root 
temperature is its potential for offsetting the photosynthetic inhibition by low air temperature 
(Gosselin and Turdel 1985, Huang et al. 1989, Jones et al. 1978), as well as offsetting 
detrimental effects of other environmental factors (Rufty et al. 1981, Radin 1990, and 
Sheppard et al. 1986).
The nature and the origin of a root temperature induced signal(s) affecting the 
physiology of the shoot is not understood. However, the rate of supply of water, ions, and 
growth substances can potentially regulate processes in the shoot in response to root 
environmental conditions.

Transport Across the Root

Studies of root transport processes must ultimately take into account the anatomical 
peculiarities of this organ. Figure 1 shows that roots can exhibit a great deal of anatomical 
heterogeneity in both axial and radial directions. Considerable diversity between species and 
numerous variations in root morphological appearances have made it difficult to simplify a 
general picture of the root along the axial direction. However, variation in transport 
functions along the length of the root is well established (Luttge 1983). In contrast, 
anatomical characterization of root cross sections and the study of radial transport of water 
and ions have received much more attention. Young roots of most species, and certainly 
of those most extensively studied for transport processes contain the following
Fig. 1 Three dimensional presentation of 4 successive zones in a typical grass root. No specific length for the zones are given since they vary greatly among species and growth conditions. (A) is the zone of primary root differentiation. (B) is a root hair zone with primary endodermis. (C) contains secondary endodermis. (D) is the zone of lateral root formation. C, CS, En, Ep, Hy, LR, MX, P, PC, Ph, Px, RC, RH, SL and XP refer to cortex. Casperian strip, endodermis, epidermis, hypodermis, lateral root, metaxylem, pericycle, passage cell, phloem, protoxylem, root cap, root hair, suberum lamella, and xylem parenchyma respectively. Figure is adopted from Luttge (1983).
morphologically distinct regions.

**Epidermis.** The outermost layer of root cells is referred to as epidermis. Epidermal cells may become modified to form root hairs and transfer cells or become suberized through chemical modification. Because they are in an immediate contact with the soil, epidermal cells may act as a limiting site for water and ion absorption into the root (Luttge 1983).

**The Cortex.** Cortical cells comprise a large portion of root cross-sectional area. These are parenchyma cells immediately below the epidermis and are highly vacuolated. Because up to 90% of the volume of cortical cells is occupied by vacuoles (Clarkson 1974) which accumulate many species of ions, the cortex can maintain the supply of nutrients to the xylem up to many hours when the ion absorption to the root has been reduced. This buffering capacity may be crucial in maintaining the short term supply of ions to the shoot when there are rapid fluctuations in their supplies to the roots. Although cortical cells must be traversed by water and solutes during their transport to the xylem, it appears though they are not universally critical for ion absorption and transport as observed in Calluna roots in which cortex is lacking (Luttge 1983).

Cortical cells provide three distinct compartments for water and ion transport. These are symplasm, apoplasm, and transcellular (vacuole to vacuole) pathways. Symplasm refers to the membrane bound protoplasmic continuum which extends from cell to cell through a network of plasmodesmatal connection. Apoplasm, on the other hand, refers to the continuum outside the plasmalemma whereas in the transcellular pathway, water must move through the cell walls, plasmalemma and tonoplast from one cell to the next. The apoplastic pathway includes mainly the cell walls and is assumed to be more readily permeable to water and ions than plasmalemma (Lauchli 1976). A question which still remains controversial is
related to the relative significance of each of these compartments to radial transport. It has traditionally been assumed that water moved predominantly in the apoplast, except at the Casparian strip where it was forced into the cytoplasm (Passiora 1988, Boyer 1985). But as early as 1970, Tyree, and later Robards and Clarkson (1976) proposed theoretical and experimental models suggesting that symplastic pathway can account for the observed tissue permeability to water and ions. Newman (1976b) also argued that symplastic pathway was the dominant transport path for water since his calculated values of the cell wall $L_p$ were too low, to account for reported tissue $L_p$.

With the advent of the pressure probe techniques, it has been possible to measure $L_p$ for individual cell and the whole root. Comparison of the root and cell $L_p$ should then provide an unequivocal quantification of relative significance of sympiasm versus apoplasm. One such study was presented by Steudle et al. (1983) where they showed that radial water flow occurred primarily through the transcellular path. These results were confirmed by Jones et al. (1983), but later this group reported that except for the root tip, water can equally move through either symplasm or apoplasm (Jones et al. 1988). Recently, Radin and Matthews (1989) reported that root $L_p$ was greater than cell $L_p$ and concluded that substantial water flow may occur in the apoplasm. These findings indicate that a simple comparison of $L_p$ calculated from cell $L_p$ and those measured from the whole root cannot unambiguously determine the path of water flow. Part of the uncertainty results from the fact that, $L_p$ of the whole root measured in osmotically induced flow is substantially lower than hydrostatically driven flow in single cells (Steudle et al. 1987, Frensch and Steudle 1989, Jones et al. 1988). Therefore, the differences in the absolute values of cell and tissue $L_p$ may reflect different driving forces rather than actual differences in the transport pathways.

In addition, factors such as the age and the length of root may influence the magnitude of the difference between cell and root $L_p$ (Jones et al 1988). In calculating
radial $L_p$ based on a single cell measurement, caution must also be taken to avoid assumptions which may not be necessarily true (such as anatomical homogeneity of the entire root), in order to allow for accurate evaluation of transport mechanism. The absolute knowledge of the radial movement in the cortical region may ultimately depend on the knowledge of cell wall $L_p$ and it may even be possible that no universal path can be assigned for different varieties of plants.

The Endodermis. The innermost layer of the cortex enclosing the stele is known as endodermis. When these cells are encrusted with suberin like compounds, which form the Casparian strip, they act as an effective barrier to passive water and ion fluxes. It is at this point that apoplastic fluxes across the cortex must be taken up into the symplasm of the endodermis for their continued passage to parenchyma of the stele. In some roots, the Casparian strip is made discontinuous by the presence of non-suberized cells known as "Passage" cells which may offer less resistance to water and solute transport (Glass 1989).

The Stele. The innermost part of the root cross section is the stele. Immediately interior to the endodermis is the pericycle which retains meristematic potential and can give rise to lateral root primordia. Two to five vascular bundles, xylem and phloem, are alternately located within the pericycle in dicots, whereas monocots characteristically have many more. Traditionally, it was believed that the stele is always under anaerobic stress resulting in physiologically inactive and "leaky" parenchyma cells (Crafts and Broyer 1938). More recent evidence, however, indicates that although the $O_2$ partial pressure is slightly lower in the stele, it is not low enough to inhibit aerobic processes (Bowling 1973). The stelar region also contains xylem parenchyma cells which surround the xylem vessels and are thought to be important in ion transfer to the xylem (Glass 1989). There is now much evidence to support active secretion of ions from parenchyma cells in the stele across the plasmalemma into the xylem vessels (Lauchli and Epstein 1971, Lauchli et al. 1971, and
Pitman 1972). The mature vessel elements (non-living xylem cells) appear a short distance from the root tip and as empty capillaries they become the main conducting elements for the long distance transport of water and solutes to the shoot.

**Ion Transport into the Xylem**

**Transport Models.** Tracer studies have shown that more than 80% of K⁺ and N, 75% of P and 97% of Ca²⁺ taken up by the root is transported to the shoot (Pitman 1972), indicating the presence of large net fluxes at the root surface as well as root-xylem interface. Solute transport studies are often interpreted based on simplified models such as those shown in Fig. 2. Figure 2A highlights the major component of the flux pathway such as symplasm, apoplasm, the barrier of endodermis, plasmodesmal connections, and the loading of ions into the xylem. Figure 2B draws attention to the major sites at which membranes can govern the rate of solute fluxes. Inorganic solutes enter the apoplasm from the external medium by either mass flow associated with transpiration or by diffusion. Depending upon the prevailing conditions, ions may penetrate the apoplasm as far as the endodermis. Influx \( \Phi_{in} \) and efflux \( \Phi_{out} \) of ions at the plasma membrane of epidermal or cortical cells determine ion transport into the cytoplasm, whereas the influx \( \Phi_{cy} \) and efflux \( \Phi_{ct} \) of ions across the tonoplast determine the amount of solute accumulation in, or mobilization from, the vacuole. In addition, Fig. 2B shows that fluxes at the interface between xylem parenchyma and xylem vessels (\( \Phi_{il} \) and \( \Phi_{il} \)) ultimately determine the amount of solutes loaded into the vessels.

According to these models and thermodynamic considerations for an active transport from the medium to the xylem, it is sufficient that the active transport occurs only at one possible site in Fig.2B. Nonetheless, the above statement does not exclude the possibility of active transport at more than one site. In fact, the exact site(s) at which active transport...
Fig. 2 (A) is a schematic presentation of radial pathways of ion transport in roots. Note the restriction of the apoplastic pathway at the Casperian strip and the symplastic connections between cells through the endoplasmic reticulum (ER). (B) is a simplified presentation of major membrane fluxes in the root radial ion transport. \( J_s \) is the net solute flux in xylem defined as \( J_s = \Phi_{dx} - \Phi_{c} \). Figures adopted from Lauchli (1984).
may occur is not clearly understood but substantial evidence points to a "two pump" transport system (Pitman 1982, Lauchli 1984, Anderson 1976) in which both ion "uptake" into the root, $\Phi_u$, and "release" into the xylem, $\Phi_x$, required active transports. An alternative hypothesis known as the "one pump" theory is also presented (Bowling 1973, 1981). The proponents of this theory argue that active ion uptake occurs only at either epidermis or cortex plasma membrane and is subsequently transported passively (along the electrochemical gradient) to the xylem through the symplastic continuum. Evidence for this hypothesis has been seriously challenged. For example, Davis and Higinbotham (1976) showed that although $\text{Cl}^-$ moves to the xylem along the electrochemical gradient, $\text{K}^+$ movement was against this gradient indicating an active loading. Further evidence for the two step regulation of ion transport comes from studies of hormones and inhibitors of protein synthesis. Pitman (1977) showed that both AZ (azetidine 2 - carboxylic acid) an analogue of proline and abscisic acid (ABA) inhibited $\Phi_u$ but enhanced $\Phi_x$, suggesting that ion transport from the external medium to the xylem is regulated at two active sites.

Transport Driven by Electrochemical Potential Gradients. One of the major ways by which charged species such as ions can traverse the cell membrane is in response to electrochemical potential gradients ($\Delta \mu$). As the term implies, this gradient results from concentration and electrical assymmetries across a cell membrane. Root cells generally exhibit a negative electrical potential difference (PD) between inside and external solution (inside negative) of 100 to 200 mV (Glass 1989). The negatively charged inside is believed to be caused by active accumulation of anions (Anderson 1975) which potentially favors the passive entry of cations into the cell but opposes anions. Thorough reviews, of the factors influencing ($\Delta \mu$) and (PD) are given by Clarkson (1974) and Higinbothom (1973), but briefly the electrochemical potential gradient for an ion is mathematically expressed by the Nernst equation:
\[ \Delta \mu = 58/Z \log_{10} \frac{C_o}{C_i} \]  

(1)

where \( Z \) is the ionic charge and \( C_o \) and \( C_i \) are concentration differences between inside and outside. If the calculated value of \( \Delta \mu \) equals that of the measured PD, then the concentration inside and outside are at thermodynamic equilibrium. If \( \Delta \mu \) is less than PD, then "active" ion transport is indicated. However, this formulation of the Nernst equation does not take into account the differences between concentrations and activities of ions as well as the variable membrane permeability coefficients between ions. Therefore, equation (1) is often replaced by the Goldman equation:

\[ \mu_0 = 58 \log_{10} \left[ \frac{\alpha [K^{+}]+[\betaNa^{+}]+[\sigma Cl^{-}]}{[\alpha K^{+}][\beta Na^{+}][\sigma Cl^{-}]} \right] \]  

(2)

where \( \alpha \), \( \beta \) and \( \sigma \) are the permeability coefficients of K, Na, and Cl, respectively. Equation (2) describes the diffusional component of the ion transport, but it has become increasingly evident that other components of cell membrane are crucial for ion transport. Among these components is the electrogenic \( H^+ \) efflux pump which allows for influx of cations with a high degree of selectivity for \( K^+ \) against \( Na^+ \) (Jeschke 1980).

The proton efflux pump is energetically powered by ATP and consequently depends on enzymatic function of ATPase (Hodges 1976, Leonard 1983 and 1984). Additionally, against the background evidence that the curves describing the relationship between the external concentration and rate of ion uptake obey the Michaelis-Menton Equation, Epstein (1953) proposed the existence of a "carrier model" of ion transport. The kinetics of root ion uptake as a function of external concentration, also known as "absorption isotherm", is identical to the kinetics of some membrane bound ATPases, leading researchers to suggest that some carriers are ATPases. Early evidence of the plasma membrane ATPase involvement in active ion transport came from Fisher et al. (1970) where they showed a high degree of correlation between \( K^+ \) influx and membrane bound ATPase in a variety of cereal roots. Whether the carrier proteins are ATPases or not, there seems to be at least two types
of carrier transport, namely: "symport" or "cotransport" in which the carrier simultaneously moves an anion and a proton into the cytosol and "antiport" in which the efflux of H$^+$ is associated with the influx of a cation (Na$^+$). When a wide range of ion concentration is used, the absorption isotherm curves show two distinct mechanisms of ion uptake referred to as System I and System II (Luttge and Higinbotham 1979). System I, which saturates at very low external concentrations, is characterized by a low Michaelis Constant, $K_m$, whereas System II saturates at substantially higher concentrations and has a high $K_m$. The two uptake systems have been suggested to represent two different carrier mechanisms (Epstein 1976). Although most of the evidence for the carrier model is still circumstantial, the concept is widely accepted among researchers and phenomena such as ion selectivity and antagonism seem both to provide evidence for such a model and be readily explained by it.

