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Root-shoot interactions in the response of sugarcane to drought

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ROOT-SHOOT INTERACTIONS IN THE RESPONSE OF SUGARCANE TO DROUGHT

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF
THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF
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To Coco and Vida for being there...

ABSTRACT

Studies using greenhouse-grown plants of sugarcane cultivars known to have varying levels of resistance to drought were conducted to identify distinctive physiological features that may confer drought resistance in sugarcane. When leaf water relations, stomatal conductance (g), and shoot growth rate (SGR) were measured concurrently during a soil drying cycle, it was found that both osmotic and elastic adjustment occurred in the leaves of all cultivars in response to soil drying and diurnal water deficit. These adjustments led to almost complete maintenance of symplast volume, but only partial maintenance of turgor. During the early stages of drought, reductions in g and SGR were not accompanied by significant reductions in bulk leaf water status, suggesting that signals originating within the roots may have regulated shoot behavior. When the hydraulic properties of entire root systems and isolated roots were characterized by the transpiration-gradient and pressure-flux techniques, cultivar differences in both root- and leaf-specific root hydraulic conductance (G_{root}) were discerned. At high soil moisture, transpiration and G_{root} differed considerably among cultivars and were positively correlated, whereas leaf water potential (ψ_L) was similar among cultivars. Within a narrow range of soil water suction (0 to 0.1 MPa), over which G_{root} and g fell to nearly zero, ψ_L remained nearly constant because the vapor phase conductance of the leaves and the liquid phase conductance of the roots declined in parallel. These patterns reinforced the suggestion that control of g in sugarcane plants exposed to drying soil was exerted primarily at the root rather than at the leaf level. Cultivar variation in water relations characteristics, especially bulk tissue elasticity, was more distinct in the roots than in the leaves, suggesting that the previously reported cultivar differences in drought resistance were likely to be root-based. It was hypothesized that coordination of g with declining G_{root} during soil drying was accomplished by a chemical signal moving from the roots to the leaves via the transpiration stream. Decreased root osmotic potential may have stimulated export of this putative substance from the roots.

TABLE OF CONTENTS

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure Page

LIST OF ABBREVIATIONS

Abbreviation	Description	Unit
ψ	Water potential	MPa
ψ_L	Leaf water potential	MPa
ψ_{CL}	Covered basal leaf water potential	MPa
ψ_π	Osmotic potential	MPa
ψ_π^{100}	Osmotic potential at full turgor	MPa
ψ_π^z	Osmotic potential at zero turgor	MPa
ψ_P	Turgor potential	MPa
ψ_R	Root water potential	MPa
ϵ	Bulk tissue modulus of elasticity	MPa
τ	Soil water suction	MPa
ABA	Abscisic acid	-
A_{cortex}	Cross-sectional area of the root cortex	mm^2
A_{stele}	Cross-sectional area of the root stele	mm^2
A_{stele}/A_{cortex}	Ratio of A_{stele} to A_{cortex}	-
cv.	Sugarcane cultivar	-
dwt	Tissue dry weight	g
E	Leaf area specific transpiration rate	$mmol\ m^{-2}\ s^{-1}$
EXP	Exponential math function	-
g	Stomatal conductance	$mol\ m^{-2}\ s^{-1}$
G_{root}	Leaf area-specific root hydraulic conductance	$mmol\ m^{-2}\ s^{-1}\ MPa^{-1}$
G_{shoot}	Leaf area-specific shoot hydraulic conductance	$mmol\ m^{-2}\ s^{-1}\ MPa^{-1}$

Abbreviation	Description	Unit
L	Root length-specific root hydraulic conductance	mmol m^{-1} s^{-1} MPa^{-1}
ln	Natural logarithmic math function	-
N_t	Total solute content	mOsmol g^{-1} dwt
N_s	Symplastic solute content	mOsmol g^{-1} dwt
r	Correlation coefficient	-
r^2	Coefficient of determination	-
RWC	Relative water content	%
RWC_z	Relative water content at zero turgor	%
RWD	Relative water deficit	%
SGR	Shoot growth rate	mm day^{-1}
SQRT	Square root math function	-
SV	Symplastic water volume	cm^3 g^{-1} dwt
SWF	Symplastic water fraction	%
T/D	Turgid to dry weight ratio	-
V_e	Cumulative symplastic water volume expressed	cm^3 g^{-1} dwt
V_s	Absolute symplastic water volume at full hydration	cm^3 g^{-1} dwt
W_f	Weight of water in the fresh tissue	kg

CHAPTER I

INTRODUCTION

Water is a predominant factor in sugarcane production and sugar yield is determined in part by the amount of water used by the crop for evapotranspiration. To produce one kilogram of sucrose, a sugarcane crop needs about 1500 kilograms of water for evapotranspiration (Gascho and Shih 1983). Under water-limited conditions, where the transpirational demand for water by the leaves is not fully satisfied, crop productivity is reduced. For example, insufficient soil moisture due to periods of severe drought that occurred in Hawaii in 1962 and 1984 caused 18 and 10 per cent reductions in sugar yields per acre in 1963 and 1985, respectively (Sheets 1986). The adverse impact of drought can be reduced by appropriate cultural management practices, but a more effective way of alleviating drought effects is by developing drought-resistant varieties. The use of varieties that can maintain relatively high levels of productivity under limited water supply is therefore advantageous. Under dry conditions, for example, higher sucrose yields of a drought-adapted over a drought-susceptible sugarcane variety were attributed to the former's faster leaf area adjustment and leaf elongation under lower leaf water status (Inman-Bamber and De Jager 1986).

If plant attributes that sustain yield under water-limited conditions could be identified and used as selection criteria, then better adapted and higher yielding genotypes could be bred more efficiently and effectively. Among the putative drought resistance traits enumerated by Ludlow and Muchow (1990), the ones that probably would enhance drought resistance in sugarcane are the following: (1) osmotic adjustment of shoots and roots, (2) greater rooting depth and density, and (3) low lethal water status (the ability of leaves to survive low water potential and relative water content). Osmotic adjustment, the active accumulation of solutes within cells, helps in the maintenance of turgor, which enhances the growth of roots in soil with increasing water deficit (Turner 1986a). Turgor maintenance also allows the continuation of photosynthesis, though at a reduced rate, with declining leaf water status (Ludlow 1987). In situations of intermittent soil water deficits, greater root depth and density would reduce the incidence and slow the development of water deficits in the shoot (Ludlow and Muchow 1990). A low lethal water status would allow for the survival of leaves until soil water becomes available and would aid in the maintenance of photosynthetic area (Ludlow and Muchow 1990).

Mechanisms of adaptation to water deficits are divided broadly into three categories: drought escape, dehydration postponement, and dehydration tolerance (Kramer 1980). Drought escape, the ability of a plant to complete its life cycle before serious soil and plant water deficits develop, is achieved through rapid phenological development. Dehydration postponement, the ability of a plant to maintain high water status when exposed to environmental water deficits, is accomplished through greater rooting depth and density. Dehydration tolerance, the ability of a plant to survive low water status without permanent injury, is attained by having a low lethal water status due to osmotic adjustment (Flower and Ludlow 1986). Drought-adaptive mechanisms may or may not entail metabolic costs to productive processes. Turner (1979, 1982) concluded that only those mechanisms that aided in drought escape, maintenance of water uptake, and maintenance of turgor did not reduce photosynthesis, crop growth, and yield.

Regardless of the type of mechanism utilized by the plant in responding to drought, there must be a sensor to activate it. It has been hypothesized that turgor pressure is the transducer of water deficits in the cell based on reports that plants adapt to drought by maintaining leaf turgor via active accumulation of solutes (Hsiao *et al* 1976; Turner and Jones 1980; Tyree and Jarvis 1982; Morgan 1984). This hypothesis has received support from studies showing stomatal closure at zero turgor (Turner 1974), abscisic acid (ABA) accumulation at zero turgor (Pierce and Raschke 1980), and a lowering of the water potential at which stomata closed and ABA accumulated because of a gradually increasing water deficit (Turner *et al* 1978; Ludlow 1980; Wright *et al* 1983; Ludlow *et al* 1985).

The use of the energy status of water (i.e., water potential) of plant tissues in relation to physiological behavior has been questioned by Sinclair and Ludlow (1985). They argued that though the thermodynamic approach may be biophysically more satisfying, no unique relationship has been shown between water potential (or the components, turgor and osmotic potential) and the physiological processes noted above. Evidence supporting their contention is found in studies demonstrating that photosynthetic activity (Kaiser 1982; Berkowitz and Kroll 1988;

Santakumari and Berkowitz 1990) and leaf survival (Flower and Ludlow 1986) are dependent upon relative cell volume. Additionally, the significance of osmotic adjustment and its turgor maintenance effect have been questioned (Munns 1988), because maintenance of leaf turgor did not sustain leaf expansion (Passioura 1988b; Passioura and Gardner 1990) and stomatal opening (Gollan *et al* 1986; Henson *et al* 1989) of plants growing in drying soil. Therefore, it was suggested that the the relationships between physiological processes and tissue volume may be more practical and meaningful, and the approach to evaluating plant water status be broadened beyond the thermodynamic reference points (Sinclair and Ludlow 1985).

Plant water status is partly determined by the stomatal regulation of the rate of water vapor loss from the leaves. In a literature review, Schulze (1986a) concluded that stomata control gas exchange by (1) a direct response to humidity, (2) a direct response to root functions, and (3) a response to mesophyll internal conditions. The first and third responses involve leaf water relations, whereas the second response deals with root water relations. The issue of whether the root or the shoot is the primary sensor of dorught-induced water deficits has been the subject of a recent series of discussions (Kramer 1988; Passioura 1988a; Schulze *et al* 1988; Boyer 1989). Passioura (1980), Termaat *et al* (1985), Gollan *et al* (1986) and Munns (1987) broke the interdependence of the water status of root and shoot by growing plants with their roots in a pressure chamber in which sufficient pressure is applied to keep the shoot turgid irrespective of the water potential of the soil. They showed, using this technique, that both growth and stomatal conductance of plants were largely unaffected by whether the leaves were turgid, but were affected by drying soil (Termaat *et al* 1985; Gollan *et al* 1986; Munns 1987). This prompted Kramer (1988) to argue that in the field, it is usual for leaves to experience water deficiency before the roots. He contended that the dependence of the behavior of the shoot on conditions in the soil was a laboratory artifact caused by the drying of the soil while the leaves were kept artificially turgid. Nevertheless, the split root technique employed by Blackman and Davies (1985) in the laboratory is a confirmation of the results by Bates and Hall (1981) who showed that even without any change in leaf water potential, stomatal closure occurred in field-grown cowpea exposed to mild soil drying. These studies, therefore, suggested that the root, instead of the shoot, is the primary sensor of soil water deficits.

The alternative mechanism involving roots, instead of the leaves, as the primary sensors of soil water deficits is consistent with the idea of Cowan (1982) that the adaptive response is feedforward rather than feedback (i.e., with respect to the shoot). A feedforward reaction to soil drying is more advantageous because it is expected to be more rapid, more efficient, and more conservative of the available soil moisture than a feedback (i.e., leaf sensing the soil water deficit) response. The response time of roots to changes in soil water potential is much smaller than that of the leaves because the leaf cuticle has a very low hydraulic conductivity which uncouples the leaf from the atmosphere (Schulze *et al* 1988). It has been emphasized that the root-shoot interactions are governed by both physical (i.e., hydraulic) and chemical (i.e., metabolic) factors (Turner 1986b), and that the relative importance of hydraulic and metabolic signals is dependent upon the physiological state of the plant (Schulze *et al* 1988). For example, when water deficit develops slowly, drought-induced metabolic signals could be integrated, which is difficult to visualize for hydraulic signals. On the other hand, if the roots are droughted severely so that hydraulic impacts occur in the shoot, then the hydraulic effects may predominate over that of metabolic effects.

As plants are exposed to drying soil, the root and leaf water deficit may increase in concert making the relative importance of the root and leaf water relations in the control of leaf growth, stomatal conductance and photosynthesis difficult to discern. Both hydraulic conductance and elasticities of root and leaf cells could determine the magnitude of changes in root and leaf water relations during periods of water deficit (Turner 1986b). Stomatal responses to humidity have been observed to arise from local changes in epidermal turgor, suggesting a role for leaf water relations independent of any changes in root water relations (Shackel and Brinckmann 1985). On the other hand, studies have shown that stomatal aperture declined and leaf expansion remained inhibited with soil drying in plants whose shoots were kept fully turgid by pressurizing the roots (Gollan *et al* 1986; Passioura 1988b). Furthermore, a positive correlation between stomatal conductance and soil/root/leaf hydraulic conductance has been observed in woody species (Kuppers 1984). Plant stems are a possible location for a mechanism controlling leaf responses since xylem vessel cavitations during drought may lead to variations in hydraulic conductance (Zimmermann and Milburn 1982; Dixon *et al*

1984; Tyree *et al* 1984). The correlation between stomatal and hydraulic conductance (Kuppers 1984), however, was independent of the leaf water potential so that the controlling mechanism was not at the stem but at the root level.

The metabolic root signal controlling stomatal opening during soil drying was originally proposed to be a reduced supply of cytokinins (Blackman and Davies 1985; Davies *et al* 1986), which are produced in the roots (Van Staden and Davey 1979) and transported via the transpiration stream to the leaf where they promote stomatal opening (Incoll and Whitelam 1977; Blackman and Davies 1983, 1984; Incoll and Jewer 1985). Subsequently, it was found that ABA was produced in the roots in response to soil and root dehydration and was transported to the leaves where it reduced stomatal conductance (Lachno and Baker 1986; Zhang and Davies 1987a; Zhang *et al* 1987; Hubick and Reid 1988). In contrast, Munns and King (1988) found in wheat that stomatal opening of droughted plants was inhibited by the presence of an unknown compound in the xylem sap. Working with maize, Zhang and Davies (1989a, 1990) provided evidence that changes in the ABA concentration of the xylem sap in response to drought can account for changes in leaf growth and stomatal conductance. It is likely then, that the root-sourced chemical message may differ between species. In a recent review (Davies *et al* 1990), ABA produced in the roots was suggested to play a central role in sensing the soil water status and regulating plant growth during periods of drought. While a hormonal signal seems probable, the possibility exists that inorganic ions or other organic compounds may also play a role (Schwartz 1985; Fitzsimons and Weyers

1986; Inoue and Katoh 1987; Ludlow *et al* 1989). An interesting conclusion made by Schulze (1986a) in his review on whole-plant responses to drought is that a direct metabolic signal from the roots to the leaves seems to regulate stomatal conductance as the soil dries but only prior to leaf wilting. This implied that leaf water status may have an important role in the regulation of stomatal aperture only when drought is already severe. It appears then, that a two-stage response to drought is occurring: firstly, chemical messages from the roots are transported to the shoots under mild to moderate soil drying, and secondly, hydraulic signals from the roots arrive at the shoots to amplify the chemical effects during moderate to severe drought.

Adverse conditions other than drought such as high soil strength (Masle and Passioura 1987), hypoxia (Smit *et al* 1989), flooding (Zhang and Davies 1987b), and salinity (Termaat *et al* 1985) also have been shown to cause roots to send chemical signals to the shoot where growth and stomatal conductance are reduced without changing the leaf water potential. These studies further reinforce the idea that under many circumstances, leaf water potential may not be important in controlling leaf growth and stomatal behavior.

The behavior of a crop species or cultivar under water-limited conditions is determined both by interactions between aerial environment and leaf water relations, and by interactions between soil environment and root water relations (Turner 1986b). Thus, investigations involving both shoot and root water relations are needed for a better understanding of crop adaptation to water deficit.

Presently, very little information is available regarding both specific features that may confer drought resistance in sugarcane and the degree of root-shoot interaction during soil drying. In the present study, comparative drought responses of three sugarcane cultivars were used to identify specific root and shoot features associated with differential responses to drought. Though these identified features may not be useful in a crop breeding program, a knowledge of such features should lead to a more efficient process of selecting the right genotype for the right environment. Additionally, these specific features may provide information about optimal irrigation regimes for sugarcane production in Hawaii. The objectives of this study were to: (1) determine the effect of drought on the water relations characteristics, stomatal conductance, and growth of leaves of three contrasting sugarcane cultivars; (2) evaluate the relative importance of leaf, root and soil water status, and plant hydraulic properties in the control of stomatal aperture and transpiration during drought; (3) assess methods for determining root hydraulic properties of sugarcane; and (4) examine specific root water relations characteristics that may regulate stomatal behavior of three contrasting sugarcane cultivars.

Three sugarcane cultivars, known to perform differently in previous experiments conducted by the Hawaiian Sugar Planters' Association (HSPA), were used. In one experiment (Ingram 1985), canopy temperature and total dry matter yield were used as criteria for evaluating drought resistance. Cultivar H65-7052 had a high difference in canopy temperature between irrigated and droughted treatments and its droughted-to-irrigated ratio for total dry matter yield was low.

On the other hand, cv. H67-5630 had a small difference in canopy temperature between irrigated and droughted treatments and its droughted-to-irrigated ratio for total dry matter yield was high. In another experiment involving salinity tolerance (Ginoza and Moore 1985), cv. H69-8235 was ranked as the most resistant whereas cv. H65-7052 was ranked as the most susceptible. Based on these tests and on the recognition that plant responses to drought and salinity are often similar, the three cultivars were classified as susceptible (cv. H65-7052), moderately resistant (cv. H67-5630), and resistant (cv. H69-8235) to drought.

CHAPTER II

LEAF WATER RELATIONS, STOMATAL CONDUCTANCE AND GROWTH OF DROUGHTED SUGARCANE CULTIVARS

ABSTRACT

Leaf water relations, stomatal conductance (*g*) and shoot growth rate (SGR) were monitored during a soil drying cycle in three sugarcane cultivars growing in pots in a greenhouse. The pressure-volume technique was used to evaluate diurnal and drought-induced variation in leaf water relations characteristics. Leaf solute content and bulk elasticity varied diurnally in both irrigated and droughted plants and were highest at midday. Solute accumulation and increased elasticity were also observed as leaf water deficits developed more slowly during soil drying. This osmotic and elastic adjustment maintained symplast volume essentially constant both diurnally and during soil drying, whereas turgor was only partially maintained. The extent of osmotic adjustment associated with drought was not reflected in the leaf osmotic potential at full turgor because the concurrent increase in tissue elasticity resulted in a larger symplast volume at full turgor. Cultivar responses over the range of leaf water deficits imposed did not provide conclusive evidence for genotypic variation in osmotic and elastic adjustment. The magnitude of osmotic and elastic adjustment in response to drought appeared to have been determined by

behavioral differences in rates of water use among cultivars. In the early stages of soil drying, reductions in SGR and *g* were not accompanied by significant reductions in bulk leaf water status. This suggested that other factors, presumably signals originating from the roots, may have regulated SGR, *g* and leaf water relations characteristics.

INTRODUCTION

Plant acclimation to adverse environmental conditions entails modification of characteristics necessary to sustain key physiological processes. Drought often induces changes in tissue water relations characteristics such as osmotic potential and elasticity. Osmotic adjustment and increased tissue elasticity are frequently cited as mechanisms enabling droughted plants to maintain turgor necessary for stomatal opening and leaf growth (Hsiao *et al* 1976; Turner 1979; Tyree and Jarvis 1982; Morgan 1984). Recent findings, however, have demonstrated that leaf growth (Passioura 1988b; Saab and Sharp 1989; Passioura and Gardner 1990) and stomatal conductance (Bates and Hall 1981; Blackman and Davies 1985; Gollan *et al* 1985, 1986; Henson *et al* 1989) are reduced in droughted plants despite complete turgor maintenance. This has led Munns (1988) to challenge the prevailing belief that the significance of osmotic adjustment lies in its effect on turgor. Other workers have proposed that the importance of osmotic and elastic adjustment lies in maintenance of protoplast volume to sustain photosynthesis

(Kaiser 1982; Santakumari and Berkowitz 1990) and to avoid lethal relative water content (Flower and Ludlow 1986).

Early emphasis on the role of osmotic and elastic adjustment in turgor maintenance (Hsiao *et al* 1976; Tyree and Jarvis 1982) may have led researchers to overlook the significance of symplast volume. Both osmotic adjustment (Sen Gupta and Berkowitz 1987) and elastic adjustment (Kikuta and Richter 1986; Richter and Kikuta 1989) can alter the water potential-protoplast volume relationship in leaves of plants subjected to drought. When osmotic adjustment occurs, the increased solute concentration promotes entry of water into the cells leading to maintenance of both symplast volume and turgor. Elastic adjustment, on the other hand, alters the relative rates at which turgor and volume decrease with declining water potential.

In a previous study on the effect of drought on field-grown sugarcane plants, it was reported that an apparent osmotic adjustment of -0.48 MPa occurred five weeks after withholding irrigation (Koehler *et al* 1982). In another investigation, Inman-Bamber and De Jager (1986) evaluated the drought sensitivity of two sugarcane varieties grown in eighty-liter bins in the greenhouse. They reported that shoot growth and stomatal opening began to decline at leaf water potential thresholds of -0.8 and -0.9 MPa, and both were completely inhibited at -1.3 to -1.7 MPa, respectively.

In the present study, the pressure-volume (P-V) technique (Tyree and Hammel 1972; Tyree and Richter 1981, 1982) was used to characterize the leaf water relations of three sugarcane cultivars during a soil drying cycle. Results from the P-V curve analyses and leaf water status measurements were used to evaluate the extent of osmotic and elastic adjustment and their influence on maintenance of turgor and symplast volume in droughted plants. Stomatal and shoot growth responses were also examined in association with changes in leaf water relations.

MATERIALS AND METHODS

Plant Material and Experimental Conditions

Three sugarcane (*Saccharum* spp. hybrid) cultivars were selected on the basis of contrasting performance in previous salinity and drought resistance tests (Ginoza and Moore 1985; Ingram 1985). The cultivars were classified as susceptible (cv. H65-7052), moderately resistant (cv. H67-5630), and resistant (cv. H69-8235) to drought.

Two single stem segments containing one lateral bud were sown in 11-liter plastic pots filled with a 4:4:1 v/v mixture of clay soil, commercial potting mix, and volcanic cinders. Plants were grown in the greenhouse and were watered automatically twice daily by drip irrigation. Greenhouse temperatures were typically 30 to 35 °C during the day and 20 to 25 °C at night. Complete fertilizer was first applied one month after planting and once a month thereafter. Plants were three months old (12 leaves) when experimental measurements were carried out.

Plants were subjected to two irrigation regimes: i) irrigated twice daily, and ii) droughted by withholding irrigation until predawn ψ_L decreased to -1.0 MPa. The three cultivars subjected to the two irrigation regimes resulted in a total of six treatments. Two plants of about the same size in each of three pots represented six replicates for each treatment.

Leaf Water Relations

Samples consisting of leaf segments, 8 to 12 cm long, were excised from the youngest fully expanded leaf at predawn (0530 to 0630 h) and midday (1200 to 1300 h). The leaf segments were obtained by enclosing a portion of an attached leaf in a small plastic bag, rapidly excising the leaf at the mouth of the bag, and sealing immediately to avoid post-excision decline in xylem pressure potential (Turner and Long 1980). The samples were immediately placed in the dark inside a cooler and brought to the laboratory for leaf water relations measurements.

Three leaf segments were sampled from each treatment approximately every other day for determination of predawn and midday leaf water potential (ψ_L) with a commercial pressure chamber (PMS Inc., Corvallis, OR). A strip of lamina, 1 cm wide, was excised longitudinally from the middle part of one side of a leaf segment, immediately inserted through the slit of a rubber stopper, and fitted to the cover of the chamber, which was lined with moist filter paper (Saliendra *et al* 1990). Pressure was increased at a rate of about 0.02 MPa s^{-1} and

ψ_L was taken as the negative value of the balancing pressure determined when liquid first appeared at the cut surface of the leaf. The remaining leaf tissue minus the midrib was resealed in the plastic bag for determination of total solute content as described below.

P-V Curve Procedures

Bulk leaf tissue water relations characteristics at predawn and midday were determined from P-V curves of samples obtained from six leaves in each treatment at the beginning and at the end of the drying cycle. Non-rehydrated leaf samples were used because preliminary P-V curve determinations indicated that rehydration of samples caused alterations in tissue water relations characteristics similar to those reported by Meinzer *et al* (1986, 1988) and Evans *et al* (1990) following rehydration treatments. Turgid weights of non-hydrated samples were estimated by multiplying their dry weights by the turgid-to-dry-weight ratios of rehydrated leaf segments. Initial balance pressure was determined from a segment of lamina excised from one side of the midrib. Additional lamina segments, 1 cm wide and 12 cm long, excised from the middle part of the other side of the midrib were used to generate P-V curves.

The basal end of each segment was inserted through a slit in a rubber stopper. A rubber balloon was placed over the portion of the segment facing the inside of the pressure chamber and sealed to the rubber stopper with a nylon cable tie (Meinzer and Moore 1988). The stopper assemblies were then sealed in a specially constructed ten-chamber apparatus lined with moist filter paper. Chamber pressure was successively raised in 0.1 to 0.2 MPa increments and each pressure was held for approximately 20 minutes depending on the hydration of the sample. Sap expressed as a result of an incremental increase in pressure was collected in small vials filled with tissue paper. The vials were weighed to the nearest 0.1 mg before and after each sap collection. In order to retard evaporation from the tissue paper between weighings, an inverted beaker lined with moist filter paper was placed over the vial and leaf blade end. In this method, each increment in pressure was taken to correspond to the new equilibrium balance pressure (Monson and Smith 1982). This was unlike the method of Tyree and Hammel (1972) in which overpressures were applied and new balance pressures determined after each sap collection. This apparatus and the procedures used permitted as many as 20 samples to be processed on each sampling day, e.g., 10 samples at predawn and 10 samples at midday. The use of predetermined levels of ψ_L during pressure dehydration facilitated comparison of leaf water relations characteristics among cultivars and treatments at comparable levels of ψ_L.

