Mycorrhiza (2008) 18:115–121
DOI 10.1007/s00572-008-0162-9

ORIGINAL PAPER

# Hydraulic conductance and water potential gradients in squash leaves showing mycorrhiza-induced increases in stomatal conductance

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Received: 31 July 2007 / Accepted: 10 January 2008 / Published online: 29 January 2008


**Abstract** Stomatal conductance ($g_s$) and transpiration rates vary widely across plant species. Leaf hydraulic conductance ($k_{leaf}$) tends to change with $g_s$, to maintain hydraulic homeostasis and prevent wide and potentially harmful fluctuations in transpiration-induced water potential gradients across the leaf ($\Delta\Psi_{leaf}$). Because arbuscular mycorrhizal (AM) symbiosis often increases $g_s$ in the plant host, we tested whether the symbiosis affects leaf hydraulic homeostasis. Specifically, we tested whether $k_{leaf}$ changes with $g_s$ to maintain $\Delta\Psi_{leaf}$ or whether $\Delta\Psi_{leaf}$ differs when $g_s$ differs in AM and non-AM plants. Colonization of squash plants with *Glomus intraradices* resulted in increased $g_s$ relative to non-AM controls, by an average of 27% under amply watered, unstressed conditions. Stomatal conductance was similar in AM and non-AM plants with exposure to NaCl stress. Across all AM and NaCl treatments, $k_{leaf}$ did change in synchrony with $g_s$ (positive correlation of $g_s$ and $k_{leaf}$), corroborating leaf tendency toward hydraulic homeostasis under varying rates of transpirational water loss. However, $k_{leaf}$ did not increase in AM plants to compensate for the higher $g_s$ of unstressed AM plants relative to non-AM plants. Consequently, $\Delta\Psi_{leaf}$ did tend to be higher in AM leaves. A trend toward slightly higher $\Delta\Psi_{leaf}$ has been observed recently in more highly evolved plant taxa having higher productivity. Higher $\Delta\Psi_{leaf}$ in leaves of mycorrhizal plants would therefore be consistent with the higher rates of gas exchange that often accompany mycorrhizal symbiosis and that are presumed to be necessary to supply the carbon needs of the fungal symbiont.

**Keywords** Arbuscular mycorrhiza · Leaf hydraulic conductance · Salinity stress · Stomatal conductance · Water potential gradients

**Abbreviations**

| | |
|---|---|
| AM | arbuscular mycorrhizal |
| $k_{leaf}$ | leaf hydraulic conductance |
| $g_s$ | stomatal conductance |
| $\Delta\Psi_{leaf}$ | transpiration-induced water potential gradient, or drawdown, across the leaf |

## Introduction

Small changes in leaf water status can have relatively large effects on critical physiological processes such as photosynthesis and water transport (Franks 2006; Taiz and Zeiger 2006). Because of this, leaves appear to be designed to maintain a certain degree of hydraulic homeostasis, both across species and across environments (Cowan and Farquhar 1977; Farquhar et al. 2002; Franks 2006). Changes in stomatal conductance ($g_s$) and transpiration are typically balanced by changes in leaf hydraulic conductance ($k_{leaf}$), keeping changes in water potential gradient across the leaf ($\Delta\Psi_{leaf}$) to a minimum (Franks 2006). If $k_{leaf}$ did not change with fluctuations in $g_s$, $\Delta\Psi_{leaf}$ would change just as dramatically as $g_s$, with potentially adverse effects on leaf physiology and metabolism.

Colonization of plant roots and soils by arbuscular mycorrhizal (AM) fungi is often accompanied by changes

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in $g_s$ and transpiration in the host plant (Augé 2001; Khalvati et al. 2005; Cho et al. 2006; Querejeta et al. 2006). There are some reports of AM-induced changes in whole-plant and root hydraulic conductance (Allen et al. 1981; Allen 1982; Augé 2001), but the possibility of AM-induced changes in $k_{leaf}$ has not been examined. We conducted this study to answer the question, is the increase in $g_s$ often observed in AM plants accompanied by increased $k_{leaf}$? Or do there tend to be larger water potential gradients across leaves of actively transpiring mycorrhizal plants, relative to non-AM controls? We examined squash (*Cucurbita pepo* L.) because its $g_s$ has previously been shown to increase with AM colonization (Augé et al. 2007) and because it represents an important crop family. We used *Glomus intraradices* because it has frequently been shown to change the $g_s$ of its host plants (Augé 2000).