In addition to the electrogenic protein pump and carriers, some of the active transport in roots may be driven by the electron transport system. A specific support for such a hypothesis comes from the study by Lin (1982), when he showed that exogenous NADH applied to corn root protoplasts and corn root segments increased K$^+$ and O$_2$ uptake by 2- and 3-fold and depolarized the protoplast PD by 20mV. Electropotential differences of a few tens of millivolts have also been recorded between the xylem exudate and the solution bathing the root (Anderson 1976). This gradient has been referred to as trans-root potential (TRP) and is often more complicated to interpret since it also involves an apoplastic pathway of transport. However, it was the observations of TRP rather than single cell (PD) that led to the hypothesis of the electrogenic pump in plants (Higinbotham et al 1970).

**Effect of Root Nutrient Status on Ion Transport.** Measured permeabilities of solutes in individual cells and the whole organ are seldom identical, suggesting that movement of ions in the tissue may be governed by additional factors than those already mentioned for
cellular transport. Ion movement in the root, for example, may largely depend on the rate of water flow, the apoplast to symplast ratio and root nutrient status. Roots grown in dilute CaSO\textsubscript{4} solution, referred to as "low-salt" roots, have a higher capacity for subsequent absorption of several ions than "salt-saturated" (also known as high-salt) roots (Pitman et al. 1976). Manipulation of root nutrient status has led to significant progress in identifying the relative importance of different components of ion transport in plant roots. For example, Pitman et al. (1968) showed that low-salt roots lose their selectivity for K\textsuperscript{+} over Na\textsuperscript{+} in contrast to a strong selectivity in salt-saturated roots in barley. They concluded that such a difference in ion selectivity was correlated with H\textsuperscript{+} efflux from the roots, i.e. low-salt roots exhibited an H\textsuperscript{+} efflux rate of 4\textmu mole gfw\textsuperscript{-1}h\textsuperscript{-1}, but the rate was almost zero in salt-saturated roots. It is still very common to use both low-salt and salt-saturated roots to study ion movement at the cellular level and salt-saturated roots to study transport across the root.

**Root Water Transport**

Water movement from the external medium to the xylem, like that of ions, is perhaps equally dependent on anatomical considerations depicted in Fig. 1. In this section, a summary of major anatomical as well as metabolic components and their relative contribution to root water transport is presented.

Axial. Experimental results indicate that the meristematic region of the root is hydraulically impervious (Frensch and Steudle 1989) perhaps due to lack of mature xylem cells and the high density protoplastic nature of this region (Kramer 1983). On the other hand, the zone immediately behind the root tip roughly equivalent to where the root hairs are present (Zone A to C, Fig.1), is highly permeable to water. The exact length of this highly conductive zone varies between species and stages of development. For example, this
zone extends from 2 to 6 cm in wheat (Boyer 1985), 1.5 to 8 cm in Vicia faba, 1.0 to 4.5 cm in barley and 1 to 9 cm from the root tip in pumpkin (Kramer 1983). The axial water flow is believed to occur almost entirely in the xylem vessels (Passiora 1988) and is therefore expected to increase as the distance increases from the root cap, because of the gradual increase in the number of conducting vessels (mature xylem).

Based on the assumption of axial flow exclusively in the xylem and based on the capillary nature of the xylem vessels, the longitudinal flow of water, $L_x$, is often mathematically described by Poissuelle's equation:

$$ L_x = \frac{Pr^4}{8\eta} \cdot t \quad (3) $$

where $L$ is the length of the conducting vessels with the radius, $r$, $P$ is the pressure on the liquid, $\eta$ is the viscosity, and $t$ is the time. This equation states that the axial flow rate is proportional to the 4th power of the radius of the vessel and is inversely proportional to the length of the xylem. In an intact plant, the driving force for axial water flow is the hydrostatic pressure generated by the transpirational demand. Axial $L_x$ estimated from the Poiseulle equation are often larger than those actually measured (Klepper 1983). It is argued that such differences are perhaps due to erroneous assumptions that the number, size and friction of the vessel elements along the conducting path remain uniform and that there is no lateral exchange of water (Klepper 1983). The equation can be more reliably utilized if the variation, particularly in terms of the size and number of xylem vessels along the length of the root, is taken into account (Frensch and Steudle 1989).

The resistance to water flow in the axial direction is often substantially lower than the resistance in the radial direction. Frensch and Steudle (1989) have shown that the axial resistance is three orders of magnitude smaller than the radial resistance to water flow in maize. However, under high suction, the continuous water column in the vessel elements
may cavitate, resulting in the introduction of air bubbles and a high axial resistance to water flow (Blizzard and Boyer 1980).

Radial. As mentioned earlier, there is much evidence that indicates both apoplastic and symplastic pathways are in use in the radial water transport, but no unequivocal results have been presented to quantify the relative importance of each pathway. A more detailed treatment of this topic was given in the previous section, however evidence for symplastic transport is often substantiated by the fact that radial water movement is sensitive to respiration inhibitors, high CO₂ concentration, anoxia, and extremes of temperature (Kramer 1983). Some of the early evidence for membrane involvement and symplastic transport was presented by Ordin and Kramer (1956). They showed that the half-time (t½) of water exchange for deuterium oxide (D₂O) was reduced from 36s in living bean roots to 6 to 12s for the same roots killed by treatment with boiling water.

One of the factors which may regulate the proportion of symplastic and apoplastic water movement is the magnitude of transpiration dependent mass flow. The evidence for this comes from a study by Raney and Vaadia (1965) when they showed that the t½ for equilibration of THO with excised roots and roots of non-transpiring plants was 30 seconds, but t½ was 15 minutes in roots of transpiring plants. Presumably at high flux rate, water can bypass some of the less conductive components of the symplasm or not get loaded in the cytoplasm altogether. On the other hand, evidence of apoplastic transport is often concluded when permeability of the root tissue is larger than those measured for single cells (Jones et al. 1988, Radin and Mathews 1989). However, until a reliable method of measuring Lp in cell walls is developed, exact quantification of radial water movement in each pathway remains unclear.
Theoretical Consideration of Simultaneous Water and Ion Transport

In studying water and solute transport, roots are often treated as a membrane system which separates the two aqueous solutions between the external medium and the xylem. In an intact plant, the flow of water can be expressed by a Darcy's analogous equation as follows:

\[
J_v = L_p(\Delta \psi) - L_p(\Delta P - \sigma \Delta \Pi)
\]  \hspace{1cm} (5)

where \( J_v \) is the flow rate, \( \Delta \psi \), \( \Delta P \), and \( \Delta \Pi \) are water potential, pressure and osmotic gradients, \( \sigma \) is the effective membrane reflection coefficient, and \( L_p \) is the hydraulic conductance of the root system.

Equation (5) can also describe the flow rate in excised roots or root systems, using applied pressure or suction to simulate the naturally occurring hydrostatic gradient. A unique characteristic of pressure induced-flow has been a greater than linear apparent increase in hydraulic flow with increasing pressure, leading many investigators to propose that root hydraulic resistance decreases with increased pressure gradient (Mees and Weatherly 1957, Lopushinsky 1964, Nulsen and Thurtell 1978). Similar changes in transpiration in relation to \( \Delta \psi \) have also been reported (Aston and Lawlor 1979, Simmelsgaard 1976). However, Fiscus and Kramer (1975) argued against the variable resistance and Fiscus (1975) and Dalton et al. (1975) provided an explanation for the non-linear nature of \( J_v \) versus \( \Delta P \) curves at low \( J_v \). According to Fiscus and Dalton, at steady state flow rate, the total solute fluxes \( J_I \) must equal the product of the concentration in the xylem \( C_I \), and volume flux \( J_v \):

\[
J_I = C_I J_v
\]  \hspace{1cm} (6)

They also adopted the equation derived by Katchalsky and Curran (1965), to express \( J_I \) at non-equilibrium conditions, as a function of its diffusive component, \( \omega \Delta \Pi \), convective component \( J_v (1-\sigma) C_I \), and the active component \( J_I^* \):
\[ J_l - \omega \Delta \pi + J_t (1 - \sigma)C + J_t^* \]  

(7)

where \( \omega \) is the osmotic permeability and \( C \) is the average ion concentration between the xylem and the medium. By combining equations (5) and (7) these two labs calculated \( J_t \) as a function of parameters such as \( \sigma \), \( J_t^* \), and \( J_t \) and concluded that the apparent change in root hydraulic resistance may be caused by the shift from simultaneous hydrostatic and osmotic transport at low gradients to predominantly hydrostatic transport at higher gradients. Such an explanation is not universally accepted and several workers have since challenged the Fiscus and Dalton model (Newman 1976a, Pitman 1982, Passiora 1988), maintaining that \( \text{L}_p \) indeed increases at higher flow rates.

**Methods of Estimating Water and Ion Fluxes**

Estimates of water and ion movement across the root in intact plants are often made by analyzing the content of the shoot at various time intervals. Short term transport characteristics can be evaluated by tracer studies, i.e. radioisotope labeling of water and ions, at much smaller time intervals. Alternatively, many studies make use of excised root or root systems, by simply sealing the cut end of the root into a capillary tube of an appropriate diameter. The rate of water flow can subsequently be determined by changes in the weight or volume of the fluid in the capillary tube, while the collected liquid can additionally be analyzed for its chemical content. From the sap concentration data and \( J_v \), the inorganic solute fluxes under osmotic and hydrostatically driven flow can be estimated from equation (6). Some investigators have used the values of osmotic pressure differences between the external medium and the xylem, \( \Delta \pi \), to draw inferences about the ion fluxes. Such inferences are not always accurate since the xylem may carry organic compounds which contribute to \( \Delta \pi \). In fact, xylem sap has often been shown to contain a significant amount
of organic solutes and in the case of leguminous plants, organic compounds can reach a concentration of 200mM (Gunning et al. 1974).

When excised roots are used, the transpirational effect can be simulated by applying pressure to the root or suction to the cut end. Under applied pressure, ion fluxes at steady state flow rate can be determined from equations (6) for equilibrium and (7) for non equilibrium conditions whereas hydraulic permeability $L_p$ can be estimated from the $J_v/\Delta\psi$ from equation (5). The analysis of transport functions under applied pressure has been challenged on the grounds that it rarely gives close estimates of events in intact roots. Applied pressure would undoubtedly force water into the intercellular spaces which may otherwise be symplastically transported. In terms of ion movement, studies with the applied pressure techniques have been shown to significantly increase ion fluxes compared to their rate of transport in intact roots (Pitman 1982) and to affect the almost universal membrane selectivity for $K^+$ and against $Na^+$ (Pitman 1965).

Excised roots of many species of plants exude fluid from the cut stump due to what is referred to as root pressure. Root exudation has been used extensively to study transport, particularly ion movement. It is now universally accepted that such a flow is chiefly a result of an osmotic flow and can be described by a modified version of equation (5):

$$J_v = L_p \sigma A \pi$$

Note that equation (8) also provides the means for estimation of $L_p$ under osmotically driven flow by evaluating the $J_v$ to $\sigma A \pi$ ratio. Such determination of $L_p$ is usually based on the assumption of perfect semipermeability of the root system and assumes a value of $\sigma$ equal to 1. The membrane reflection coefficient can also assume a value of zero if the membrane is equally permeable to water and ions. Other methods of $L_p$ determination using the osmotically induced flow and the principle of equation (8), include the "reverse flow method" which was developed by Arisz and Helder (1951) and improved by Pitman and
Wellfare (1978). In this method, exudation rate \( \dot{J}_v \) is measured as described earlier and when sufficient exudate is collected from the capillary tube sealed to the cut stump, the medium bathing the root is rapidly changed to a solution of considerably lower osmotic potential. The low osmotic potential can be obtained by adding mannitol or other non-permeating osmoticum, to the root medium. By reversing the concentration gradients, water in the capillary will move downward indicating efflux from the root and the rate of flow is measured as \( J_{\text{in}} \). Root hydraulic conductance can be calculated as:

\[
L_p = \frac{\dot{J}_v + \dot{J}_{\text{in}}}{\sigma (\pi_a + \pi_v)}
\]

where \( \pi_a \) and \( \pi_v \) are the osmotic pressure of the mannitol and nutrient solutions.

Root hydraulic conductance in an intact plant can also be estimated from the ratio of transpirational flow to water potential gradient \( \frac{\dot{J}_v}{\Delta \psi} \), where the gradient is the water potential differences between the shoot and the root surface (Kramer 1983). Finally, permeability to water in a single cell can be determined by the pressure probe technique, which has been treated in detail by Zimmermann and Steudle (1978, 1980). Briefly, in this technique, a microcapillary filled with an oil is inserted into the cell after a period of which the oil hydrostatic pressure equilibrates with that of the cell. This pressure can be recorded by a pressure transducer. Cell volume and its hydrostatic pressure can be modified instantaneously by pushing the oil inside the capillary backward or forward which results in a change in the cell water potential and the cell volume and means that water has to either exit or enter the cell to compensate for pressure changes. The half time of water exchange \( t_{\Delta} \) and changes in cell volume \( \Delta V \), are recorded and used to calculate \( L_p \) from:

\[
L_p = \frac{V \ln 2/A}{2A \left( \varepsilon - \Psi \right)}
\]

where \( \Delta \psi \) is the cell osmotic potential obtained by subtracting \( \Psi_{\text{root}} \) from its turgor, \( A \) is cell surface area, and \( \varepsilon \) is the volumetric elastic modulus which can be determined from:

\[
\varepsilon = \frac{V \Delta P}{V \Delta V}
\]
where $V$ is the mean cell volume and $\Delta v$ is change in cell volume associated with changes in hydrostatic pressure, $\Delta P$.

It is worth mentioning that $L_p$ estimates show considerable variability based on techniques used. We have already mentioned the variability which exists between single cell and whole root $L_p$, but variation in $L_p$ estimates have also been reported in techniques using hydrostatic versus osmotic flow as well (Frensch and Steudle 1989, Pitman 1982, Steudle et al. 1987). Such information warrants against the universal comparison of $L_p$ determined by different techniques. Let us now evaluate how excision and elimination of transpiration can influence ion fluxes in the root.