P-V Curve Analysis

Data from each leaf segment were plotted as the reciprocal of balance pressure on the ordinate versus relative water deficit (**RWD**). RWD was calculated as follows:

$$\mathrm{RWD} = 100 - \mathrm{RWC}$$

$$\mathrm{RWC} = ((\text{fresh wt} - \text{dry wt})/(\text{turgid wt} - \text{dry wt}))*100$$

where **fresh wt** is the initial fresh weight of the leaf segment minus cumulative weight of the sap expressed; **dry wt** is the weight of the leaf segment after oven-drying for 24 h; and **turgid wt** is the estimated weight of the fully hydrated leaf segment. Values of osmotic potential at full turgor (ψ_π^{100}) and symplastic water fraction (SWF) were obtained by extrapolating the linear portion of the plot to the ordinate and abscissa, respectively. Osmotic potential at zero turgor (ψ_π^{z}) and relative water content at zero turgor (RWC_z) were determined graphically. Turgor (ψ_P) was calculated as the difference between the incremental decrease in ψ_L obtained during the pressure dehydration and the corresponding osmotic potential (ψ_π) obtained by extrapolating the linear portion of the plot to RWD=0. The tissue bulk modulus of elasticity (ϵ) was calculated after Tyree (1981):

$$\epsilon = (\Delta\psi_P/\Delta W)*W$$

where $\Delta\psi_P$ is the difference in ψ_P between increments of pressure; ΔW is the difference in weight of water in the symplast between increments of pressure; and W is the average weight of water in the symplast. Absolute symplastic water volume (SV) was calculated by the equation:

$$\mathrm{SV} = V_s - V_e$$

where V_s, the absolute symplastic water volume at full hydration ($\mathrm{cm}^3\ \mathrm{g}^{-1}$ dwt), is the turgid-to-dry-weight ratio multiplied by SWF, and V_e, the cumulative volume of water expressed from the symplast with increasing pressure, is the total volume of sap expressed multiplied by SWF then divided by dry weight.

Leaf Solute Content

Samples for determination of total solute content were prepared from leaf tissue remaining after segments had been obtained for ψ_L or P-V curve determinations. The middle portions of the remaining sides of the lamina, excluding the midrib, were excised and immediately sealed in small pre-weighed plastic tubes. The tubes containing the tissue were weighed to obtain the tissue fresh weight and were frozen immediately at -80 °C. The frozen samples were thawed for about 30 minutes and sap was expressed by squeezing each tube in a hydraulic press (Carver Inc.). A filter paper disc was immersed for about 3 to 5 s in the expressed sap and was sealed immediately in the psychrometer chamber of a vapor pressure osmometer (Wescor 5100B) to determine osmolality. Tissue dry weight was recorded after oven-drying the samples at 70 °C for 24 h. Total solute content on a dry weight basis (N_t) was calculated with the following equation:

$$\mathbf{N_t = (W_f * mOsmol\ kg^{-1})/DWT}$$

where $\mathbf{W_f}$ is the total weight of water (kg) in the fresh tissue, **DWT** is the tissue dry weight, and **mOsmol kg^{-1}** is the osmolality of the expressed sap determined by vapor pressure osmometry. Symplast solute content on a dry weight basis (N_s) was determined from P-V curve data after Tyree *et al* (1978) as:

$$N_s = ((W_f{*}SWF){*}(-\psi_\pi/0.00245))/DWT$$

where **0.00245** is a factor used to convert MPa to mOsmol kg^{-1}.

Stomatal Conductance and Shoot Growth

Stomatal conductance (g) of the abaxial surface of the youngest fully expanded leaf was monitored with a steady-state porometer (LI-COR 1600, LI-COR Inc., Lincoln, NE). Measurements of g were taken hourly between 1000 to 1500 h and were restricted to sunny periods when photosynthetically active radiation exceeded 1000 μmol m^{-2} s^{-1}.

Shoot growth rate was monitored by making a small pinhole through the leaf elongation zone above the shoot apex (Mongelard and Mimura 1971). The distance of the innermost pinhole from the soil surface was recorded to the nearest millimeter. Shoot growth rate, **SGR** (mm day^{-1}), was obtained as the difference between successive measurements divided by the number of days between measurements.

RESULTS

Predawn and midday ψ_L did not differ significantly among droughted and irrigated plants until the fifth day after withholding irrigation. Predawn ψ_L of droughted plants then began to decline precipitously (Fig. 1). The midday ψ_L was much lower than predawn value. After five days from withholding irrigation, the decline in midday ψ_L was generally less rapid than that of predawn values. The predawn and midday ψ_L of droughted plants of cv. H69-8235 declined more rapidly than those of the other two cultivars. SGR declined sooner than g upon withholding irrigation, indicating that it was more sensitive to water deficit than g.

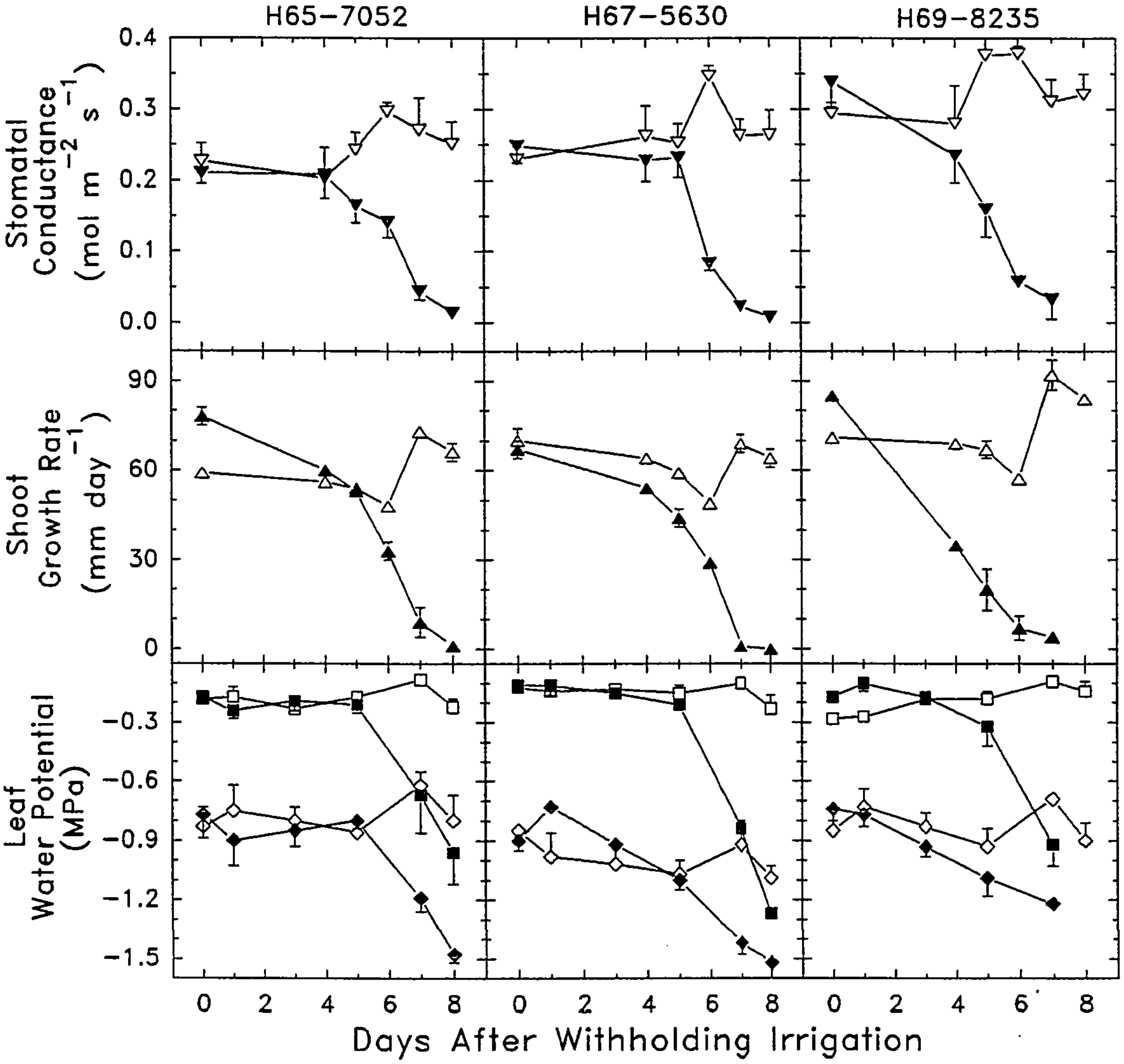

Figure 1. The course of stomatal conductance, g (▽,▼), shoot growth rate, SGR (△,▲), and predawn (□,■) and midday (◇,◆) leaf water potential, ψ_L, in irrigated (open symbols) and droughted (closed symbols) plants of three sugarcane cultivars during a soil drying cycle. Data points are means (± standard error) of 12 to 24, 6, and 3 to 6 observations for g, SGR, and ψ_L, respectively.

SGR and *g* in cv. H69-8235 were exceptionally sensitive since they began to decline even before there were detectable differences in ψ_L among droughted and irrigated plants. Among all cultivars, the rates of decline in *g* and SGR upon withholding irrigation appeared to be positively correlated with their initial *g* and SGR values according to the ranking: H69-8235 > H67-5630 > H65-7052.

Leaf water relations characteristics of all the sugarcane cultivars studied changed diurnally in irrigated plants and in response to drought. Graphical examination of P-V curves indicated that these changes were associated primarily with alterations in the non-linear rather than the linear part of the curve (Fig. 2). Alterations in the non-linear portion of the curve are usually associated with changes in tissue elasticity, which would influence the parameters ψ_π^z and RWC_z obtained from the P-V curve. The nearly coincident linear portions of the curves in both predawn versus midday and irrigated versus droughted comparisons suggest that adjustments in ψ_π^{100} did not occur.

When leaf water relations parameters were evaluated in detail, variations in ψ_π^{100} in response to drought (Table 1) and diurnal water deficit (Table 2) generally were small and not significant in all cultivars. Since a decrease in ψ_π^{100} is often taken as an indication of solute accumulation or osmotic adjustment, the data from P-V analyses suggested that drought and diurnal variation in water deficit did not induce osmotic adjustment. On the other hand, both ψ_π^z and RWC_z decreased considerably in all cultivars in response to drought and diurnal water deficit (Tables 1 and 2). The reductions in ψ_π^z and RWC_z associated with

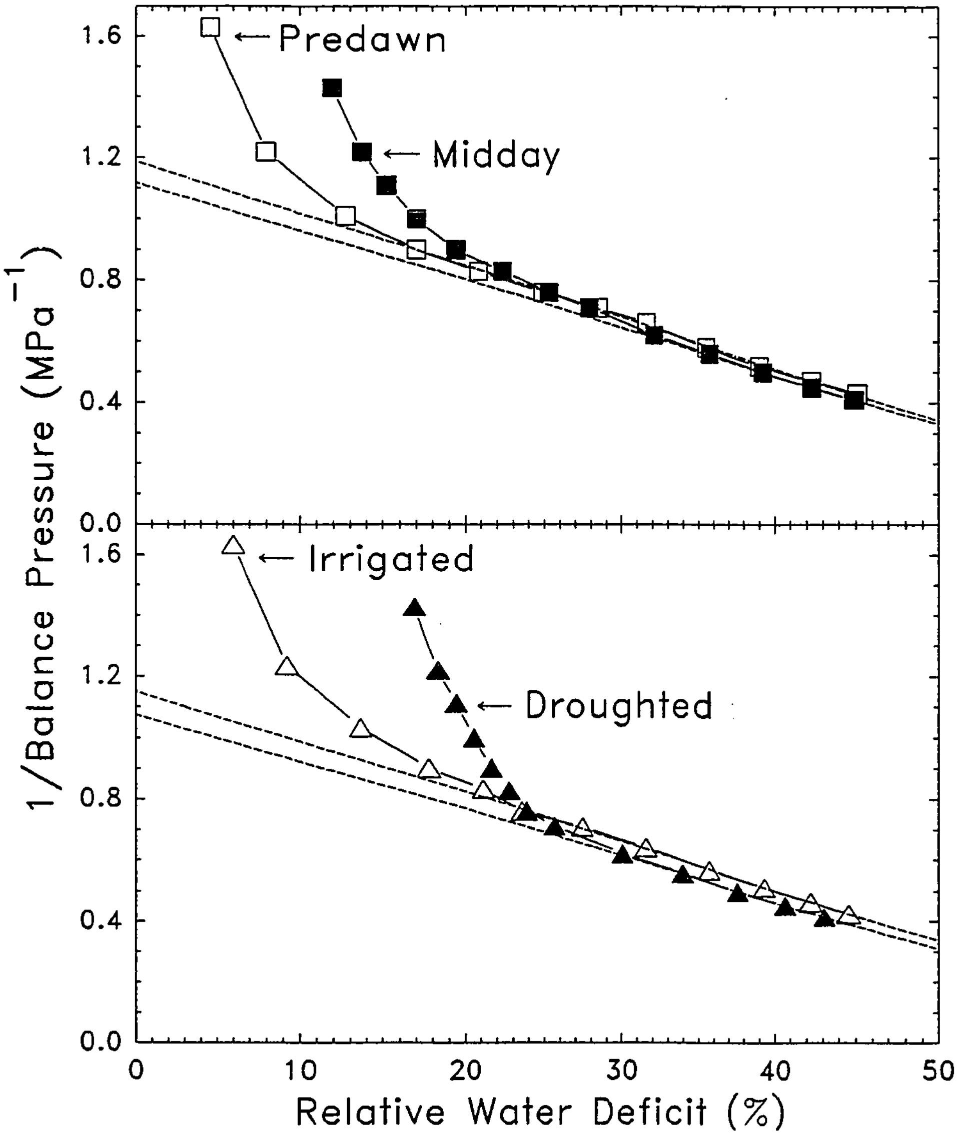

Figure 2. Representative pressure-volume (P-V) curves for leaf segments sampled from irrigated plants at predawn (□) and midday (■); and from irrigated (△) and droughted (▲) plants at predawn. Each P-V curve was generated from a leaf segment obtained from sugarcane cultivar H69-8235. Equations for dashed lines fitted to the linear portion of the curves are:

$$\text{predawn, } 1/\psi_L = 1.184 - 0.017(\text{RWD}),\ r^2 = 1.00^{**}$$

$$\text{midday, } 1/\psi_L = 1.148 - 0.016(\text{RWD}),\ r^2 = 1.00^{**}$$

$$\text{irrigated, } 1/\psi_L = 1.149 - 0.016(\text{RWD}),\ r^2 = 1.00^{**}$$

$$\text{droughted, } 1/\psi_L = 1.091 - 0.016(\text{RWD}),\ r^2 = 1.00^{**}.$$

Table 1. Osmotic potential at full turgor (ψ_π^{100}), osmotic potential at zero turgor (ψ_π^{z}), symplastic water fraction (SWF), and relative water content at zero turgor (RWC_z) of leaves excised at predawn from irrigated (I) and droughted (D) plants of three sugarcane cultivars.

Data are means (± standard error) of six observations obtained from pressure-volume curves.

Cultivar	ψ_π^{100}		ψ_π^{z}		SWF		RWC_z	
	I	D	I	D	I	D	I	D
	(MPa)				(%)			
H65-7052	**-0.98**	**-1.01**	**-1.60**	**-1.79**	**64.6**	**69.0**	**75.1**	**69.8**
	(±0.03)	(±0.02)	(±0.07)	(±0.02)	(±2.2)	(±1.4)	(±1.7)	(±1.5)
H67-5630	**-0.92**	**-1.11**	**-1.51**	**-1.79**	**62.0**	**67.9**	**75.6**	**74.3**
	(±0.03)	(±0.03)	(±0.06)	(±0.02)	(±1.0)	(±1.2)	(±1.4)	(±0.9)
H69-8235	**-0.86**	**-0.91**	**-1.32**	**-1.78**	**70.5**	**72.6**	**76.0**	**66.6**
	(±0.01)	(±0.04)	(±0.05)	(±0.05)	(±1.6)	(±1.7)	(±1.8)	(±2.3)

drought (Table 1) were generally greater than those associated with diurnal water deficit (Table 2). The largest changes in these parameters were observed in cv. H69-8235 (Tables 1 and 2).

The preceding pattern suggested that the tissue elasticity may have increased in response to drought and more rapid diurnal fluctuations in water deficit, and that cv. H69-8235 may have experienced larger changes in leaf elasticity than the other cultivars. Increased SWF observed in droughted leaves and in leaves collected at midday (Tables 1 and 2) was consistent with adjustments in tissue elasticity. Rehydration of leaves collected from droughted

Table 2. Osmotic potential at full turgor (ψ_π^{100}), osmotic potential at zero turgor (ψ_π^{z}), symplastic water fraction (SWF), and relative water content at zero turgor (RWC$_z$) of leaves excised at predawn (PD) and midday (MD) from irrigated plants of three sugarcane cultivars.

Data are means (± standard error) of twelve observations obtained from pressure-volume curves.

Cultivar	ψ_π^{100}		ψ_π^{z}		SWF		RWC$_z$	
	PD	MD	PD	MD	PD	MD	PD	MD
	(MPa)				(%)			
H65-7052	**-0.96** (±0.03)	**-1.01** (±0.04)	**-1.54** (±0.06)	**-1.69** (±0.07)	**67.4** (±1.9)	**71.0** (±2.0)	**74.7** (±1.0)	**71.4** (±1.7)
H67-5630	**-0.93** (±0.02)	**-0.95** (±0.03)	**-1.55** (±0.03)	**-1.70** (±0.05)	**66.8** (±1.9)	**69.4** (±2.4)	**73.2** (±1.3)	**69.5** (±1.0)
H69-8235	**-0.86** (±0.02)	**-0.87** (±0.03)	**-1.42** (±0.06)	**-1.60** (±0.06)	**76.1** (±2.0)	**78.8** (±2.0)	**70.7** (±1.4)	**64.1** (±1.2)

plants resulted in a significant decrease in SWF and significant increases in ψ_π^{100}, ψ_π^{z}, and RWC$_z$ (Table 3). This was consistent with the results presented in Tables 1 and 2 wherein more hydrated leaves collected from irrigated plants and leaves collected prior to dawn had higher ψ_π^{100}, ψ_π^{z}, and RWC$_z$ but lower SWF. This provided additional evidence that relatively rapid changes in hydration produced similarly rapid changes in elasticity and other leaf water relations parameters, and indicated the necessity of using tissue in its natural state of hydration to evaluate diurnal and drought-induced alterations in leaf water relations characteristics. The extent of elastic adjustment to drought and diurnal

Table 3. Osmotic potential at full turgor (ψ_π^{100}), osmotic potential at zero turgor (ψ_π^z), symplastic water fraction (SWF), and relative water content at zero turgor (RWC_z) of non-rehydrated and rehydrated leaves excised at predawn from droughted plants of cv. H65-7052.

Data are means (± standard error) of six observations obtained from pressure-volume curves.

Treatment	ψ_π^{100}	ψ_π^z	SWF	RWC_z
	-----------(MPa)-----------		----------(%)-----------	
Non-rehydrated	**-1.37** (±0.06)	**-2.04** (±0.10)	**76.0** (±3.8)	**75.1** (±3.0)
Rehydrated	**-1.22** (±0.06)	**-1.70** (±0.06)	**66.0** (±2.5)	**81.0** (±1.6)

water deficit was quantified by calculating ϵ. Since ϵ varies with turgor, a reference turgor of 0.6 MPa was used to obtain values of ϵ for comparisons among treatments. Drought and midday water deficit caused substantial reductions in ϵ of all of the cultivars (Tables 4 and 5). This pattern confirmed that increased tissue elasticity contributed to the reductions in ψ_π^z and RWC_z associated with increased water deficit (Tables 1 and 2).

Symplast solute content increased in response to drought (Table 4) and midday water deficit (Table 5). These drought and diurnal effects on N_s, as calculated from ψ_π derived from P-V curves, were corroborated by similar changes in N_t, which was calculated from independent measurements of bulk tissue ψ_π by osmometry. The increases in N_s and N_t associated with drought were greater than

Table 4. Bulk tissue modulus of elasticity at 0.6 MPa turgor ($\epsilon_{0.6}$), turgid to dry weight ratio (T/D), and symplastic (N_s) and total (N_t) solute content of leaves excised at predawn from irrigated (I) and droughted (D) plants of three sugarcane cultivars.

Data are means (± standard error) of six observations.

Cultivar	$\epsilon_{0.6}$		**T/D**		N_s		N_t	
	I	**D**	**I**	**D**	**I**	**D**	**I**	**D**
	----(MPa)----				--------(mOsmol g^{-1} dwt)-------			
H65-7052	**5.68** (±0.46)	**3.17** (±0.41)	**3.42** (±0.08)	**3.91** (±0.05)	**0.65** (±0.04)	**0.89** (±0.01)	**0.62** (±0.04)	**0.92** (±0.02)
H67-5630	**5.99** (±0.47)	**4.06** (±0.42)	**3.13** (±0.11)	**3.38** (±0.05)	**0.60** (±0.05)	**0.80** (±0.03)	**0.60** (±0.03)	**0.74** (±0.01)
H69-8235	**6.58** (±0.64)	**3.68** (±0.26)	**3.61** (±0.16)	**4.24** (±0.11)	**0.71** (±0.03)	**0.91** (±0.07)	**0.65** (±0.03)	**0.97** (±0.05)

those associated with diurnal water deficit. This evidence of osmotic adjustment did not appear to be consistent with the small and non-significant decreases in ψ_π^{100} observed in leaves of droughted plants and in leaves collected at midday (Tables 1 and 2). Examination of the leaf turgid-to-dry-weight ratios (T/D) (Tables 4 and 5) resolved this apparent contradiction. There was a significant increase in the T/D, and therefore in the symplast volume of all cultivars when droughted and during midday (cf. Tables 1 and 4, and Tables 2 and 5), which was associated with a concurrent increase in tissue elasticity. The combined influence of solute accumulation and increased tissue elasticity on symplast volume,

Table 5. Bulk tissue modulus of elasticity at 0.6 MPa turgor ($\epsilon_{0.6}$), turgid to dry weight ratio (T/D), and symplastic (N_s) and total (N_t) solute content of leaves excised at predawn (PD) and midday (MD) from irrigated plants of three sugarcane cultivars.

Data are means (± standard error) of twelve observations.

Cultivar	$\epsilon_{0.6}$		T/D		N_s		N_t	
	PD	MD	PD	MD	PD	MD	PD	MD
	----(MPa)----				--------(mOsmol g^{-1} dwt)--------			
H65-7052	**7.10**	**3.52**	**3.52**	**3.56**	**0.71**	**0.82**	**0.64**	**0.68**
	(±0.56)	(±0.22)	(±0.12)	(±0.08)	(±0.05)	(±0.03)	(±0.03)	(±0.03)
H67-5630	**6.98**	**2.50**	**3.24**	**3.47**	**0.65**	**0.69**	**0.61**	**0.63**
	(±0.48)	(±0.20)	(±0.11)	(±0.15)	(±0.06)	(±0.09)	(±0.02)	(±0.03)
H69-8235	**7.43**	**2.19**	**3.73**	**4.01**	**0.79**	**0.81**	**0.71**	**0.76**
	(±0.51)	(±0.18)	(±0.17)	(±0.17)	(±0.05)	(±0.06)	(±0.03)	(±0.03)

therefore, concealed the effect of solute accumulation on ψ_π^{100} during drought and at midday.

Osmotic and elastic adjustment influenced both turgor and symplast volume. By comparing the behavior of ψ_P and SV with declining ψ_L imposed during P-V curve dehydrations, it was possible to assess the extent to which turgor and volume were maintained as ψ_L declined during drought. When both SV and ψ_P were plotted as a function of ψ_L for leaf segments excised at predawn from irrigated and droughted plants, all cultivars exhibited similar patterns in SV and ψ_P across the range of ψ_L imposed (Fig. 3). As expected, the rate of decline in ψ_P as ψ_L decreased was much faster than that of SV, indicating better maintenance of

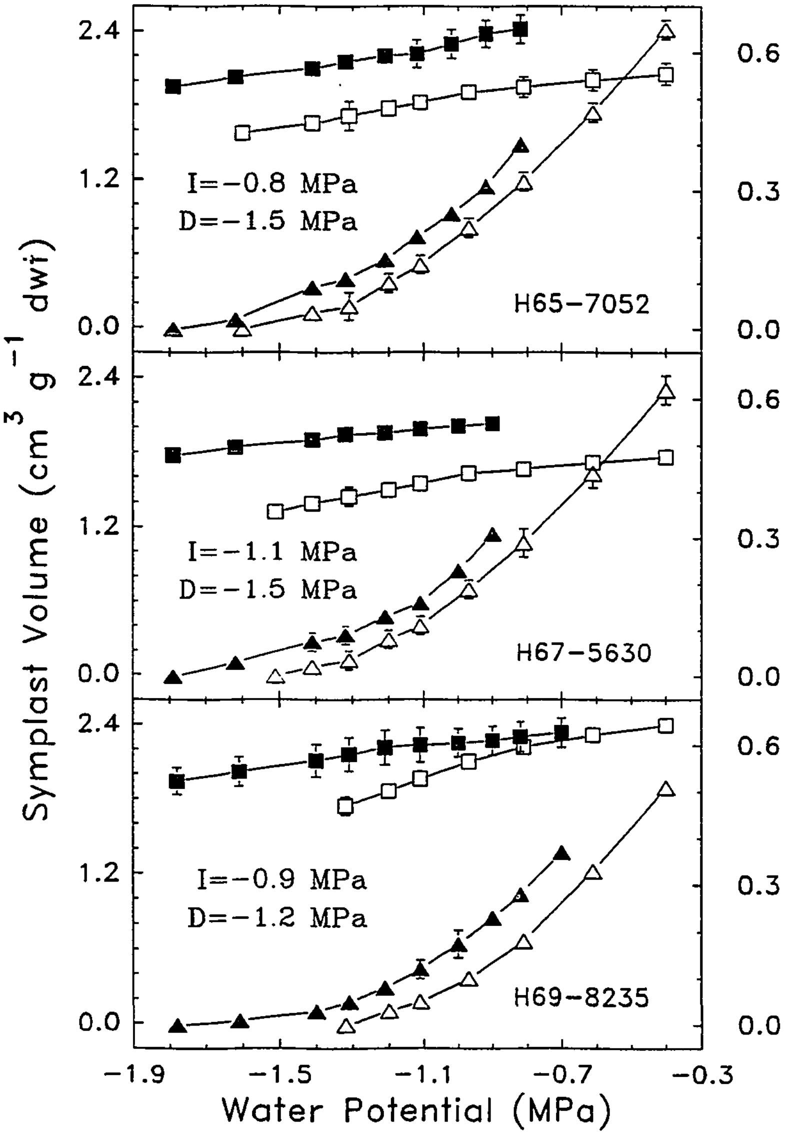

Figure 3. Symplast volume (□,■) and turgor (△,▲) in relation to water potential of leaf segments sampled at predawn from irrigated (open symbols) and droughted (closed symbols) plants of three sugarcane cultivars. Irrigated (I) and droughted (D) midday leaf water potentials are listed for each cultivar. Data points, connected by straight lines, are means (± standard error) of two to six observations obtained during pressure-volume dehydration.