We tested four hypotheses: (1) AM symbiosis modifies $g_s$ of its host plants, (2) AM symbiosis modifies $k_{leaf}$, (3) $\Delta\Psi_{leaf}$ is similar in AM and non-AM plants showing different $g_s$, and (4) changes in $k_{leaf}$ approximate changes in $g_s$, whether changes in $g_s$ are induced by AM symbiosis or by exposure to stress. We tested the hypotheses under conditions of high transpiration (amply watered, unstressed plants) and lower transpiration (exposure to two salinity treatments that caused $g_s$ to decline to about one half and one quarter of $g_s$ before exposure), allowing testing of $g_s$, $k_{leaf}$, and $\Delta\Psi_{leaf}$ relationships under differing strengths of hydrodynamic drawdown.

## Materials and methods

### Plant culture

Thirty 2-L plastic pots were seeded with *C. pepo* L. cv. White Bush Scallop on 7 November 2006 and thinned after germination to one plant per pot. The potting medium was calcined montmorillonite clay (Turface, Industrial Materials, Deerfield, IL). Pots were inoculated with 200 mL of fresh pot culture, banded beneath seeds: half of the pots with *G. intraradices* Schenck & Smith isolate IA509 and the other half with non-AM pot culture. AM and non-AM pot cultures were established on sorghum in a Turface/sand (1:1) mixture and were 4 months old at the time of inoculation. In addition to using non-AM pot cultures grown under similar conditions as AM pot cultures, similar soil microbial populations were encouraged among treatments by also applying water filtrates (60 mL; 44-μm sieve) of each inoculum to each pot.

Plants were fertilized weekly with a water-soluble fertilizer at 100 ppm N (Peters Professional Dark Weather Feed, N/P/K=15:0:15, Scotts-Sierra Horticultural Product, Marysville, OH) and twice with a micronutrient solution at 0.02 mM Fe (Microplex, Miller Chemical & Fertilizer, Hanover, PA) during the growth phase preceding hydraulic measurements. Phosphorus was supplied once per week as 0.6 mM $KH_2PO_4$ to AM plants and as 1.2 mM $KH_2PO_4$ to non-AM plants. Plants were grown in a glasshouse in Knoxville, TN, with temperature maintained at 22–25/18–22°C (day/night) under natural light.

### Leaf hydraulic measurements

Leaf hydraulic conductance normalized to leaf area was measured in mol $m^{-2}$ $s^{-1}$ $MPa^{-1}$, following the electrical analogue approach (Tyree and Cheung 1977; Schulte 1993) using the procedures of Franks (2006). Franks described the quantity $k_{leaf}$ as the bulk hydraulic conductance to water flow between the water storage tissues of leaves, mostly mesophyll and epidermis, and the petiole xylem, which includes the entire leaf vasculature. In a transpiring leaf, water moves from the petiole, through the xylem, to the storage tissues (bulk capacitance) to replace water lost through transpiration. To obtain $k_{leaf}$, the lamina of a detached, fully hydrated leaf is pressurized so that a small amount of water moves in the opposite direction, from the storage tissue to the xylem:

$$k_{leaf} = J_{w0}/\Delta P \quad (1)$$

where $J_{w0}$ (mol $m^{-2}$ $s^{-1}$) is the initial, maximal rate of water efflux from the tip of the petiole of the pressurized leaf and $\Delta P$ (in MPa) is the difference between the initial and final pressure of the step change.

$J_{w0}$ can be estimated as the volume of xylem sap expressed ($\Delta v$, in moles) per unit leaf area ($A_{leaf}$, in $m^2$) over a time interval $t$, provided that $t$ is much less than the time required to discharge the leaf's stored water (Franks 2006). Franks computed a time constant for discharge as 63% of the total sap discharge (60–120 s for the species used in his study). The time constant for 63% discharge for squash in our experiment was 45 s. $J_{w0}$ was estimated using

$$J_{w0} \approx \Delta v/10A_{leaf} \quad (2)$$

where $\Delta v$ was the volume of sap expressed in the first 10 s after the step increase in pressure.