**Effect of Excision and Transpiration on Solute Fluxes**

An important question regarding analysis of transport functions using excised roots is whether or not they behave like roots of intact plants. It is evident that over extended periods, excision may introduce problems with respect to the decline in reserves and respiration. Excision may have a more immediate effect on uptake of ions into the root than their release into the xylem. Bloom and Caldwell (1988) showed that net potassium uptake was reduced within 2 hours after excision in barley roots, but the flux returned to control levels within 6 hours after excision. A similar reduction in $NO_3^-$ uptake did not occur until at least 6 hours and no recovery was observed up to 11 hours after excision. Others, however, have shown that in maize $K^+$ transport in excised roots was unaffected up to 24 hours (Anderson and House 1967). Excision also eliminates transpiration and convective flow resulting from transpiration. Schulze and Bloom (1984) showed that $NH_4^+$ and $NO_3^-$ influx in the intact roots of tomato and radish seedlings were independent of transpiration rate. Greenway (1965) measured $Cl^-$ transport to shoots in barley and concluded that transpiration rate does not affect the active component of ion flux, but affects the passive
component. Increased transpiration rate in barley was reported to increase Na\(^+\) uptake and transport to the shoot at the expense of K\(^+\) (Pitman 1965). The exact relationship between the transpirational rate and ion movement across the root appears to depend on the ionic and plant species. However, the prevailing feeling is that for most ions the radial transport is relatively independent of the transpiration rate, but the upward movement of ions in the xylem is a function of the flow rate in the xylem (Sutcliffe 1976).

**Effect of Root Temperature on Water Transport**

Root temperature has been shown to have profound effects in uptake and transport of water in intact plants (Kuiper 1964, Brouwer 1953, Kaufmann 1976, Radin 1990, Shirazi et al. 1975), excised roots under pressure (Markhart et al. 1979) and excised roots exuding by root pressure (O’Leary 1966, Clarkson 1976, Anderson and Reilly 1968, Collins and Morgan 1980). In general, plants adapted to warmer environments exhibit a higher sensitivity to low temperature than plants from cooler regions. According to Kramer (1983) the principal factors involved in root chilling sensitivity are: a) Decreased root hydraulic conductance, b) Decreased metabolic activities leading to decreased salt accumulation and decreased osmotic water uptake, and c) Decreased growth leading to reduced absorbing surfaces. At extremely low temperatures, increased viscosity of water can also be a factor for reduced water transport (Kuiper 1964). Clarkson (1976) and Markhart et al. (1979) showed that roots acclimated to cool temperature exhibited a higher hydraulic conductance when transferred to 25°C than did the non-acclimated roots. Later Markhart et al. (1980) suggested that cold acclimation enhancement of root hydraulic conductance was due to increase in membrane saturated fatty acids. Root temperature effects on water uptake and transport can be mediated by production of plant growth regulators such as ABA and cytokinins which have been shown to influence root hydraulic properties (to be covered in
a later section). The amount of a number of growth regulators such as ABA, cytokinin, GA and IAA in the xylem exudate was found to be dependent on the root growth temperature (Atkin et al. 1973).

Studies of temperature effects on volume flux in excised roots have produced variable results. Anderson and Reilly (1968) reported that root temperature alters exudation rate by an effect on $\Delta n$, while Clarkson (1976) suggested that enhanced $J_v$ in precooled barley and rye roots was primarily due to changes in root hydraulic conductivity. Still others have proposed that both hydraulic ($L_p$), and osmotic $\Delta n$, components of the root transport can be altered by changes in temperature (Collins and Morgan 1980).

**Effects of Root Temperature on Ion Transport**

As stated in the preceding section, temperature can influence root pressure exudation by affecting the osmotic gradient and since it is generally assumed that this gradient is largely produced by ion concentration differences, the temperature induced changes in $J_v$ may be a result of changes in inorganic solute fluxes $J_i$. Temperature sensitive processes such as respiration which partially regulate ion movement can mediate $J_i$ response to variation in root temperature. The $Q_{10}$ for the uptake of many ionic species such as $PO_4^{-3}$, $H^+$, $K^+$, $Cl^-$, and $SO_4^{2-}$ is between 2 and 3, corresponding to a similar $Q_{10}$ expected for respiration, whereas $Ca^{2+}$ influx has a $Q_{10}$ of close to 1 indicating a passive process (Glass 1989).

Various investigators have demonstrated a strong dependence of ion transport on root temperature (Cumbus and Nye 1982, Cumbus and Nye 1985, Clarkson et al. 1986) where the apparent mechanism may be the ensuing changes in the absorbing surfaces and/or development of conducting tissues. On the other hand, short term experiments have revealed changes at the cellular level which may affect root ion transport. Kennedy and
Gonzalves (1988) showed that proton efflux was maximum at 30°C for control and 32°C for pre-cooled corn roots. In another study, cell membrane potential in corn was also found to be temperature dependent and it increased in a biphasic manner, up to a temperature of around 35°C (Bravo and Uribe 1981). This group also implied that a reduction in $K^+$ and $PO_4^{-3}$ transport at low temperature is not closely associated with respiration and membrane permeability. Instead they suggested that the low temperature inhibition of ion transport resulted from a direct effect on the protein carrier systems.

Clarkson (1976) reported that when barley and rye roots were pre-cooled for 12 to 72 hours and then transferred to the control temperature, the fluxes of $K^+$, $Ca^{2+}$, $H_2PO_4^-$ were more than doubled compared to the nonprecooled roots. Clarkson suggested that an increase in solute transport was a likely result of an increase in the number of ion-transporting sites rather than increased activity of the individual sites. However, the exact mechanism of cold-acclimation enhancement of ion transport is still a matter of considerable debate. Some studies of root temperatures and ion transport have been carried out with intact plants, but often over relatively long periods. Gosselin and Trudel (1986) showed that leaf supply of N, P, and K increased gradually with increasing root temperature from 12° to 36°C, but the content of Ca and Mg was reduced in the same range in pepper. Such studies average the leaf content of ions over several days and give no information about short term mechanistic interpretations of temperature induced changes in ion fluxes. Ion movement across the root has seldom been characterized at high temperature (supra-optimal), and as a result, the mechanisms of high temperature induced changes in $I_I$ and its potential contribution to volume flow in excised roots have rarely been evaluated.
Regulation of Ion and Water Transport by Abscisic Acid (ABA)

There is an impressive body of evidence indicating that exogenously applied ABA has a marked influence on transport properties of roots. It is therefore logical to consider if induced responses in water and ion movement to an environmental stimulus e.g. temperature could potentially be regulated by ABA. Although much of the literature shows stimulation of volume flow, $J_v$, in ABA treated roots (Ludewig et al. 1988, Van Steveninck et al. 1988, Glinka 1980, Karmoker and Van Steveninck 1978), ABA inhibition of $J_v$ has also been reported (Pitman and Wellfare 1978). The mechanism(s) of action of ABA is not clearly understood, and the evidence indicates that the response is mediated by either changes in root $L_p$ (Glinka 1980, Fiscus 1981, Collins and Morgan 1980) or by changes in $J_I$ (Pitman and Wellfare 1978).

Hydraulic Conductivity. Collins (1974) reported that in corn root, ABA increased $L_p$ by more than two-fold compared to the untreated roots and suggested that ABA must affect the symplastic pathway of water transport since any effect on the apoplastic pathway was unlikely. Fiscus (1981) and Markhart et al. (1979) reported that ABA decreased $L_p$ in bean roots. In these studies, however, the reduction in $L_p$ may have been caused by unreasonably high concentration of ABA ($5 \times 10^{-5}$ to $10^{-4}$ M) applied to the roots. Glinka (1977, 1980) showed that ABA stimulated $L_p$ in ABA treated sunflower roots and suggested that such a change is a result of increased permeability at the endodermal level. Hydraulic conductance was also shown to increase in both pressurized and non-pressurized ABA-treated excised sunflower roots (Ludewig et al. 1988). The results reported by Ludewig et al. confirmed the earlier report by Glinka (1977) that the magnitude of ABA effect did not depend on the hydrostatic gradients. The exact mechanism by which ABA changes root permeability to water is a focus of an ongoing debate, but the consensus is that some membrane functions may be responsible for changes in $L_p$. 
Solute Fluxes. In addition to its effect on root hydraulic permeability, ABA has a great influence on root ion transport. In fact ABA enhancement of \( J_y \) in root exudation experiments has often been attributed to an increase in the osmotic gradient resulting from increased ion release, particularly \( K^+ \), into the xylem (Karmoker and Van Steveninck 1978). Most investigators have reported enhanced solute fluxes in ABA treated roots (Glinka 1980, Fiscus 1981, Ludewig et al. 1988, Collins and Morgan 1980). Pitman and coworkers (1972, 1974, and 1978) found that solute fluxes along with \( J_y \) were inhibited in ABA treated barley roots and the inhibition was equally induced in excised roots and intact transpiring plants, indicating that the ABA effect on ion transport was independent of water flow. In another work, Pitman et al. (1974) suggested that stimulation or inhibition could occur depending upon the conditions at which the plants are grown, i.e. root temperature and nutrient status. Several reports have indicated that ABA may differentially affect the different components of ion flux. For example, Cram and Pitman (1972) found that ABA inhibited ion transport (\(^{86}\)Rb and \(^{35}\)Cl) to the xylem, but uptake to barley roots was unaffected. Similarly, Pitman (1977) found that in ABA treated barley roots, a reduction in \( \Phi_t \) was accompanied by either no change or a slight increase in \( \Phi_o \). These observations were instrumental in the development of the "two pump" hypothesis for the radial ion transport. Pitman and his coworkers have since examined the effect of cycloheximide, a protein synthesis inhibitor, and two amino acid analogues, DL-P-Fluorophenylalanine (FPA) and L-azetidine-2-carboxylic acid (AZ), on ion fluxes in roots. The amino acid analogues are not inhibitors, but they lead to the formation of ineffective proteins. These studies indicated a preferential inhibition of \( \Phi_t \) by these compounds similar to that reported from ABA treated roots. Pitman (1977, 1982) has therefore suggested that ABA affects the release of ions from the xylem parenchyma cells into the xylem by perhaps affecting membrane-bound transport proteins.
Ion supply can be wholly or partially supplied by internal sources (Jarvis and House 1970, Hodges and Vaadia 1964). When the external source of an ion is eliminated, the symplastic transport of that ion to the xylem is likely to be supplied by the vacuoles of the cortical cells (Anderson 1975). In sunflower, Glinka (1980) reported an increase in both $K^+$ and $NO_3^-$ fluxes to the xylem in ABA treated roots, even when the external supplies of these ions were cut off. He therefore suggested that ABA may increase ion fluxes by increasing the vacuolar release of ions into the symplasm.
MATERIALS AND METHODS

Growth Conditions

Seeds of barley (*Hordium vulgare* L. cv 'Arivat') and sorghum (*Sorghum bicolor* L. cv 'Funks') were germinated in vermiculite and transferred to modified half-strength Hoaglands' solution (Hoagland and Arnon 1938) at 4 and 6 days of age, respectively. Before and after transplanting, seedlings were grown at 25±2°C with 13 h daily light (250 to 300 μmol m⁻² s⁻¹ PAR). In some experiments, the nutrient solution was modified to obtain a K⁺ deficient medium as described by Kurtz and McEwan (1960). This solution was found to have an osmotic pressure identical to that of the unmodified solution.

Measurement of Exudation Rate, Osmotic Pressure Gradient and Solute Fluxes

At 5 and 8 days after germination, shoots of barley and sorghum seedlings, respectively, were excised about 0.5 cm above the meristem located near the base of the leaf. Preliminary work in this lab had established that growth rate in barley and sorghum seedlings were maximum at these corresponding ages, therefore root temperature effects on growth of the shoot were expected to be magnified and consequently more detectable for short term experiments. A 100 μl capillary pipet was then fitted to the stump of the root with a flexible tygon tube and roots were placed in vigorously aerated controlled temperature nutrient solution. Temperature control was obtained by immersing the nutrient solution in several water baths, each set at a different but constant temperature. In each experiment, a maximum of four treatments were tested in the 15 to 40°C ranges. Temperature increments were 3 to 5°C for the most part and data from several experiments had to be combined.
Abscisic acid (Calbiochem) was applied to the nutrient solution at various temperatures by first dissolving it with 1 ml of 95% ethanol and then adding it to the solution to a final concentration of 10μM. In ABA studies, similar amounts of ethanol were added to solutions which did not contain the hormone, to account for the possible effects of the alcohol. Rate of exudation \( (J_v) \) was determined from the changes in the level of fluid in the capillary tube. All data reported here were collected between 2 to 4 h after a steady state value of \( J_v \) was obtained within 1/2 h after excision. Osmotic pressure of the exudate \( \pi_e \) and the medium were determined with a Wescor model 5500 vapor pressure osmometer. The osmotic pressure gradient \( (\Delta \pi) \) was calculated from \( (\pi_e - \pi_m) \) at any given temperature. Exudate samples were sent to the Soil and Water Testing Laboratory at the University of Arizona where they were analyzed by ion chromatography for anions and atomic absorption and flame emission for cations. Solute fluxes were calculated as the product of \( J_v \) and ion concentrations \( C_i \) as described by equation (6).

**Growth, Volume Flow, and Leaf Water Status**

Growth was evaluated from the average leaf elongation rate between 2 to 4 h after initiation of root temperature treatments at a constant 25°C ambient (air) temperature. Elongation rate was determined from changes in the length of the youngest leaf, simply measured with a ruler and was expressed per unit time. To estimate the flow rate in the intact seedlings, the transpirational water loss was measured gravimetrically by wrapping the leaf basal region of 10 seedlings in a 25 x 100 mm polyurethane foam and inserting their roots in a 25 ml plastic centrifuge tube containing the nutrient solution. Replicates of centrifuge tubes containing the seedlings were then placed in several water baths, each set at a different but constant temperature. Air temperature was constant at 25°C and at the end of each run, roots were severed from the seedlings and blotted with paper towels for the
fresh weight determination. The total volume flow in intact seedlings was then calculated as μl gfw⁻¹ h⁻¹ at the same time intervals as growth rate measurements were made.