SV than ψ_P. SV in leaves from droughted plants was higher than that in leaves from irrigated plants over the range of ψ_L imposed, whereas ψ_P in leaves from both droughted and irrigated plants eventually reached zero at the lowest values of ψ_L imposed. Comparison of SV and ψ_P in leaves from droughted and irrigated plants (Fig. 3) at the corresponding values of midday ψ_L observed at the end of the soil drying cycle (Fig. 1) revealed that while ψ_P dropped to values near zero, SV was relatively unchanged.

When diurnal changes in SV and ψ_P of droughted plants were examined as a function of time elapsed since withholding irrigation, it was apparent that SV remained nearly constant both diurnally and during soil drying (Fig. 4). In contrast, ψ_P exhibited substantial diurnal variation and in two of the cultivars had fallen to near zero by the end of the drying cycle. The ranking of the cultivars according to the rates of decline in ψ_P and SV after withholding irrigation corresponded to the ranking according to the initial rates of growth and *g* (Fig. 1). This suggested that higher *g* was associated with more rapid depletion of soil water.

Droughted and irrigated sugarcane plants differed with respect to the apparent dependence of SGR and *g* on ψ_P (Fig. 5). In the case of midday ψ_P, SGR and *g* of irrigated plants were independent of ψ_P over a 0.35 MPa range while SGR amd *g* of droughted plants fell nearly to zero over that same range. A similar pattern was observed when predawn ψ_P was used, although minimum and maximum values of ψ_P were higher for both droughted and irrigated plants.

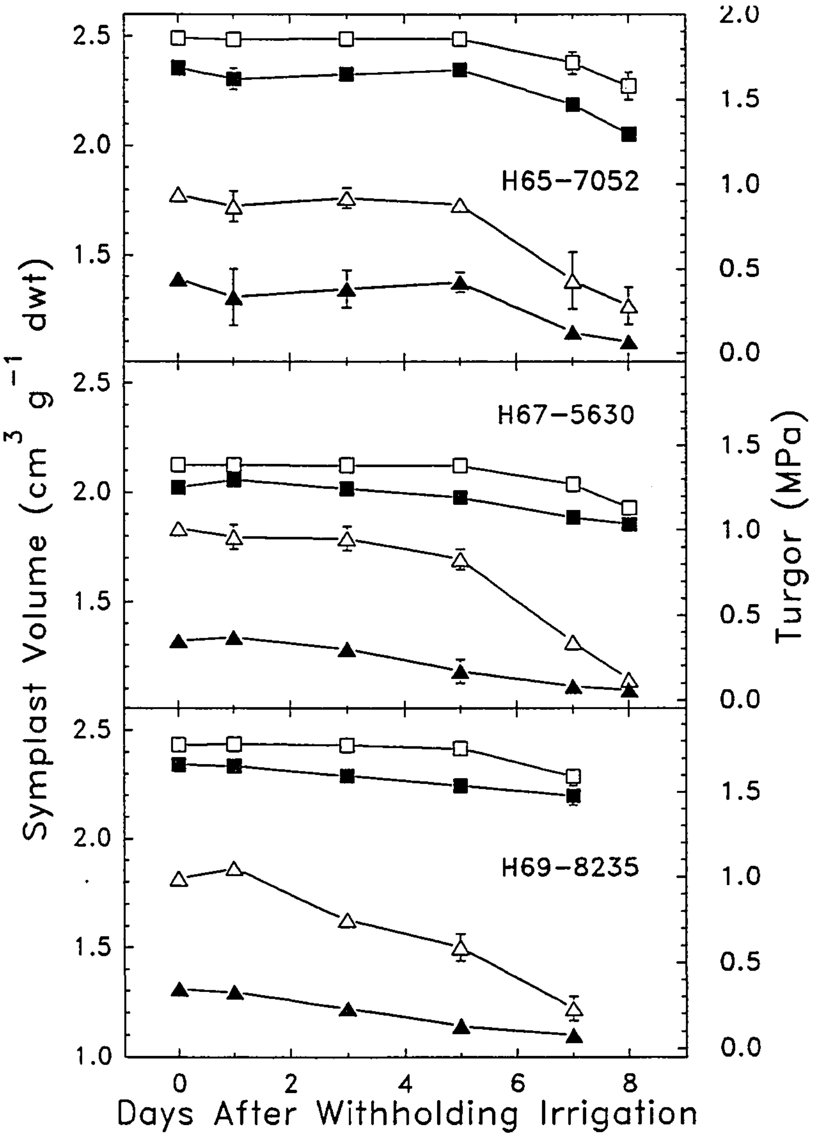

Figure 4. The course of symplast volume (□,■) and turgor (△,▲) at predawn (open symbols) and midday (closed symbols) in leaves of plants of three sugarcane cultivars under a drying soil. Data points are means (± standard error) of three to six observations.

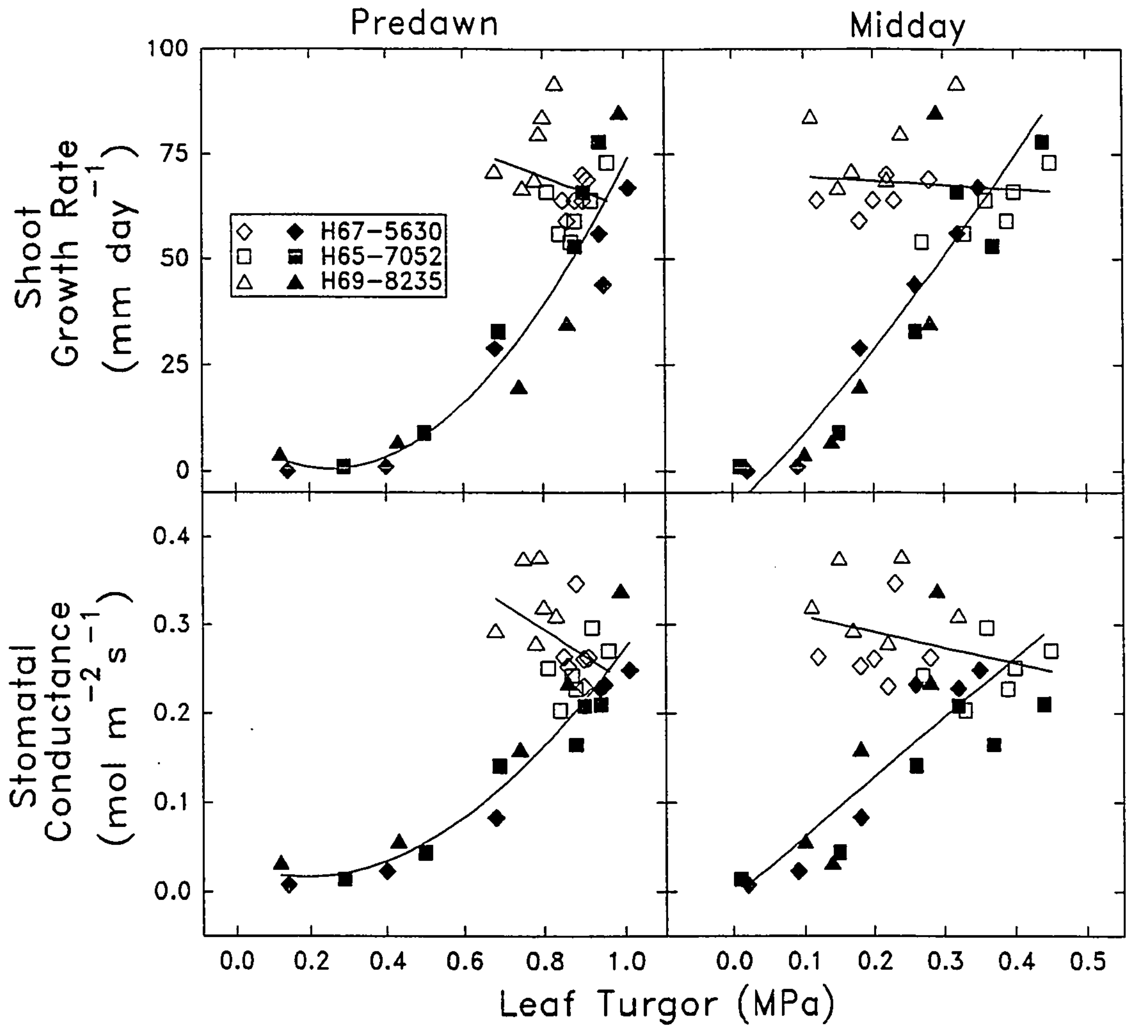

Figure 5. Shoot growth rate, SGR, and stomatal conductance, *g*, as functions of predawn and midday leaf turgor in droughted (closed symbols) and irrigated (open symbols) plants of three sugarcane cultivars. Data points are means of 6, and 12 to 24 observations for SGR and *g*, respectively. Equations for the lines fitted by regression are:

predawn, irrigated - $SGR = 97.9 - 35.3(\psi_P)$, $r^2 = 0.06^{ns}$

$g = 0.53 - 0.29(\psi_P)$, $r^2 = 0.16^{ns}$

droughted - $SGR = 8.2 - 62.2(\psi_P) + 126.4(\psi_P)^2$, $r^2 = 0.89^{**}$

$g = 0.05 - 0.22(\psi_P) + 0.45(\psi_P)^2$, $r^2 = 0.92^{**}$

midday, irrigated - $SGR = 70.6 - 10.1(\psi_P)$, $r^2 = 0.01^{ns}$

$g = 0.33 - 0.18(\psi_P)$, $r^2 = 0.12^{ns}$

droughted - $SGR = -8.8 - 166.1(\psi_P) + 104.3(\psi_P)^2$, $r^2 = 0.83^{**}$

$g = -0.005 + 0.67(\psi_P)$, $r^2 = 0.68^{**}$.

DISCUSSION

The data presented here indicate that symplastic volume remained nearly constant in sugarcane leaves exposed to drought and to diurnal variations in water deficit, whereas ψ_P was only partially maintained under these conditions. It is proposed that symplast volume maintenance was attained through the combined influence of osmotic and elastic adjustment on leaf T/D and SWF. Solute accumulation and increased tissue elasticity acted synergistically, with the former providing an increased gradient for influx of water and the latter allowing a greater volume of water in the symplast at a given ψ_L. This response resembles the influx of water associated with solute uptake and irreversible cell wall relaxation in growing tissues (Cosgrove 1986). In mature sugarcane leaves, however, reversible changes in tissue elastic extensibility contributed to regulation of symplast volume.

Osmotic adjustment and increased tissue elasticity are often cited as mechanisms for maintenance of turgor in droughted plants (Hsiao *et al* 1976; Tyree and Jarvis 1982; Morgan 1984). In the present study, the diurnal variation in ψ_P was approximately 0.4 MPa, and after 7 to 8 days after withholding irrigation, midday ψ_P had fallen to near zero. Over these ranges of variation, absolute symplast volume remained nearly constant. Comparative information on volume maintenance in droughted plants cannot be readily obtained from most published studies because the impact of osmotic and elastic adjustment on ψ_P has been emphasized in the presentation of results.

It has been proposed that plant acclimation to low leaf ψ_L may involve a reduced degree of cell shrinkage at a given ψ_L, which would allow for the maintenance of relatively higher photosynthetic capacity during drought (Berkowitz and Kroll 1988; Santakumari and Berkowitz 1990). In contrast, Bunce (1986) reported that even when symplast volume was maintained, photosynthesis in a range of species decreased as ψ_L declined. In sugarcane, photosynthetic gas exchange was probably not maintained as ψ_L declined because symplastic volume maintenance (Fig. 4) was not accompanied by maintenance of g (Fig. 1). The physiological and adaptive significance of symplastic volume maintenance in sugarcane is thus uncertain but it may postpone reaching a lethal symplastic volume, a response similar to the delayed occurrence of lethal relative water content attributed to osmotic adjustment in pigeonpea leaves (Flower and Ludlow 1986).

Other workers (Tyree and Hammel 1972; Turner 1981) have suggested the use of rehydrated samples in generating P-V relationship of a plant tissue. Rehydration of excised sugarcane leaf segments substantially altered several parameters subsequently derived from P-V curves (Table 3). In a recent study of rehydration-induced changes in P-V relationships, Evans *et al* (1990) predicted that changes due to rehydration would be less likely to occur in plants of mesic environments and more likely to occur in plants of arid environments. It is probable that upon examination of additional species, rehydration effects on P-V relationships will be detected in plants adapted to a wide range of environments.

Cultivar responses over the range of leaf water deficits imposed diurnally and during soil drying did not provide conclusive evidence for genotypic differences in solute accumulation. Diurnal and drought-induced variations in ϵ were largest in cv. H69-8235 (Tables 4 and 5), suggesting that this cultivar may possess a somewhat higher capacity for elastic adjustment. Thus, it appeared that the magnitude of osmotic and elastic adjustment in response to drought was due to differences among sugarcane cultivars in the rates of water use or in efficiency of extracting soil water. For example, upon withholding irrigation, the rates of decline in SGR and *g* were most rapid in plants of cv. H69-8235 (Fig. 1), which also had the highest SGR and *g* at the start of the drying cycle. In accord with its presumably higher rate of water use, the rate of development of plant water deficit was fastest in this cultivar.

There were indications that responses of SGR and *g* to soil drying were independent of ψ_L. For example, 5 days after withholding irrigation, the SGR and *g* of irrigated plants were, in general, significantly higher than those of droughted plants, but predawn and midday ψ_L in irrigated and droughted plants were similar (Fig. 1). In addition, SGR and *g* were apparently independent of bulk leaf turgor in irrigated plants but appeared to be turgor-dependent in droughted plants (Fig. 5). These patterns suggest that factors other than leaf water status may control growth and stomatal behavior in sugarcane. Other workers have shown convincingly that leaf growth (Passioura 1988b; Saab and Sharp 1989; Passioura and Gardner 1990) and stomatal aperture (Bates and Hall 1981; Blackman and

Davies 1985; Gollan *et al* 1985, 1986; Henson *et al* 1989) during soil drying were governed by soil water status rather than bulk leaf water status. It has been proposed that information concerning root and soil water status is communicated to the shoot by chemical signals in the transpiration stream (Blackman and Davies 1985; Zhang *et al* 1987; Munns and King 1988; Saab and Sharp 1989; Zhang and Davies 1989a, 1990). Prior to dawn, shoot water status is assumed to be in equilibrium with root water status. Therefore, root water status could have influenced shoot growth and stomatal behavior in sugarcane because SGR and *g* were better correlated with predawn than with midday leaf turgor (Fig. 5). In a drying soil, roots have been proposed to be able to measure the soil water potential and send chemical messages to the shoot via the transpiration stream. These root chemical messages ultimately control leaf growth and stomatal aperture.

CONCLUSIONS

Changes in leaf water relations characteristics of three sugarcane cultivars occurred in response to both drought and diurnal variation in water deficit. These changes resulted in nearly complete maintenance of symplast volume but only partial maintenance of turgor. Symplastic volume maintenance at a given ψ_L was attributable to the combined influence of osmotic adjustment on the gradient for water uptake and of increased tissue elasticity on symplast volume.

Rehydration of excised leaves before P-V curve determination caused alterations in leaf water relations characteristics similar in magnitude to those resulting from drought and diurnal variation in leaf water deficit. This indicated that determination of pressure-volume relationships in rehydrated leaves was not a satisfactory technique for characterizing leaf water relations *in vivo*.

SGR and *g* declined independently of bulk leaf water status during the early stages of drought and continued to decline throughout a soil drying cycle despite complete maintenance of leaf symplast volume and partial maintenance of turgor. This suggests that signals originating in the roots rather than in the leaves may regulate growth and stomatal behavior in sugarcane during soil drying.

CHAPTER III

A COMPARISON OF METHODS FOR CHARACTERIZING HYDRAULIC PROPERTIES OF ENTIRE ROOT SYSTEMS AND ISOLATED ROOTS OF SUGARCANE

ABSTRACT

An evaluation of the different methods of measuring root system hydraulic properties is a primary step in relating root water relations to shoot water relations. In the present study, transpiration-gradient and pressure-flux techniques were employed to characterize the hydraulic properties of entire root systems and isolated roots of sugarcane cultivars H69-8235, H67-5630 and H65-7052. The cultivars were ranked in the same order on the basis of leaf area-specific total root system hydraulic conductance (G_{root}, mmol m^{-2} s^{-1} MPa^{-1}), regardless of the method of measurement. However, values of G_{root} were overestimated using the pressure-flux method presumably because of movement of water through paths not followed by transpiration water flow. Cultivar ranking in G_{root} were reflected as similar ranking in root length-specific root hydraulic conductance (L, mmol m^{-1} s^{-1} MPa^{-1}) of individually excised shoot-roots, which were observed to differ anatomically among cultivars. Cultivar differences in L were most obvious near the root tip and tended to diminish as the distance from the tip increased, disappearing completely 15 cm from the tip. This suggested that the cultivar

differences in G_{root} may have been due to cultivar variations in root morphology, specifically root branching. Very large differences between the L of excised shoot-roots and entire sett-root systems were observed. These differences were attributed to much higher axial conductance in shoot-roots. It was hypothesized from drought and reirrigation experiments using both intact plants and detopped sugarcane root systems, that xylem cavitation may have occurred in roots exposed to drying soil. A rapid dissolution of gas emboli in the root xylem of reirrigated plants may have occurred when their detopped root systems were pressurized.

INTRODUCTION

An important function of the plant root system is to absorb water from the soil for transport to the shoot. The roots, however, are usually the site of the highest resistance in the pathway for liquid-phase movement of water through the soil-plant-atmosphere continuum (Kramer 1983). Therefore, the efficiency of soil water uptake by the root system is an important factor in determining the balance between transpiration and shoot water status.

Root water transport efficiency is generally expressed as hydraulic conductance (mmol m^{-2} s^{-1} MPa^{-1}), the ratio of water flux (mmol m^{-2} s^{-1}) through the roots to the water potential gradient (MPa) between the root xylem and the soil at the root surface (Passioura 1988c; Markhart and Smit 1990). This gradient has an osmotic and a hydrostatic pressure component, which account for water movement into the roots by osmosis and mass flow, respectively (Dalton *et al* 1975; Fiscus

1975). Mass flow predominates at high transpiration rates when solute concentrations in the xylem are negligible. Osmotic flow predominates at low transpiration rates when active solute uptake may lead to the development of root pressure and guttation (Fiscus 1977).

Water movement through the soil-root-shoot continuum follows a radial pathway from the soil-root interface to the root xylem, and an axial pathway from the root xylem to the apoplast of the leaf (Markhart and Smit 1990). The axial pathway consists of dead xylem elements connecting the root and the shoot, while the radial pathway consists of the soil-root interface, epidermis, cortex, endodermis and stele. The total root hydraulic conductance, therefore, has a radial and an axial component. It is widely believed that the Casparian bands of the root endodermis in the radial pathway are the most important barrier to liquid-phase water transport in the entire plant. At the outer edge of the cortex, one or two cell layers beneath the epidermis is called the hypodermis. In the roots of some species (e.g., maize and onion), Casparian bands have also been observed in the hypodermis and when they occur there, the tissue is called exodermis (Peterson 1988).

The physical location of this rate-limiting barrier to water movement is important because of its role in controlling root shrinkage, which affects soil-root interfacial resistance (Passioura 1988c). If the barrier is in the endodermis, then the water in the cortex (the tissue likely to shrink) should equilibrate more readily with water in the soil than with water in the xylem. The cortex, therefore, should not shrink as plant water deficits develop because it is in better hydraulic communication with the soil than with the rest of the plant. On the other hand, if the barrier is in the hypodermis, the water in the cortex should tend to equilibrate with that in the rest of the plant resulting in cortical shrinkage as plant water deficits develop. It has been reported that increased plant water deficits at high transpiration rates cause root shrinkage, resulting in a large interfacial resistance between the roots and the surrounding soil in cotton (Huck *et al* 1970) and sunflower (Faiz and Weatherley 1978, 1982). In contrast, in wheat (Hansen 1974a), Italian ryegrass (Hansen 1974b) and soybean (Blizzard and Boyer 1980), decreased hydraulic conductivity observed during soil drying originated within the plant and not in the soil or at the soil-root interface. These contrasting observations may be attributable to differences among species in the location of the rate-limiting barrier to radial movement of water through the roots.

Several techniques have been employed to measure root hydraulic properties, namely, i) determination of transpiration-gradient relationships in intact plants (Hansen 1974b; Black 1979; Meyer and Ritchie 1980), ii) determination of pressure-flux relationships in detopped (Fiscus 1977) and intact plants (Passioura 1980), iii) pressure probe techniques (Steudle and Jeschke 1983), and iv) osmotically induced back-flow techniques (Pitman and Wellfare 1978). Each of these techniques has its own merits and drawbacks. The transpiration-gradient technique uses intact plants and is the least prone to the development of artifacts during measurement, but it has been criticized for its inability to measure accurately the effective osmotic pressure gradient at the soil-root interface (Markhart and Smit

1990). The reverse is true with the pressure-flux technique; the osmotic and hydrostatic pressure can be accurately imposed around the roots, but the use of high hydrostatic pressures may force the solution to flow through paths different from those followed by the transpiration stream (Salim and Pitman 1984; Koide 1985). In addition, elimination of phloem transport when plants are detopped disrupts the movement of substances from the shoot to the root (Bowling *et al* 1985), thereby affecting osmotically-driven water uptake. The pressure probe technique, on the other hand, is advantageous because only a small amount of water is moved across the root so that osmotic gradients within the root are unaltered and interactions between solute uptake and water flow are accurately detected (Steudle and Jeschke 1983). The arguments against the use of pressure probe are that excision of roots might change their transport properties and that the positive pressures used during measurements do not represent the tension occurring in the xylem of a transpiring plant (Steudle *et al* 1987). The osmotically-induced backflow technique has similar merits and drawbacks as the pressure-flux technique, but an additional disadvantage of this technique is that the direction of water flow through the roots is the reverse of that occuring in the intact plant.

Root/soil hydraulic properties differ substantially among sugarcane cultivars both under well-irrigated conditions and during soil drying (see Chapter III). However, the extent to which these differences arise within the roots, or at the soil-root interface is not known. The objectives of the present study were to compare different methods for characterizing root hydraulic properties in three contrasting sugarcane cultivars and to determine whether properties manifested by the entire root system were observable in individual excised roots.

MATERIALS AND METHODS

Plant Material and Experimental Conditions

Three sugarcane (*Saccharum* spp. hybrid) cultivars were selected on the basis of contrasting performance in previous salinity and drought resistance tests (Ginoza and Moore 1985; Ingram 1985). The cultivars were classified as susceptible (cv. H65-7052), moderately resistant (cv. H67-5630), and resistant (cv. H69-8235) to drought.

Single stem segments containing one lateral bud were sown in 11-liter plastic pots filled with 4:4:1 v/v mixture of clay soil, commercial potting mix, and volcanic cinders. Plants were grown in the greenhouse and were watered automatically twice daily by drip irrigation. Greenhouse temperatures were typically 30 to 35 °C during the day and 20 to 25 °C at night. Complete fertilizer was first applied one month after planting and once a month thereafter. The plants were three to four months old when experimental measurements were carried out. One-month old plants whose shoot-roots had not yet developed, were used for whole sett-root system measurements. These plants were grown from stem segments sown in 250 ml pots which were fertilized once, two weeks after planting.

Hydraulic Properties of Entire Root Systems

Hydraulic properties of entire (shoot plus sett) root systems of intact and detopped plants were measured by the transpiration-gradient and pressure-flux techniques, respectively. To estimate total root system hydraulic conductance (G_{root}) by the transpiration-gradient technique, transpiration rate (E), soil water suction (τ) and covered leaf water potential (ψ_{CL}) of intact, well-irrigated plants were measured at hourly intervals from 0800 to 1500 h on clear days in the greenhouse. E was determined gravimetrically by sealing pots in plastic bags, then recording weights to the nearest 0.1 g and time intervals to the nearest s. At the end of the experiment, total uncovered leaf area per plant was measured with a leaf area meter (model 3100, LI-COR), permitting E to be expressed on a unit leaf area basis. τ was measured by a tensiometer installed in each pot. Tensiometers were installed with their tips near the bottoms of the pots where previous observations indicated most of the roots were located. ψ_{CL}, the xylem pressure potential of a covered, non-transpiring leaf, was measured with a pressure chamber. The difference between τ and ψ_{CL} was taken to represent the hydrostatic pressure gradient across the entire root system. Root xylem pressure potential was estimated from the xylem pressure potential of the oldest green leaf which had been covered with aluminum foil on the previous day. This covered, non-transpiring leaf was attached at the base of the stalk near the origin of the shoot-roots. The water potential of this leaf was influenced by the water potential of the root cortex and axial resistance of the xylem, as well as the water potential of the soil immediately adjacent to the root. G_{root} was calculated as the ratio of E to the hydrostatic pressure gradient between the soil and the root xylem using the Ohm's law analogy as modified from Meyer and Ritchie (1980):

$$G_{\text{root}} = E/(\tau - \psi_{\text{CL}})$$

Thus, G_{root} as defined here is a leaf-specific hydraulic conductance (Zimmermann 1978). Finally, G_{root}, obtained as the slope of the line fitted by linear regression to a plot of E on the ordinate axis versus the pressure gradient between τ and ψ_{CL} (Markhart and Smit 1990), was used to compare with G_{root} obtained from pressure-flux relationship.