Plants were kept amply watered during the experiment. On the morning that a plant's leaf hydraulics was examined, the plant was watered at 9 A.M. to bring it to full hydration. The most recently expanded, unshaded leaf was selected from each plant for characterization of $k_{leaf}$ and $\Delta\Psi_{leaf}$. First, its $g_s$ was measured, using a diffusion porometer (AP4, Delta-T Devices, Cambridge, UK). The porometer cuvette was placed parallel to the midrib in the center of the lamina on the abaxial side of the leaf. Stomatal conductance was measured on each side of the midrib, and those two values were pooled, to obtain a more accurate estimate of

leaf $g_s$ ($g_s$ varies across the lamina in many species). Clamping the porometer cuvette to the leaf did not affect stomatal opening or cause a decrease in $g_s$ for the second measurement of the same leaf; across all days and mycorrhizal treatments for unstressed plants, average $g_s$ was 185 mmol $m^{-2}$ $s^{-1}$ for the first measurement and 194 mmol $m^{-2}$ $s^{-1}$ for the second measurement. After the $g_s$ measurements, the leaf was excised, placed in a pressure chamber (Soilmoisture Equip., Santa Barbara, CA), and brought to the balance pressure. After allowing the leaf to equilibrate at a balance pressure for 45 s, the chamber pressure was quickly increased by 0.5 MPa. Sap expressed from the leaf was collected for 10 s and weighed on an analytical balance to obtain $\Delta v$. Leaf area was measured with an Epson Expression 1680 flatbed scanner and Winfolia software (version 2006a, Regent Instruments, Quebec City, Canada). $J_{w0}$ and $k_{leaf}$ were computed from Eqs. 1 and 2.

The $\Delta\Psi_{leaf}$ was calculated as:

$$\Delta\Psi_{leaf} = g_s \Delta w / k_{leaf} \quad (3)$$

with $\Delta w$ as the leaf-to-air water vapor concentration difference (kg $kg^{-1}$) computed from leaf and air temperatures and relative humidities. $\Delta\Psi_{leaf}$ has been variously referred to as the water potential gradient across the leaf, hydrodynamic (transpiration-induced) water potential drawdown across the leaf, or hydrodynamic pressure gradient (Franks 2006). Relative humidity of air was measured with a resistive sensor (U10-003, Hobo datalogger, Onset Computer, Bourne, MA). Relative humidity of fully hydrated leaves was near 100% (balance pressures were above −1.0 MPa in all cases), and a value of 100% was used in the calculations. Equation 3 applies when leaf boundary layer conductance is much larger than stomatal conductance (Franks 2006), as was the case in the stirred greenhouse air.

Water relations measurements began 8 weeks after planting. Stomatal conductance of all AM and non-AM plants was compared eight times over 3 weeks (5–26 January 2007), at 9:00–11:30 EST and 1 h after plants had been watered. Stomatal conductance, $k_{leaf}$, $J_{w0}$, and $\Delta\Psi_{leaf}$ of unstressed plants were measured from 17 to 26 January 2007. Equal numbers of replicates of each of the two mycorrhizal treatments were measured on each day, and measurements of AM and non-AM plants were alternated throughout the day to avoid diurnal bias. A total of 60 leaves were measured from unstressed plants.

Plants were then subjected to salinity stress during the next week, and all water relations measurements were repeated. Pots received an 180-mM NaCl drench 1 day and a 360-mM NaCl drench the following day, and $g_s$, $k_{leaf}$, $J_{w0}$, and $\Delta\Psi_{leaf}$ were measured on that second day. All pots were then flushed with tap water and 3 days later given 180 mM NaCl, after which water relations were again measured. A total of 60 leaves were measured from salinized plants.

### Shoot, root, and soil characters

Shoots were excised in each pot after completing stomatal and hydraulic conductance measurements, and dry weights determined. Leaves used for hydraulic measurements were pooled for each plant (four leaves per plant, two before and two after exposure to NaCl), and their elemental analyses were performed using inductively coupled plasma mass spectroscopy (Agilent 7500ce Series, Agilent Technologies, Santa Clara, CA). Immediately after harvesting shoots, soil was removed from each pot, sealed in a plastic bag, and frozen, for subsequent root and soil measurements.

