

# Genotypic Variability in Vulnerability of Leaf Xylem to Cavitation in Water-Stressed and Well-Irrigated Sugarcane<sup>1</sup>

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## ABSTRACT

Genotypic variability in vulnerability of leaf xylem to water-stress-induced cavitation was determined in four sugarcane (*Saccharum* sp.) clones using detached leaf segments in a hydraulic conductivity apparatus. Vulnerability curves were constructed by plotting the percentage of maximum conductivity versus leaf water potential ( $\psi_l$ ) and fitting curves using a Weibull function. The  $\psi_l$  at which each clone lost 10, 50, and 80% of maximum conductivity was determined. Maximum conductivity per unit of leaf width was positively associated with metaxylem vessel diameter. The commercial clone H65-7052 exhibited the highest and the nondomesticated *S. spontaneum* exhibited the lowest conductivity. All four clones lost substantial conductivity at values of  $\psi_l$  less negative than  $-1.4$  MPa, but H65-7052 was able to maintain 50% conductivity to lower  $\psi_l$  than the other clones. *S. spontaneum* sustained the most negative  $\psi_l$  ( $-1.99$  MPa) before reaching the 80% conductivity loss point. Clone H69-8235 was consistently the most vulnerable to initial loss of conductivity. These vulnerability functions were used in conjunction with field measurements of  $\psi_l$  to estimate diurnal losses in leaf hydraulic conductivity under irrigated and droughted conditions. H69-8235 lost up to 50% of its conductivity during the day, even when well irrigated, and more than 80% when subjected to drought. The other clones exhibited lower conductivity losses. These losses are apparently reversed overnight by root pressure. Despite their close genetic relationships, these clones exhibited large differences in conductivity, in the vulnerability of their xylem to cavitation, and in gas exchange behavior. The potential for altering water relations by selecting for particular hydraulic characteristics is discussed.

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There is a growing body of evidence, particularly for woody species, that  $g_s$ <sup>5</sup> is maintained at a level that maintains xylem pressure potential above the point at which cavitation, em-

bolism, and loss of xylem conductivity occur (28). The hydraulic architecture associated with these varying operational points is found to vary among species, representing a compromise between maximizing conductance to water in the liquid phase at high xylem water potentials and minimizing susceptibility to cavitation at low water potentials (23–25, 28, 31). These interacting constraints on the anatomy of the xylem have led to suggestions that the range of viable xylem anatomies is limited by environmental selection pressures (28). This hypothesis remains to be tested experimentally using closely related genotypes.

Sugarcane (*Saccharum* sp.) clones vary in the sensitivity of their gas exchange responses to soil drying (17), due in part to differences in root hydraulic conductivity. Recently, we showed that  $g_s$  in individual sugarcane leaves is regulated according to the ratio of total plant hydraulic conductance to total transpiring leaf area, resulting in an asymptotic value of whole plant transpiration as leaf areas per plant exceed  $0.2$  m<sup>2</sup> (11). This coordination between shoot and root water fluxes appears to be mediated by chemical signals transported from the roots to the leaves in the xylem sap (12) and not directly by  $\psi_l$  or changing root hydraulic conductivity. Although the potentially limiting movement of water through sugarcane leaves has been assessed in relation to these developmental changes (10), the role of leaf hydraulic properties in resistance of sugarcane or other grass species to water stress has not been evaluated with respect to loss of conductivity and cavitation phenomena.

The objectives of this study were to assess the range of genotypic variation in the vulnerability of the xylem of sugarcane leaves to water-stress-induced cavitation and to characterize the relationship between this xylem vulnerability and the operating ranges of  $\psi_l$  for well-irrigated and droughted plants. Experiments were performed using leaf tissue because evidence with other species (22) suggested that cavitation may be confined to the relatively expendable leaf xylem, conserving water transport through the stem.

## MATERIALS AND METHODS

### Plant Material and Growing Conditions

Four sugarcane (*Saccharum* sp.) clones were studied. Cv H65-7052 is a current, and cv H69-8235 a former, commercial clone in Hawaii. Both clones were derived from crosses between *Saccharum officinarum* and *Saccharum spontaneum* (7). H69-8235 has been shown (17) to exhibit different

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<sup>5</sup> Abbreviations:  $g_s$ , stomatal conductance;  $\psi_l$ , leaf water potential.

hydraulic, gas exchange, and drought tolerance behavior than H65-7052. Cv AP85-0068, a pure *S. spontaneum* clone, is representative of one of the parent species of the commercial clones and is smaller, with narrower leaves, than the other clones studied. *S. spontaneum* germplasm has been a source of multiple stress resistance in sugarcane-breeding programs. Cv Cavengerie, a pure *S. officinarum* clone, is representative of the other parent species and is characterized by relatively large, wide leaves. *S. officinarum* germplasm has been the traditional source of high stalk sugar content in sugarcane-breeding programs.

All four clones were grown in the same experimental field at the Kunia research farm, near Waipahu, Oahu, Hawaii (latitude 21°21' N, longitude 158°2' W, elevation 100 m) and were approximately 6 months old at the time of sampling. Water was withheld from half of the plants in late September, and irrigation was resumed in mid-November, although all plots received occasional light rainfall. Irrigated plants were irrigated and fertilized weekly through drip lines. Soil water potentials in the upper 15 cm of the irrigated plot were maintained above  $-0.02$  MPa, as determined with soil tensiometers (Soil Moisture Corp., Santa Barbara, CA), whereas those in the dry plot were approximately  $-0.1$  MPa by the end of the experiment. This range of soil drying has been observed to cause effects on leaf gas exchange in greenhouse experiments (17).

### Hydraulic Conductivity

Hydraulic conductivity per unit of leaf width was determined on leaf blades excised from irrigated plants in the field. Leaves were excised before dawn, their basal ends were immediately immersed in water, and the laminae were enclosed in a plastic bag.  $\psi_i$  values remained above  $-0.05$  MPa until imposition of dehydration in the laboratory.