Water potentials of the leaves were determined psychrometrically with Merrill 75-13 Psychrometers and a Wescor MJ-55 microvoltmeter after 3 to 4 h of equilibration in a water bath at a constant temperature of 25°C. Each psychrometer was calibrated individually using NaCl solutions of various concentrations. More specifically, a 0.7 cm circle of Whatman No. 1 filter paper was dipped into the solution of known concentration (known osmotic pressure) and then placed into the chamber. After the equilibration period, 15 s cooling was applied and the induced voltage was recorded. The correction factor to convert the voltage reading to water potential (MPa) varied slightly between the psychrometers and the range was between 0.18 to 0.21. For tissue water potential determinations, five 0.5 cm long segments from the leaf (growing or expanded blade regions) and the root were excised and placed in the psychrometer chambers. Root segments were excised from 5 to 5.5 cm from the tip in each plant while the growing and expanded segments of the leaf were excised at 0 to 0.5 cm and 5 to 5.5 cm from the meristematic region of the shoot respectively. After \( \Psi_r \) determination, the chambers were inserted in liquid N for 30 s and then allowed to thaw before they were placed back in the water bath for equilibration (1/2 h) at 25°C to measure \( \Psi_t \). Tissue turgor \( \Psi_t \) was then calculated from \( (\Psi_L - \Psi_r) \). In barley, the water potential of the growing region is substantially lower than the expanded blade (Riazi et al 1985, Matsuda and Riazi 1981), therefore water status measurements of both regions were made. Such differences were not observed in sorghum leaf (youngest) based on our preliminary observations, therefore only the water potential measurements of the expanded blades were made.
Hydraulic Conductance

Due to experimental limitations, root hydraulic conductances were estimated in root pressure exudation studies from the ratio of $J_p / \Delta\pi$ (according to equation 8) assuming a value of 1 for $\sigma$. The estimated $L_p$ from this method was, however, compared to several other methods at 25°C in sorghum and barley roots. Whenever possible, the comparisons were extended to include temperatures other than 25°C. Estimates of the root hydraulic conductance for intact plants were made from $J_{\text{intact}} / \Delta\psi$ from equation (5). Hydraulic conductance determinations were made under applied pressure only for sorghum roots at 25°C. In these studies, sorghum roots were placed inside a Scholander chamber containing the nutrient solution, with the cut end protruding outside the chamber through a tightly sealed rubber stopper. The pressure was applied at 1 to 2 bar increments. Sap was collected inside a capillary tube attached to the cut end of the root and weighed. The ratio of the volume flow, $J_v$, to applied hydrostatic pressure, $\Delta P$, was used to calculate root $L_p$.

Finally, $L_p$ was also determined under an osmotically driven flow by utilizing the reverse flow method. In these experiments, exudation rate ($I_e$) was determined as described in the earlier part of this chapter. When sufficient sap was collected, often 2 to 4 h after exudation had started, the solution around the root was rapidly changed to a solution containing mannitol with an osmotic pressure of 3.9 to 4.3 bars. This induced a reverse flow out of the root and its flow rate was determined by the change in the level of the fluid in the capillary. Ideally, the rate of loss to mannitol should be measured instantaneously upon transfer to mannitol, in order to avoid the possible changes in the internal osmotic concentration because apparently water flows out of the xylem vessels more rapidly than the solutes do (Pitman and Wellfare 1978). In this study, an interval of 5 min. was used to measure the flow out of the root. Hydraulic conductance in this method was calculated without the knowledge of $\pi_{hyd}$ using equation (9) from the previous chapter. In addition
to using the "reverse flow method" to estimate $L_p$ at various temperatures in response to an osmotic gradient of 3.9 to 4.3 bars, this method was also employed to estimate the $L_p$ at various mannitol concentrations (various $\Delta n$) for both plants at 25°C.
RESULTS AND DISCUSSION

**Plant Growth Response**

Short term growth rate of both barley and sorghum seedlings showed a strong root temperature dependence. Figure 3 shows that the maximum growth rate, measured within 4 to 5 h after the treatments, occurred at 35°C in sorghum and 25°C in barley seedlings. The differences in optimum root temperature (ORT) between the two species are typical of growth responses of cool- and warm-season species to root temperature (Cooper 1973, Neilsen 1974). Leaf elongation rate, at ORT, was slightly higher in barley than sorghum and in both plants, when the root temperature was elevated to 40°C, growth rates were inhibited to about 60% of the maximum (Fig 3). In some studies with barley seedlings, shoot growth responses were monitored for 4 days at various root temperatures. At the end of the fourth day, it was noted that 30, 50 and 65% of the plants had a visible new leaf in seedlings grown at 15, 25 and 35°C root temperatures respectively. Such observations were not made for sorghum, but the results in barley indicated that root temperature may differentially influence shoot metabolic processes, i.e. in barley at supra optimal temperatures, cell division and/or cell differentiation may have been enhanced in spite of an apparent reduction in cell expansion. These observations are consistent with the findings of Power (1970) who showed that in barley, increasing the root temperature above the ORT (27°C) led to reduced time to maturity, but also reduced what they referred to as "yield potential". He suggested that elevated root temperature did not allow for all the plant factors necessary for maximum growth.

Although root temperature-induced variation in shoot growth has been reported by numerous investigators, the literature has rarely offered a mechanism(s) for such a response. Seedling growth is ultimately regulated by the rate of water and ion supply from the root.
Fig 3. Short term growth rate as influenced by root temperature in 5- and 8-d-old barley and sorghum seedlings respectively. Growth was measured on the 1st leaf in barley and 2nd leaf in sorghum within 4 h after root temperature treatment. Values are means of 15 to 20 readings ±SD.
Limited water supply to the shoot leads to reduced leaf water and turgor potential hence inhibiting growth (Hsiao 1973). Consequently, seedling water status was analyzed to evaluate whether root temperature alteration of growth rate was mediated by changes in seedling water status and/or by the rate of water supply to various parts of the plants.

**Seedling Water Status and Volume Flow**

*Water Potentials.* Leaf and root water potentials ($\Psi_l$ and $\Psi_r$) were found to be independent of root temperature within the range tested in this study (Table 1). The results indicated that the bulk leaf water potential, determined psychrometrically, did not mediate the short term root temperature induced changes in growth rate of the seedlings (Fig 3). Such observations have also been made by Milligan and Dale (1988) when they reported that long term root cooling in *Phaseolus vulgaris* reduced growth but not $\Psi_l$. Similarly Benzioni and Dunstone (1988) showed that root cooling reduced $J_r$ but not $\Psi_l$ in jojoba. Other investigators, however, have observed relatively rapid responses in $\Psi_l$ at suboptimal root temperatures (Radin 1990, Wilson 1983, and McWilliam 1983) particularly when root temperatures were substantially lower than 15°C.

Matsuda and Riazi (1981) and Riazi et al. (1985) demonstrated that in barley seedlings the leaf water potential in the growing region ($\Psi_{Gl}$) was significantly lower than the expanded blade ($\Psi_{Ed}$) and that leaf elongation rate was more closely associated with $\Psi_{Gl}$ than $\Psi_{Ed}$. The results in Table 1 confirmed such differences in barley, but root temperature did not alter the water status of either region. In sorghum, leaf water potential determinations were made only in the expanded blade, since the $\Psi_{Ed}$ was similar to $\Psi_{Gl}$ at 25°C root temperature (Table 1) and were assumed to be equal for both region at other temperatures as well. Leaf water potential in sorghum was also unchanged at various
Table 1. Influence of root temperature on leaf and root water status in 5- and 8-d-old barley and sorghum seedlings respectively. Medium water potential was constant at -0.1 MPa at all temperature when measured with the vapor pressure osmometer. In sorghum, water potential ($\psi_v$) of the expanded leaf (EB) and the growing region (GR) were identical at 25°C and assumed to be identical at other temperatures. Leaf water potential used for calculating the gradients between the leaf and the medium ($\psi_v - \psi_m$) and leaf and the root ($\psi_v - \psi_R$) are the water potentials of the growing regions in both plants. Values are means of 5 replicates ± SD.

<table>
<thead>
<tr>
<th>WATER POTENTIAL (MPa)</th>
<th>15</th>
<th>25</th>
<th>35</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sorghum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\psi_v$</td>
<td>.30 ± .02</td>
<td>.28 ± .03</td>
<td>.29 ± .03</td>
<td>.31 ± .06</td>
</tr>
<tr>
<td>$\psi_v$ (EB)</td>
<td>.62 ± .07</td>
<td>.54 ± .04</td>
<td>.62 ± .14</td>
<td>.61 ± .14</td>
</tr>
<tr>
<td>$\psi_v$ (GR)</td>
<td>-</td>
<td>.54 ± .14</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$\psi_m$ (GR)</td>
<td>-</td>
<td>.10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>($\psi_v - \psi_m$)</td>
<td>.20</td>
<td>.18</td>
<td>.19</td>
<td>.21</td>
</tr>
<tr>
<td>($\psi_l - \psi_v$)</td>
<td>.52</td>
<td>.44</td>
<td>.52</td>
<td>.51</td>
</tr>
<tr>
<td>($\psi_l - \psi_R$)</td>
<td>.32</td>
<td>.36</td>
<td>.32</td>
<td>.31</td>
</tr>
<tr>
<td>$J_v$ ($\mu g^{-1} h^{-1}$)</td>
<td>311 ± 36</td>
<td>580 ± 34</td>
<td>840 ± 43</td>
<td>276 ± 23</td>
</tr>
<tr>
<td><strong>Barley</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\psi_v$</td>
<td>.36 ± .05</td>
<td>.28 ± .09</td>
<td>.31 ± .06</td>
<td>-</td>
</tr>
<tr>
<td>$\psi_v$ (EB)</td>
<td>.40 ± .08</td>
<td>.37 ± .05</td>
<td>.47 ± .03</td>
<td>-</td>
</tr>
<tr>
<td>$\psi_v$ (GR)</td>
<td>.72 ± .08</td>
<td>.62 ± .09</td>
<td>.68 ± .08</td>
<td>-</td>
</tr>
<tr>
<td>($\psi_v - \psi_m$)</td>
<td>.26</td>
<td>.18</td>
<td>.21</td>
<td>-</td>
</tr>
<tr>
<td>($\psi_l - \psi_v$)</td>
<td>.62</td>
<td>.52</td>
<td>.58</td>
<td>-</td>
</tr>
<tr>
<td>($\psi_l - \psi_R$)</td>
<td>.32</td>
<td>.34</td>
<td>.370</td>
<td>-</td>
</tr>
<tr>
<td>$J_v$ ($\mu g^{-1} h^{-1}$)</td>
<td>517 ± 56</td>
<td>1075 ± 124</td>
<td>1145 ± 60</td>
<td>-</td>
</tr>
</tbody>
</table>
root temperatures (Table 1). Since short term changes in growth were unrelated to tissue water status, turgor in the growing region was considered to regulate growth, but \( \Psi_p \) determination in limited experiments (Table 2) indicated that turgor did not change significantly with changing root temperature. The lack of a close association between cell turgor and leaf growth has been reported by many investigators (Michelena and Boyer 1982, Mason and Matsuda 1985, Van Volkenburgh and Boyer 1985, Termaat et al. 1985). Boyer (1987) interpreted such results in terms of the Lockhart equation (Lockhart 1965) restated as follows:

\[
G = \frac{mL_p}{m + L_p(\Psi_X - \Psi_w - Y)}
\]

where \( G \) is growth rate, \( m \) is cell wall extensibility, and \( Y \) is wall yield threshold.

Data in Table 2 indicate that like \( \Psi_h \), leaf solute or osmotic potential (\( \Psi_v \)) was also unaffected by root temperature. Because \( \Psi_{EB}, \Psi_{EF} \) and \( \Psi_k \) remained unaffected by changes in root temperature (Table 1), it can reasonably be assumed that \( \Psi_X \) was also unchanged ruling out \( \Psi_X \) as a potential cause of shoot growth response to root temperature. According to equation 12, cell wall extensibility, yield threshold, and the rate of water supply to the growing tissue may have mediated root temperature responses in growth rate. In this study, only root hydraulic conductance was evaluated in detail as a signal from the root which may possibly lead to changes in shoot growth.

**Volume Flow.** The amount of water passing through the seedlings, \( J_v (\mu l \ gfw^{-1} \ h^{-1}) \), depended strongly on root temperature. More specifically, \( J_v \) increased in intact sorghum seedlings up to 35°C (also optimum temperature for shoot growth) and then declined at higher temperatures, whereas in barley, \( J_v \) increased with increasing root temperature up to 25°C and remained relatively unchanged at elevated temperature (Table 3). Variations in \( J_v \), when \( \Psi_v \) remains constant, are often indicative of a variable root hydraulic conductance, but in intact plants, root temperature-induced variation in \( J_v \) may also lead to no change in
Table 2. Effects of root temperature on leaf ($\psi_r$), osmotic $\psi_m$ and turgor potential $\psi_p$ in 5 and 8d old barley and sorghum seedlings respectively. Leaf water potentials were determined psychrometrically for the growing region in both plants. Values are means of 5 replicates ±SD.

<table>
<thead>
<tr>
<th>$\psi_r$(GR)</th>
<th>$\psi_m$</th>
<th>$\psi_p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 Root Temp (C°)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>0.72 ± .13</td>
<td>1.08 ± .04</td>
</tr>
<tr>
<td>25</td>
<td>0.62 ± .05</td>
<td>0.95 ± .05</td>
</tr>
<tr>
<td>35</td>
<td>0.69 ± .07</td>
<td>0.99 ± .04</td>
</tr>
<tr>
<td>25</td>
<td>0.72 ± .12</td>
<td>0.97 ± .04</td>
</tr>
<tr>
<td>35</td>
<td>0.58 ± .07</td>
<td>0.94 ± .09</td>
</tr>
</tbody>
</table>
Table 3. Effect of root temperature on volume flow ($I_v$) and hydraulic conductance ($L_p$) for 5 and 8d old barley and sorghum seedlings respectively. $L_p$ is calculated based on water potential gradients at various points between the nutrient solution ($\psi_o$), roots ($\psi_r$) and the water potential of the growing region ($\psi_{GR}$). In sorghum, the water potential of growing and expanded blade are assumed to be equal. Values are means of 5 replicates ± SD.