Hyphal, arbuscular, and vesicular colonization of roots was determined for each plant, on one grid intersection on each of 50–0.5-cm root pieces, after clearing with boiling 10% KOH for 10 min, acidifying with 2% HCl for 1.5 h, staining with 0.05% Trypan blue for 1 h, and destaining in a lactoglycerol solution. Soil hyphal density was measured as described previously (Augé et al. 2001; Augé et al. 2003), on 10-g subsamples obtained from the soil after thorough mixing. Roots were carefully excavated from another 25 g of soil of each pot, for the measurement of root length, using scanning equipment and imaging software (WinRhizo, Regent Instruments).

### Experimental design and statistical analysis

Pots were arranged in a completely randomized design. There were 15 replicates for each mycorrhizal treatment. Analysis of variance was performed using mixed models (SAS Institute, Cary, NC). Correlations among water relation parameters and other plant and soil variables were tested by computing Pearson correlation coefficients.

## Results

The first requirement for determining if AM-induced increases in $g_s$ were associated with AM-induced changes in $k_{leaf}$ or $\Delta\Psi_{leaf}$ was to substantiate that *G. intraradices* would affect $g_s$ in this experiment. We measured $g_s$ of all plants eight times over a 3-week period under unstressed conditions and did observe a consistent mycorrhizal promotion of $g_s$ (Fig. 1). During this period, $g_s$ in *G. intraradices* plants averaged 27% higher than $g_s$ in non-AM plants, with the AM-induced increase on individual days ranging from 14% (day 24) to 48% (day 5). Hypothesis 1 was proven, for unstressed plants.

For hydraulic conductance measurements, $g_s$ was measured immediately before each measurement of $k_{leaf}$. Under high transpirational demand (amply watered, unstressed plants), $g_s$ associated with $k_{leaf}$ and $\Delta\Psi_{leaf}$ measurements was increased 18% by *G. intraradices*, less than the 27%

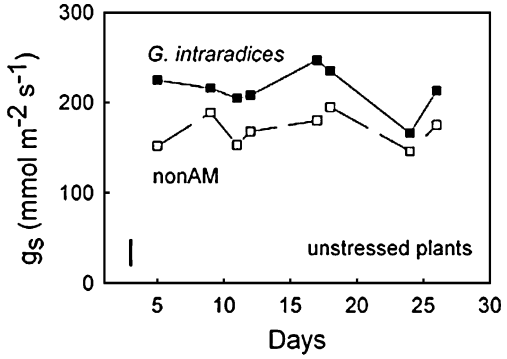


**Fig. 1** Stomatal conductance of unstressed squash plants. *Days* refers to date in January 2007. Each *symbol* represents the mean of 30 measurements: 2 leaves from 15 plants. *Closed symbols*, plants colonized by *G. intraradices*; *open symbols*, nonmycorrhizal plants. *Vertical bar* represents two times the pooled SE of the means (32 mmol $m^{-2}$ $s^{-1}$)

observed over the longer course in unstressed plants but still significantly higher than non-AM plants (Table 1).

The two conditions of NaCl stress reduced $g_s$ to about one fourth and one half of values seen before exposure to the stress (Table 1). Exposure to 180 mM NaCl followed the next day by 360 mM NaCl resulted in $g_s$ of 52 mmol $m^{-2}$ $s^{-1}$ averaged across mycorrhizal treatments, a decline of 72% relative to $g_s$ before exposure to NaCl. Stomatal conductance averaged 97 mmol $m^{-2}$ $s^{-1}$ after rinsing soils with tap water and again exposing plants to 180 mM NaCl, a decline of 48% relative to $g_s$ before exposure to NaCl. Mean $g_s$ was not significantly different in AM vs. non-AM plants at 180 and 360 mM NaCl. Hence, hypothesis 1 did not prove true for $g_s$ under saline conditions.

Values for $k_{leaf}$ were similar in leaves of AM and non-AM plants before and after exposure to each NaCl treatment (Table 1). Therefore, hypothesis 2 was not true under either the high or low transpiration conditions. Leaf hydraulic conductance was markedly reduced when plants were exposed to NaCl (Table 1, Fig. 2a). Averaged over mycorrhizal treatments, $k_{leaf}$ was 20.6 mmol $m^{-2}$ $s^{-1}$ $MPa^{-1}$ in the absence of NaCl, 6.9 mmol $m^{-2}$ $s^{-1}$ $MPa^{-1}$ in plants exposed to 360 mM NaCl, and 12.1 mmol $m^{-2}$ $s^{-1}$ $MPa^{-1}$ in plants exposed to 180 mM NaCl following the water rinse.