Conductivity estimates were obtained using a pressure apparatus similar to that described by Sperry et al. (23), modified slightly to measure grass leaf segments. Degassed oxalic acid (10 mM) was used in the apparatus and for the bathing solution in which segments were cut from the leaves. A  $0.2\text{-}\mu\text{m}$  in-line filter was used to remove particles from the solution in the conductivity apparatus. Leaf segments 7 cm long from the middle section of each leaf were cut under solution. Strips approximately 0.7 cm wide were then detached under the solution, midway between midrib and leaf margin. Segments were installed in slit rubber stoppers and placed in the conductivity apparatus with their distal ends protruding.

Leaf segments were allowed to equilibrate at low pressure for 15 min before measuring conductivity. Solution flow through the leaf segments was then measured at the low hydrostatic pressure of 0.01 MPa for 2 min, followed by a 2-min high-pressure flush at 0.10 MPa (to remove any embolisms), followed by another 2-min measurement at low pressure. Preliminary experiments showed that 2 min at 0.10 MPa was sufficient to eliminate embolisms and that higher pressures or longer flush times resulted in decreases in leaf conductivity.

Solution passing through the leaf segments was collected in small preweighed plastic vials containing absorbent paper.

Hydraulic conductivity per unit of leaf width ( $\text{mg s}^{-1} \text{MPa}^{-1}$ ) was calculated as the mass flow rate of solution ( $\text{mg s}^{-1}$ ), determined by weighing the vials, divided by the hydrostatic pressure gradient along the length of the segment ( $\text{MPa cm}^{-1}$ ), and by the segment width (cm).

Conductivity loss functions were generated for each clone by expressing hydraulic conductivity at various levels of  $\psi_i$  as a percentage of that obtained after refilling embolized xylem vessels by pulses of high hydrostatic pressure (0.10 MPa), according to the method of Sperry et al. (23). Individual leaves were used for only one conductivity measurement. Some leaves were measured immediately following equilibration in water. Other leaves were allowed to dehydrate on the laboratory bench for varying durations. Conductivity was then measured when the leaves reached target levels of  $\psi_i$ .  $\psi_i$  was monitored by sampling small sections from the tips of the leaves for measurements with a pressure chamber (18). These cuts for  $\psi_i$  determination were always farther from the section to be sampled for conductivity than the length of the longest vessels (see below) to avoid artifactual introduction of embolisms. Sequential sampling did not reveal any gradients in  $\psi_i$  along the leaf blade following dehydration on the bench top.

Percent conductivity readings were grouped into water potential classes of 0.2 MPa and then averaged within classes and plotted against the midpoint of each water potential class to obtain the xylem vulnerability curves. Conductivity loss functions were determined from these data using a Weibull function (Eq. 1; 16) to estimate the  $\psi_i$  at which 10, 50, and 80% losses in conductivity occurred:

$$H = \alpha \cdot e^{-(\psi_i/\phi)^\sigma} \quad (1)$$

where  $H$  is the percentage of maximum conductivity at particular values of  $\psi_i$ ,  $\alpha$  is an estimate of maximum conductivity,  $\phi$  is the  $\psi_i$  at which  $\alpha$  is reduced to  $0.37\alpha$ ,  $\sigma$  is a dimensionless parameter controlling the shape of the curve, and  $\psi_i$  is leaf water potential in MPa. Curves were fit using a nonlinear procedure (20).

### Acoustic Measurements

Ultrasonic acoustic emissions were measured in the laboratory on the most recently fully expanded leaves, excised from field-grown plants as previously described. A sample leaf was held vertically on the laboratory bench with the basal end in water, and a model I15I ultrasonic acoustic sensor (Physical Acoustics Corp., Princeton, NJ) attached to the midblade region. Acoustic emissions were monitored in real time with a model 4615 drought stress meter (Physical Acoustics Corp.) connected to a portable computer. Leaves were allowed to equilibrate in water for 20 min (or until measurement of the leaf tip showed  $\psi_i > -0.05$  MPa).

There were few acoustic emissions as long as the leaf base remained immersed in water. Emissions were monitored for approximately 45 to 60 min with the leaf fully hydrated, after which time the water was withdrawn. At periodic intervals as the leaf dried,  $\psi_i$  measurements were made at the remaining leaf tip, including specifically the time at which the first acoustic emissions above background were noted. Represent-

ative emission data for the two commercial clones, H65-7052 and H69-8235, are presented.

### Morphological Measurements

Maximum vessel lengths were estimated using compressed air (6). Leaf blades were stripped to approximately 1 cm width, and the basal end was inserted into a stopper, with the distal end immersed in water. An air pressure of 0.07 MPa was applied to the basal end, and approximately 1-cm segments were sequentially excised from the distal end until air bubbles appeared. The remaining leaf length was a measure of maximum vessel length. Leaf segments used for the conductivity measurements were observed under a microscope ( $\times 400$ ) for determination of the frequency distribution of bundles in small, medium, and large size classes and for the diameters of the largest vessels in each class of bundles (1, 3).

### $\psi_i$ and $g_s$

Diurnal courses of  $\psi_i$  and  $g_s$  were measured periodically throughout the imposed drought. Water potential measurements were made on the uppermost fully expanded leaf using a pressure chamber (18). A portion of the leaf blade was inserted in a small plastic bag, detached, and quickly sealed in the bag. Bags with leaf segments were kept in a darkened insulated container until all leaves had been sampled. Measurements of  $\psi_i$  were made during the next hour in a nearby laboratory. Previous experiments showed no significant changes in  $\psi_i$  of the sealed leaf segments during this period.

Photosynthesis and  $g_s$  measurements were made on the uppermost fully expanded leaves of additional plants using a portable photosynthesis system (model 6200, Li-Cor, Inc., Lincoln, NE) with a 0.25-L chamber. Only the conductance data are reported in this paper. Water potential and gas exchange measurements were made every 2 to 3 h during the day, from dawn to late afternoon on three to five plants per clone. These data were subjected to a one-way analysis of variance and clone and treatment means were separated using Duncan's Multiple Range test (20).