Three- to four-month old plants were watered manually just before G_{root} was determined by the pressure-flux technique. Green leaf blades were first excised at their ligule and immediately sealed in long plastic bags for subsequent measurement of their area with a leaf area meter. The plants were then detopped by cutting through the stem at approximately 10 cm above the soil surface with a sharp razor blade. The stump of the cut stem was fitted through a hole in the center of a rubber stopper and the entire rubber stopper-root-soil-pot assembly was placed inside a specially constructed cylindrical pressure vessel measuring 28.5 cm in diameter and 29.5 cm in height. The protruding stump of the stem was inserted through a hole at the center of the cover of the pressure vessel which was designed to form a gas-tight seal against the rubber stopper and stem. The cover was then tightened and a short section of tygon tubing was fitted over the end of the protruding cut stem. This tube was used to collect the root system xylem exudate when the vessel was pressurized.

Pressure-flux relationships were determined by pressurizing the detopped (shoot plus sett) roots with compressed nitrogen gas at approximately 0.15 MPa and raising the pressure in 0.15 MPa increments until a final pressure of 0.60 MPa had been attained. Root xylem exudate flow rate increased initially with each pressure increase, but after approximately 20 minutes, a steady-state flow rate was obtained. Xylem exudate was collected from the tygon tube every three to five minutes and transferred to a pre-weighed vial, which was reweighed immediately to the nearest 0.1 mg. No evidence of hypoxia was observed using nitrogen as the pressurizing gas over the entire two-hour period (from 0.15 to 0.60 MPa pressure applied) required to attain steady-state flow. G_{root} was calculated as the slope of the line fitted by linear regression to a plot of flux on the ordinate axis versus pressure applied (Markhart and Smit 1990). The total leaf area of the shoot corresponding to each root system was measured with a leaf area meter so that G_{root} could be expressed on a leaf-specific basis for comparison with results obtained with the transpiration-gradient method.

The pressure-flux technique was also used to characterize the hydraulic properties of sett-root systems. One-month old plants were removed from their small pots, their root systems were washed carefully with water, and they were immediately placed in 25 per cent Hoagland solution. The plants were detopped by cutting the stem 3 cm above the shoot-root axis. A specially constructed ten-chamber apparatus was used for pressure-flux measurements. Cylindrical plastic filled with 25 per cent Hoagland solution containers were placed inside the chambers. Detopped sett-root systems were prepared for pressurization by inserting the stump of the cut stem through the hole at the center of a rubber stopper with the aid of an insertion tool. The root system-stopper assemblies were sealed inside the chambers so that the portion of the root system sealed inside was immersed in the solution during the entire period of measurements. The apparatus was pressurized with compressed air in increments of 0.05 MPa from 0.05 to 0.25 MPa. The solution and the roots were aerated during measurements. Root system exudate was collected in small vials filled with tissue paper weighed to the nearest 0.1 mg before and after each sap collection. An inverted beaker lined with moist filter paper was placed over the vial and cut stem to prevent evaporation from the tissue paper between weighings. Preliminary measurements indicated that steady-state flow rates were attained at approximately ten minutes after applying each new pressure. Upon completion of the pressure-flux measurements, total lengths of the sett root systems were measured with an area meter (Delta-T Area Meter Sytem, Delta-T Devices, Cambridge, England), set on a length mode, permitting flux and hydraulic conductance to be expressed on a unit root length basis. Root length-specific hydraulic conductance (*L*) was calculated as the slope of the pressure-flux relationships as described above.

The pressure-flux and transpiration-gradient techniques were used to observe changes in G_{root} during soil drying and following reirrigation. The same set of four plants was used for all measurements with the transpiration-gradient method. Soil drying was monitored with tensiometers and irrigation was resumed when soil suction had attained a value of 0.07 MPa. It was previously determined that G_{root} and stomatal conductance declined substantially at or below this value of soil suction (see Chapter III). Since determination of G_{root} with the pressure-flux method required detopping, different sets of plants were used before and during soil drying, and following reirrigation. Procedures to calculate G_{root} of intact plants were identical to those described above, whereas flux of detopped root systems was determined at a single pressure of 0.4 MPa. G_{root} of detopped root systems was calculated as the flux divided by 0.4 MPa. The G_{root} calculated this way may not be identical to the G_{root} calculated from the slope of the pressure-flux relationship.

Hydraulic Properties of Isolated Roots

Individual shoot-roots were obtained by removing the entire root-soil mass from the pot exposing the white, active shoot roots located along the sides and the bottom of the pot. White shoot-roots approximately 2 mm in diameter and 8 cm long measuring from their tip were rapidly selected and excised. Excised roots were immediately placed in a 25 per cent Hoagland solution and brought to the laboratory for determination of L with the pressure-flux method. Individual shoot roots were prepared for pressurization by inserting the cut end of a root through a small hole at the center of a rubber stopper with the aid of an insertion tool. The same apparatus and procedures were used as described above in sett-root system measurements.

Exudate was collected at two- to three-minute intervals for ten to fifteen minutes after steady-state flow rates were attained. A new set of roots was used at each pressure increment. Flux was expressed on a unit root length basis. L was calculated as the slope of a line fitted by linear regression as described above.

To evaluate the effect of root length on L, shoot roots having lengths of 5, 10, and 15 cm from the tip were excised as previously described. These roots were pressurized at a single pressure of 0.1 MPa and L was calculated as the flux per unit length divided by 0.1 MPa. The L calculated this way may not be identical to the L calculated from the slope of the line in pressure-flux relationships.

Root Anatomy

Individual shoot roots of the three sugarcane cultivars were selected and excised near the tip as described in the preceding section. Anatomical specimens were prepared as described by Luza and Polito (1988). Briefly, excised roots were fixed with 4% glutaraldehyde in 0.05 M phosphate buffer, pH 6.8, and dehydrated through an ethanol series. Samples were embedded in glycol methacrylate (Sorvall) and sectioned at 3 μm with glass knives on a Sorvall JB-4 microtome. Sections were stained with aniline blue-black, periodic acid-Schiff's reaction, and decolorized aniline blue.

Cross sectional diameters of the stele and the root were measured under a light microscope, and their cross sectional areas were calculated. The area of the cortex was calculated from the difference between the entire root area and the stele area. In addition, radii and areas of individual metaxylem elements were determined from stele photographs using a digitizer (Sigma-Scan, Jandel Scientific, Corte Madera, CA) linked to a personal computer.

RESULTS

The hydrostatic pressure gradient between the soil and the root xylem of intact sugarcane plants was linearly related to the transpirational flux of water (Fig. 6). There were consistent differences among cultivars in the relationship between transpiration rate and hydrostatic pressure gradient. At a given transpiration rate, the ranking of cultivars according to the pressure gradient was H65-7052 > H67-5630 > H69-8235. Therefore, the root system of cv. H69-8235 was able to sustain a greater transpirational flux of water at a given hydrostatic pressure gradient between the bulk soil and the root xylem (Fig. 6).

When the pressure-flux technique was used to determine the hydraulic properties of detopped root systems of cv. H65-7052 and cv. H69-8235, patterns similar to those obtained with the transpiration-gradient technique were observed (Fig. 7). At all pressures applied, water flux from roots of cv. H69-8235 was greater than that from roots of cv. H65-7052. For both cultivars, water flux at a given pressure applied (Fig. 7) was always less than the transpiration rate at a corresponding soil-root hydrostatic pressure gradient (Fig. 6). However, the slopes of the pressure-flux relationships were steeper than those of the transpiration-gradient relationships indicating that the pressure-flux method yielded higher

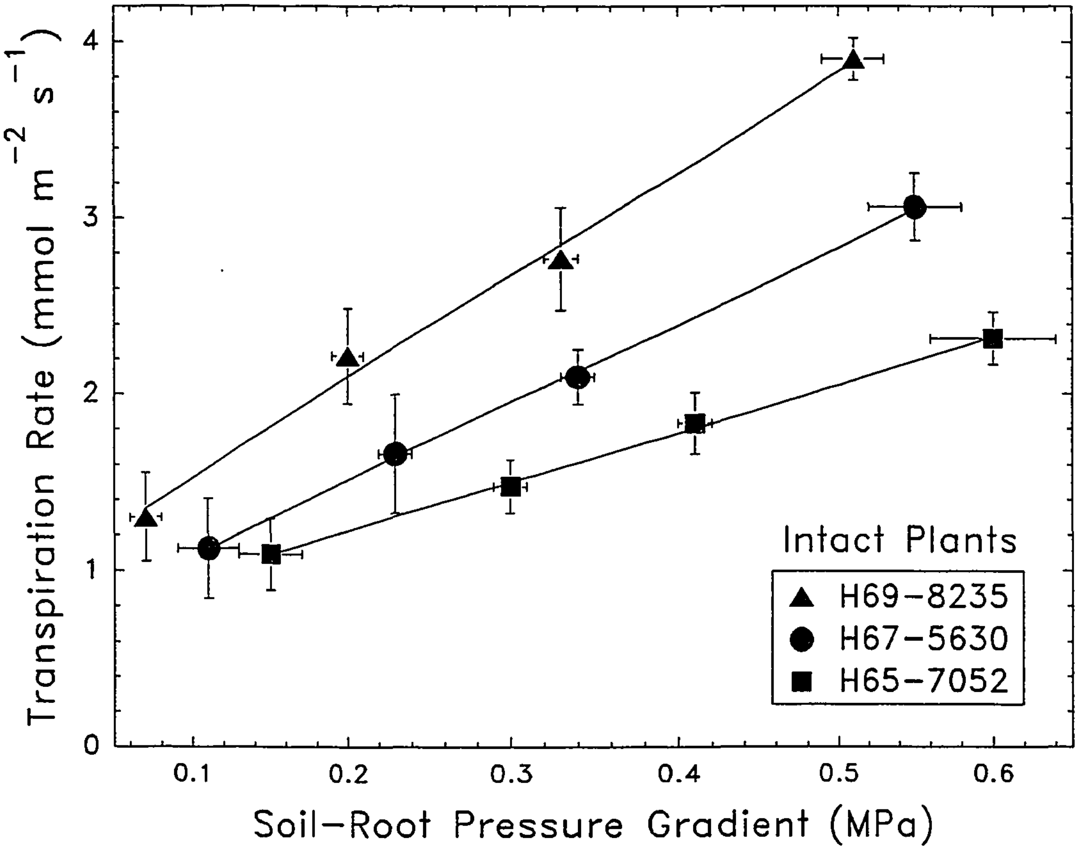

Figure 6. The relationship between transpiration rate and hydrostatic pressure gradient between the soil and the root xylem in intact, irrigated plants of three sugarcane cultivars. Transpiration rates are expressed on a unit leaf area basis. Data points are means (± standard error) of 5 to 10 observations from 4 plants of each cultivar. Equations for the lines fitted by regression are:

H69-8235, $E = 0.97 + 5.75(\tau - \psi_{\mathrm{CL}})$, $r^2 = 0.99^{**}$

H67-5630, $E = 0.65 + 4.37(\tau - \psi_{\mathrm{CL}})$, $r^2 = 1.00^{**}$

H65-7052, $E = 0.68 + 2.75(\tau - \psi_{\mathrm{CL}})$, $r^2 = 1.00^{**}$.

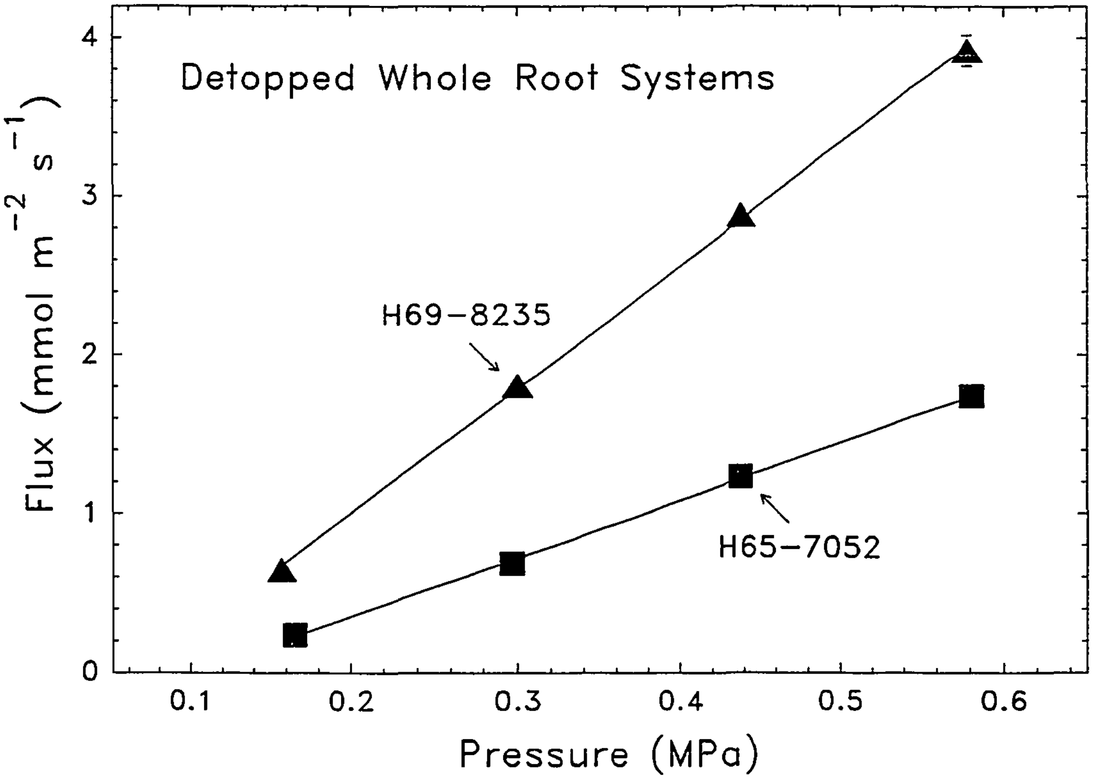

Figure 7. Xylem exudate flux versus root chamber pressure for whole root systems of detopped, irrigated plants of two sugarcane cultivars. Fluxes are expressed per unit leaf area of the excised shoot. Data points are means (± standard error) of ten observations from two root systems of each cultivar. Most of the standard errors are smaller than the symbols. Equations for the lines fitted by regression are:

H69-8235, Flux = -0.55 + 7.77(Pressure), $r^2 = 1.00^{**}$

H65-7052, Flux = -0.37 + 3.64(Pressure), $r^2 = 1.00^{**}$.

estimates of G_{root} than the transpiration-gradient method (Table 6). Regardless of the method employed, cv. H69-8235 exhibited the highest values of G_{root} which were roughly twice the values obtained for cv. H65-7052.

Table 6. Leaf area-specific hydraulic conductance of entire sugarcane root systems determined with the transpiration-gradient and pressure-flux techniques.

Hydraulic conductances are the slopes (± standard error) of the lines fitted to the data in Figures 6 and 7.

CULTIVAR	HYDRAULIC CONDUCTANCE	
	Transp.-Gradient	Pressure-Flux
	-----(mmol m^{-2} s^{-1} MPa^{-1})-----	
H69-8235	5.75 (±0.31)	7.77 (±0.12)
H67-5630	4.37 (±0.08)	-
H65-7052	2.75 (±0.07)	3.64 (±0.06)

The pressure-flux technique was also used to characterize the hydraulic properties of individual excised shoot-roots and whole sett-root systems (Fig. 8). At a given applied pressure, the water flux per unit root length was highest in cv. H69-8235 and lowest in cv. H65-7052. The ranking among cultivars with respect to the pressure-flux relationships was thus the same regardless of whether entire root systems (Figs. 6 and 7), individual shoot-roots (Fig. 8, top), or entire sett-root systems (Fig. 8, bottom) were used. However, water fluxes per unit root length

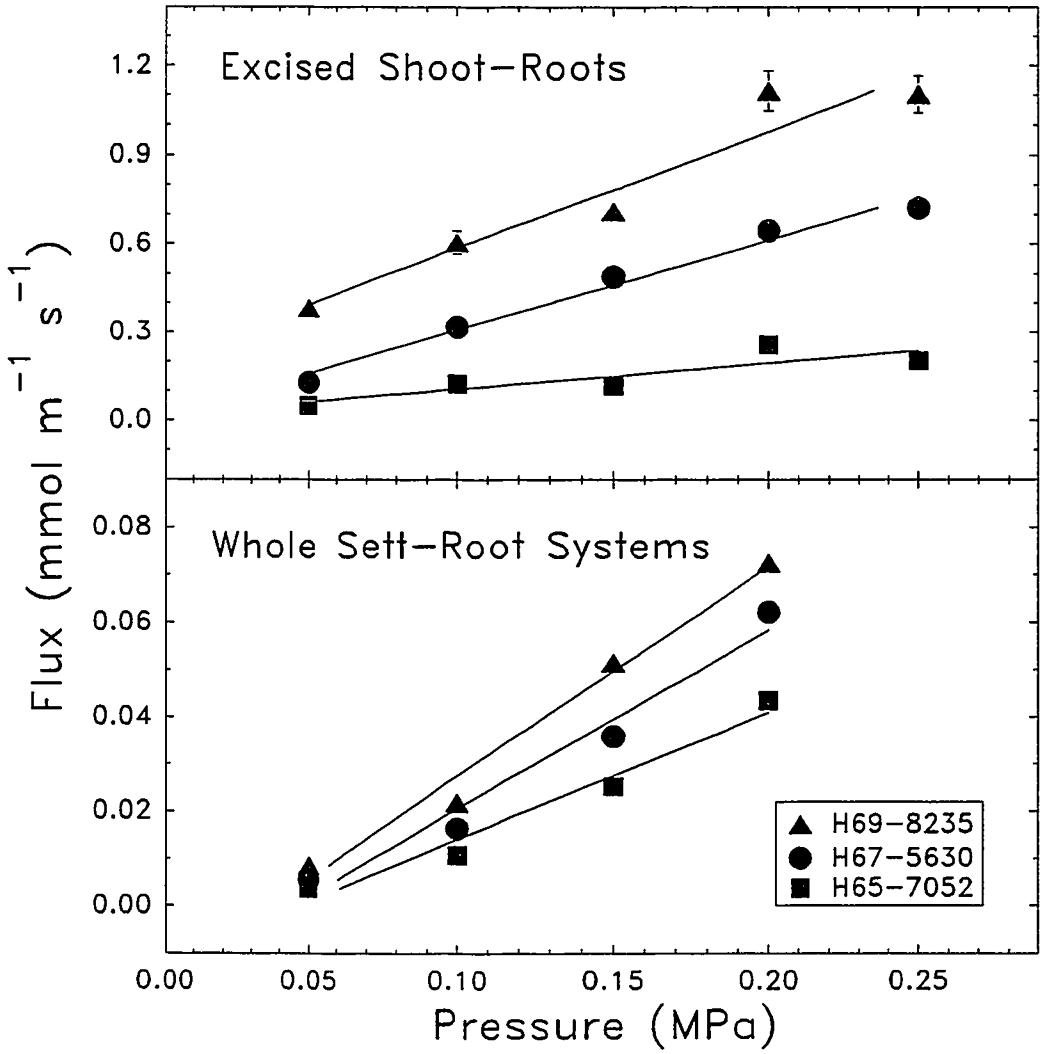

Figure 8. Xylem exudate flux versus root chamber pressure for single excised shoot-roots and whole sett-root systems of three sugarcane cultivars. Data points are means (± standard error) of three observations from three shoot-roots or sett-root systems of each cultivar. Most of the standard errors are smaller than the symbols. Lines were fitted by regression using the following equations:

excised shoot-roots, H69-8235, Flux = 0.20 + 3.90(Pressure), $r^2 = 0.93^{**}$
H67-5630, Flux = 0.01 + 3.03(Pressure), $r^2 = 0.98^{**}$
H65-7052, Flux = 0.02 + 0.88(Pressure), $r^2 = 0.74^{ns}$

whole sett-root systems, H69-8235, Flux = -0.03 + 0.60(Pressure), $r^2 = 0.93^{*}$
H67-5630, Flux = -0.01 + 0.39(Pressure), $r^2 = 0.98^{**}$
H65-7052, Flux = -0.01 + 0.27(Pressure), $r^2 = 0.96^{*}$.

(Fig. 8) and the corresponding hydraulic conductances (Table 7) were approximately an order of magnitude greater in excised shoot-roots than those in the whole sett-root systems. Shoot roots used were excised at 8 cm from their tips, whereas sett root systems contained roots in the range of 20 to 40 cm long. The larger proportion of immature tissue in shoot-roots, therefore, may have contributed to their higher hydraulic conductance on a unit length basis (Table 7).

Table 7. Root length-specific hydraulic conductance of single excised sugarcane shoot-roots and entire sett-root systems determined with the pressure-flux technique.

Hydraulic conductances are the slopes (± standard error) of the lines fitted to the data in Figure 8.

CULTIVAR	HYDRAULIC CONDUCTANCE	
	Shoot-Roots	Sett-Roots
	-----(mmol m^{-1} s^{-1} MPa^{-1})-----	
H69-8235	3.90 (±0.62)	0.60 (±0.11)
H67-5630	3.03 (±0.25)	0.39 (±0.04)
H65-7052	0.88 (±0.30)	0.27 (±0.04)

Subsequent determinations of hydraulic conductance in single roots excised at various distances from their tips revealed that conductance decreased precipituously with increasing root length beyond 5 cm (Fig. 9). The difference in

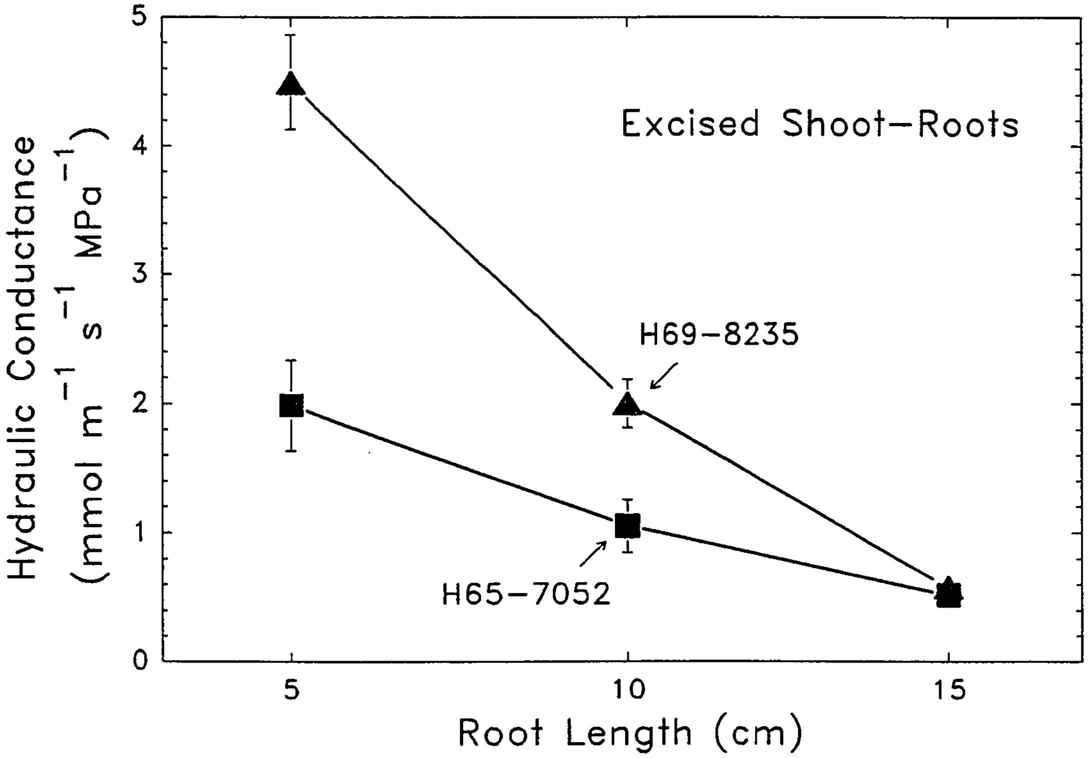

Figure 9. The effect of root length on the hydraulic conductance of excised shoot-roots of two sugarcane cultivars. Hydraulic conductance on a unit length basis was calculated from xylem sap flow rates of roots pressurized at 0.1 MPa. Data points are means (± standard error) of 5 to 8 observations.

conductance between roots of cv. H69-8235 and cv. H65-7052 was greatest in 5 cm long roots and diminished with increasing root length until there was no difference detected in 15 cm long roots (Fig. 9).

Shoot root anatomical measurements at approximately 8 cm from the tip revealed that the area of the stele (Table 8) and the total metaxylem area (Table 9) of cv. H69-8235 were greater than those for the other two cultivars. The A_{stele}/A_{cortex} ratio was also highest in this cultivar (Table 8), which suggested that for a unit of vascular tissue (stele) the radial route of water through the cortex was shortest in this cultivar. Furthermore, the metaxylem radius and area of cv. H69-8235 was larger than the other two cultivars (Table 9) implying that its root axial conductance was the highest of the three cultivars. For a given root diameter, this cultivar would also have higher endodermal surface area which should lower the its radial resistance.