<table>
<thead>
<tr>
<th>Root Temp (°C)</th>
<th>$I_v$ (µl g⁻¹ h⁻¹)</th>
<th>$L_p$ (µl g⁻¹ h⁻¹ MPa⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorghum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>0.311 ± 0.036</td>
<td>598</td>
</tr>
<tr>
<td>25</td>
<td>0.580 ± 0.034</td>
<td>1318</td>
</tr>
<tr>
<td>35</td>
<td>0.840 ± 0.043</td>
<td>1615</td>
</tr>
<tr>
<td>40</td>
<td>0.276 ± 0.023</td>
<td>541</td>
</tr>
<tr>
<td>Barley</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>0.517 ± 0.056</td>
<td>834</td>
</tr>
<tr>
<td>25</td>
<td>1.075 ± 0.124</td>
<td>2067</td>
</tr>
<tr>
<td>35</td>
<td>1.145 ± 0.060</td>
<td>1974</td>
</tr>
</tbody>
</table>
ψ, if other sites in the shoot offer variable conductances to water flow. Experimental conditions under which \( J_v \) was calculated, low light intensity as well as a small transpiring surface in these seedlings, were conducive of relatively low transpiration rates. Under such conditions it is likely that a considerable resistance to water flow is offered at the site of the growing region of the shoot (Boyer 1974, Radin and Boyer 1982), leading to inaccurate quantification of root \( L_p \) alone.

Root Hydraulic Conductance

Intact plant method. Root \( L_p \) was calculated using various \( \Delta \psi \) (from nutrient solution to root, solution to leaf, and root to leaf) supplied from Table 1. In sorghum, regardless of which \( \Delta \psi \) was chosen for calculation, \( L_p \) was found to increase with increasing root temperatures up to 35°C, and then to decline sharply when the temperature was raised to 40°C (Table 3). Similar response was observed in \( J_v \) (Table 3) and shoot growth rate (Fig. 3) indicating that the root temperature–induced response in growth is perhaps regulated by a hydraulic signal. Root temperature effects on \( J_v \) in barley were also accompanied by similar responses in \( L_p \) (Table 3). Although in sorghum supraoptimal root temperature inhibited both growth and \( L_p \) (Fig. 1 and Table 3), nonetheless, in barley inhibition of growth rate between 25 and 35°C coincided with no changes in \( L_p \). The results indicate that growth inhibition in barley at elevated temperature is not likely to be mediated by root volume flow and the rate of water supply to the shoot. Non-hydraulic signals such as rate of ion supply are more likely a potential mechanism.

There are some concerns about the use of intact plants for root \( L_p \) determination. Bunce (1978) reported that leaf water potential does not rapidly equilibrate with changes in \( J_v \). He showed that \( \Delta \psi \) increased linearly with increased \( J_v \) if \( \psi \) measurements were taken 3 h after steady state \( J_v \) had been reached, but a non-linear relationship was observed when
were measured 1 h after steady state \( J_v \) was reached. Another concern is that, as \( J_v \) increases, the nature of the driving force changes from "osmotic" to "hydrostatic" (Fiscus 1975, Dalton 1975) which has been suggested to induce variable \( L_p \) (Frensch and Steudle 1989). Finally, in calculating root \( L_p \) from \( J_v/Δψ \), a major difficulty is to determine exactly the effective driving force for root water transport. In Table I, three water potential gradients between the medium and various components of the seedlings are listed. Traditionally, \( (\psi_m - \psi_a) \), has been used as the effective gradient to calculate root \( L_p \) with the implicit assumption that the bulk leaf water potential \( \psi_l \) is a close approximation of the xylem water potential \( \psi_x \). This approximation of the effective gradient can be challenged on two grounds, first, in many monocot species such as barley and corn, \( \psi_l \) is substantially lower in the growing region than the expanded blade. This difference raises the question which \( \psi_l \) exactly represents the \( \psi_x \) (Rayan and Matsuda 1988). Second, even if leaf water potential were ideally uniform, the assumption that \( \psi_l \) is a close estimate of \( \psi_x \) is questionable. Using \(^3\text{H}\)-labeled water, Rayan and Matsuda (1988) showed that water in the xylem does not readily exchange with the mesophyll cells in the growing and expanded region. In the growing region, the specific radioactivity (SR) of the apoplastic water (including the xylem water) reached that of the nutrient solution in about 2 h, whereas SR in the growing tissue was about 60% of that in the root medium after 5 h. The SR was even lower in the expanded blade after 5 h of exposure to \(^3\text{H}\)-labeled water. Their results indicate that water status of leaf tissues can be partially or fully uncoupled from changes in the water status of the xylem. Accordingly, it is strongly suspected that neither \( (\psi_{GR} - \psi_0) \) or \( (\psi_{EB} - \psi_0) \) can accurately describe the effective gradient for radial water transport in these roots.

Alternatively, \( (\psi_R - \psi_0) \) can be used to estimate \( L_p \). If \( \psi_R \) is largely a measure of the water status of root cortical cells, then it can be argued that \( \psi_R \) does not necessarily
represent \( \Psi_R \) and \( (\Psi_R - \Psi_0) \) does not adequately describe the driving force across the major barrier to radial water flow. However, Rayan (1989) has shown that in barley roots, within 15 minutes of exposure to \( ^3 \text{H} \)-water, root tissue and xylem contained a similar amount of radioactivity. These data lend support to the idea that the resistance to water equilibration across the root is small, and therefore \( \Psi_R \) is perhaps a close approximation of \( \Psi_\text{x} \). In this case \( (\Psi_R - \Psi_0) \) must account for the drop in water potential across the site of the largest resistance to radial water flow in the root, perhaps the Casparian strip. In addition, the assumption that \( \Psi_R \) closely estimates \( \Psi_\text{x} \) also fulfills the thermodynamic requirements of a higher water potential in the xylem than in the mesophyll cells of the growing region and the expanded blade to ensure a flow from the xylem to the cells. In the following section, \( L_p \) values obtained by other methods will be compared to \( L_p \) determined in intact plants from \( (I_p/\Psi_R - \Psi_0) \).

**Comparing Effects of Temperature on Root \( L_p \) by Different Methods**

As has been mentioned, the difficulty of exactly determining the driving force for root water transport, the shift from osmotic to hydrostatic flow at high \( I_p \), and existence of substantial resistances to water flow elsewhere in the plant, can potentially affect the accuracy of intact plant determinations of root \( L_p \). Consequently, by using excised roots and various other methods, effects of temperature on root hydraulic conductance were evaluated independently of hydraulic conductances elsewhere in the seedlings.

**Osmotic uptake method (OUM).** As stated previously, the rate of root pressure exudation is chiefly governed by \( \Delta \Pi (\Pi_\text{x} - \Pi_\text{g}) \) and root hydraulic conductance, \( L_p \). In barley roots, the exudation rate \( (I_p) \) increased almost linearly with root temperature from 15 to 25°C, with an average \( Q_{10} \) of 4 (Fig. 4A). Since the osmotic potential gradient \( (\Delta \Pi) \) was virtually unaffected in this temperature interval (Fig. 4B), the increases in exudation rates
Fig. 4 Temperature induced response of $J_v$(A) and its major components $\Delta \pi$(B) and $L_p$(C) in excised roots of 5-d barley and 8-d-old sorghum. Measurements of $J_v$ and $\Delta \pi$ were made during steady state flow, often within 4 h following excision. Values are means of 6 measurements ±SD.
resulted primarily from increased hydraulic conductivity (Fig. 4C). Above 25°C, however, $J_v$ (Fig. 4A) and also $\Delta \Pi$ (Fig. 4B) decreased by similar amounts with increasing temperature, and values of both measures at 40°C were about half those at 25°C. These data suggest that decreased exudation at high temperature was not limited by $L_p$. Although $J_v$ responded differently to high temperature in osmotic uptake versus intact method (Table 3 and Fig. 4A), $L_p$ calculated by either method showed similar responses at supraoptimal temperature.

Temperature responses of sorghum roots differed somewhat from those of barley. At the lower temperatures (e.g. 15 to 25°C), the osmotic potential gradients in sorghum and barley roots were alike (Fig. 4B), but exudation rates of sorghum roots were lower than those of barley (Fig. 4A). In sorghum roots, exudation rates increased linearly with an average $Q_{10}$ of 3.4 from 15 to 35°C, but exudation dropped nearly in half when the temperature was raised from 35 to 40°C. Because the osmotic gradient remained constant at all measured temperatures (Fig. 4B), the high temperature-induced decrease in exudation can be attributed to a drop in hydraulic conductivity (Fig. 4C) rather than a decrease in osmotic pressure gradient, as was found for barley (Fig. 4B).

Volume flow, $J_v$, was significantly lower in freely exuding excised roots than intact-plants (Table 3 and Fig. 4A) most likely because flow in intact-plants is driven by both osmotic and hydrostatic gradients. The volume flow in intact sorghum increased by almost three-fold between 15 and 35°C, which was accompanied by almost a three-fold increase in $L_p$. In the OUM, $J_v$ increased by seven-fold with more than a six-fold increase in $L_p$. Because flow in intact-plants is driven by both hydrostatic and osmotic forces, the substantially larger increase in $J_v$ and $L_p$ in the OUM versus intact methods, indicates that the osmotic water permeability is more sensitive to increased temperature than osmotic plus hydrostatic water permeability. Temperature-induced responses of $L_p$ were identical between the intact and OUM (Table 3 and Fig. 4C) which indicates that OUM can reliably
characterize physiological responses of intact roots to changing temperature. Hydraulic properties of barley roots responded in much the same way to root temperature changes as sorghum with the exception that the increase in $J_v$ and $L_p$ between OUM and intact was not as large as those reported in sorghum.

Absolute values of $L_p$ estimated by OUM, were also in good agreement with those estimated by the intact method (Table 3 and Fig. 4). At optimum and supraoptimum temperatures, the $L_p$ estimated by either method are practically identical. The $L_p$ estimated OUM at suboptimal temperatures, however, is about two-fold smaller than intact $L_p$ in both plants. This is because at low root temperature, e.g. 15°C, the osmotic flow is only 10 and 14% of total flow occurring in intact $J_v$ for barley and sorghum respectively, and indicates that at cooler temperatures osmotic water permeability does not significantly influence the total $L_p$ in intact roots. However, at optimum temperature, e.g. 25°C in barley and 35°C in sorghum, at least 30% of flow in intact roots can be accounted for by the osmotic water flow and as a result, a substantial portion of the root water permeability is regulated by the osmotic $L_v$.

At higher temperatures tested (35°C), the rise in $L_p$ in both species can possibly be attributed to the breakdown of the plasma membrane, eliminating a potential site of resistance to water flow. To test this hypothesis, excised barley roots were given 2 h of treatment at 35°C, and then quickly transferred to a solution at 25°C. The $L_p$ was determined just before transfer to 25°C and within 2 h after the new $J_v$ was reached at 25°C. High temperature-induced variations in $J_v$, $\Delta\pi$, and $L_p$ were completely reversible in barley roots (Table 4). Thus permanent membrane damage can not explain the maintenance of relatively high $L_p$ at supraoptimal temperatures. A similar study conducted with sorghum roots but only $J_v$ was analyzed. Results indicated that at the optimum root temperature, the
rise in \( J_v \) and perhaps \( L_p \) was also fully reversible when roots were transferred into a lower root temperature (Table 4).

The increase in \( J_v \) with rising temperature in the suboptimal temperature range is mediated by \( L_p \) in both species which is consistent with the findings of others (Clarkson 1976). However, the sensitivity of \( L_p \) to temperature differs between the two species (\( Q_{10} \) of 3.6 in barley between 15 and 25\(^\circ\)C and an average \( Q_{10} \) of 2.6 in sorghum between 15 and 35\(^\circ\)C) (Fig 2). These are significantly higher values than those expected from changes in viscosity of water (\( Q_{10} \) of 1.2 to 1.3) at these temperature ranges (Kuiper 1964) and indicate that \( L_p \) is not mediated by simple changes in physical properties of the system.

Dependence of \( L_p \) upon biological, rather than physical, factors in exuding roots has been noted by others (Ginsburg 1971, Pitman et al. 1981, Zimmerman and Steudle 1978). Various sites of such dependence may include cytoplasmic streaming, plasmodesmata, and the plasma membrane. It is unknown which one of these sites may be responsible for the greater response to temperature in barley than in sorghum at suboptimal temperatures. However, in this interval, the generally higher water permeability in barley than sorghum can be explained by several factors which may intrinsically be different between these species, including ratios of surface area to weight or structural properties of their plasma membranes. Increased unsaturation of membrane fatty acids is often associated with cold adaptation and increased fluidity of the lipid bilayer (Clarkson and Hall 1977, Pike and Berry 1980). A higher degree of membrane fluidity, as might be expected for the more cold tolerant barley at these temperature intervals, has been suggested to be associated with increased water movement (Clarkson and Hall 1977). Another partial explanation for differential response in \( L_p \) between barley and sorghum, up to the optimum temperature, may be presented by \( \sigma \). If the membranes are not ideally semipermeable, then the assumption that \( \sigma \) is unity is
Table 4. Changes in $J_v$, $\Delta n$, and $L_p$ in barley roots as well as $J_v$ in sorghum roots treated at 35°C and then transferred to 25°C are completely reversible. In barley, values are means of 3 to 5 readings ±SD, for the same roots at 25 and 35°C. In sorghum, $J_v$ values are for roots treated at 25 and 35°C only, as well as roots which were treated at 35°C for 2 hours and then transferred to 25°C.