## RESULTS

### Morphological Measurements

There were no differences in maximum vessel length among the clones (Table I). *S. spontaneum* had significantly more large bundles than the other three clones ( $\chi^2$  analysis,  $P = 0.01$ , 6 *df*), whereas *S. officinarum* had significantly more medium and small size bundles per cm of blade width. Large and medium bundle vessel element diameters were smallest in *S. spontaneum* and largest in H65-7052 (Table I).

### Hydraulic Conductivity versus $\psi_i$

Losses in conductivity were observed (Fig. 1, Table II) at rather moderate levels of water stress. Although H65-7052 was the clone most resistant to initial loss of conductivity at high  $\psi_i$ , *S. spontaneum* was most resistant at low  $\psi_i$  and was able to maintain 20% of maximum conductivity (i.e. 80% loss; Table II) at lower  $\psi_i$  than the other clones. The most susceptible clone at high  $\psi_i$  was H69-8235.

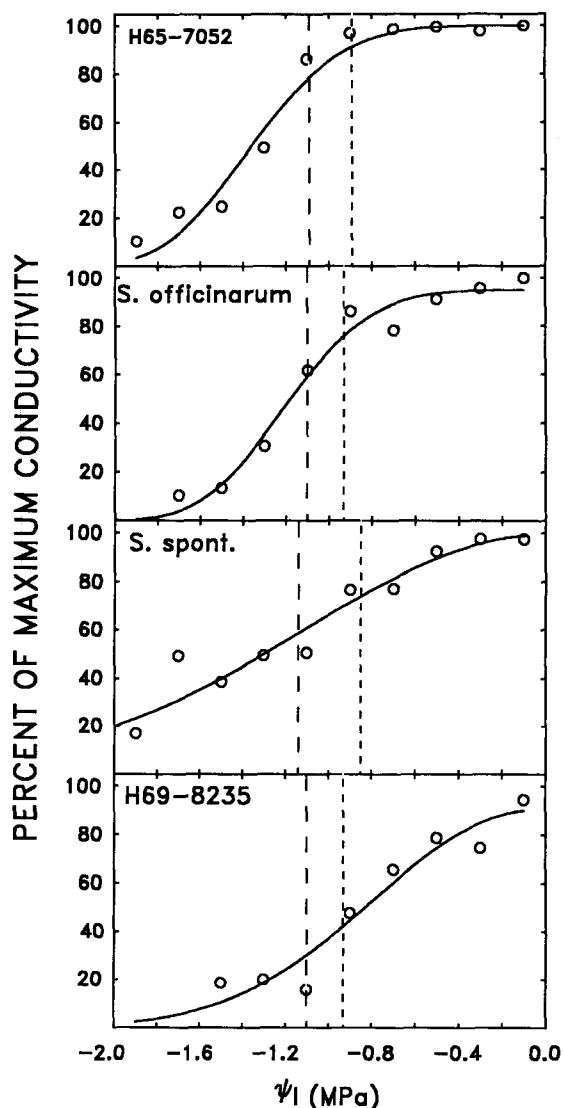
Not only were there differences in the magnitude of conductivity losses at specific  $\psi_i$  values but the pattern of loss with decreasing  $\psi_i$  varied among the clones (Fig. 1). H65-7052 and *S. officinarum* both exhibited threshold responses, whereas *S. spontaneum* and H69-8235 both exhibited little or no threshold response. This is specifically reflected in the  $\sigma$  shape parameter of Equation 1 (Table II).

Clone H65-7052, with the largest vessel diameters, had the highest maximum hydraulic conductivity, whereas *S. spontaneum*, with the smallest vessel sizes, had the lowest conductivity (Table III). However, neither maximum hydraulic conductivity nor vessel diameter was consistently correlated with xylem vulnerability (cf. Fig. 1, Tables I-III). For instance, clone H69-8235 had the second highest maximum hydraulic conductivity but was the most susceptible to cavitation at all levels of  $\psi_i$ . Likewise, clone H65-7052 was the most resistant to cavitation at high levels of  $\psi_i$  yet had the largest vessel sizes of all the clones.

**Table I.** Comparison of the Vascular Anatomy of the Leaves of Four Sugarcane Clones

All values are means ( $\pm$ SE). Means within a column not followed by the same letter are significantly different at  $P < 0.05$ . Sample sizes are: 10 to 13 leaves for vessel lengths, 8 to 13 leaf segments for bundle size classes, and 24 bundles for vessel diameters.

Clone	Vessel Length	Bundle Size Class Distribution			Diameters of Three Largest Xylem Vessels		
		Large	Medium	Small	Large	Medium	Small
	cm	No./cm blade width			$\mu$ m		
H65-7052	7.78a (0.76)	3.2a (0.4)	31.1a (1.7)	32.1a (1.7)	54.2a (2.4)	22.9a (2.0)	12.6a (1.0)
<i>S. officinarum</i>	12.14a (1.61)	3.4a (0.2)	45.2b (1.6)	54.4b (1.7)	34.9b (1.5)	18.8b (2.1)	7.4b (0.6)
<i>S. spontaneum</i>	11.66a (1.16)	10.3b (1.3)	32.5a (1.5)	45.6c (4.0)	24.4c (1.4)	12.1c (0.7)	7.3b (1.1)
H69-8235	10.93a (0.96)	4.0a (0.4)	32.5a (0.7)	43.6c (0.9)	32.1b (1.5)	17.2b (1.5)	7.7b (0.3)



**Figure 1.** Relationship between relative leaf hydraulic conductivity and  $\psi_l$  in four sugarcane clones. Points represent the means over ranges of 0.2 MPa. Curves are derived from the Weibull function (see text). Vertical broken lines indicate average daily midday minimum  $\psi_l$  for each clone (short dashed line, irrigated plants; long dashed line, droughted plants).

**Table III.** Maximum Hydraulic Conductivity of Leaves in Four Sugarcane Clones

Values are means  $\pm$  SE. Means not followed by the same letter are significantly different at  $P < 0.05$ .