The effect of a soil drying and reirrigation cycle on G_{root} was assessed with both the pressure-flux and transpiration-gradient techniques. Soil drying to 0.07 MPa soil suction caused reductions of 20 to 50% in G_{root} assessed with the pressure-flux technique (Table 10). Recovery of G_{root} upon reirrigation was rapid, occurring within 1 h after reirrigation. In that time, G_{root} of cv. H65-7052 had recovered completely and that of cv. H69-8235 had recovered to more than 80% of its initial value (Table 10). In contrast to the foregoing, G_{root} measured by the transpiration-gradient technique in soil dried to 0.08 MPa soil suction was nearly zero (Fig. 10). Recovery of G_{root} measured by the pressure-flux technique was rapid, while

Table 8. Average area of the stele (A_{stele}), cortex (A_{cortex}), and their ratio as determined from cross-sections of shoot-roots excised from irrigated plants of three sugarcane cultivars.

Data are means (± standard error) of 13 to 17 observations.

Cultivar	A_{stele}	A_{cortex}	A_{stele}/A_{cortex}
	-------(mm^2)-------		
H69-8235	1.33	3.56	0.37
	(±0.34)	(±0.89)	(±0.02)
H67-5630	1.18	5.70	0.21
	(±0.19)	(±0.94)	(±0.02)
H67-7052	0.52	2.03	0.26
	(±0.05)	(±0.26)	(±0.02)

Table 9. Average number, radius, and total area of metaxylem as determined from cross-sections of shoot-roots excised from irrigated plants of three sugarcane cultivars.

Data are means (± standard error) of 13 to 17 observations.

CULTIVAR	METAXYLEM CHARACTERISTICS		
	Number	Radius	Total Area
		(μm)	(10^5 μm^2)
H69-8235	11.2	64.3	1.46
	(±1.3)	(±3.8)	(±0.22)
H67-5630	13.9	51.9	1.18
	(±0.9)	(±4.6)	(±0.21)
H65-7052	9.5	58.2	1.01
	(±0.7)	(±2.2)	(±0.15)

Table 10. Leaf area-specific root hydraulic conductance determined with the pressure-flux technique from detopped sugarcane root systems subjected to three irrigation treatments.

Hydraulic conductances were calculated as the ratio of the flux to the constant pressure applied (0.4 MPa). Data are means (± standard error) of 18 to 24 observations from three to four root systems of each cultivar.

TREATMENT	ROOT HYDRAULIC CONDUCTANCE H65-7052	 H69-8235
	---(mmol m^{-2} s^{-1} MPa^{-1})---	
Irrigated	**2.9** (±0.1)	**6.3** (±0.2)
Droughted	**0.6** (±0.1)	**3.2** (±0.3)
Reirrigated	**3.4** (±0.1)	**5.2** (±0.3)

24 to 72 h were required for full recovery of G_{root} following reirrigation when measured by the transpiration-gradient technique even though root xylem pressure potential recovered fully within 1 h (Fig. 10).

DISCUSSION

The higher G_{root} for pressure-flux relationships than for transpiration-gradient relationships (Table 6) may be attributable to the nature of the driving forces in the transpiration- and pressure-induced water movement through the root systems of intact and detopped plants, respectively. In detopped root systems, the

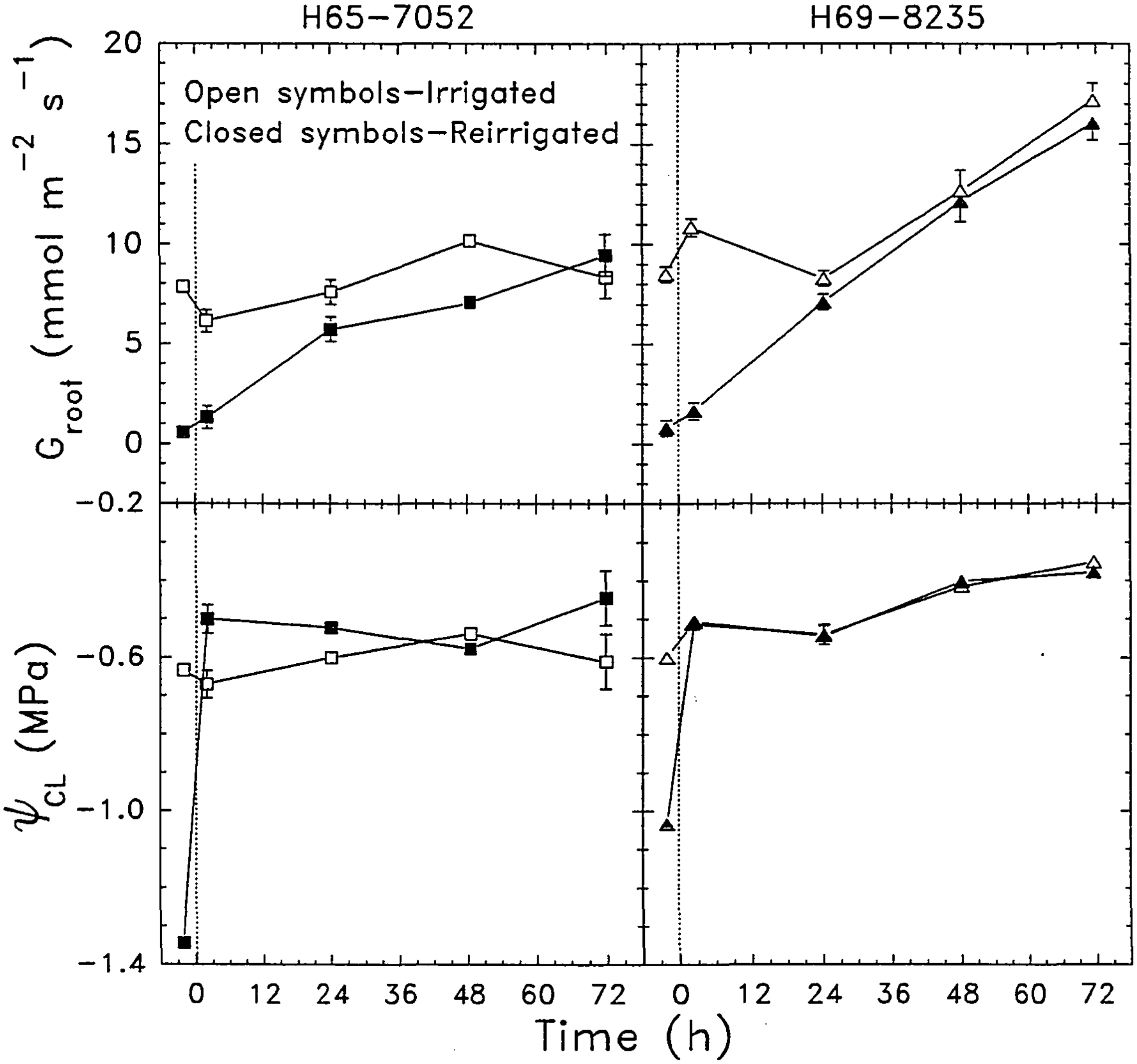

Figure 10. Time course of root hydraulic conductance (G_{root}) estimated by the transpiration-gradient technique and covered leaf water potential (ψ_{CL}) for well-irrigated plants (open symbols) and plants growing in soil dried to $\tau = 0.08$ MPa and reirrigated at Time = 0 (closed symbols). Data points are means (± standard error) of three to five observations from four plants of each cultivar.

osmotically-induced water flow predominating under low flow rates (Fiscus 1977) may have declined. Removal of the shoot also may have inhibited the transport of carbohydrates from the shoot to the root (Bowling *et al* 1985) so that reductions in osmotically-driven water flow in detopped root systems may have occurred. On the other hand, the similarity in the maximum values of transpiration (Fig. 6) and pressure-induced flow (Fig. 7) of each cultivar could have been a consequence of artifactually high fluxes through detopped root systems caused by application of high pressure. Evidence that pressure-induced water flow through roots can follow a route different from that of transpiration-induced flow has been provided by other studies. Koide (1985) reported that formerly air-filled spaces were added as a new flow path of water transport when roots were pressurized, while Salim and Pitman (1984) demonstrated that significant amounts of water movement occurred in the cortex when pressure was applied to detopped root systems. Despite the possible artifacts associated with the pressure-flux technique, it proved to be reliable for characterizing differences in root hydraulic properties among cultivars.

When the pressure-flux technique was applied to individual excised shoot-roots (Fig. 8, top), the results were consistent with those obtained with entire root systems (Figs. 6 and 7). Differences among cultivars in whole root system transport properties, therefore may have been attributable to variations in intrinsic hydraulic properties of single roots. The decrease in L with increasing root length (Fig. 9) appears to confirm the assumption that water absorption is maximum near root tips where endodermal suberization has not yet developed (Kramer 1983) and where radial conductance is high. The rapid decrease in L of cv. H69-8235 with increasing length relative to the response of cv. H65-7052 (Fig. 9) suggested that cv. H69-8235 may have a more compressed maturation zone than does cv. H65-7052. Also, the rate of development of the endodermal suberization may have been faster (Kramer 1983) or the rate of increase in number of xylem cross-walls may have been greater (Sanderson *et al* 1988) in cv. H69-8235 than in cv. H65-7052. Thus, the more rapid rate of increase in both radial and axial resistance in cv. H69-8235 as the root got longer resulted into having its L the same as that in cv. H65-7052 with 15 cm long roots.

The similarity in L among cultivars when single roots were 15 cm long (Fig. 9) was inconsistent with cultivar differences in G_{root} of entire root systems (Table 6). It was possible that differences in cultivar root system morphology may have existed. It may be hypothesized that cv. H69-8235 had more root tips or more short roots so that its higher G_{root} may have arisen from greater root branching. In addition, the excised shoot roots used in the present study were without laterals, which may have overlooked the possibility that the proximal end of mature roots are capable of substantial amounts of water and nutrient uptake via the laterals (McCully and Canny 1988).

The patterns of cultivar differences in $A_{\mathrm{stele}}/A_{\mathrm{cortex}}$ and total metaxyleam area (Tables 8 and 9) were similar to the cultivar ranking in shoot-root hydraulic conductance (Table 7). A greater $A_{\mathrm{stele}}/A_{\mathrm{cortex}}$ could have resulted in greater radial conductance, while a higher total metaxylem area could have led into higher axial conductance. Cultivar variations in shoot-root hydraulic conductance, therefore may be partially attributable to intrinsic differences in anatomical characteristics among genotypes. A faster rate of movement of water from the epidermis to the stele may have occurred in cv. H69-8235 because its higher $A_{\text{stele}}/A_{\text{cortex}}$ implied that water molecules would traverse a shorter path from the epidermis to the stele. Upon reaching the stele, water would have reached the xylem at a faster rate in this cultivar as well, because its higher A_{stele} (Table 9) denoted greater stelar circumference, thus the avenues for the influx of water were also greater. In addition, there could have been cultivar differences in membrane permeability in both endodermis and hypodermis. Preliminary data indicated that the hypodermis of sugarcane roots may be suberized (Welbaum, unpublished data), similar to the exodermis described by Peterson (1988).

The wide variations in L between the shoot-roots and sett-roots (Table 7) could have been due to substantial differences in their axial conductances. If the xylem vessels are treated as circular tubes with constant diameter according to Poiseuille equation, the axial conductance of the root is directly proportional to the number of conducting xylem elements and to the fourth power of their radii (Frensch and Steudle 1989). Based on visual observations, the diameter of the sett roots were estimated to be 0.10 to 0.25 times the diameter of the shoot-roots. Although this may imply a greater radial conductance in the sett-roots, much bigger radii and greater number of xylem elements in the shoot-roots probably determined the observed differences in the total hydraulic conductance between these two types of root. A very conservative estimate of just doubling the radii of xylem elements between sett-roots and shoot-roots would have resulted in axial flow rates of the shoot roots that are 16 times greater than those for the sett roots (McCully and Canny 1988).

Decreases in the hydraulic conductance of root systems subjected to drought has been attributed to xylem cavitation in cotton (Byrne *et al* 1977), decreased cell permeability in lemon and citrus (Ramos and Kaufmann 1979; Levy and Syvertsen 1983), rectifier-like activities in desert succulents (Nobel and Sanderson 1984), and increased resistance at the soil-root interface (Herkelrath *et al* 1977; Faiz and Weatherley 1978). In the present study, however, the slow recovery of G_{root} measured by the transpiration-gradient technique upon reirrigation of droughted intact plants (Fig. 10), suggested that in sugarcane, changes in hydraulic properties within the root system, rather than at the soil-root interface (Faiz and Weatherley 1978) occurred in response to soil drying. However, when G_{root} was measured by pressurizing detopped root systems, its recovery upon reirrigation of droughted plants was rapid (Table 10). This discrepancy in the rates of recovery of G_{root} measured by the transpiration-gradient and pressure-flux methods can be explained if the decline in G_{root} during soil drying was caused by embolism formation. Recovery from embolism formation likely would be much slower when the driving force was transpiration pull than if it was by pressurization. Schultz and Matthews (1988) and Tyree *et al* (1986) have suggested that formation of embolism in stems may be a common occurrence in both droughted and irrigated plants, and that the root pressure generated by the concentration of solutes in the root xylem may be important in dissolving emboli and in refilling of vapor-filled vessels during the night. Therefore, it is also possible that a more immediate role of root pressure is in the repair of drought-cavitated xylem in the roots themselves.

CONCLUSIONS

Both the transpiration-gradient and pressure-flux techniques proved adequate for determining differences in the hydraulic properties of entire root systems of contrasting sugarcane cultivars. The pressure-flux technique, however, may force water movement through paths different from that pulled by transpiration so that G_{root} may be overestimated. Sugarcane genotypes could be separated consistently on the basis of differences in hydraulic conductance of entire root systems and of individually excised shoot-root tips. Cultivar differences in hydraulic conductance in individual roots disappeared as the distance from root tip approached 15 cm suggesting that root morphological differences, particularly in the degree of branching, may have existed among root systems of the sugarcane cultivars studied. This may have contributed to cultivar differences in hydraulic conductance observed in their entire root systems. The substantially higher fluxes and conductances for shoot-roots than for sett-roots may have been attributable to the greater radii of the xylem elements of the shoot-roots, which would result in their higher axial conductances. Slow recovery of G_{root} measured by the transpiration-gradient technique relative to the recovery when G_{root} was measured by the pressure-flux technique is consistent with the occurrence of cavitation in the root xylem of droughted sugarcane plants.

CHAPTER IV

RELATIONSHIP BETWEEN ROOT/SOIL HYDRAULIC PROPERTIES AND STOMATAL BEHAVIOR IN SUGARCANE

ABSTRACT

Stomatal conductance, leaf and soil water status, transpiration, and root hydraulic conductance were measured during soil drying cycles for three sugarcane cultivars growing in containers in a greenhouse. At high soil moisture, transpiration and root hydraulic conductance were positively correlated and differed considerably among cultivars, whereas leaf water potential was similar among cultivars. In one cultivar, fluctuations in stomatal conductance were associated with parallel variations in root hydraulic conductance, but not with leaf water potential which remained relatively constant during the day. In drying soil, stomatal and root hydraulic conductance approached zero over a narrow range (0.0 to 0.1 MPa) of soil suction. The vapor phase conductance of the leaves and the liquid phase conductance of the root system declined in parallel, thus leaf water potential remained nearly constant during soil drying. A large increase in the hydrostatic pressure gradient between the soil and the root xylem occurred due to the decline in root hydraulic conductance with soil drying. These results suggested that control of stomatal conductance in sugarcane plants exposed to drying soil was exerted primarily at the root rather than at the leaf level.

INTRODUCTION

Leaf water potential has often been cited as a major factor governing stomatal aperture. Stomatal closure in plants subjected to declining soil moisture has been reported to occur at a threshold level of bulk leaf water potential near the bulk leaf osmotic potential at the turgor loss point (Parker *et al* 1982; Syverten 1982). Decreases in the water potential threshold for stomatal closure observed during acclimation to drought have been attributed to a lowering of osmotic potential at the turgor loss point (Turner *et al* 1978; Hinckley *et al* 1980). Recently, however, stomatal conductance in a variety of herbaceous and woody species growing in drying soil has been shown to decline even in the absence of significant changes in bulk leaf water status (Bates and Hall 1981; Blackman and Davies 1985; Gollan *et al* 1985; Gollan *et al* 1986). From these observations it has been suggested that, in drying soil, a chemical message originates in the roots and acts on the stomata (Davies *et al* 1986). Abscisic acid and cytokinins, which are known to affect stomatal aperture, have both been proposed to function in this role (Davies *et al* 1986; Zhang *et al* 1987a; Zhang and Davies 1989).

Plant hydraulic properties have also been shown to correlate with stomatal conductance independently of leaf water status. In a number of species, prevailing levels of stomatal conductance were positively correlated with the levels of apparent hydraulic conductance of the soil/root/shoot pathway (Aston and Lawlor 1979; Sanchez-Diaz and Mooney 1979; Kuppers 1984; Meinzer *et al* 1988). In a more direct demonstration of this relationship, it was shown that wounding of root or stem xylem, or cooling of *Abies amabilis* roots, caused rapid stomatal closure independently of factors such as evaporative demand, leaf water potential or root water status (Teskey *et al* 1983). From the rapidity of the effect, it was concluded that the stimulus was physical rather than chemical.

The purpose of this study was to evaluate the relative importance of factors such as leaf, root and soil water status, and plant hydraulic conductance in the control of stomatal aperture and transpiration in three sugarcane cultivars subjected to soil drying cycles. An additional objective was to determine whether reported differences in drought resistance among the cultivars were associated with differences in stomatal regulation during soil drying.

MATERIALS AND METHODS

Plant Material and Experimental Conditions

Three sugarcane (*Saccharum* spp. hybrid) cultivars were selected on the basis of contrasting performance in previous salinity and drought resistance tests (Ginoza and Moore 1985; Ingram 1985). The cultivars were classified as susceptible (cv. H65-7052), moderately resistant (cv. H67-5630), and resistant (cv. H69-8235) to drought.

Single stem segments containing one lateral bud were sown in eleven-liter plastic pots filled with a 4:4:1 v/v mixture of clay soil, commercial potting mix, and volcanic cinders. Plants were grown in the greenhouse and watered automatically twice daily by drip irrigation. Greenhouse temperatures were typically 30 to 35 °C during the day and 20 to 25 °C at night. Complete fertilizer was first applied one month after planting and once a month thereafter. Plants were 2.5 to 3 months old (8 to 12 leaves) when experimental measurements were carried out.

Plant and Soil Measurements

Plant and soil measurements were carried out hourly between 1000 and 1500 h local time in the greenhouse and were restricted to sunny periods during which photosynthetically active radiation exceeded 1000 μmol m^{-2} s^{-1}. For experiments in which irrigation was withheld, the same set of plants was monitored as the soil was allowed to dry.

Stomatal conductance (g) of the abaxial surface of the two youngest fully expanded leaves was measured with a steady-state porometer (model 1600, LI-COR Inc., Lincoln, Nebraska, USA). Since these two leaves were near the top of the plant, incident solar radiation was always high during the measurements. The porometer measurements coincided with the portion of the day during which g had previously been determined to be maximal when water and light were not limiting.

Leaf water potential (ψ_L) was measured with a pressure chamber in the next leaf above the youngest used for porometry. To minimize post-excision water loss (Turner and Long 1980), samples were obtained by rapidly enclosing an 8 cm segment of the leaf in a plastic bag and excising at the mouth of the bag which was sealed immediately. Successive 8 cm segments of the same leaves were used. Comparisons of ψ_L measurements between leaf segments and intact leaf ends indicated that ψ_L could be estimated over time with acceptable accuracy by sequential excision of sugarcane leaf segments (Saliendra *et al* 1990). The ψ of the oldest green leaf, which was covered with aluminum foil (ψ_{CL}) on the previous day was also measured. This covered, nontranspiring leaf was attached near the origin of the shoot-roots and the difference between its ψ and that of the soil was taken to be an estimate of the gradient in ψ across the entire root system. In addition to being influenced by the ψ of the root cortex and the axial resistance of the xylem, the ψ of this leaf was also influenced by the ψ of the soil immediately adjacent to the root.

Bulk soil water suction (τ) was monitored by installing a small tensiometer in each pot. The tensiometer was long enough to reach near the bottom of the pot, where previous observations indicated most of the roots were located. A soil drying cycle was imposed by withholding irrigation until τ reached about 0.08 MPa. This required about four to seven days.

Transpiration rate (E) was measured gravimetrically. The pots were sealed in plastic bags during measurements of g and ψ, and weighed hourly. Weights were recorded to the nearest 0.1 g and time intervals to the nearest second. At the end of the experiment total leaf area per plant was measured destructively with a leaf area meter (model 3100, LI-COR Inc.). The decline in leaf area due to leaf sampling was also accounted for. This permitted E to be expressed on a unit leaf area basis.

Root and shoot hydraulic conductance were calculated from measurements of E, τ and ψ_{CL} using an Ohm's law analogy. The total root system hydraulic conductance (G_{root}) was estimated by the equation modified from Meyer and Ritchie (1980):

$$G_{root} = E/(\tau - \psi_{CL})$$

where E, τ and ψ_{CL} were as previously defined. In a similar manner total shoot hydraulic conductance (G_{shoot}) was estimated as:

$$G_{shoot} = E/(\psi_{CL} - \psi_L) .$$

Total root system dry weight was determined at the end of a soil drying cycle. The roots were washed carefully with tap water in order to remove as much soil as possible and were then dried in an oven at 80 °C. The dried roots were then cleaned further to remove remaining soil debris and were re-dried before dry weight was finally determined.

RESULTS

In an initial experiment conducted with well-irrigated plants, average E was significantly higher in cv. H69-8235 than in either cv. H67-5630 or cv. H65-7052 but ψ_L measured when transpiration was maximal did not differ significantly (Table 11). Since transpiration rate and hydraulic conductance are assumed to determine leaf water potential the pattern in Table 11 suggested that there were differences in hydraulic conductance among cultivars. When plant hydraulic conductance was partitioned between above- and below-ground components using

ψ_{CL}, it was clear that there were large differences in G_{root} among cultivars (Table 11), and that E and G_{root} were positively correlated. However, in this experiment, it was not possible to determine whether the differences in the G_{root} were due to intrinsic root hydraulic properties, or other factors such as root/shoot ratio, root length and root surface area. G_{shoot} did not differ significantly among cultivars (Table 11).

In a subsequent experiment, leaf area, root system dry weight, and the ratio of total leaf area to root dry weight were similar for well-irrigated individuals of all cultivars (Table 12). When average G_{root} was expressed on a root system dry

Table 11. Average transpiration rate (E), covered leaf water potential (ψ_{CL}), exposed leaf water potential (ψ_L), root hydraulic conductance (G_{root}), and shoot hydraulic conductance (G_{shoot}) for three-month old plants of three sugarcane cultivars.

Data are means (± standard error) of six to nine measurements obtained from three well-irrigated individuals of each cultivar.

Cultivar	E	ψ_{CL}	ψ_L	G_{root}	G_{shoot}
	(mmol m^{-2} s^{-1})	------(MPa)-----		(mmol m^{-2} s^{-1} MPa^{-1})	
H69-8235	**3.8** (±0.2)	**-0.34** (±0.04)	**-1.18** (±0.03)	**11.6** (±1.2)	**4.6** (±0.4)
H67-5630	**3.2** (±0.2)	**-0.42** (±0.05)	**-1.08** (±0.03)	**7.7** (±0.6)	**4.9** (±0.5)
H65-7052	**2.7** (±0.1)	**-0.53** (±0.05)	**-1.11** (±0.02)	**5.5** (±0.6)	**5.1** (±0.5)

Table 12. Average leaf area, root dry weight, and root hydraulic conductance on a dry weight basis for three-month old plants of three sugarcane cultivars.

Leaf area and root dry weight are means (± standard error) for four individuals of each cultivar. Root hydraulic conductances are means of 22 observations obtained on consecutive days from the same individuals used for leaf area and root dry weight measurements.

Cultivar	Leaf Area	Root Dry Weight	Root Hydraulic Conductance
	(m^2)	(g)	(mmol g^{-1} s^{-1} MPa^{-1})
H69-8235	**0.266** (±0.003)	**12.2** (±0.4)	**0.39** (±0.08)
H67-5630	**0.222** (±0.004)	**11.6** (±0.3)	**0.16** (±0.02)
H65-7052	**0.205** (±0.002)	**11.6** (±0.3)	**0.12** (±0.01)

weight basis, there was a 3.25-fold difference between cv. H69-8235 and cv. H65-7052. This suggested that the observed differences in G_{root} for well-irrigated plants were due to intrinsic root properties.

During a soil drying cycle, ψ_L of all cultivars remained relatively constant above a soil suction of approximately 0.01 MPa (Fig. 11). However, this was not true for ψ_{CL}, which exhibited a steady decline over the entire range of τ attained (Fig. 11). After ψ_L initially declined by 0.2 MPa below a τ of 0.01 MPa, it showed an additional decrease of only 0.2 MPa.