<table>
<thead>
<tr>
<th></th>
<th>$J_v$ (µg·h⁻¹)</th>
<th>$\Delta n$ (MPa)</th>
<th>$L_p$ (µg·h⁻¹·MPa⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BARLEY</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4h continuous at 35°C</td>
<td>198 ± 36</td>
<td>.04 ± .005</td>
<td>4950 ± 260</td>
</tr>
<tr>
<td>2h continuous at 35°C, then transferred to 25°C for 2h</td>
<td>293 ± 47</td>
<td>.06 ± .007</td>
<td>4950 ± 590</td>
</tr>
<tr>
<td>4h continuous at 25°C</td>
<td>304 ± 17</td>
<td>.07 ± .001</td>
<td>4300 ± 180</td>
</tr>
<tr>
<td><strong>SORGHUM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4h continuous at 35°C</td>
<td>237 ± 24</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2h continuous at 35°C, then transferred to 25°C for 2h</td>
<td>158 ± 26</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4h continuous 2h at 25°C</td>
<td>142 ± 21</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
incorrect and the \( J_{v} \Delta \pi \) ratio is more appropriately \( \pi \) \( \Delta \) than \( \pi \) alone. It has been noted that different species as well as different portions of a single root might exhibit variable \( \sigma \) (Anderson et al. 1970); however, an increase of two-fold in \( \sigma \), required to completely account for the two-fold increase in \( J_{v} \Delta \pi \), seems to be unreasonably high based on typical values cited in the literature (Fiscus 1986, Miller 1985, Steudle et al. 1987).

The "Reverse Flow Method" (RFM). Temperature changes had similar effects on the \( L_{p} \) of barley roots as determined by RFM, compared to the previously mentioned method, i.e. \( L_{p} \) increased from 15 to 25°C and remained relatively unchanged at higher temperatures (Fig. 5; compare to Fig. 4C and Table 3). In sorghum, however, the characteristic inhibition of \( L_{p} \) between 35 and 40°C, observed in the intact-plant and osmotic methods, did not occur in the RFM (Fig. 5). This enhancement of \( L_{p} \) between 35 and 40°C occurred in spite of a decrease in influx \( L_{p} \) at the same temperature range and indicated that at higher temperatures, root permeability to water may exhibit a polar behavior (influx and efflux \( L_{p} \)s respond differently to elevated root temperatures). Steudle et al. (1987) indicated the existence of polar water transport across the maize root and suggested that the direction of this polarity was affected by the nature of the driving force. Additionally, in sorghum, the absolute values of \( L_{p} \) reported for the RFM were substantially lower than those reported for the other methods (Fig. 5; compare to Fig. 4 and Table 3). The differences between reverse flow and intact plant methods were consistent with the differences reported between the osmotic and hydrostatic \( L_{p} \) (Steudle et al. 1987, Frensch and Steudle 1989, Steudle and Frensch 1989, Jones et al. 1988). Steudle et al. (1987) reported that \( L_{p} \) determined by a pressure probe technique (hydrostatic) was an order of magnitude larger than the values obtained by the reverse flow method. Such differences have been argued to be caused by differences in transport mechanisms, i.e. apoplasm offering a major pathway of transport in hydrostatic but not in osmotic flow (Steudle et al. 1987, Frensch and Steudle 1989). This
Fig 5. Hydraulic conductance as a function of temperature in 5- and 8-d-old excised barley and sorghum roots determined by the "reverse flow method". Back flow was induced by replacing the external solution with a 1/2 strength Hoagland solution containing mannitol to give a final osmotic potential of 0.39 to 0.42MPa. $L_p$ was calculated based on 10 minute reversed flow rate and 2 to 4 hour exudation rate. Values are means of 3 to 5 replicates ±SD.
group has also suggested that the differences in hydrostatic and osmotic flow characteristics are not universal since $L_p$ in barley root was similar using either method (Steudle et al. 1987). In this study, both species exhibited lower $L_p$ with the RFM than the intact-plant method, but the magnitude of the differences reported here is significantly smaller than those reported by Steudle's group.

The differences in absolute values of $L_p$ between the intact and reverse flow methods can be partially explained by the fact that potential gradients ($\Delta n$ in osmotic and $\Delta \psi$ in intact method) were unequal in the two methods. In RFM, $\Delta n$ was about 0.4MPa, whereas the effective gradient for water transport across the intact root ($\psi_{\text{R}} - \psi_{\text{O}}$) was only 0.18MPa (Table 2). When excised sorghum roots were exposed to different $\Delta n$ at a constant temperature of 25°C, it was found that $L_p$ decreased with increasing osmotic gradient (Fig. 6). The results indicate that if more comparable gradient forces were applied in RFM, e.g. if $\Delta n$ values closer to 0.18MPa were chosen, substantially larger $L_p$ values would have resulted. However, the possibility that $L_p$ responds to hydrostatic and osmotic gradients differently cannot be eliminated. It should be noted that increasing hydrostatic gradients commonly lead to increasing values of $L_p$ (Passioura 1988). The RFM produced the opposite response to increasing $\Delta \psi$.

The RFM method also produced substantially lower $L_p$ values than those calculated for OUM (Fig. 4C and 5). Considering the fact that in both methods, the driving force is osmotic, the differences do not appear to be due to a shift in the pathway of transport (symplast versus apoplast). Figure 6 shows that, if $\Delta n$ of 0.11 vs 0.4MPa is used, $L_p$ values determined by RFM would be larger by more than two-fold. Since the pressure gradient for OUM in exuding barley and sorghum roots did not exceed 0.08MPa (Fig. 4B) it is suspected that if similar $\Delta n$ were utilized, the absolute values of $L_p$ calculated by RFM would
Fig 6. Hydraulic conductance as a function of osmotic driving force ($\Delta \pi$) in 8-d-old excised sorghum roots at 25°C. Various $\Delta \pi$ are obtained by preparing nutrient solutions containing different amounts of mannitol. Values are means of 2 replicates.
have been closer to those determined by OUM. It should, however, be noted that even when allowance was made to correct for the high $\Delta n$ in RFM, with an increase of 250% in $L_p$ values, RFM produced slightly smaller absolute numbers than OUM.

**Pressurization Method.** Pressure induced $J_v$ was substantially larger than intact $J_v$ in sorghum roots at 25°C and at comparable driving forces (Tables 3 and 5). As a result, the pressurized $L_p$ was more than two-fold greater than those estimated in intact-plants. Such a difference may indicate that root pressurization alters the path of water movement through the root. In fact, the accuracy of this method has been challenged on the grounds that under positive pressure, intercellular spaces in the cortical region may become water filled and act as a conduit for water transport (Passioura 1988). In intact roots under negative (suction) pressure, water is not likely to have access to this pathway. Applied pressure has also been reported to alter ion fluxes across the root compared to those observed in intact plants under similar driving force (Salim and Pitman 1984).

The force/flux response observed in Table 5 led to a relatively constant $L_p$ in the range of $\Delta P$ tested in this experiment, which is consistent with the Fiscus model (1975, 1983). The response of $J_v$ to $\Delta P$ in sorghum was similar to Fiscus’s observations in yet another way. Fiscus (1983) suggested that the typical hysteretic response observed by many investigators (Radin and Eidenbock 1984, Shirazi et al. 1975, Mees and Weatherly 1957), could be avoided when $\Delta P$ was increased gradually, often for a period of several hours. Although, in this experiment, the step wise increase in $\Delta P$ was not employed, increasing and decreasing pressures produced relatively similar $J_v$ (except at 0.6MPA). Fiscus (1983) has taken the lack of hysteresis as an indication of a steady state $J_v$ which he regards as absolutely crucial for a reliable estimate of $L_p$.

Assuming that the intact method provided the most representative values for $L_p$, this study indicated that reliable estimates of root hydraulic conductance can be obtained by the
Volume flow ($J_v$) and hydraulic conductance ($L_p$) in excised roots of 8-d-old sorghum as a function of applied pressure at 25°C. I and D subscripts refer to "Increasing" and "Decreasing" pressure. Osmotic gradient ($\Delta_{p}$) and $\Delta P$ - O is equal to 0.066 MPa as determined by V.P.O. $J_v$ is in $\mu$L $g^{-1}$ h and $L_p$ is in $\mu$L $g^{-1}$ h$^{-1}$ MPa. Values are means of 2 measurements.

<table>
<thead>
<tr>
<th>$\Delta P$ (MPa)</th>
<th>$J_{vI}$</th>
<th>$L_{pI}$</th>
<th>$J_{vD}$</th>
<th>$L_{pD}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>160</td>
<td>2420</td>
<td>160</td>
<td>2420</td>
</tr>
<tr>
<td>0.15</td>
<td>1028</td>
<td>6850</td>
<td>943</td>
<td>6280</td>
</tr>
<tr>
<td>0.30</td>
<td>1886</td>
<td>6290</td>
<td>1886</td>
<td>6290</td>
</tr>
<tr>
<td>0.45</td>
<td>2914</td>
<td>6470</td>
<td>2871</td>
<td>6380</td>
</tr>
<tr>
<td>0.60</td>
<td>4286</td>
<td>7140</td>
<td>3257</td>
<td>5430</td>
</tr>
<tr>
<td>0.80</td>
<td>5142</td>
<td>6450</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
OUM. The $L_p$ estimated by the OUM is a particularly reliable measure of intact root $L_p$ when a substantial inwardly driving force is osmotic in nature, e.g. the seedlings used in this study at near ORT. Compared to the intact method, the accuracy of $L_p$ as a measure of the root physiological behavior seems to be diminished when alternative methods (reverse flow and pressurization) are used. The present study indicated that the absolute value of $L_p$ estimated by RFM depended strongly on the magnitude of the driving force ($\Delta n$) with $L_p$ decreasing with increasing $\Delta n$. This method produced an accurate qualitative account of $L_p$ response to temperature in barley at all temperatures tested and in sorghum at suboptimal temperatures only. However, in both plants, RFM underestimated the value of $L_p$ compared to the intact-plant method. It is suggested that the exclusive use of the osmotic gradient combined with polar water transport may affect the accuracy of the reverse flow method. The pressurized root method, on the other hand, produced the largest $L_p$ values and although it was studied for sorghum and only at a constant temperature of $25^\circ$C, represented a relatively inaccurate account of intact $L_p$.

**Root Pressure Exudation Analysis of Ion Transport**

As previously mentioned, root $L_p$ responses did not always explain the observed changes in growth rate and $J_v$ in intact-plants as well as $J_y$ in excised roots. Even when $L_p$, $J_v$ and growth rates changed similarly in response to root temperature, the results are considered correlative at best and do not establish a cause and effect relationship. It has already been argued that the osmotic gradient, $\Delta n$, is a significant driving force inducing flow in intact-plants and the sole driving force in root pressure exudation. Therefore, short term analysis of ion release to the xylem will provide a mechanistic interpretation of temperature induced changes in $J_v$, while also allowing assessment of the potential role of the rate of ion supply in temperature induced growth rate responses.
Results presented in the following section are based on sap analysis obtained from root pressure exudation studies associated with Fig 4.

Effects of root temperature on ion release into the xylem. The xylem profile of the major osmolytes showed that NO$_3^-$ and K$^+$ together accounted for about 70% of the total ions analyzed in both plants at either 25 or 35°C (Table 6). The sum of cations was roughly equal to that of anions at the temperatures tested for both species. The PO$_4^{3-}$ and Cl$^-$ ions were about 20% while Ca$^{2+}$, Mg$^{2+}$, and Na$^+$ together comprised only 10% of the total ions measured. Three of these ionic species (K$^+$, NO$_3^-$ and PO$_4^{3-}$) were studied over the entire temperature range. In barley roots, the xylem concentration of K$^+$, NO$_3^-$ and total ions decreased precipitously with increasing temperature (Fig. 7A, B and D), while ion concentrations in sorghum roots were reduced only at temperatures exceeding 25°C. Compared to K$^+$ and NO$_3^-$, PO$_4^{3-}$ concentration was relatively insensitive to short term root temperature treatment in both species (Fig. 7C).

Rate of ion transport to the xylem, $J_v$, can not be deduced solely from $C_i$. According to Equation (6), variation in $J_v$ resulting from changes in $L_p$ can also affect $C_i$. Therefore solute fluxes were calculated and the result indicated that in roots of both species, $J_K$, $J_{NO_3}$, and $J_{total}$ were enhanced by increased temperature in the 18 to 25°C range (Fig. 8A, B and D). Within this range, although the rate of ion release to the xylem increased at a similar rate in both species, barley consistently showed significantly higher fluxes perhaps due to a larger number of ion transporting sites. Between 25 and 35°C, total ion transport to the xylem dropped by more than 50% in barley roots (Fig. 8D) whereas these fluxes increased slightly or remained constant in sorghum (Fig. 8A, B and D). Ion fluxes in sorghum were severely inhibited only when the root temperature exceeded 35°C. $J_{PO_4}$ was
Table 6. Ionic composition of exudate sap at 25 and 35°C temperatures for excised roots of barley and sorghum at 5- and 8-d-old respectively. Values are means of 6 measurements ±SD. Saps were collected during the steady state \( I_v \) within 4 hours following excision.