Clone	Maximum Hydraulic Conductivity
	$mg\ s^{-1}\ MPa^{-1}$
H65-7052	$173.6 \pm 21.4a$
H69-8235	$81.1 \pm 8.6b$
<i>S. officinarum</i>	$58.0 \pm 11.2b,c$
<i>S. spontaneum</i>	$46.7 \pm 8.4c$

**Acoustic Measurements**

Representative acoustic emission curves for clones H65-7052 and H69-8235 are shown in Figure 2. Emissions for clone H65-7052 did not begin until after approximately 35 min following withdrawal of water, when  $\psi_l$  reached about  $-0.95$  MPa, nearly identical with the value of  $\psi_l$  at which cavitation began as indicated by the vulnerability curve (Fig. 1). For clone H69-8235, emissions substantially increased after approximately 35 min, at a  $\psi_l$  of about  $-0.65$  MPa, which corresponds to the point of substantial increase in cavitation observed in this clone (Fig. 1). Emissions continued for many hours (Fig. 2), even after leaves had begun to curl and dry, indicating that some of the acoustic emissions may have been from cells other than conducting elements of the xylem. Emissions (Fig. 2) and conductivity loss (Fig. 1) are consistent in identifying H65-7052 as more resistant to conductivity loss at high  $\psi_l$  than H69-8235 but subject to a more rapid rate of conductivity loss as  $\psi_l$  further declines.

**Diurnal Water Relations**

The average daily minimum  $\psi_l$  of all clones in the well-irrigated plants never decreased below  $-0.95$  MPa (Fig. 3). Clonal differences in minimum  $\psi_l$  were generally less than 0.25 MPa. The lowest values of  $\psi_l$  observed in the droughted plants for H69-8235 and H65-7052 were only  $-1.21$  and  $-1.26$  MPa, respectively, whereas *S. spontaneum* had a lower minimum  $\psi_l$  of  $-1.28$  MPa (Fig. 3). By November 3 (data not shown), droughted plants of all clones exhibited significantly lower  $\psi_l$  ( $P < 0.05$ ) than the irrigated plants, although the

**Table II.** Critical  $\psi_l$  for Losses of Hydraulic Conductivity in Four Sugarcane Clones

Clone	Losses in Hydraulic Conductivity <sup>a</sup>				Weibull Parameter Estimates <sup>b</sup>		
	Incipient	10%	50%	80%	$\alpha$	$\phi$	$\sigma$
		%			MPa	MPa	
H65-7052	-0.88	-0.91	-1.36	-1.63	100.0	14.7	4.72
<i>S. officinarum</i>	-0.20	-0.67	-1.18	-1.44	94.90	13.04	4.42
<i>S. spontaneum</i>	-0.20	-0.49	-1.30	-1.99	99.40	15.71	1.98
H69-8235		-0.11	-0.83	-1.28	90.59	10.53	2.19

<sup>a</sup> Calculated from the Weibull function (see text). No threshold detected for clone H69-8235. <sup>b</sup> Parameter estimates obtained using absolute values of  $\psi_l$ .

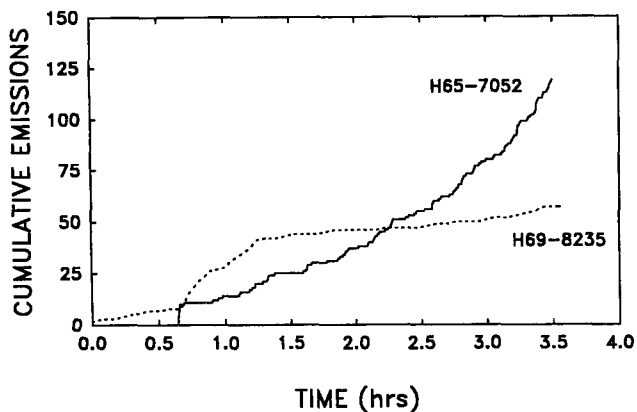


Figure 2. Representative ultrasonic acoustic emission curves for sugarcane clones H65-7052 and H69-8235.

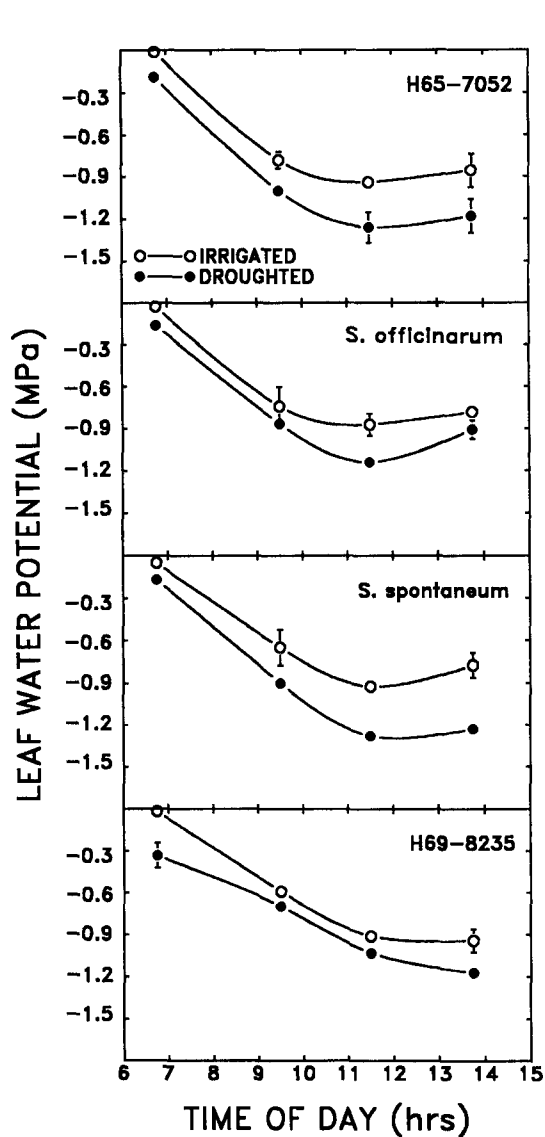


Figure 3. Diurnal patterns of  $\psi_l$  for irrigated and droughted sugarcane clones on November 8. Values are means  $\pm$  SE.

differences never exceeded 0.4 MPa. *S. spontaneum* exhibited the largest differences between irrigated and droughted plants, whereas *S. officinarum* and H69-8235 had the smallest differences (Fig. 3).