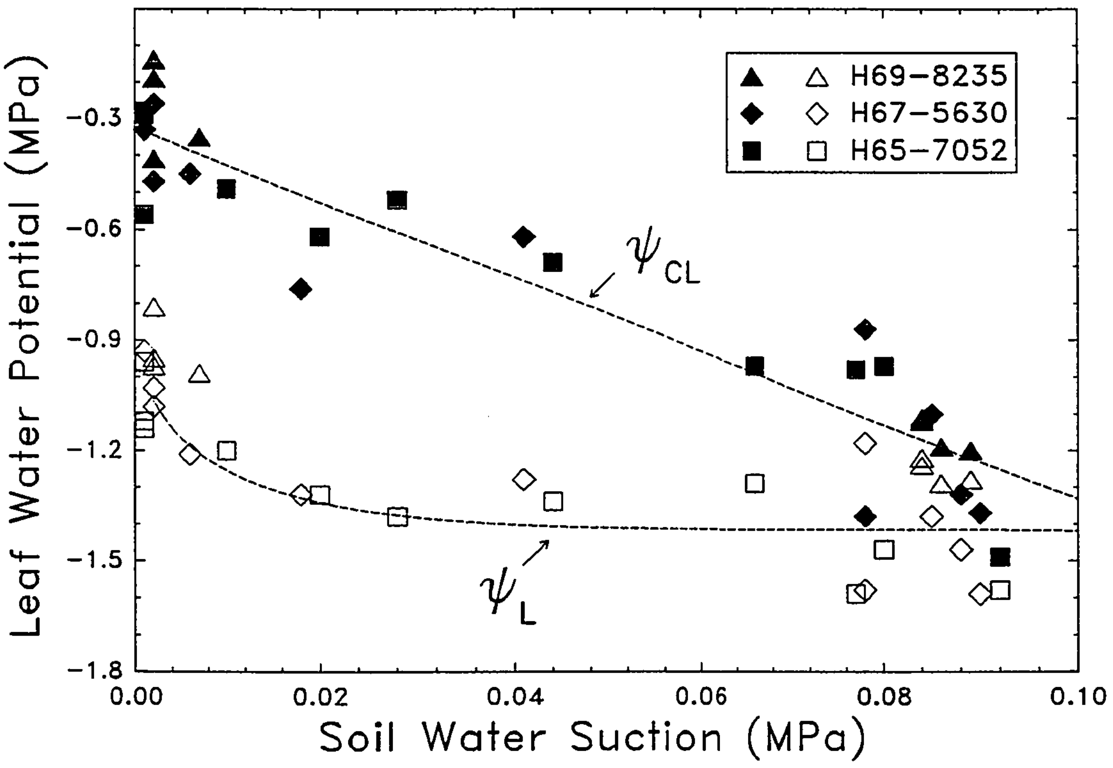

Figure 11. Water potential of exposed upper leaves (ψ_L, open symbols) and covered basal leaves (ψ_{CL}, closed symbols) of three sugarcane cultivars as a function of soil water suction (τ). ψ_L was measured on three plants of each cultivar during soil drying. Each point is the mean of four observations. Equations for the lines fitted by regression are:

$$\psi_{CL} = -0.32 - 10.07(\tau),\ r^2 = 0.88^{**}$$

$$\psi_L = -1.70 - 0.1(\ln(\tau)),\ r^2 = 0.74^{**}.$$

Stomatal conductance also declined with increasing τ and approached zero as τ approached 0.09 MPa (Fig. 12). The relationship between g and τ varied among sugarcane cultivars, with cv. H69-8235 exhibiting pronounced stomatal closure over a very small range of τ. The behavior of G_{root}, which also approached zero over this range of τ, mirrored that of g in each cultivar (Fig. 12). In wet soil, maximum values of G_{root} for cv. H69-8235 were higher than in the two cultivars and declined more precipitouosly with slight soil drying.

Through its large effect on G_{root}, the relatively small 0.1 MPa range of τ observed during soil drying (Fig. 12) was manifested during periods of high transpiration as a much larger 1.3 MPa range in root ψ as estimated from ψ_{CL} (Fig. 13). The relationship between ψ_{CL} and g (Fig. 14) was not as strikingly different among cultivars as that between τ and g (cf. Fig. 12). However, cv. H69-8235 exhibited a linear relationship between g and ψ_{CL}, while the other two cultivars exhibited an apparent threshold response. When g was plotted as a function of G_{root}, differences in stomatal response among cultivars appeared to diminish even further (Fig. 15). At maximum values of G_{root} attained by cultivars H65-7052 and H67-5630 growing in wet soil, g of all three cultivars was similar (Fig. 15). Nevertheless, maximum g and G_{root} were highest in plants of cv. H69-8235 growing in wet soil. This was in agreement with data presented in Tables 11 and 12.

Even when the soil was wet, the opening and partial closure of stomates during the day was mirrored by similar changes in G_{root} as exhibited by a plant of

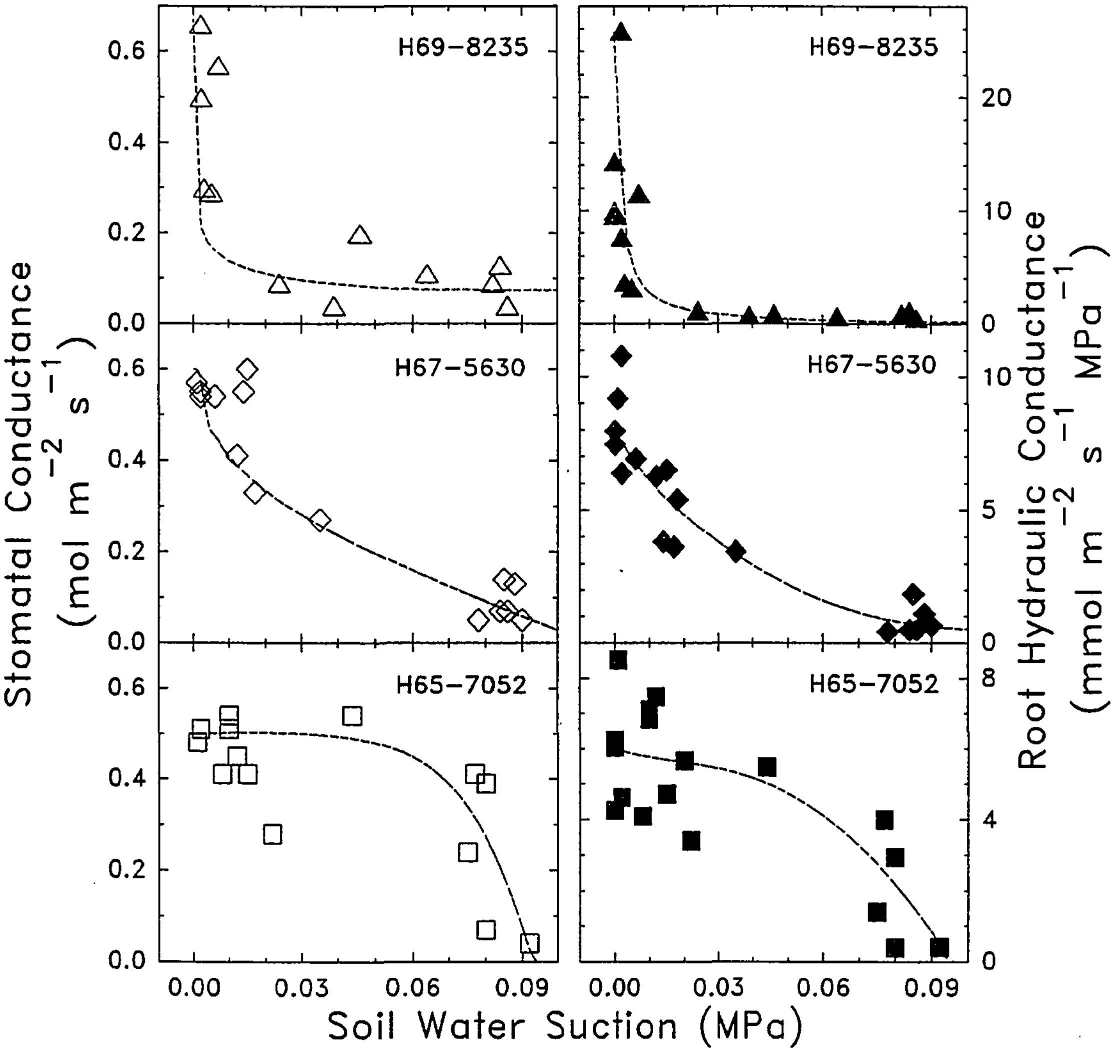

Figure 12. Stomatal conductance (g, open symbols) and root hydraulic conductance (G_{root}, closed symbols) of three sugarcane cultivars as a function of soil water suction (τ). Each point is the mean of three to ten observations. The same plants were monitored during the course of soil drying. Equations for the curves fitted by regression are:

H69-8235, $g = -0.18 - 0.10\ln(\tau)$, $r^2 = 0.87^{**}$
$G_{root} = -3.37 - 1.86\ln(\tau)$, $r^2 = 0.68^{**}$

H67-5630, $g = \mathrm{EXP}(-0.51 - 21.5(\tau))$, $r^2 = 0.97^{**}$
$G_{root} = \mathrm{EXP}(2.13 - 29.4(\tau))$, $r^2 = 0.96^{**}$

H65-7052, $g = 0.47 - 456.1(\tau)^3$, $r^2 = 0.94^{**}$
$G_{root} = 5.77 - 7267(\tau)^3$, $r^2 = 0.93^{**}$.

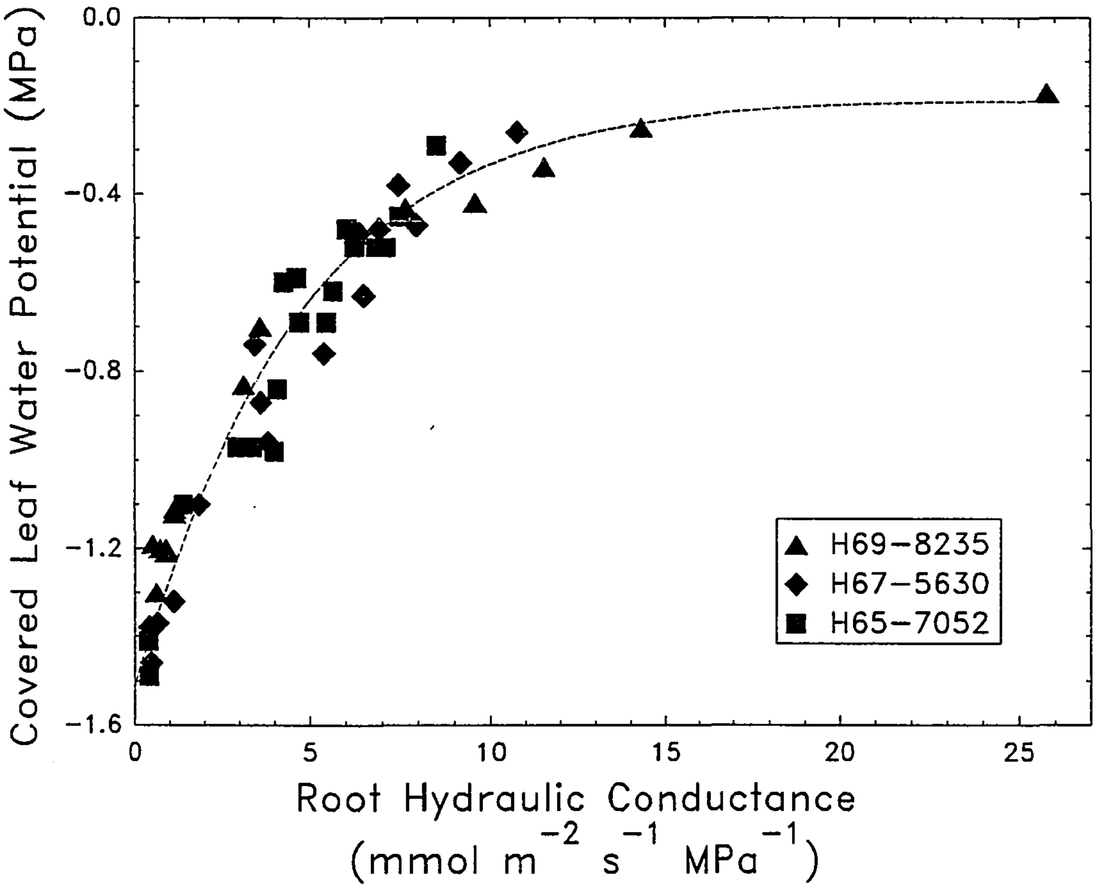

Figure 13. The relationship between covered leaf water potential (ψ_{CL}) and root hydraulic conductance (G_{root}) during soil drying. The water potential of a covered basal leaf was an estimate of the drop in potential across the entire root system. Each point is the mean of three to ten observations. The equation for the curve fitted by regression is:

$$\psi_{CL} = 1/((-0.59 - 0.21G_{root})/(1 - 0.003G_{root})),\ r^2 = 0.92^{**}.$$

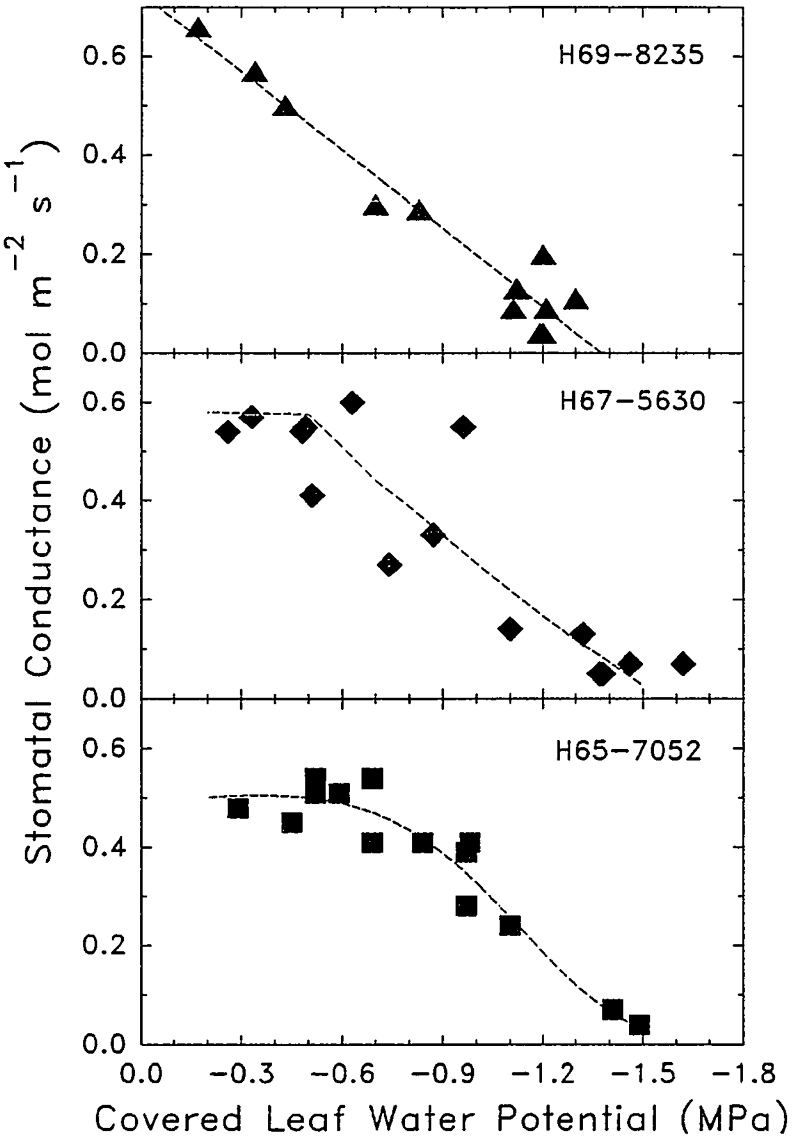

Figure 14. Stomatal conductance (*g*) of three sugarcane cultivars in drying soil in relation to the water potential of a covered basal leaf (ψ_{CL}). Each point is the mean of four to ten observations. Equations for the lines fitted by regression are:

H69-8235, $g = 0.73 + 0.53(\psi_{CL})$, $r^2 = 0.94^{**}$

H67-5630, $g = 1.31 + 0.74(\psi_{CL}) - 0.73\mathrm{EXP}(\psi_{CL})$, $r^2 = 0.82^{**}$

H65-7052, $g = 1.15 - 0.24\mathrm{EXP}(-\psi_{CL}) - 0.45\mathrm{EXP}(\psi_{CL})$, $r^2 = 0.91^{**}$.

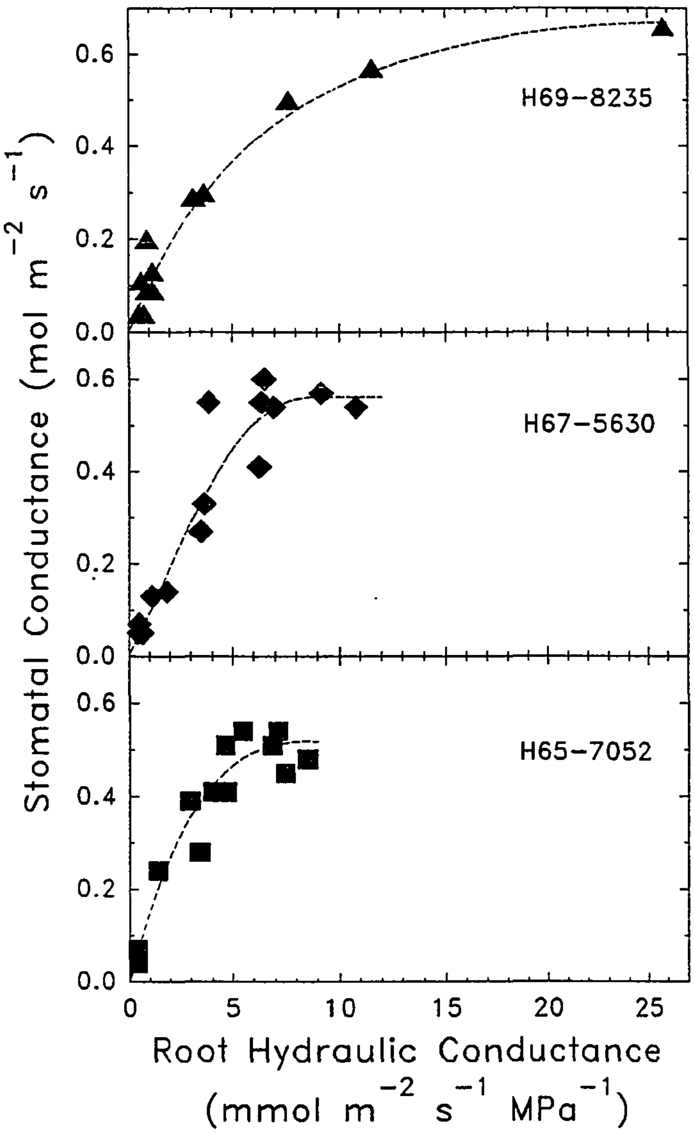

Figure 15. Stomatal conductance (g) in three sugarcane cultivars in relation to root hydraulic conductance (G_{root}) in drying soil. Each point is the mean of four to ten observations. Equations for the curves fitted by regression are:

H69-8235, $g = 0.13 + 0.16(\ln(G_{root}))$, $r^2 = 0.95^{**}$

H67-5630, $g = 0.16 + 0.18(\ln(G_{root}))$, $r^2 = 0.87^{**}$

H65-7052, $g = 0.20 + 0.16(\ln(G_{root}))$, $r^2 = 0.90^{**}$.

cv. H69-8235 (Fig. 16). In contrast, ψ_L remained relatively constant from 1015 h until the end of measurements. These were further indications that the stomatal conductance to water vapor loss from the leaves was closely associated with the liquid phase conductance to water movement through the roots, and that ψ_L did not regulate stomatal aperture.

DISCUSSION

The results presented here suggest that control of stomatal conductance in sugarcane plants exposed to drying soil may be exerted primarily at the root rather than at the leaf level. This observation is in agreement with those of several earlier studies with other species in which stomatal control of transpiration enabled leaf ψ to remain nearly constant when root water uptake was impeded by various treatments (Aston and Lawlor 1979; Coutts 1980; Briggs and Wiebe 1982; Masle and Passioura 1987). It is interesting that in the present study, G_{root} and root xylem ψ were more adequate than bulk soil water status in describing patterns of g and transpiration in both wet and dry soil for all three sugarcane cultivars studied. It is difficult to visualize how the hydraulic conductance *per se* of the roots and soil could function as a physical signal controlling g. However, at a given bulk soil water status and transpiration rate, root ψ will be determined by both the hydraulic conductance of the soil surrounding the roots and the radial conductance of the roots themselves. If root water status directly influenced g, this could act to keep liquid and vapor phase conductances in balance and to

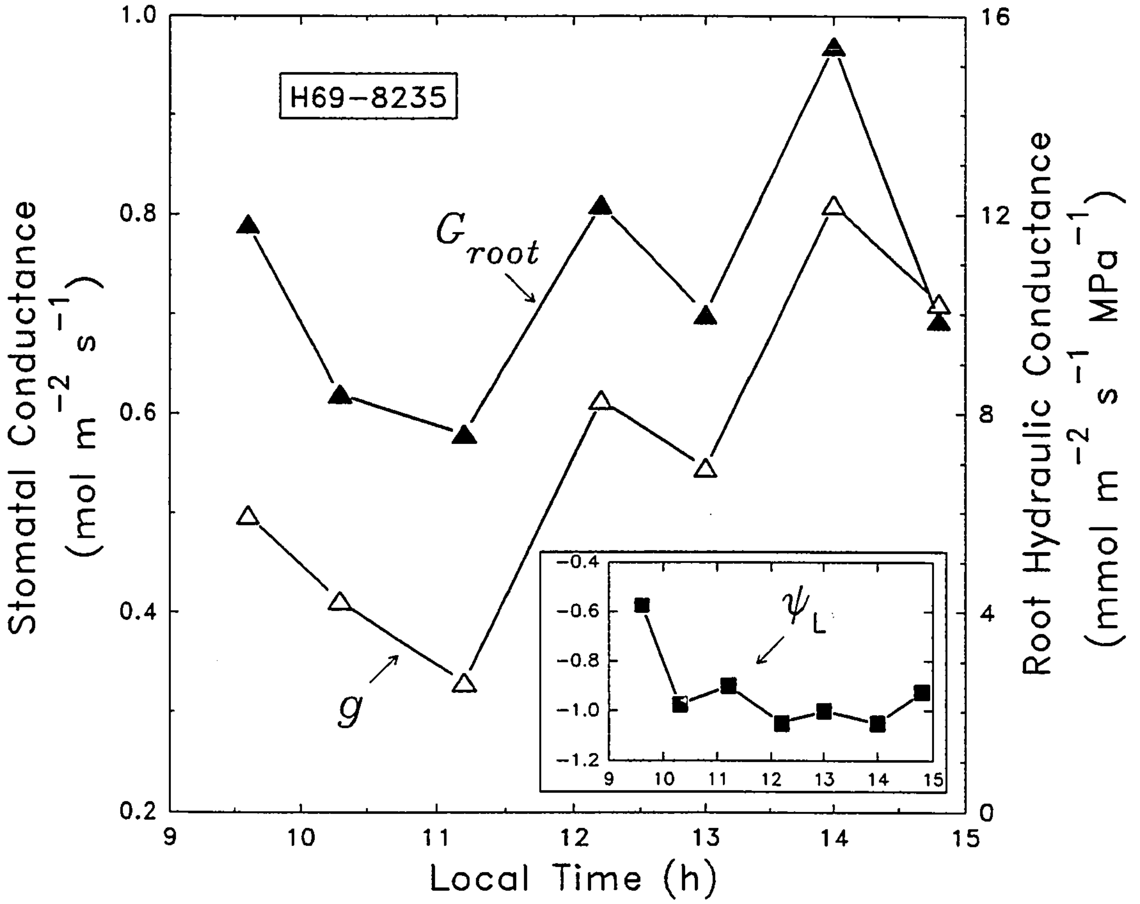

Figure 16. Time course of variation in stomatal conductance (g), root hydraulic conductance (G_{root}), and leaf water potential (ψ_L, inset) during the day for a well-irrigated plant of cv. H69-8235. Each point is the mean of two observations.

maintain nearly constant leaf water status over a large range of soil moisture availability.

The specific mechanism by which root water status might regulate *g* in sugarcane cannot be determined from the data presented here. However, recent studies suggest that increased abscisic acid (ABA) synthesis in roots under drying soil may serve as a chemical signal responsible for reductions in *g* (Zhang *et al* 1987; Zhang and Davies 1989). Observations that ABA decreases the hydraulic conductance of root systems when rates of water uptake are relatively high (Fiscus 1981; Davies *et al* 1982) suggest the additional possibility that ABA could act synergistically to amplify the drop in ψ across roots in drying soil. The lack of a unique relationship between *g* and root ψ among sugarcane cultivars (Fig. 14) may reflect cultivar-specific differences in root turgor or ψ thresholds for increased ABA synthesis. Further studies of root ABA levels in relation to root water potential components during soil drying are needed to evaluate this possibility.

The use of Ohm's law analogy to describe water transport into and through plants has been criticized because of its inability to account for observed deviations from linearity in the water potential force-water flux relationship (Fiscus *et al* 1983; Passioura 1984). These deviations from linearity have been attributed principally to water uptake driven by active solute uptake (Fiscus 1975) and partitioning of water flux between expansive growth and transpiration (Boyer 1985). However, the effect of both of these factors becomes negligible at high transpiration rates and relatively large hydrostatic pressure gradients such as those that prevailed when hydraulic conductance measurements were made in the present study. In this study, water flux was described as a function of gradients in hydrostatic pressure rather than of total ψ, since the pressure chamber was used for plant measurements and tensiometers were used for soil measurements. This is not problematic if the osmotic component of the gradient remains constant as has been suggested (Passioura 1988c).

The results presented here raise questions concerning the exact location of the apparent change in root resistance with soil drying. Considering the small range of τ attained, it seems unlikely that the change in resistance occurred in the bulk soil. However, several workers have suggested that large increases in apparent root resistance associated with soil drying may be the result of increasing resistance at the interface between root and soil due, in part, to root shrinkage with declining water content (see Passioura 1988c for review). Although the tensiometers used in the present study were in intimate contact with both soil and roots, the hydrostatic pressure gradient measured for calculation of hydraulic conductance probably represented the gradient between the bulk soil and the root xylem. It was, therefore, not possible to isolate changes in interfacial resistance from changes in resistance within the root. Since large changes in root conductance coincided with relatively small changes in soil and root ψ, it seems somewhat unlikely that significant changes in interfacial resistance due to root shrinkage could have occurred. If the roots were highly elastic, however, relatively large changes in root volume would occur with small changes in ψ. Differences in hydraulic conductance within the roots, rather than differences in interfacial conductance were probably responsible for the differences in below-ground hydraulic conductance among sugarcane cultivars growing in wet soil (Tables 11 and 12). Regardless of the exact location of the major portion of this below-ground resistance, during soil drying it may become the functionally limiting resistance to water loss in sugarcane through its effects on root water status and g.