<table>
<thead>
<tr>
<th>ROOT TEMPERATURE (°C)</th>
<th>Sorghum</th>
<th>Barley</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>35</td>
<td>25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CATIONS</th>
<th>CONCENTRATION (mM)</th>
<th>Sorghum</th>
<th>Barley</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K^+ )</td>
<td>17.40 ± 2.00</td>
<td>13.00 ± 0.7</td>
<td>23.90 ± 1.0</td>
</tr>
<tr>
<td>( Na^+ )</td>
<td>1.80 ± 0.20</td>
<td>1.30 ± 0.05</td>
<td>2.60 ± 0.2</td>
</tr>
<tr>
<td>( Ca^{2+} )</td>
<td>2.20 ± 0.30</td>
<td>1.70 ± 0.05</td>
<td>1.85 ± 0.2</td>
</tr>
<tr>
<td>( Mg^{2+} )</td>
<td>1.90 ± 0.30</td>
<td>1.60 ± 0.05</td>
<td>2.20 ± 0.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ANIONS</th>
<th>Sorghum</th>
<th>Barley</th>
</tr>
</thead>
<tbody>
<tr>
<td>( NO_3^- )</td>
<td>19.20 ± 3.00</td>
<td>13.10 ± 1.5</td>
</tr>
<tr>
<td>( Cl^- )</td>
<td>3.05 ± 2.50</td>
<td>3.30 ± 0.7</td>
</tr>
<tr>
<td>( PO_4^{3-} )</td>
<td>3.80 ± 1.70</td>
<td>4.30 ± 0.4</td>
</tr>
<tr>
<td>Total</td>
<td>48.63</td>
<td>38.3</td>
</tr>
</tbody>
</table>

\[ \frac{NO_3 + K}{\text{Total}} \] 75.0 | 69  | 75  | 65  |
\[ \frac{Cl + Mg + Na + Ca}{\text{Total}} \] 10.5 | 20  | 17  | 24  |
\[ pH \] 6.0 ± 0.04 | 5.97 ± 0.01 | 6.41 ± 0.09 | 6.52 ± 0.09
Fig. 7 Exudate concentration of major ions as a function of temperature in excised roots of barley and sorghum. Results at 25 and 35°C in D are based on measured sum of all ions from Table 1. At other temperatures, total ions were estimated based on the assumption that sum of measured NO₃⁻, K⁺ and PO₄³⁻ are 80% of the sap inorganic ions. Values in A, B, and C are means of 6 measurements ±SD.
Fig. 8  Effect of temperature on fluxes of major ions in excised roots of barley and sorghum. Results at 25 and 35°C in D are sum of calculated $J_i$ with ion concentration supplied from Table 1. At other temperatures, total ion concentration used in calculating $J_i$ are estimated in a similar manner as in Fig. 2. All values are means of 6 measurements ±SD.
less affected by root temperature than \( J_{NO_3} \) and \( J_k \), but \( PO_4^{3-} \) concentrations in the exudate were much lower than \( K^+ \) and \( NO_3^- \) concentrations.

It is generally assumed that \( J_i \) is largely regulated by symplasmic release of ions to the xylem (\( \Phi_{cy} \)) and by the rate of ion supply to the symplast from either vacuoles (\( \Phi_{cv} \)) or bathing medium (\( \Phi_{cm} \)) (Pitman 1982). An experiment was designed to identify the relative effects of temperature on these components of the transport pathway. Roots grown with full nutrients were excised and incubated at several temperatures in +K or -K nutrient solution. Upon excluding \( K^+ \) from the medium (-K roots), \( J_k \) becomes primarily a function of ion release to the xylem supplied mainly by the vacuoles (Glinka 1980). In barley roots, increased temperature up to 25°C enhanced ion release to the xylem (Table 7). This response was independent of the \( K^+ \) status of the medium and therefore was almost entirely supplied by endogenous sources, presumably the vacuoles. At 35°C, \( J_k \) was decreased from the peak at 25°C, but the inhibition was 60% greater in -K than in +K roots (Table 7). This additional reduction in flux may partly be caused by decreased vacuolar contribution to the symplasm and/or increased \( K^+ \) efflux to the medium. In contrast, \( J_k \) in sorghum was unaffected by \( K^+ \) content of the medium at all temperatures tested (Table 8). The data indicate that short-term response of \( J_k \) (and perhaps other ion fluxes) to temperature are largely independent of uptake, except for barley roots at supraoptimal temperatures. Presumably the fluxes are mainly supplied by the vacuoles.

Temperature may induce changes in \( J_v \) by simultaneously affecting \( L_p \) and \( J_i \). In some temperature ranges, the components of \( J_v \) are differentially influenced and as a result their relative significance in regulating volume flow may be assessed. For example, in barley roots, the inhibition of \( J_v \) at elevated temperatures is a direct consequence of reduced solute fluxes (Fig 4A and 8D). The inhibition is caused by a significant reduction of endogenous
Table 7  Effect of K⁺ status of the nutrient solutions on $J_v$ and $J_k$ in 5-d-old excised barley roots at different temperatures. Values are means of 6 to 9 measurements ±SD.

<table>
<thead>
<tr>
<th>Root Temperature (°C)</th>
<th>Treatment</th>
<th>$J_v$ (µl g⁻¹ h⁻¹)</th>
<th>$J_k$ (µmol g⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>+K</td>
<td>84 ± 11</td>
<td>2.04 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>-K</td>
<td>85 ± 08</td>
<td>1.96 ± 0.60</td>
</tr>
<tr>
<td>20</td>
<td>+K</td>
<td>140 ± 23</td>
<td>3.45 ± 1.30</td>
</tr>
<tr>
<td></td>
<td>-K</td>
<td>146 ± 35</td>
<td>3.22 ± 1.20</td>
</tr>
<tr>
<td>25</td>
<td>+K</td>
<td>231 ± 34</td>
<td>4.40 ± 1.20</td>
</tr>
<tr>
<td></td>
<td>-K</td>
<td>246 ± 28</td>
<td>4.10 ± 0.95</td>
</tr>
<tr>
<td>35</td>
<td>+K</td>
<td>172 ± 43</td>
<td>2.70 ± 1.00</td>
</tr>
<tr>
<td></td>
<td>-K</td>
<td>160 ± 32</td>
<td>1.10 ± 0.55</td>
</tr>
</tbody>
</table>
Table 8. Effect of K\(^+\) status of nutrient solutions on \(J_v\) and \(J_k\) in 8-d-old excised sorghum roots at different temperatures. Values are means of 3 to 6 measurements ±SD.

<table>
<thead>
<tr>
<th>Root Temperature (°C)</th>
<th>Treatment</th>
<th>(J_v) (μl g(^{-1}) h(^{-1}))</th>
<th>(J_k) (μmol g(^{-1}) h(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>+K</td>
<td>93 ±06</td>
<td>.89 ±.10</td>
</tr>
<tr>
<td></td>
<td>-K</td>
<td>92 ±08</td>
<td>.90 ±.08</td>
</tr>
<tr>
<td>25</td>
<td>+K</td>
<td>149 ±18</td>
<td>1.82 ±.50</td>
</tr>
<tr>
<td></td>
<td>-K</td>
<td>165 ±12</td>
<td>2.00 ±.40</td>
</tr>
<tr>
<td>35</td>
<td>+K</td>
<td>232 ±22</td>
<td>1.95 ±.30</td>
</tr>
<tr>
<td></td>
<td>-K</td>
<td>230 ±22</td>
<td>1.70 ±.26</td>
</tr>
<tr>
<td>40</td>
<td>+K</td>
<td>117 ±20</td>
<td>.90 ±.55</td>
</tr>
<tr>
<td></td>
<td>-K</td>
<td>129 ±18</td>
<td>.94 ±.60</td>
</tr>
</tbody>
</table>
(presumably vacuolar) supply of ions (Table 7). In sorghum, a separation between \( J_i \) and \( L_p \) occurs only between 25 to 35°C where \( J_f \) is primarily regulated by \( L_p \).

It is generally assumed that \( J_i \) affects \( J_v \) through \( \Delta n \). Our data indicate that large fluctuations in ion release to the xylem did not necessarily result in proportional changes in \( \Delta n \), i.e. an increase of 100\% in \( J_i \) at suboptimal temperatures in both species led to no to no appreciable changes in \( \Delta n \) (Fig. 4B). Based on sap ion concentration and by using the Vant’ Hoff equation, the osmotic pressure of xylem sap can be calculated and compared to their measured values at various temperatures. Such a comparison showed that in barley calculated \( \Delta n \) was a close approximation of the measured values (i.e. at 15, 18, 35 and 40°C). In sorghum, however, calculated \( \Delta n \) was substantially lower than the measured values at all temperatures. Concentrations and fluxes of inorganic salts may therefore contribute largely to \( \Delta n \) in barley, but other solutes may be partially responsible for xylem osmotic content in sorghum roots. The fact that the inorganic solutes in the xylem do not totally account for the measured xylem sap osmotic pressure has been previously observed in barley and rye (Clarkson 1976, Pitman and Wellfare 1978). This discrepancy can be simply explained by the presence of organic solutes. Andersen & Brodbeck (1989) reported that organic acids and amino acids accounted for 2/3 of solutes in the exudate from excised grape roots while Triplett et. al. (1980) noted that the concentration of organic acids in the xylem sap from excised wheat roots was inversely proportional to inorganic ions. Sucrose, glucose and fructose together were very low in sorghum xylem exudate and accounted for only 1 to 2\% of the solutes in the exudate (data not shown), implying that organic acids and amino acids may significantly contribute to the xylem osmotic pressure of sorghum. A higher organic acid content may also explain the consistently lower pH observed in sorghum than barley roots (Table 6). Ions are often assumed to be the major solutes which generate the osmotic gradient for the volume flow. However, our study shows that changes in \( J_i \) do not produce
proportional changes in $\Delta n$ at all temperatures (Figs. 4B and 8D). In such cases, the relative importance of $J_1$ and its contribution to osmotically driven flow cannot be ascertained from $\Delta n$ or vice versa.

In sorghum, ion uptake appears to be inconsequential in the short-term temperature effects on $J_1$, since removing external $K^+$ did not affect the release of ion to the xylem at all temperatures (Table 8). Presumably vacuoles were the primary source of ion released to the xylem at all temperatures tested. As mentioned before, $J_k$ is not closely related to $\Delta n$ over the entire temperature range tested, and its contribution to temperature effects on $J_y$ appears to be insignificant.

Like sorghum, in barley, vacuolar supply of ion to the flux pathway ($\Phi_v$) also appears to be the major source of ions for short term transport at 15 to 25°C (Table 7). A major difference between these species is exhibited at supra optimal temperature, where inhibition of $J_k$ in barley (Table 7) results from a combined reduction in ion release to the xylem and vacuolar release of ion to the symplasm. In sorghum, high temperature has no effect on $\Phi_v$ (Table 8). These results are consistent with the findings of others (Anderson et al. 1974, Jarvis and House 1970, Pitman 1971). Pitman (1971) found that in barley roots, reduced Cl$^-$ absorption led to no change in $J_{cl}$, and he concluded that the continued export of Cl$^-$ to the xylem was supplied mainly from vacuoles of cortical cells.

In the previous section it was concluded that root $L_p$ may (sorghum at all temperatures tested) or may not (barley at higher than 25°C) correlate to root temperature induced changes in growth rate. Even when a good correlation existed between $L_p$ and growth rate, the possibility could have not been excluded that non-hydraulic signals may have also been involved in regulating growth. The results of solute fluxes at various root temperatures showed that growth inhibition at elevated temperatures in barley seedlings may have resulted from a decreased supply of $K^+$ and $NO_3^-$ to the growing tissues. Inhibition
of $J_k$ and $J_{\text{H}^+}$ at high temperature also explains reduced exudation rate ($J_v$) at these temperatures in excised barley roots. In both plants at the 18 to 25°C temperature range, the increased growth rate was associated with both solute and water fluxes. However, in sorghum, the rapid enhancement of growth between 25 and 35°C root temperature range was associated with similar changes in $L_p$ but not solute fluxes.

**Effect of ABA on Temperature Induced Changes in Solute and Water Flow**

In addition to hydraulic and osmotic signals, root temperature induced variation in shoot growth may be mediated by phytohormones. Recently, Creelman et al (1989) demonstrated that ABA and water stress induce similar growth rates. Moreover, numerous reports have documented that ABA affects $L_p$ and $J_i$ which can in turn lead to changes in shoot elongation. Although the exact site of ABA production in an intact plant is still unclear, roots have been known to transport ABA to the shoot. Atkin et al. (1973) reported that the amount of ABA transported in the maize root was temperature dependent. Therefore, the interaction of temperature and ABA was studied in excised roots of barley and sorghum seedlings in an effort to assess the possible role of ABA in temperature induced responses of $L_p$, $J_i$, and ultimately $J_v$.

**ABA and Temperature Effects on $J_v$, $L_p$, $J_i$, and $J_{\text{H}^+}$**. Volume flow responded strongly to temperature. In barley roots $J_v$ peaked at 25°C, and 10μM ABA enhanced $J_v$ at all temperatures (Fig 9). The enhancement of $J_v$ by ABA was slightly, but reproducibly, greater at 15 to 25°C than 25 to 35°C. Similar results were reported by Collins & Morgan (1980) in corn roots where maximum enhancement of $J_v$ occurred at 20°C. This group concluded that ABA decreased the activation energy of water flow by increasing membrane permeability to water.
Fig. 9 Effect of 10μM ABA on temperature induced changes in $J_v$ of 5-d-old excised barley roots. The values are means of 6 measurements ±SD. Inset is the hydraulic conductance at various temperatures in the presence or absence of ABA. Values are means of 6 measurements.
Ion transport properties of the root have also shown different responses to ABA at various temperatures. In barley roots, ABA inhibited $K^+$ flux at 28°C after a lag period of 2.5 hours, but stimulated it at 22°C (Pitman et al. 1974). In our experiments $K^+$ and $NO_3^-$ together accounted for 70% of total xylem ion content, and thus we have limited our studies to these two major ions. Although ABA decreased both $K^+$ and $NO_3^-$ concentrations at 20°C, $J_k$ and $J_{NO_3}$ remained unaffected due to enhanced $J_v$ (Table 9). Similarly, at other temperatures tested, ABA treatment had no significant effect on $J_k$ and $J_{NO_3}$. Hydraulic conductance was calculated as the ratio of $J_v$ to $\Delta P$ (Fig. 9, inset). During the first few hours after ABA treatment, increased $L_p$ and not ion fluxes induced higher volume flow in barley roots. Elevation of $L_p$ by ABA is relatively rapid (Glinka 1980, Ludewig et al. 1988) and may not persist for long periods of treatment (Pitman and Wellfare 1978). This increase is probably regulated by the membrane that is normally rate-limiting to $L_p$ (Glinka 1980), most likely the endodermal membrane. At 35°C, ABA lost most of its promotive effect on $J_v$ because it had little effect on water permeability at this temperature (Fig. 9).