Maximum  $g_s$  ranged between 0.100 and 0.250  $\text{mol m}^{-2} \text{s}^{-1}$  during the course of the season (Fig. 4 and data not shown). By the peak of the drought on November 8, clones *S. spontaneum* and *S. officinarum* exhibited the largest depression of  $g_s$  in the droughted plots, whereas the commercial clones H65-7052 and H69-8235 exhibited much smaller declines (Fig. 4). Values of  $\psi_l$  in clone H69-8235 were slightly lower in the droughted as compared to the irrigated plants, at similar levels of  $g_s$  (cf. Figs. 3 and 4), as expected from the differences in hydraulic properties.

The diurnal course of  $\psi_l$  on November 8 (45 d after imposition of the drought), in conjunction with the data in Figure

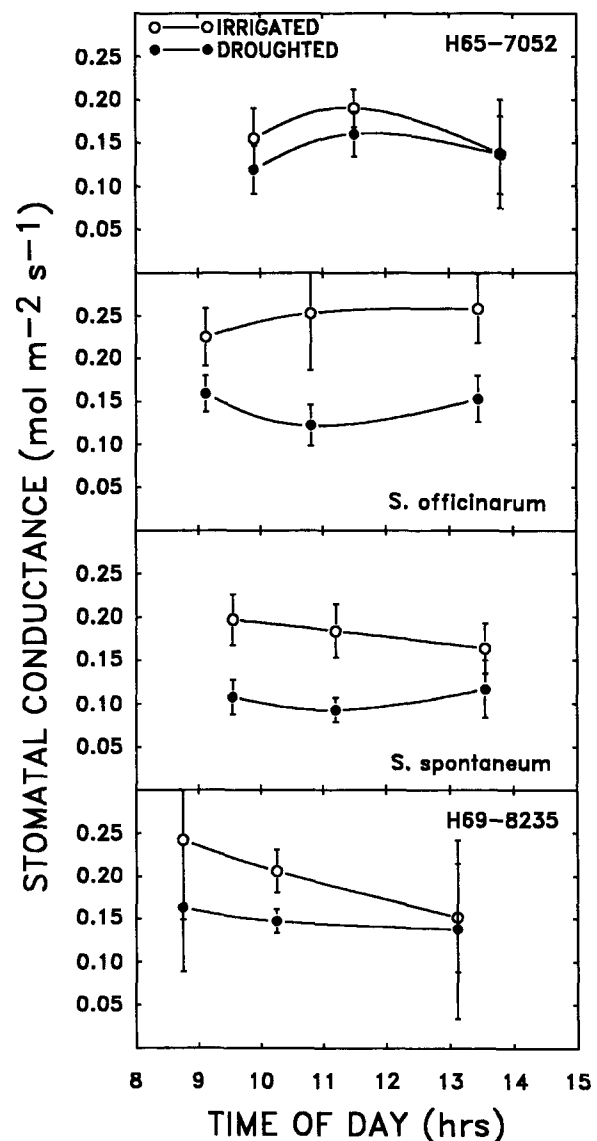


Figure 4. Diurnal patterns of  $g_s$  for irrigated and droughted sugarcane clones on November 8. Values are means  $\pm$  SE.

1, can be used to estimate diurnal losses in hydraulic conductivity (Fig. 5). If we assume that November 8 represented a typical day, the leaves of irrigated plants would rarely be expected to suffer more than a 30% loss in hydraulic conductivity, with the exception of H69-8235, which would likely experience diurnal losses of up to 50% (Fig. 5). Droughted plants, however, exhibited larger losses in conductivity of approximately 50% and up to 81% in H69-8235. The largest difference in diurnal loss of conductivity between droughted and irrigated plants was observed in H65-7052, in which irrigated plants were predicted to experience less than a 10% loss in conductivity at midday and droughted plants were predicted to lose 50% (Fig. 5).

Differences in maximum pit membrane pore sizes and the frequency distribution of smaller pore sizes within the xylem

of each clone may be responsible for the genotypic variability in the vulnerability curves generated. If we make the assumption that the  $\psi_i$  at which conductivity loss in sugarcane is first detected indicates the maximum pore size in pit membranes, we can use the formula for the retention of liquid in a capillary to gain an estimate of maximum pore sizes in sugarcane xylem (14). If we assume a contact angle of  $0^\circ$  (that is a hydrophilic vessel wall), circular pores, and  $20^\circ\text{C}$ :

$$r = 2\theta/\psi_i \quad (2)$$

where  $r$  is the radius of the pore,  $\theta$  is the surface tension of water, and  $\psi_i$  is the leaf water potential at which cavitation is observed. Pore sizes estimated from the value of  $\psi_i$  at which conductivity was reduced by 10% range from 1324 nm in H69-8235 to 160 nm in H65-7052, whereas at the 50% loss point, pore size estimates range from 175 nm in H69-8235 to 107 nm in H65-7052 (Table IV). As losses reach 80%, pore sizes range from 114 to 73 nm, with *S. spontaneum* having the smallest.