The period of oscillations in g and G_{root} observed in this study (Fig. 16) was similar to the oscillations in g and plant hydraulic conductance reported by other workers (Barrs 1971; Passioura and Tanner 1985). In addition, the relatively unchanged ψ_{L} (Fig. 16) concurrent with fluctuating g and G_{root} was in support of the idea that the root water relations, rather than the leaf water relations, may regulate stomatal behavior (Passioura and Munns 1984).

The patterns of hydraulic and stomatal conductance associated with soil drying in the three sugarcane cultivars studied may be of ecological significance. All three cultivars exhibited drought avoidance behavior in that stomatal closure tended to maintain a constant shoot water status as water uptake from the soil became increasingly difficult. Pronounced stomatal closure associated with relatively small depletion in soil water content has been noted previously for field-grown sugarcane (Ingram and Moore 1986; Meinzer and Grantz, unpublished observations). In the present study, cv. H69-8235 was most extreme in its manifestation of classic drought avoidance behavior. Stomatal closure in this cultivar not only occurred over a very narrow range of τ, but in wet soil stomatal conductance and transpiration were significantly higher in cv. H69-8235 than in the other two cultivars. This behavior correlates well with the reputation of H69-8235 as a drought (Ingram 1985) and salinity (Ginoza and Moore 1985) resistant cultivar.

CONCLUSIONS

Although there is much evidence that changes in soil and root water status control stomatal conductance, a hydraulically based measure of the ability of the root system to supply the shoot with water may better predict stomatal conductance. Conductance to water loss in the vapor phase comprises the major physical conductance controlling transpiration, but hydraulic conductance below-ground may funtionally limit water loss by controlling root water status and stomatal conductance. This feedforward form of regulation would permit leaf water status to remain nearly constant over a range of soil water status.

The results of the present study suggest a physiological basis for the differential performance of the three sugarcane cultivars previously observed during soil drying. The classical drought avoidance behavior of cv. H69-8235 is manifested through the maintenance of higher gas exchange in wet soil and rapid conservation of water when soil begins to dry. The observation that there is a unique relationship between stomatal conductance and root hydraulic conductance among the cultivars studied suggests that below-ground hydraulic properties are a significant factor in regulating water use in sugarcane.

CHAPTER V

ASSOCIATIONS BETWEEN ROOT WATER RELATIONS AND STOMATAL RESPONSE TO SOIL DRYING IN CONTRASTING SUGARCANE CULTIVARS

ABSTRACT

Recent investigations using various species have demonstrated that in drying soil, stomatal conductance (*g*) is controlled by chemical signals coming from the roots. This study was conducted to compare leaf and root water relations among sugarcane cultivars known to have varying levels of drought resistance, and to examine specific root water relations characteristics that may be associated with the regulation of stomatal behavior during drought. Cultivar variation in water relations characteristics, especially bulk tissue elasticity were more distinct in the roots than in the leaves, suggesting that the cultivar differences in drought resistance may have been root-based. Cultivar variation in bulk root tissue elasticity was consistent with the proposal that the decline in root hydraulic conductance (G_{root}) during soil drying occurred within the roots rather than at the soil-root interface. It was hypothesized that coordination of *g* with declining G_{root} during soil drying was accomplished by a chemical signal moving from the roots to the leaves in the transpiration stream. Decreased root osmotic potential may have stimulated export of this putative substance from the roots.

INTRODUCTION

In most studies of plant responses to soil water deficits, the role of leaf water relations has received more attention than that of water relations of the roots. This is partly attributable to the difficulty and inconvenience in gaining access to roots compared to the relative ease with which leaves can be sampled and studied. The belief that reduced stomatal aperture during soil drying is invariably associated with reductions in leaf water content and turgor (Kramer 1983) has also contributed to the emphasis placed on leaf water relations. However, several recent studies have demonstrated that soil drying can induce stomatal closure independently of alterations in leaf water status (Bates and Hall 1981; Blackman and Davies 1985; Gollan *et al* 1986; Henson *et al* 1989). This has led to the suggestion that other factors, specifically chemical signals transported from the roots to the leaves via the transpiration stream, are involved in stomatal regulation of water use by plants growing in drying soil. These recent observations indicate the need for more emphasis on understanding the role of root water relations in modifying shoot behavior during soil drying.

It has been suggested that plants may possess a sensor that can measure the water content of the soil (Turner *et al* 1985) which can lead to optimization of gas exchange during drought (Cowan 1982). In sugarcane, stomatal opening and root hydraulic conductance declined in parallel during soil drying (see Chapter IV). However, it is not clear how root hydraulic conductance *per se* could act as a signal transmitted via changes in xylem sap tension because bulk leaf water potential remained relatively unchanged with mild soil drying. Coupling of stomatal behavior to root water transport efficiency during soil drying may be achieved by modified chemical composition of the xylem sap (Cornish and Radin 1990).

In sugarcane, the specific root water relations characteristic that serves as a sensor of declining soil water status and triggers the alteration in transpirational sap composition remains to be identified. In maize plants with split root systems, the turgor of root tips growing in drying soil to declined while turgor was constant in root tips growing in wet soil (Davies *et al* 1986). Zhang and Davies (1989a) speculated that the more rapid dehydration of fine roots of maize in shallow soil may promote the production of a chemical signal that inhibits stomatal opening and leaf growth, thus enhancing the accumulation of carbohydrate in roots growing in deeper, wetter soil. On the other hand, changes in root cell volume in cotton (Huck *et al* 1970), soybean (Taylor and Willatt 1983), and sunflower (Faiz and Weatherley 1982) have been suggested to induce a direct effect on root metabolism reflected in altered phytohormone release or production (Turner 1986b).

The hydraulic, osmotic and elastic properties of both leaf and root tissues determine the relative changes in leaf and root water relations that occur as the soil water supply becomes limiting (Turner 1986b). Genetic differences in root water relations characteristics and in their response to soil drying can be expected to result in genotypic variation in the intensity of root-shoot interactions and therefore in stomatal sensitivity to soil drying. The objectives of the present study were to compare leaf and root water relations among contrasting sugarcane cultivars reported to differ in their drought resistance, and to examine specific root water relations characteristics that may be associated with different patterns of regulation of stomatal behavior during drought.

MATERIALS AND METHODS

Plant Material and Experimental Conditions

Three sugarcane (*Saccharum* spp. hybrid) cultivars were selected on the basis of contrasting performance in previous salinity and drought resistance tests (Ginoza and Moore 1985; Ingram 1985). The cultivars were classified as susceptible (cv. H65-7052), moderately resistant (cv. H67-5630), and resistant (cv. H69-8235) to drought.

Single stem segments containing one lateral bud were sown in eleven-liter plastic pots filled with a 4:4:1 v/v mixture of clay soil, commercial potting mix, and volcanic cinders. Plants were grown in the greenhouse and were watered automatically twice daily by drip irrigation. Greenhouse temperatures were typically 30 to 35 °C during the day and 20 to 25 °C at night. Complete fertilizer was first applied one month after planting and once a month thereafter. Plants were 2.5 to 3 months old (8 to 12 leaves) when experimental measurements were carried out.

Root and Leaf Water Relations

Water potential isotherms of individual sugarcane shoot-roots were determined for excised from well-irrigated sugarcane plants by removing the entire root-soil mass from the pot. White shoot roots approximately 2 mm in diameter and located along the sides and the bottom of the pot were rapidly selected and excised at a length of 10 cm from their tips. Excised roots were immediately placed in distilled water and brought to the laboratory for determinations of their water potential isotherms. The roots were blotted dry with paper towels and rapidly sealed in pre-weighed psychrometer chambers (Merrill 81-500, Logan, Utah), i.e., one root per chamber. The chambers were then reweighed to the nearest 0.1 mg, and the root turgid weights were obtained by subtracting the weight of the empty chamber. Psychrometers (Merrill 83) were sealed inside the chambers and root water potential (ψ_R) was measured with a microvoltmeter (Merrill 85-12V) in the psychrometric mode after equilibration for 30 minutes. Preliminary measurements indicated that a 30-minute equilibration period at constant temperature was sufficient for the psychrometers to attain vapor equilibrium with the gas phase inside the chambers. This equilibration period was similar to the one used in generating water potential isotherms of fern roots (Auge *et al* 1989). Subsequent ψ_R measurements were made after samples had dried for 15 minutes in the open at room temperature, and new fresh weights were recorded. Drying, weighing, and ψ_R measurement were repeated until ψ_R reached approximately -2.0 MPa. The resulting root water potential isotherms contained 10 to 14 data points. Water relations characteristics of leaves were determined with the pressure-volume (P-V) method following the procedures described in Chapter II (see pages 16 and 17).

The reciprocals of leaf balancing pressure or root water potential were plotted on the ordinate versus relative water deficit (RWD) for each individual leaf or root sample. Various leaf and root water relations characteristics, namely, ψ_P, ψ_π, ψ_π^{100}, ψ_π^{z}, RWC_z, SWF, ϵ, and SV were calculated as described in Chapter II (see pages 17 to 19). Leaf and root symplast solute content on a dry weight basis (N_s) (Tyree *et al* 1978) were calculated by the equation:

$$N_s = ((W_t * SWF)*(-\psi_\pi^{100}/0.00245))/DWT$$

where W_t is the total weight of water (kg) in the sample at full hydration, **0.00245** is a factor to convert MPa to mOsmol kg^{-1}, and **DWT** is the sample dry weight.

Root and Shoot Responses to Soil Drying

Stomatal conductance, transpiration and root hydraulic conductance were determined during soil drying from experiments described in Chapter IV. The change in ψ_R as the soil dried was estimated from the ψ of the oldest leaf which was covered (ψ_{CL}) to suppress transpiration (see Chapter IV, page 71). The estimates of ψ_R were used to predict root ψ_P, ψ_π and SV from root water potential isotherms. In this procedure, it was implicitly assumed that the response of intact roots to soil drying was similar to that of excised roots during dehydration for determination of water potential isotherms. Since excised roots lack two potential sources of solutes, namely, the soil solution and the shoot, the estimated root ψ_P, ψ_π and SV do not take into account the effects of osmotic adjustment that may occur in intact roots exposed to a drying soil. In a recent review (Sharp 1990), data on primary roots of maize indicated that osmotic adjustment occurred only within the 1 cm tip where root elongation proceeded, though at a reduced rate, at vermiculite water potentials as low as -1.6 MPa. Intact roots of the sugarcane cultivars used in the present study may have varying capacities for osmotic adjustment with drying soil. Assuming that osmotic adjustment would occur only in the 1 cm tip of sugarcane roots, the 10 cm long roots used in determining water potential isotherms should provide a comparison of responses among cultivars largely independent of osmotic adjustment.

RESULTS

Differences among cultivars in tissue water relations characteristics were greater in roots than in leaves (Fig. 17, Table 13). Cultivar ranking in ψ_π^{100}, ψ_π^{z} and N_s for the roots was the reverse of that in the leaves. Both values of ψ_π were higher in roots than in leaves though N_s was much higher in roots because the SWF and symplast volume per unit dry weight were larger in the roots than in the leaves.

When ϵ of the roots and leaves was plotted as a function of ψ_P, differences in ϵ among cultivars were observed in the roots but not in the leaves (Fig. 18). A lower ϵ value implies that a tissue is more elastic, thus root tissue elasticity was

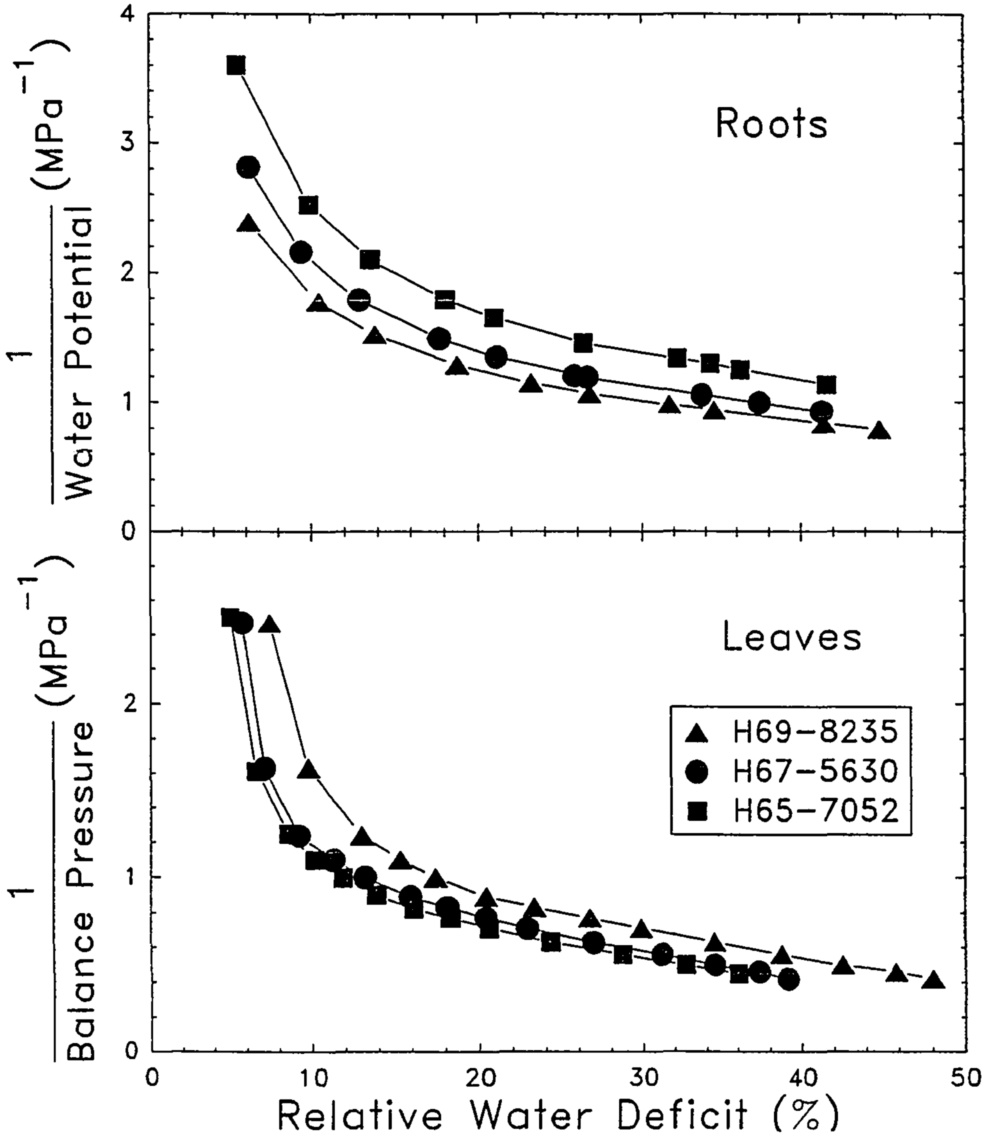

Figure 17. Water potential isotherms of roots and leaves excised at predawn from well-irrigated plants of three sugarcane cultivars. Data points, connected by straight lines, are from representative individual root and leaf samples from each cultivar.

Table 13. Osmotic potential at full turgor (ψ_π^{100}), osmotic potential at zero turgor (ψ_π^{z}), relative water content at zero turgor (RWC_z), symplastic water fraction (SWF), and symplastic solute content (N_s) derived from water potential isotherms of roots and leaves excised at predawn from well-irrigated plants of three sugarcane cultivars.

Data are means of 8 and 12 observations for roots and leaves, respectively.

Cultivar	ψ_π^{100}	ψ_π^{z}	RWC_z	SWF	N_s
	---------(MPa)--------		--------(%)-------		(mOsmol g^{-1} dwt)
			Roots		
H69-8235	**-0.67**	**-1.05**	**69.7**	**86.0**	**2.48**
	(±0.05)	(±0.08)	(±1.9)	(±1.7)	(±0.08)
H67-5630	**-0.59**	**-0.99**	**70.0**	**78.6**	**1.82**
	(±0.05)	(±0.10)	(±3.4)	(±3.2)	(±0.33)
H65-7052	**-0.46**	**-0.81**	**67.5**	**77.4**	**1.20**
	(±0.02)	(±0.05)	(±2.1)	(±1.9)	(±0.22)
			Leaves		
H69-8235	**-0.86**	**-1.42**	**70.7**	**76.1**	**0.71**
	(±0.02)	(±0.06)	(±1.4)	(±2.0)	(±0.09)
H67-5630	**-0.93**	**-1.55**	**73.2**	**66.8**	**0.75**
	(±0.02)	(±0.03)	(±1.3)	(±1.9)	(±0.03)
H65-7052	**-0.96**	**-1.54**	**74.7**	**67.4**	**0.82**
	(±0.03)	(±0.06)	(±1.0)	(±1.9)	(±0.05)

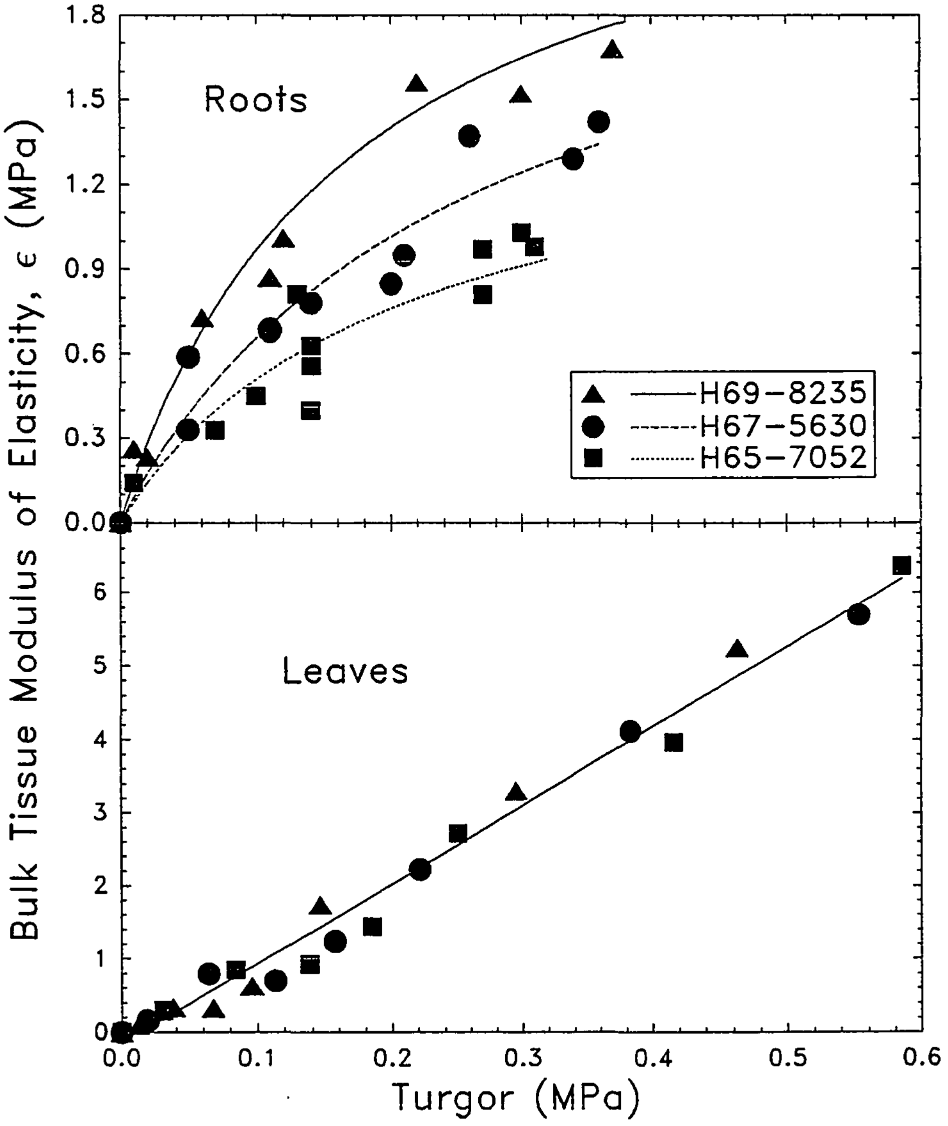

Figure 18. Bulk tissue modulus of elasticity (ϵ) of roots and leaves as a function of turgor (ψ_P) for three sugarcane cultivars. Data points are means of 2 to 12 observations from 8 root water potential isotherms and 12 leaf pressure-volume curves. Equations for the lines fitted by regression are:

roots, H69-8235: $\epsilon = 1/((-6.84E23 - 4.19E24\psi_P)/(1 - 1.07E25\psi_P))$, $r^2 = 0.98^{**}$
H67-5630: $\epsilon = 1/((-1.20E24 - 4.96E24\psi_P)/(1 - 1.11E25\psi_P))$, $r^2 = 0.93^{**}$
H65-7052: $\epsilon = 1/((-1.43E24 - 7.35E24\psi_P)/(1 - 1.10E25\psi_P))$, $r^2 = 0.89^{**}$

leaves, $\epsilon = -0.16 + 10.8\psi_P$, $r^2 = 0.98^{**}$.

greatest in cv. H65-7052 and least in cv. H69-8235. In addition, ϵ of root tissue was lower than that of leaf tissue except at values of ψ_P below about 0.1 MPa indicating that root tissue was generally more elastic than leaf tissue. These differences in bulk tissue elastic properties between root and shoot tissue and among cultivars are consistent with observed patterns of tissue ψ_π and SWF.

In addition to the influence of inherent differences in tissue biophysical properties, root water relations in intact sugarcane plants will be influenced by the operating range of root ψ. In well-irrigated sugarcane plants transpiring at maximum rates, prevailing root ψ as estimated by ψ_{CL} was highest in cv. H69-8235 and lowest in cv. H65-7052 (Table 14). This diminished the differences in

Table 14. Covered leaf water potential (ψ_{CL}), root osmotic potential (ψ_π), root symplast volume (SV), and root hydraulic conductance (G_{root}) for well-irrigated plants of three sugarcane cultivars transpiring at maximum rates.

Data are means (± standard error) of 6 to 9 observations obtained from three well-irrigated plants of each cultivar.

Cultivar	ψ_{CL}	ψ_π	ψ_P	SV	G_{root}
	----------------(MPa)----------------			(cm^3 g^{-1} dwt)	(mmol m^{-2} s^{-1} MPa^{-1})
H69-8235	**-0.34** (±0.03)	**-0.72** (±0.01)	**0.38** (±0.03)	**9.1** (±0.1)	**11.6** (±1.2)
H67-5630	**-0.42** (±0.05)	**-0.67** (±0.02)	**0.25** (±0.03)	**7.2** (±0.2)	**7.7** (±0.6)
H65-7052	**-0.53** (±0.05)	**-0.64** (±0.02)	**0.11** (±0.02)	**5.6** (±0.1)	**5.5** (±0.6)

root ψ_π among cultivars predicted to occur in intact plants and increased the differences in prevailing root ψ_P (cf. Tables 13 and 14). Differences in symplast volume were also predicted to occur in intact roots (Table 14). Root hydraulic conductance,which was highest in cv. H69-8235 and lowest in cv. H65-7052, was positively correlated with prevailing ψ_{CL}, ψ_P and SV.

Both stomatal conductance and transpiration, which were measured independently, declined in parallel with G_{root} (Fig. 19) during soil drying. The relationship between g and G_{root} appeared to be the same for all cultivars. Thus, during soil drying, the vapor phase conductance of the leaves remained in balance with the hydraulic conductance of the roots, resulting in maintenance of nearly constant leaf water status (Chapter IV, Fig. 11). This implied that the nature of the signal was not hydraulic, and that other factors such as altered root water relations characteristics were possibly involved in the release or production of a chemical signal.