Volume and ion fluxes were reported previously to be inhibited by ABA in barley roots (Pitman and Wellfare 1978) but only after a lag of at least 4 h. Our observations were usually made within 4 to 5 h after ABA treatment. Therefore, the possibility cannot be excluded that inhibition of $J_i$ might develop later. However, the effect of ABA on $J_i$ was observed within 1/2 h in our experiments. If there were subsequent changes in $J_i$ after several hours, these changes might be secondary to the initial change in $J_v$.

Sorghum roots responded somewhat differently to temperature and ABA. In this species, the optimum temperature was 35°C (Fig. 10). The ABA stimulated $J_v$ at temperatures up to 25°C, but inhibited it at 35°C (Fig. 10). As a result, ABA lowered the optimum temperature from 35 to 25°C (Fig. 10). At the cooler temperatures, virtually all
Table 9. Concentration in and fluxes to the xylem of K⁺ and NO₃⁻ in 5-d-old excised barley roots at various temperatures in the presence and absence of 10μM ABA. Values are means of 6 measurements ±SD.

<table>
<thead>
<tr>
<th>Root Temp. (°C)</th>
<th>Treatment</th>
<th>$J_\text{v}$ (μg g⁻¹ h⁻¹)</th>
<th>Concentration (mM)</th>
<th>Solute Fluxes (μmol g⁻¹ h⁻¹)</th>
<th>$J_\text{K}^+$</th>
<th>$J_\text{NO}_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>-ABA</td>
<td>106 ± 28</td>
<td>38.5 ± 4.4</td>
<td>35.0 ± 4.30</td>
<td>3.90 ± 1.10</td>
<td>3.75 ± 1.20</td>
</tr>
<tr>
<td></td>
<td>+ABA</td>
<td>154 ± 17</td>
<td>29.0 ± 6.8</td>
<td>28.0 ± 4.70</td>
<td>4.50 ± 1.20</td>
<td>3.95 ± 0.95</td>
</tr>
<tr>
<td>25</td>
<td>-ABA</td>
<td>248 ± 33</td>
<td>24.6 ± 5.0</td>
<td>23.3 ± 3.30</td>
<td>6.10 ± 1.70</td>
<td>5.75 ± 1.40</td>
</tr>
<tr>
<td></td>
<td>+ABA</td>
<td>320 ± 64</td>
<td>22.6 ± 3.0</td>
<td>20.4 ± 3.40</td>
<td>7.20 ± 1.90</td>
<td>6.55 ± 2.15</td>
</tr>
<tr>
<td>35</td>
<td>-ABA</td>
<td>147 ± 34</td>
<td>8.5 ± 2.0</td>
<td>9.1 ± 1.30</td>
<td>1.22 ± 0.30</td>
<td>1.32 ± 0.32</td>
</tr>
<tr>
<td></td>
<td>+ABA</td>
<td>170 ± 32</td>
<td>7.4 ± 3.0</td>
<td>7.6 ± 0.75</td>
<td>1.13 ± 0.50</td>
<td>1.30 ± 0.25</td>
</tr>
</tbody>
</table>
Fig. 10  Effect of 10μM ABA on temperature induced changes in $J_v$ of 5-d-old excised sorghum roots. The values are means of 6 measurements ±SD. Inset is the hydraulic conductance at various temperatures in the presence or absence of ABA. Values are means of 6 measurements.
of the increase resulted from enhanced $L_p$ (Fig. 10, inset), with no significant changes in $J_l$ (Table 10). ABA elicited greater increases in $J_v$ and $L_p$ at 25°C than at either 15 or 20°C (Fig. 10), whereas maximum ABA enhancement of $J_v$ occurred at 20°C in barley roots (Fig. 10). Volume flow showed a marked reduction between 25 and 35°C in the presence of ABA in sorghum roots (Fig. 10). In this temperature range, $L_p$ remained relatively constant (Fig. 10, inset), indicating that inhibition of $J_v$ must have resulted from a reduction in $J_l$. The analysis of ion fluxes confirmed that both $J_k$ and $J_{NO_3}$ were severely inhibited at 35°C in ABA treated roots (Table 10). The total $K^+$ and $NO_3^-$ concentrations dropped by about 48% in ABA treated roots at 35°C (Table 10). At this temperature, $\Delta n$ dropped by 40% from .048 ± .005 in -ABA to .029 ± .005MPa in +ABA exudates, while $J_v$ decreased by 45% in ABA treated roots. The slight discrepancy between $\Delta n$ and the ion concentrations may be an artificial effect due to the detection limits of vapor pressure osmometry or it may be related to the presence of organic solutes (Andersen and Brodbeck 1989). Nonetheless, it is likely that $NO_3^-$ and $K^+$ are major solutes regulating ABA induced inhibition of $J_v$ in sorghum at 35°C. Inhibition of solute transport at elevated temperature in ABA treated roots was suggested to result from a disturbance of internal hormonal balance (Pitman et al. 1974). They postulated that ABA may increase the production of growth substances such as cytokinins which have an inhibitory effect on ion transport through the roots. Limited observations (data not shown) also indicate that 10μM cytokinin (kinetin) inhibits $J_v$ at 35°C. However, ABA reduction of $J_v$, $J_k$, and $J_{NO_3}$ at 35°C is almost immediate (within 1/2 h). This is not consistent with the data of Pitman et al. which reveal a lag time of 2 to 3 1/2 h before ABA inhibits ion fluxes at elevated temperatures. Therefore, a Kinetin mediated ABA effect on $J_v$ and $J_l$ does not appear plausible.

In one experiment we treated sorghum roots at 35°C with ABA but simultaneously transferred them to a -$K^+$ nutrient solution. This provided means of evaluating the effect...
Table 10. Concentration in and fluxes to xylem of K⁺ and NO₃⁻ in 8-d-old excised sorghum roots at various temperatures in the presence and absence of 10μM ABA. Values are means of 6 measurements ±SD.

<table>
<thead>
<tr>
<th>Root Temp. (°C)</th>
<th>Treatment</th>
<th>Jᵥ (μg g⁻¹ hr⁻¹)</th>
<th>Concentration (mM)</th>
<th>Solute Fluxes (μmol g⁻¹ hr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>K⁺</td>
<td>NO₃</td>
<td>Jₖ</td>
</tr>
<tr>
<td>20</td>
<td>+ABA</td>
<td>110 ± 18</td>
<td>11.5 ± 3.40</td>
<td>11.0 ± 1.00</td>
</tr>
<tr>
<td></td>
<td>-ABA</td>
<td>85 ± 10</td>
<td>12.3 ± 0.60</td>
<td>15.1 ± 2.30</td>
</tr>
<tr>
<td>25</td>
<td>+ABA</td>
<td>172 ± 18</td>
<td>11.8 ± 1.20</td>
<td>17.8 ± 3.00</td>
</tr>
<tr>
<td></td>
<td>-ABA</td>
<td>156 ± 12</td>
<td>12.4 ± 3.30</td>
<td>15.6 ± 2.70</td>
</tr>
<tr>
<td>35</td>
<td>+ABA</td>
<td>117 ± 17</td>
<td>6.6 ± 1.58</td>
<td>4.8 ± 1.10</td>
</tr>
<tr>
<td></td>
<td>-ABA</td>
<td>260 ± 18</td>
<td>11.0 ± 1.00</td>
<td>11.1 ± 1.40</td>
</tr>
<tr>
<td></td>
<td>+ABA (-K)</td>
<td>135 ± 15</td>
<td>4.2 ± 0.30</td>
<td></td>
</tr>
</tbody>
</table>
of ABA on the release of K\(^+\) to the xylem (\(q_{xt}\)) in the absence of uptake to the root. The ABA inhibited \(J_\text{t}\) similarly in ±K\(^+\) roots (Table 10), indicating that inhibition of \(J_\text{t}\) and perhaps other ions did not result from reduced uptake. Presumably release into the xylem was independent of uptake, and ABA may have affected the process at the xylem parenchyma cells. These results are consistent with earlier studies of the site of action of ABA (Lauchli 1984, Pitman 1977, 1982).

Data presented here indicate that actions of ABA on root transport properties were both species and temperature dependent. At lower temperature, e.g. 15 to 25°C, ABA stimulated \(I_\text{v}\) by enhancing \(L_\gamma\) in both species. However, the maximum effect on \(L_\gamma\) was at 25°C for sorghum and 20°C for barley, a warm and a cool season plant respectively. The ABA induced changes in \(L_\gamma\) have been reported to result from alteration in root plasma membranes (Ludewig et al. 1988, Markhart 1984, 1986). The strong interaction of temperature and ABA on root water transport reported here also indicates the possibility of a membrane mediated response.

The response of \(I\_v\) to changes in temperature, in barley roots, were identical for both ±ABA experiments with ABA application inducing a higher \(I\_v\) than no ABA treatment at any given temperature. These observations indicated that temperature induced changes were not mediated by ABA, but were enhanced by it. It was also concluded that in barley roots, both temperature and ABA affected \(I\_v\) at the same site, perhaps the membrane which regulates osmotic water permeability. Similarly, ABA did not appear to mediate temperature response of \(J\_v\) in sorghum roots. At 35°C, ABA severely inhibited ion fluxes in sorghum root, resulting in significant inhibition of \(J\_v\). Results of this work indicate that responsiveness of root water and ion transport to ABA depends largely on factors such as root temperature and the species. Other factors such as the nutrient status of the bathing medium (Pitman et al. 1974) and the hydrostatic pressure gradient (Glinka 1977) may also influence the
responsiveness of Jv to the hormone. The dependence of ABA effects on temperature and species may be responsible for some of the conflicting responses reported in the literature.
CONCLUDING REMARKS

Root zone temperature does not fluctuate nearly as much as the air temperature. In fact, temperature fluctuations in roots similar to those experienced by the aerial portions of the plant, could result in killing the plant (Nielsen 1974). Many investigators have reported that the severity of physiological responses to various environmental stresses are dependant upon the root zone temperature (Radin 1990, Sheppard et al 1986, Huang et al. 1989, Milligan and Dale 1988). Detrimental effects of adverse environmental conditions are often exacerbated as root temperature deviates from the optimum. Despite all the evidence showing a strong coupling of shoot physiological performance to root temperature, neither the nature of the signal between root and shoot, nor its kinetic response to a wide range of root temperature has been characterized in detail.

This study has provided evidence that short term root temperature induced changes in shoot growth were most immediately mediated by changes in root permeability to water and ions. Detailed analysis of root water and ion fluxes between 15 to 40°C, indicated that optimum root temperature for maximum shoot growth varied between barley and sorghum seedlings primarily because root transport properties of these two species responded quite differently to root temperature. For example, solute fluxes were severely inhibited in barley root at temperatures higher than 25°C resulting in inhibition of both growth and root pressure exudation rate, $J_p$. Similar inhibitions were not observed in sorghum until the root temperature was raised above 35°C. Solute fluxes, particularly $J_k$ and $J_{NO_3}$, showed a relatively wide optimum between 25 to 35°C in sorghum roots (Fig. 8), and it was only in this range where $L_p$ alone was responsible for ensuing changes in the growth rate (Fig. 4). Although changes in $L_p$ led to changes in the amount of water supplied to the leaves, measurements of bulk water, osmotic and turgor potential in various parts of either plants.
were found to be unaffected by the root zone temperature (Table 1 and 2). It is suggested that undetectable changes in the xylem of the shoot may couple the observed short term correlation between Lp and shoot growth in response to root temperature.

Plant species exhibit greatly variable responses to changes in temperature with regards to their metabolic processes. The extent of the metabolic responses to temperature variation is subject to both environmental and genetic control. With the increasing interest in crop production under controlled environmental conditions, characterization of ORT for root water and ion transport such as those examined in this study are indispensible for maximizing yield return. At a physiological level, a more immediate application of this study is to provide an alternative approach to interpret the mechanism(s) of the observed differential tolerances exhibited by different species or by different cultivars within the same species to environmental stresses. Similarly, adaptation and natural selection of many ecological plants to their particular environmental limitations, may also be interpreted in terms of responses of root transport properties to the prevailing soil temperature ranges. The desirable root physiological responses to the prevalent root zone temperature regime can be identified and genetic lines exhibiting such characteristics can be developed by either traditional breeding or by molecular techniques.

It is first necessary, however, to better identify the cellular factor(s) which may regulate temperature induced changes in root water and ion permeability. Water and solute fluxes showed a relatively large Q_{10} in response to root temperature, therefore indicating that metabolic regulation and most likely regulation at the membrane level may be suspected. This study has provided evidence that fluxes of NO₃ and K⁺ to the xylem, in response to root temperature, were independent of the status of these ions in the nutrient solution (Table 7 and 8) suggesting that the site of short term temperature effect is at $\Phi_\text{t}$ rather than $\Phi_\text{v}$ and that during this time, vacuoles become the major supplier of these ions to the
symplasm. The exact site at which \( L_p \) is being regulated is more difficult to ascertain, primarily because the exact nature of the pathway of water transport is unknown. Potentially, membranes in the cortical region, cyclosis, and the endodermal membrane can act as limiting sites which regulate \( L_p \) response to temperature.

In the ABA study, it was concluded that the hormone was unlikely to mediate the qualitative responses of water and solute fluxes to short term temperature treatment. In barley, ABA perhaps acted at the same site as temperature to increase osmotic water permeability, \( L_p \) (Fig. 9). In sorghum, ABA inhibited \( J_q \) and \( J_{NO_3} \) at 35°C leading to a severe reduction of root pressure exudation rate, \( J_v \) (Fig. 10). At lower temperatures, ABA had little or no effect on solute fluxes in sorghum roots, but tended to enhance \( L_p \). The results also indicated that the mechanism of ABA effects on \( L_p \) and \( J_q \) depends very strongly on the plant species and the temperature at which ABA experiments are conducted.
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