## DISCUSSION

There were clearly detectable differences in the vulnerability curves relating hydraulic conductivity to  $\psi_i$  among the sugarcane clones studied (Fig. 1). Clone H65-7052 appeared to be the most resistant to initial cavitation, whereas H69-8235 was the most susceptible. Interestingly, despite relatively large differences in  $\psi_i$  at the points of incipient and 10% conductivity losses, there was a convergence toward a common  $\psi_i$  at which three of the clones exhibited a 50% loss. The one exception was H69-8235, which reached 50% loss in conductivity at only  $-0.83$  MPa, compared to  $-1.18$  to  $-1.36$  MPa for the other three clones. Clones H65-7052 and *S. officinarum* also showed much steeper declines in conductivity between the 10 and 50% loss points than H69-8235 and *S. spontaneum*. Compared at the point at which they have lost 80% of their initial conductivity, differences among the clones become more apparent, with a range of approximately 0.7 MPa between the most and least resistant (Table II). The magnitude of the differences, particularly at the 80% loss point, are striking, considering the close genetic relationships among these sugarcane clones. Although the differences in vulnerability to cavitation among the clones are generally small throughout the normal operating range of  $\psi_i$ , they become accentuated in all clones at the low  $\psi_i$  values that can occur during times of extreme water stress. Differences in leaf hydraulic conductivity at these extreme  $\psi_i$  values may play a factor in yield recovery and, ultimately, survival of the different clones and may be of more significance than conductivities at the more moderate levels of  $\psi_i$  typical of well-irrigated plants.

Anatomical observations (Table I) demonstrated that *S. spontaneum* has more large vessel bundles in proportion to the total number of bundles but the smallest individual vessel diameters of the four clones. Maximum vessel diameters are approximately one-half those of H65-7052. Salleo and Lo Gullo (19) found a strong correlation between vessel diameter and susceptibility to cavitation. On the other hand, pore sizes

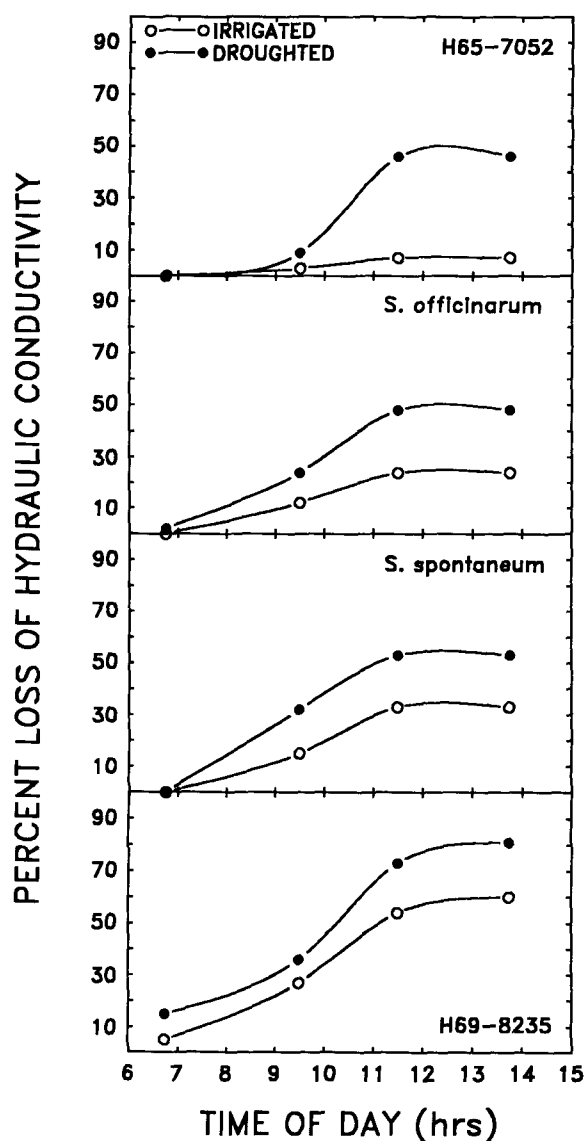


Figure 5. Diurnal courses of calculated losses of hydraulic conductivity for leaves of irrigated and droughted sugarcane clones on November 8. Values computed from data in Figures 1 and 3.

**Table IV.** Maximum Estimated Radii of Wall Pores of Sugarcane Xylem at Specific Loss Points for Hydraulic Conductivity

Clone	Pore Radii <sup>a</sup>		
	10%	50%	80%
		<i>nm</i>	
H65-7052	160	107	89
<i>S. officinarum</i>	217	123	101
<i>S. spontaneum</i>	297	112	73
H69-8235	1324	175	114

<sup>a</sup> Calculated from Equation 2.

in conduit walls, most likely the pit membranes, may be the crucial factors in determining the susceptibility of the xylem to cavitation (15, 31). Maximum pore size would primarily determine the  $\psi_i$  threshold for cavitation, whereas the range of pore sizes would determine the distribution of cavitation events across a range of  $\psi_i$ . Calculated values of the pore sizes cavitating at each level of  $\psi_i$  in sugarcane clones (Table IV) fall within the ranges reported for other species (5, 21, 29). Tyree and Dixon (25) suggested that differences in susceptibility to cavitation may vary with lumen size, as observed by Salleo and Lo Gullo (19), not only because of effects on capillarity but also because larger cells have greater wall surface areas with larger numbers of wall imperfections that contribute to cavitation and, hence, embolism formation.

It is interesting to note that H65-7052 was the most resistant to initial cavitation, despite having the largest metaxylem lumen diameters, yet hydraulic conductivities declined more rapidly than for the other clones as  $\psi_i$  decreased. This may reflect sequential cavitation of large xylem vessels, or it may indicate that flow in the xylem is not well described by the Hagen-Poiseuille law (flux proportional to  $r^4$ ; 2, 8). In the latter case, the largest vessel elements may cavitate first but reduce total conductivity less than predicted (31). Further studies are needed to determine which specific cells cavitate at given levels of water stress.