Stomatal conductance and G_{root} were subsequently plotted as functions of root ψ_P, SV and ψ_π in order to identify root water relations characteristics most closely associated with coordination of g and G_{root} during soil drying. Although g and G_{root} declined with root ψ_P in drying soil, the relationship between these variables and ψ_P appeared to be different in each cultivar (Fig. 20). Similarly, the relationship between g or G_{root} and root SV was unique for each cultivar, with the differences between cultivars being determined chiefly by initial values of SV in well-irrigated plants (Fig. 21). In contrast to the foregoing, a single relationship

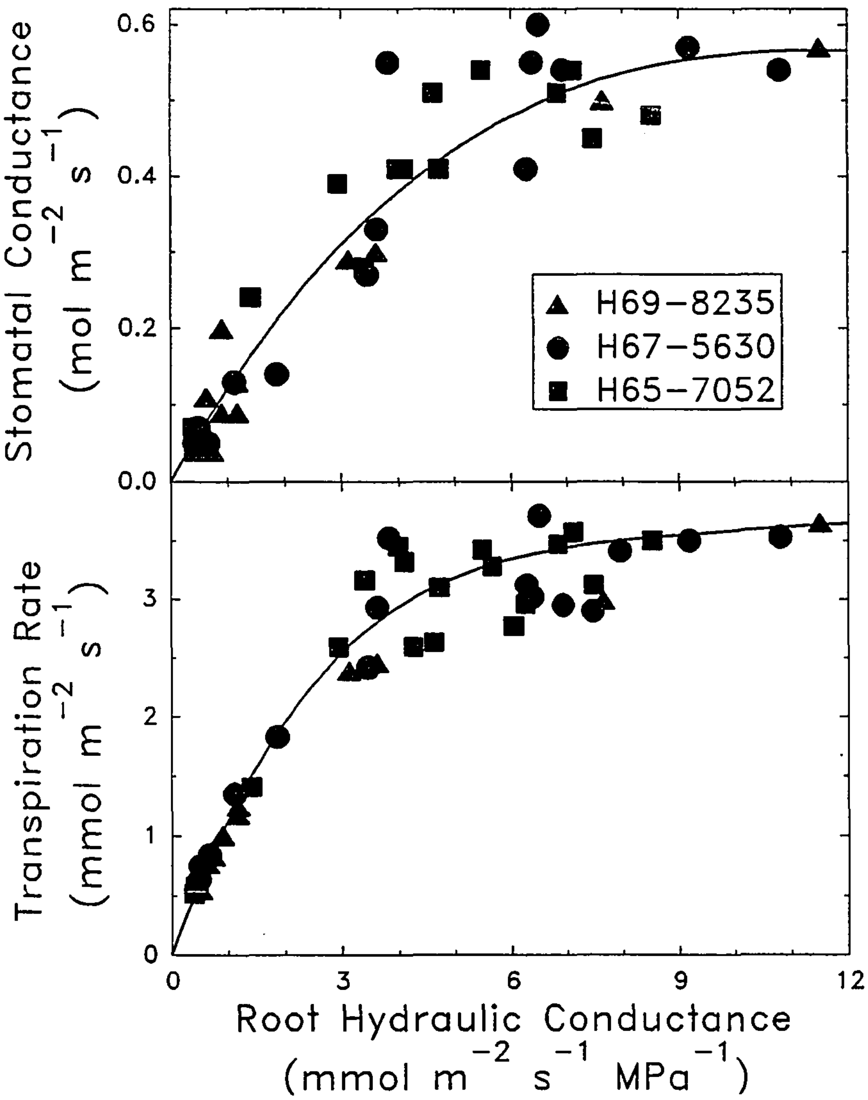

Figure 19. Stomatal conductance (g) and transpiration rate (E) in relation to root hydraulic conductance (G_{root}) of three sugarcane cultivars growing in a drying soil. Data points are means of four to ten observations. Equations for the curves fitted by regression are:

$$g = \mathrm{EXP}((-5.52 - 0.55G_{root})/(1 + 1.92G_{root})),\ r^2 = 0.96^{**}$$

$$E = \mathrm{EXP}((-6.71 - 8.61G_{root})/(1 + 6.40G_{root})),\ r^2 = 0.98^{**}.$$

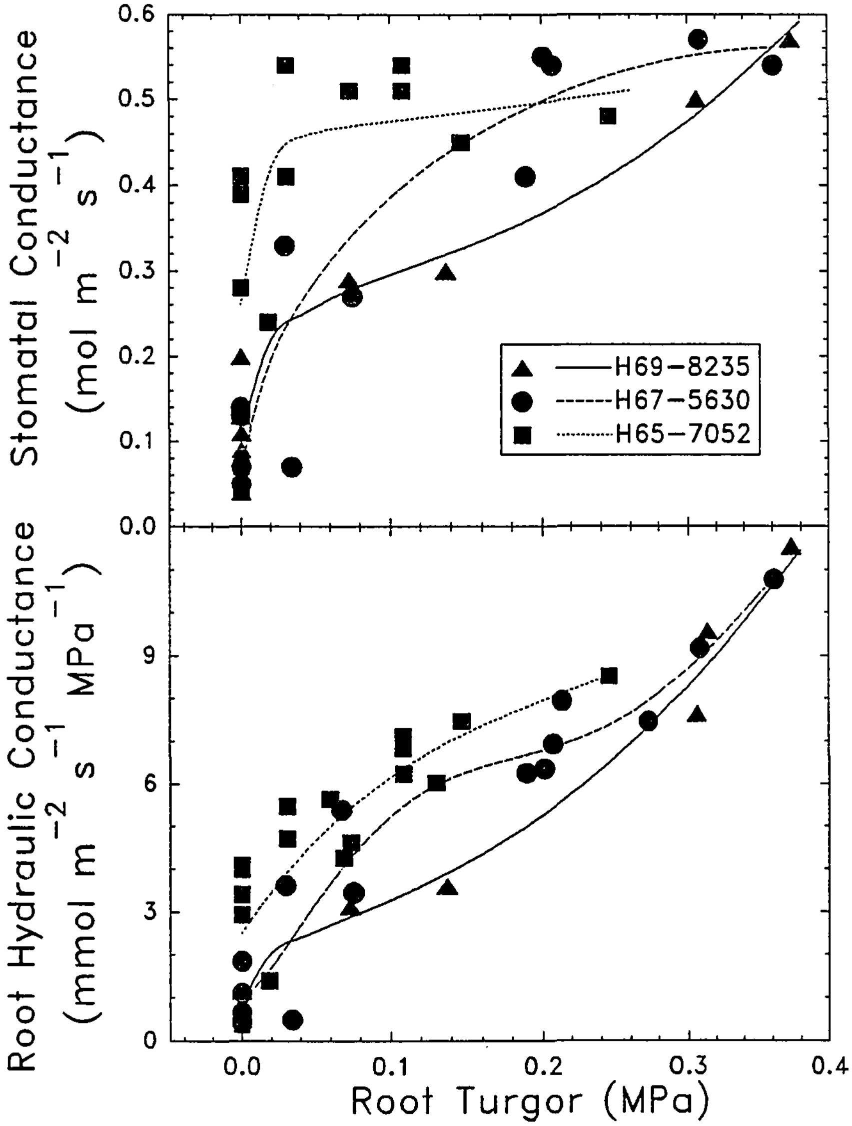

Figure 20. Stomatal conductance (g) and root hydraulic conductance (G_{root}) as functions of root turgor (ψ_P) of three sugarcane cultivars growing in a drying soil. Data points are means of 4 to 10 observations. Equations for the curves fitted by regression are:

H69-8235, $g = -8.15 + 6.42\psi_P + 1.07\mathrm{SQRT}(\psi_P) + 8.25\mathrm{EXP}(-\psi_P)$, $r^2 = 0.94^{**}$
H67-5630, $g = \mathrm{SQRT}(0.005 + 1.65\psi_P - 2.22(\psi_P)^2)$, $r^2 = 0.88^{**}$
H65-7052, $g = 0.28 + 0.18\mathrm{EXP}(\psi_P)$
$-0.43(1/(1+\mathrm{EXP}(-(\psi_P+0.002)/-0.01)))\psi_P$, $r^2 = 0.91^{**}$
H69-8235, $G_{root} = -164 + 148\psi_P + 10.5\mathrm{SQRT}(\psi_P) + 165\mathrm{EXP}(-\psi_P)$, $r^2 = 0.94^{**}$
H67-5630, $G_{root} = 0.9 + 30\psi_P + 606(\psi_P)^2 - 7312(\psi_P)^3 + 31501(\psi_P)^4$
$-59036(\psi_P)^5 + 41133(\psi_P)^6$, $r^2 = 0.94^{**}$
H65-7052, $G_{root} = 1698 - 1644\psi_P + 673(\psi_P)^2 - 1696\mathrm{EXP}(-\psi_P)$, $r^2 = 0.95^{**}$.

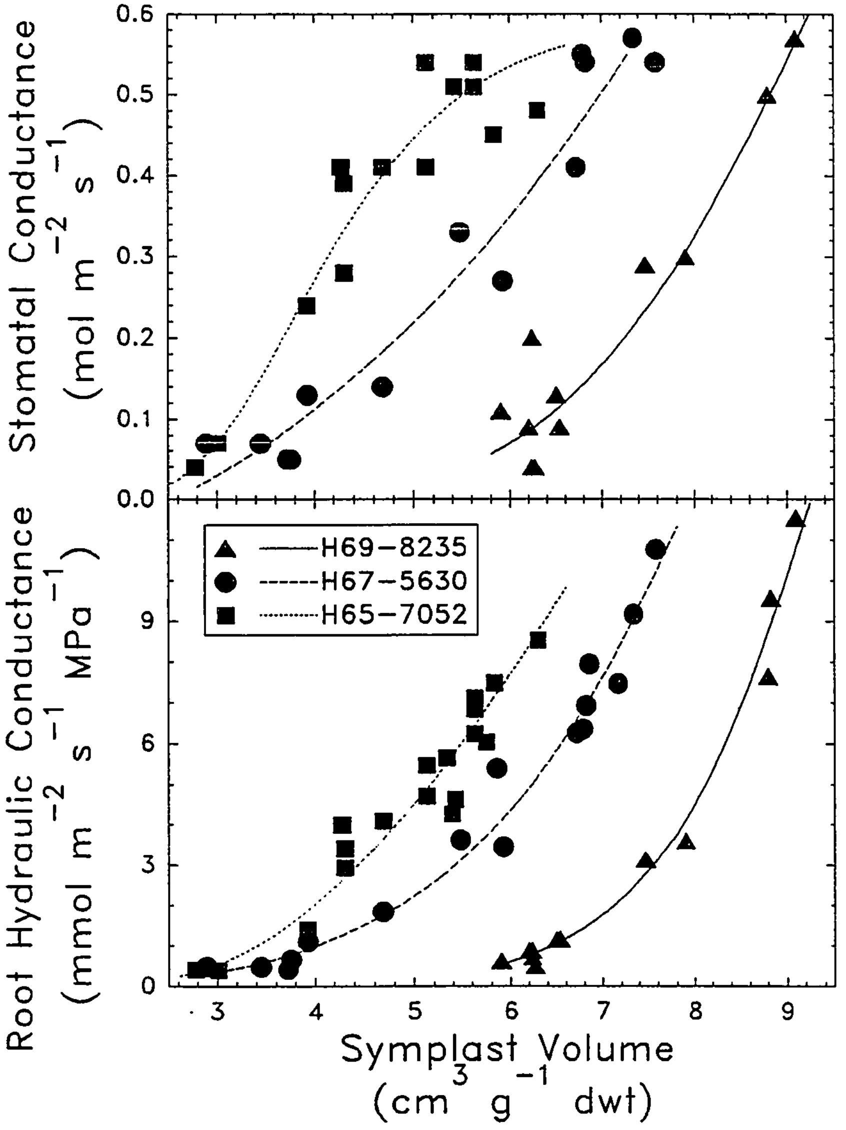

Figure 21. Stomatal conductance (g) and root hydraulic conductance (G_{root}) as functions of root symplast volume (SV) of three sugarcane cultivars growing in a drying soil. Data points are means of 4 to 10 observations. Equations for the curves fitted by regression are:

H69-8235, $g = \mathrm{EXP}(3.49 - 37.0/\mathrm{SV})$, $r^2 = 0.93^{**}$
H67-5630, $g = -0.08 + 0.012(\mathrm{SV})^2$, $r^2 = 0.94^{**}$
H65-7052, $g = \mathrm{EXP}(-0.52 - 43.2\mathrm{EXP}(-\mathrm{SV}))$, $r^2 = 0.90^{**}$

H69-8235, $G_{root} = \mathrm{EXP}(-13.0 + 6.98(\ln(\mathrm{SV})))$, $r^2 = 0.99^{**}$
H67-5630, $G_{root} = \mathrm{EXP}(-5.07 + 3.65(\ln(\mathrm{SV})))$, $r^2 = 0.97^{**}$
H65-7052, $G_{root} = \mathrm{EXP}(4.71 - 16.0/\mathrm{SV})$, $r^2 = 0.91^{**}$.

could be used to describe the association between *g* and root ψ_π for all cultivars (Fig. 22). There were no unique cultivar responses between G_{root} and root ψ_π after root ψ_π had fallen below about -0.8 MPa. Above this value, the relationships between G_{root} and root ψ_π appeared to diverge among sugarcane cultivars (Fig. 22).

DISCUSSION

Results from the present study provided additional evidence to reinforce the idea that stomatal conductance of sugarcane plants exposed to drying soil is largely controlled at the root rather than at the leaf level. Cultivar differences in ψ_π^{100}, ψ_π^{z}, N_s and ϵ (Table 13 and Fig. 18) were larger in roots than in leaves suggesting that the previously observed differences in drought (Ingram 1985) and salinity (Ginoza and Moore 1985) resistance among these cultivars are likely to be root-based.

Cultivar differences in root ϵ (Fig. 18) may play an important role in determining the location of the observed changes in G_{root} with soil drying (Chapter IV). The data presented here strengthened the idea that the decrease in G_{root} with increasing soil water deficits was attributable primarily to intrinsic changes in the roots, rather than to increases in the resistance at the soil-root interface (Huck *et al* 1970; Herkelrath *et al* 1977; Faiz and Weatherley 1978). Sugarcane cultivars differed in their bulk root tissue elasticities with cv. H65-7052 being the most elastic and cv. H69-8235 the least elastic (Fig. 18). This indicated

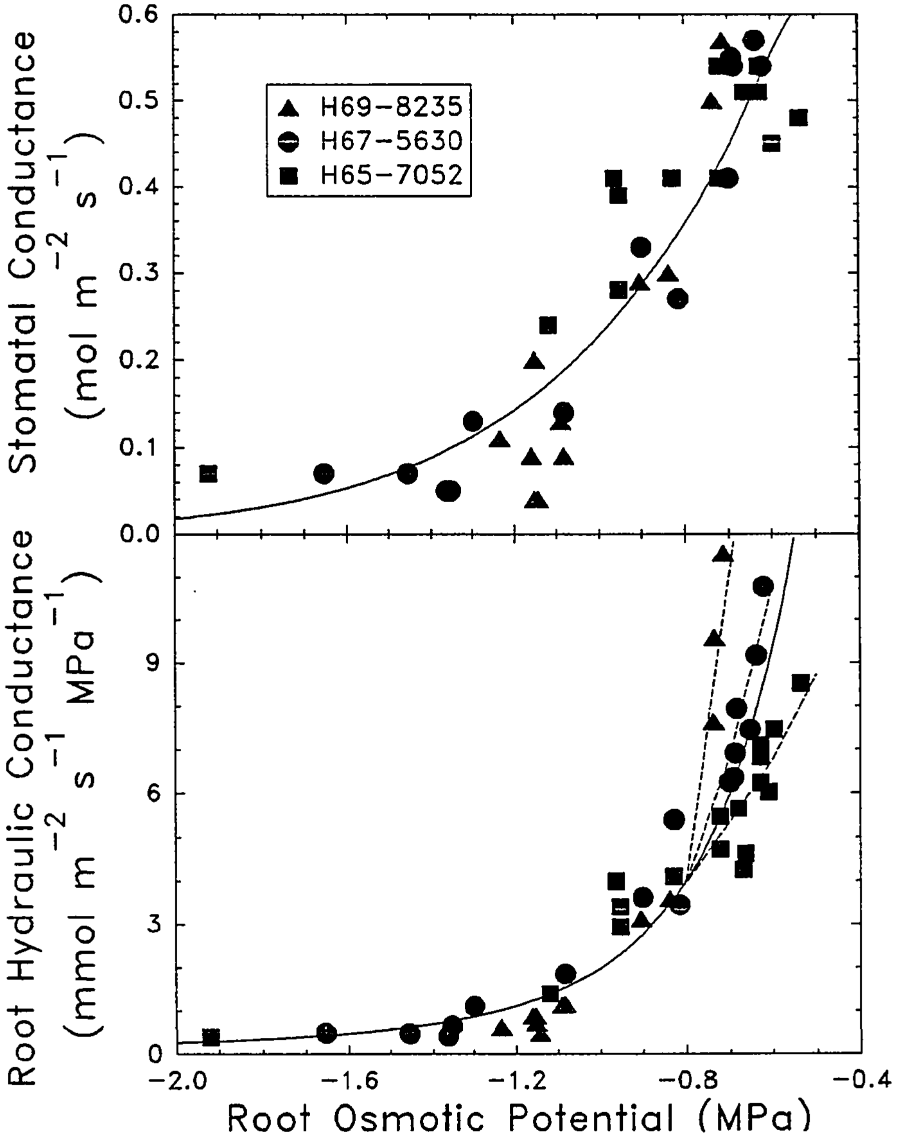

Figure 22. Stomatal conductance (g) and root hydraulic conductance (G_{root}) in relation to osmotic potential (ψ_π) of roots during soil drying. Data are means of 4 to 10 observations. Equations for the curves fitted by regression are:

$$g = (-0.07 + 1.49\mathrm{EXP}(\psi_\pi))^2,\ r^2 = 0.95^{**}$$

$$G_{root} = \mathrm{EXP}(-1.27 + 6.24\mathrm{EXP}(\psi_\pi)),\ r^2 = 0.88^{**}.$$

that for a corresponding decrease in root ψ, the volume of water lost from the root tissue of cv. H65-7052 should have been greater than that of cv. H69-8235. Thus, if roots should shrink due to dehydration, the roots of cv. H65-7052 should have shrunk more than the roots of cv. H69-8235. Preliminary observations of sugarcane roots indicated that suberization may be present in the hypodermis (Welbaum, unpublished data) so that the cortical tissue may have shrunk with declining ψ_R. The resulting soil-root interfacial resistance due to root shrinkage should have been smaller in cv. H69-8235 so that its G_{root} should have declined at a slower rate as ψ_R declined. Such was not the case, however, because the G_{root} of cv. H69-8235 declined faster than the G_{root} of cv. H65-7052 as root ψ_P declined (Fig. 20). This indicated that mild soil drying (Chapter IV) may not have caused significant alterations in root diameter so that changes in soil-root interfacial resistance may not have occurred. This is in agreement with the findings that the limiting resistance in the transport of water in the soil-plant-atmosphere continuum was located in the plant, rather than at the soil or soil-root interface (Hansen 1974a, 1974b; Taylor and Klepper 1975; Reicosky and Ritchie 1976; Blizzard and Boyer 1980). Thus, in sugarcane, intrinsic changes in the root may have caused the reductions in G_{root} with increasing soil water deficits.

Decreased root hydraulic conductance in drying soil has been attributed to a decline in root membrane permeability (Ramos and Kaufmann 1979), which is influenced by the endogenous concentrations of ABA and cytokinins (Itai and Benzioni 1976). Cytokinins have been reported to reduce root permeability to water (Collins and Kerrigan 1974), whereas ABA has been reported to either increase or decrease root permeability to water depending on the rate of water flux through the root sysem (Glinka 1980; Fiscus 1981). In a recent review, Cornish and Radin (1990) concluded that the typical root response to ABA may be a decrease in hydraulic conductance coupled with an increase in ion transport.

Although both stomatal conductance and transpiration rate were closely correlated with G_{root} (Fig. 19), the absence of significant changes in ψ_{L} during mild soil drying (Chapter IV, Fig. 11) indicated that the nature of the root-sourced signal was metabolic instead of hydraulic. The existence of three unique associations, i.e., one for each cultivar, for the plots of g versus root ψ_{P} (Fig. 20) and g versus root SV (Fig. 21) seems to preclude the possibility that g was regulated by root ψ_{P} or SV. In contrast, the presence of a unique, close association between g and root ψ_{π} of all cultivars (Fig. 22) suggested that changes in root ψ_{π} may have been the means by which soil and root water deficits were sensed and transduced into altered xylem sap composition. It has been demonstrated that changes in xylem sap composition in well-irrigated sugarcane cause g to change as a predictable function of total leaf area (Meinzer, Grantz and Smit 1991). Thus, it is conceivable that in response to soil drying, the declining root ψ_{π} exhibited by all sugarcane cultivars in the present study may have induced an alteration in the chemistry of the transpirational sap which subsequently regulated stomatal behavior. This hypothesized root ψ_{π}-induced change in xylem sap composition is consistent with the observed changes in the biochemical pathways and enzyme activity caused by high cellular ionic concentrations in response to salinity (Kylin and Quatrano 1975). Additionally, it has been demonstrated that photosynthetic photophosphorylation is directly influenced by increased concentration of cellular ions when the leaf tissue is dehydrated (Younis, Weber and Boyer 1983).

CONCLUSIONS

Cultivar differences in root bulk tissue elasticity were in agreement with the suggestion that changes in hydraulic conductance during soil drying occurred in the roots rather than at the soil-root interface. It is hypothesized that in response to soil drying, declining root ψ_π induced changes in the metabolic pathways of the roots resulting in decreased root hydraulic conductance and modified xylem sap composition. In drying soil, stomatal conductance and transpiration rate may have been regulated by metabolic root signals transmitted to the leaves via the transpiration stream.

CHAPTER VI

SUMMARY AND CONCLUSION

The use of drought-resistant sugarcane cultivars in water-limited environments can partly reduce the adverse effect of water deficits on sucrose yield of the crop. In order to breed genotypes resistant to drought and to identify genotypes appropriate for a water-limited environment, it is necessary to have a sufficient understanding of specific features that sustain productive physiological processes in the presence of soil water deficits. There is currently very little information regarding specific physiological characteristics that may confer drought resistance in sugarcane. An important aspect determining drought adaptation and acclimation is the coordination of the physiological responses of shoots and roots when plants are exposed to soil water deficits. In the present study, root-shoot interactions were examined in sugarcane cultivars previously observed to possess different levels of drought resistance.

When plants are exposed to drought, adjustments in osmotic and elastic properties of the leaf tissue may occur. The P-V technique was used to evaluate diurnal and drought induced variation in leaf water relations characteristics, then *g* and SGR responses were examined in association with changes in leaf water relations. Both osmotic and elastic adjustment occurred in leaves of all sugarcane cultivars in response to both diurnal water deficit and soil drying. These adjustments caused symplast water volume to remain nearly constant with increasing leaf water deficits, while turgor was only partially maintained. Cultivar responses did not provide conclusive evidence for genotypic variation in osmotic and elastic adjustment within the range of leaf water deficits imposed. Behavioral differences in rates of water use seem to have determined the degree of osmotic and elastic adjustment in response to drought. Bulk leaf water status did not vary significantly between irrigated and droughted plants during the early stages of drought, whereas significant reductions in g and SGR occurred. Thus, signals originating from the roots were suggested as a possible mechanism controlling SGR, g and leaf water relations.

The efficiency of the roots to absorb and transport the available soil water determines in part the prevailing water relations in the shoot. The transpiration-gradient and pressure-flux techniques were employed to characterize the hydraulic properties of entire root systems and of isolated roots. Cultivar differences were discerned by both of these techniques, though it is cautioned that when detopped root systems were pressurized, values of G_{root} were overestimated, presumably due to movement of water through unnatural paths. Cultivar differences in entire root system hydraulic conductance were mirrored by similar variations in hydraulic conductance of individual excised roots, which were observed to differ anatomically among cultivars. The frequency of root branching was postulated to have differed among cultivars, because cultivar differences in L tended to disappear with increasing root length. Comparison of changes in G_{root} determined in irrigated, droughted, and reirrigated plants by employing the transpiration-gradient and pressure-flux techniques led to a conclusion that xylem cavitation may have occurred in sugarcane roots exposed to drying soil.

Early reports indicate that leaf water status determines the degree of stomatal opening in plants under soil water deficits. Recent investigations, however, suggest that it is the stomatal behavior, as influenced by root-sourced metabolic signals, that regulates leaf water status. Concurrent measurements of leaf and soil water status, g, E and G_{root} were done to evaluate the relative roles of soil, root and leaf water status, and plant hydraulic conductance in the control of g and E in sugarcane cultivars subjected to soil drying. In wet soil, E and G_{root} differed between cultivars and were positively correlated, whereas ψ_L was similar among cultivars. Additionally, fluctuations in g were associated with parallel variations in G_{root}, but not with ψ_L which stayed relatively constant during the day. In drying soil, ause the vapor phase conductance of the leaves and the liquid phase conductance of the roots declined in parallel, thus ψ_L remained nearly constant. These results indicated that the regulation of g in sugarcane plants exposed to drying soil is exerted primarily at the root rather than at the leaf level.

Other workers have proposed that chemical signaling via the transpiration stream plays a central role in the regulation of water use by plants exposed to soil water deficits. A study was conducted to compare leaf and root water relations, and to examine specific root water relations characteristics that may be associated with the regulation of g in contrasting sugarcane cultivars exposed to drying soil. Cultivar differences in water relations characteristics and bulk tissue elasticity were larger in the roots than in the leaves indicating that the previously reported cultivar differences in drought resistance may have been attributable to root properties. Cultivar responses in bulk root tissue elasticity were consistent with the idea that reductions in the rate of root water transport during drought are due to changes in intrinsic root hydraulic properties rather than increased soil-root interfacial resistance. In drying soil, the decline in both g and E were closely correlated with G_{root} but not with ψ_L. This implied that the nature of the g-regulating signal from the roots was metabolic rather than hydraulic. Subsequently, it was found that both g and G_{root} were closely associated with root ψ_π suggesting that osmotic changes in the root symplast could have influenced the production or release of a chemical signal causing reductions in g and G_{root}.

The studies presented here suggest that interactions between roots and shoots of sugarcane plants are involved in the regulation of water use under soil water deficits. Results indicate that a metabolic signal coming from the roots may govern stomatal behavior and shoot growth of sugarcane plants during drought. It is hypothesized that in a drying soil, the declining soil water status is sensed by the plants through changes in their root water relations, specifically root ψ_π. This feedforward (i.e., roots as sensors of soil water deficits) mode of regulating shoot behavior should be more efficient than a feedback (i.e., leaf as sensors of soil water deficits) mode of regulation, and should provide an efficient way of maintaining homeostasis in shoot water status.

There was a relationship between the patterns of physiological responses and presumed levels of drought resistance among sugarcane cultivars. The drought-resistant cultivar (H69-8235) had the highest SGR, g and G_{root} when well-irrigated, indicating that it probably had the greatest rate of carbon assimilation and water use. Consequently, when irrigation was withheld, this cultivar had the fastest rate of decline in g and G_{root}, though the relationship between g and G_{root} was similar among cultivars. The patterns of g and G_{root} associated with soil drying in the three cultivars studied may be of ecological significance. All three cultivars exhibited drought avoidance behavior in that g tended to maintain constant ψ_L as water uptake from the soil became increasingly difficult. The extreme, classical drought avoidance behavior of cv. H69-8235 was manifested through the maintenance of higher gas exchange in wet soil and rapid conservation of water when soil begins to dry. This type of strategy is expected to be beneficial in areas where prolonged periods of drought may occur. On the other hand, the maintenance of g, though at a reduced level, by cv. H65-7052 in drying soil, appeared to be favorable in areas where brief, intermittent shortage of irrigation water or precipitation may occur.

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