The relatively minor differences in leaf water relations between droughted and irrigated individuals of H65-7052 and between irrigated individuals of H69-8235 and H65-7052 (Figs. 3 and 4) appear difficult to reconcile with the substantial differences in their predicted patterns of conductivity loss in the field (Fig. 5). For example, the large difference in hydraulic conductivity between droughted and irrigated leaves at midday, particularly evident for H65-7052, did not result in a large difference in  $\psi_i$  at midday (Fig. 3) even though  $g_s$  and, therefore, presumably, transpiration were similar in droughted and irrigated plants (Fig. 4). However, if shedding of lower leaves occurred in the droughted plants (as observed in other experiments; D.A. Grantz and F.C. Meinzer, unpublished data) total transpiration per plant would be lower even though transpiration per unit of leaf area remained constant with constant  $g_s$ . The resulting reduction in total water flux through the plant would diminish the effect on  $\psi_i$  of diurnal losses in leaf conductivity. Similarly, midday  $\psi_i$  was nearly identical in irrigated individuals of H65-7052 and H69-8235 (Fig. 3) despite the 50% greater midday loss in conductivity in the latter (Fig. 5). This may

have been attributable to a smaller total leaf area and water flux in H69-8235 or to a higher root hydraulic conductivity in H69-8235 than in H65-7052 (18). Both of these factors would have resulted in a smaller gradient in water potential between the soil and the leaf bases in H69-8235.

There are several possible alternative explanations that require further study of the patterns of conductivity loss and leaf water relations. These include more rapid drying of detached leaves in the laboratory than in the field, which might have induced greater numbers of embolisms at higher  $\psi_i$ . Alternatively, lateral transfer of water to adjacent vessels may occur at vessel branch points in the field, enhancing water transport to distal leaf segments despite cavitation of some xylem vessels. Lateral transfer would have been largely prevented in our laboratory measurements using narrow leaf segments. In addition, the influence of embolisms in leaf xylem on total soil-plant hydraulic conductivity remains to be investigated. If leaf conductivity is high relative to other parts of the pathway (i.e. roots [10]), or if more substantial embolisms occur simultaneously in other tissues as conductivity declines in leaves, then the relationships among  $\psi_i$ ,  $g_s$ , and leaf conductivity are likely to be somewhat weak.

In *S. officinarum*, differences in  $\psi_i$  between droughted and irrigated plants were small because of reductions in  $g_s$  and restriction of transpiration in the droughted plants.  $\psi_i$  in *S. spontaneum* was significantly lower in the droughted as compared with the irrigated plants, even though  $g_s$  was also reduced. This may reflect a low intrinsic hydraulic conductivity in this clone, particularly at very low  $\psi_i$  that could result during severe drought. The greater tolerance of this clone to water-stress-induced cavitation at low  $\psi_i$  (Fig. 1) may, therefore, be an adaptation for avoiding catastrophic xylem failure under these conditions.

The four clones exhibited a range of predicted conductivity losses under well-irrigated field conditions (Fig. 5). *S. spontaneum* and H69-8235 exhibited more shallow conductivity loss curves (Fig. 1) than *S. officinarum* or H65-7052. Simulation studies (9), under these conditions, indicate that operating points of  $\psi_i$  that lie below levels causing some degree of cavitation should lead to maximum sustainable levels of stomatal conductance. Under droughted conditions all except H69-8235 exhibited predicted losses of approximately 50%, whereas clone H69-8235 exhibited an approximately 80% loss near midday (Fig. 5). All clones except H69-8235 exhibited a narrow range of  $\psi_i$  at which 50% loss occurs, ranging only from -1.18 to -1.36 MPa (Fig. 1). This may represent the operational  $\psi_i$  for these three clones. Although the minimum  $\psi_i$  reached for clone H69-8235 during the drought was similar to those of the other clones (-1.28 MPa), suggesting a similar operating point for  $\psi_i$ , the 50% loss point was approximately -0.8 MPa, which was much higher than for the other clones. The loss at minimum  $\psi_i$  was also considerably greater, more than 80%. The tight stomatal control over water loss by the leaves of sugarcane and the developmental changes in hydraulic architecture that maximize water flow to upper leaves (8, 10, 11) combine to enable sugarcane plants to avoid extremely low  $\psi_i$  under most conditions. The results of the vulnerability curve analyses presented here indicate that these stomatal and hydraulic properties in these clones tend to maintain  $\psi_i$  above the point of catastrophic

xylem failure. The point of massive cavitation, at which loss of conductivity leads to reduced  $\psi_i$  and further cavitation, has been previously suggested as a general set point for stomatal control (27). The present data are consistent with, but do not provide definitive proof of, such a control system in sugarcane.

Diurnal losses in conductivity for herbaceous plants in the field and for leaves of the palm *Rhapis excelsa* were reported previously (13, 22, 26). In maize, the restoration of leaf water status on a diurnal basis involved refilling of embolized xylem conduits by the generation of root pressure (26). The onset of each diurnal course at high levels of relative conductivity (Figs. 3 and 5) and high levels of absolute conductivity (Table III) suggest that substantial refilling of conducting elements (dissolution of embolisms) occurs in sugarcane (as suggested in other species [13, 26]) overnight. Sugarcane plants generate up to 0.3 MPa of root pressure because of the high solute concentration in the root xylem sap (12), apparently enough to restore conductivity diurnally during the dark period. Guttation, at leaf margins and between the ligule and stem, of sugarcane is nearly always observed in early morning. Sperry (22) found that embolisms in the leaves of the palm *R. excelsa* could even be dissolved at atmospheric pressure, as during periods of low transpiration. The ability to restore leaf hydraulic conductivities on a diurnal basis through root pressure may reduce the negative impacts of large losses in hydraulic conductivity on the water relations of the droughted plants and of the well-watered plants of clone H69-8235. The hydraulic architecture of sugarcane (10) presents a high resistance to flow from the node to the leaf sheath, which confines embolisms to the leaves, conserving water flow through the stem and preserving survival of the meristematic zones and the photosynthetic capacity of non-cavitating leaves. Potentially lethal values of  $\psi_i$  are seen mainly in senescing leaves, which are soon shed from the plant.

There have been few direct determinations of the susceptibility of the xylem in leaf tissue to cavitation (4, 12, 22, 30), and no others have generated vulnerability curves for grass leaves. Other studies, particularly in woody plants, have shown that vulnerability curves can differ in shape with some showing threshold responses and others showing no threshold (23, 24). Both types of responses were observed in the present study, along with surprising variation in the magnitude of conductivity loss, among closely related grass genotypes.

The differences among the clones detected in this study suggest that there may be a rather large potential to modify xylem anatomy through plant breeding. Pit membrane pore sizes may be more crucial in determining susceptibility to cavitation of individual vessels than the vessel lumen size itself, although relative conductivity loss with declining  $\psi_i$  may be more critical and reflect the distribution rather than absolute magnitude of either vessel or pit sizes. We observed no correlation between maximum conductivity and vulnerability to cavitation, although selection for small pore sizes could result in lower overall hydraulic conductivities. These possible trade-offs between reduced vulnerability and maximal conductivity could have significant implications for plant selection. A complete understanding of the physiological

ecology of relationships among hydraulic architecture, carbon gain, and water use efficiency awaits further study.

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#### LITERATURE CITED

1. Artschwager E (1928) Anatomy of the vegetative organs of sugarcane. *J Agric Res* **60**: 197-221
2. Calkin HW, Gibson AC, Nobel PS (1986) Biophysical model of xylem conductance in tracheids of the fern *Pteris vittata*. *J Exp Bot* **37**: 1054-1064
3. Colbert JT, Evert RF (1982) Leaf vasculature in sugarcane (*Saccharum officinarum* L.). *Planta* **156**: 136-151
4. Crombie DS, Hipkins MF, Milburn JA (1985) Gas penetration of pit membranes in the xylem of *Rhododendron* as the cause of acoustically detectable sap cavitation. *Aust J Plant Physiol* **12**: 445-453
5. Crombie DS, Milburn JA, Hipkins MF (1985) Maximum sustainable xylem sap tensions in *Rhododendron* and other species. *Planta* **163**: 27-33
6. Ewers FW, Fisher JB (1989) Techniques for measuring vessel lengths and diameters in stems of woody plants. *Am J Bot* **76**: 645-656
7. Heinz DJ (1987) Sugarcane Improvement through Breeding. Developments in Crop Science, Vol 11. Elsevier Publishers, Amsterdam, The Netherlands
8. Jeje AA, Zimmermann MH (1979) Resistance to water flow in xylem vessels. *J Exp Bot* **30**: 817-827
9. Jones HG, Sutherland RA (1991) Stomatal control of xylem embolism. *Plant Cell Environ* **14**: 607-612
10. Meinzer FC, Goldstein G, Neufeld HS, Grantz DA, Crisosto GM (1992) Hydraulic architecture of sugarcane in relation to patterns of water use during plant development. *Plant Cell Environ* **15**: 471-477
11. Meinzer FC, Grantz DA (1990) Stomatal and hydraulic conductance in growing sugarcane: stomatal adjustment to water transport capacity. *Plant Cell Environ* **13**: 383-388
12. Meinzer FC, Grantz DA, Smit B (1991) Root signals mediate coordination of stomatal and root hydraulic conductance in growing sugarcane. *Aust J Plant Physiol* **18**: 329-338
13. Milburn JA, McLaughlin ME (1974) Studies of cavitation in isolated vascular bundles and whole leaves of *Plantago major* L. *New Phytol* **73**: 861-871
14. Nobel PS (1991) Physiochemical and Environmental Plant Physiology. Academic Press, San Diego, CA, pp 96-98
15. Pickard WF (1981) The ascent of sap in plants. *Prog Biophys Mol Biol* **37**: 181-229
16. Rawlings JO, Cure WW (1985) The Weibull function as a dose-response model to describe ozone effects on crop yields. *Crop Sci* **25**: 807-814
17. Saliendra NZ, Meinzer FC (1989) Relationship between root/soil hydraulic properties and stomatal behavior in sugarcane. *Aust J Plant Physiol* **16**: 241-250
18. Saliendra NZ, Meinzer FC, Grantz DA (1990) Water potential in sugarcane measured from leaf segments in a pressure chamber. *Agron J* **82**: 359-361
19. Salleo S, Lo Gullo MA (1986) Xylem cavitation in nodes and internodes of whole *Chorisia insignis* H.B. et K. plants subjected to water stress: relations between xylem conduit size and cavitation. *Ann Bot* **58**: 431-441
20. SAS Institute, Inc (1990) SAS for Linear Models, a Guide to the ANOVA and GLM Procedures. SAS Institute, Cary, NC
21. Siau JF (1984) Transport Processes in Wood, Chap 4. Springer-Verlag, Berlin, Germany
22. Sperry JS (1986) Relationship of xylem embolism to xylem pressure potential, stomatal closure, and shoot morphology in the palm *Rhapis excelsa*. *Plant Physiol* **80**: 110-116



23. **Sperry JS, Donnelly JR, Tyree MT** (1988) A method for measuring hydraulic conductivity and embolism in xylem. *Plant Cell Environ* **11**: 35–40
24. **Sperry JS, Tyree MT, Donnelly JR** (1988) Vulnerability of xylem to embolism in a mangrove vs an island species of Rhizophoraceae. *Physiol Plant* **74**: 276–283
25. **Tyree MT, Dixon MA** (1986) Water stress induced cavitation and embolism in some woody plants. *Physiol Plant* **66**: 397–405
26. **Tyree MT, Fiscus EL, Wullschlegel SD, Dixon MA** (1986) Detection of xylem cavitation in corn under field conditions. *Plant Physiol* **82**: 597–599
27. **Tyree MT, Sperry JS** (1988) Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? Answers from a model. *Plant Physiol* **88**: 574–580
28. **Tyree MT, Sperry JS** (1989) Vulnerability of xylem to cavitation and embolism. *Annu Rev Plant Physiol Mol Biol* **40**: 19–38
29. **Van Alfen NK, McMillan BD, Turner V, Hess WM** (1983) Role of pit membranes in macromolecule-induced wilt of plants. *Plant Physiol* **73**: 1020–1023
30. **West DW, Gaff DF** (1976) Xylem cavitation in excised leaves of *Malus sylvestris* Mill. and measurement of leaf water status with the pressure chamber. *Planta* **129**: 15–18
31. **Zimmermann MH** (1983) *Xylem Structure and the Ascent of Sap*. Springer-Verlag, Berlin