Values for $\Delta\Psi_{leaf}$ were statistically similar in leaves of AM and non-AM plants when compared at each NaCl exposure (Table 1). However, there was a tendency for $\Delta\Psi_{leaf}$ to be higher in AM leaves before exposure to NaCl and at the 180 mM NaCl stress (Table 1, Fig. 2b). In addition, when averaged across all soil water treatments (0, 180, and 360 mM NaCl), $\Delta\Psi_{leaf}$ was statistically higher in AM than in non-AM leaves, if extending the probability criterion to the 7% level ($P$=0.07) as seems reasonable for a parameter with as much inherent variation as $g_s$ (used to compute $\Delta\Psi_{leaf}$). This small change in $\Delta\Psi_{leaf}$ is biologically significant, as $\Delta\Psi_{leaf}$ should change only very slightly over the $g_s$ range examined (Franks 2006). Therefore, hypothesis 3 was disproved, averaged across the high and low transpiration conditions: the higher $g_s$ of AM plants appeared to be linked to higher $\Delta\Psi_{leaf}$. The NaCl treatments themselves did not have much effect on $\Delta\Psi_{leaf}$. Averaged

**Table 1** Water relations parameters of leaves of non-AM plants and plants colonized by *G. intraradices*

| | *G. intraradices* | non-AM | *P* |
|---|---|---|---|
| Before NaCl exposure (unstressed) | | | |
| $g_s$ (mmol $m^{-2}$ $s^{-1}$) | 202±15 | 171±11 | 0.05 |
| $k_{leaf}$ (mmol $m^{-2}$ $s^{-1}$ $MPa^{-1}$) | 20.8±1.4 | 20.5±1.4 | 0.89 |
| $\Delta\Psi_{leaf}$ (MPa) | 0.150±0.037 | 0.102±0.011 | 0.21 |
| 360 mM NaCl exposure | | | |
| $g_s$ (mmol $m^{-2}$ $s^{-1}$) | 55±9 | 48±6 | 0.48 |
| $k_{leaf}$ (mmol $m^{-2}$ $s^{-1}$ $MPa^{-1}$) | 6.5±0.7 | 7.4±0.9 | 0.61 |
| $\Delta\Psi_{leaf}$ (MPa) | 0.108±0.021 | 0.100±0.016 | 0.76 |
| 180 mM NaCl exposure | | | |
| $g_s$ (mmol $m^{-2}$ $s^{-1}$) | 105±11 | 88±5 | 0.19 |
| $k_{leaf}$ (mmol $m^{-2}$ $s^{-1}$ $MPa^{-1}$) | 11.8±1.1 | 12.4±1.1 | 0.78 |
| $\Delta\Psi_{leaf}$ (MPa) | 0.161±0.035 | 0.104±0.013 | 0.14 |
| Before and after NaCl exposure | | | |
| $g_s$ (mmol $m^{-2}$ $s^{-1}$) | 143±10 | 122±8 | 0.10 |
| $k_{leaf}$ (mmol $m^{-2}$ $s^{-1}$ $MPa^{-1}$) | 15.2±1.0 | 15.4±1.0 | 0.85 |
| $\Delta\Psi_{leaf}$ (MPa) | 0.144±0.026 | 0.102±0.008 | 0.07 |

$g_s$ stomatal conductance was measured immediately before beginning leaf hydraulic measurements. For unstressed plants, $n$=30 for $k_{leaf}$, $n$=60 for $g_s$, and $n$=60 for $\Delta\Psi_{leaf}$. For plants exposed to NaCl, $n$=15 for $k_{leaf}$, $n$=30 for $g_s$, and $n$=30 for $\Delta\Psi_{leaf}$. Values for "Before and after NaCl exposure" represent all data: three soil solution treatments combined. $P$ is probability that AM and non-AM means were not significantly different. $k_{leaf}$ Leaf hydraulic conductance, $\Delta\Psi_{leaf}$ $\Psi$ gradient across the leaf

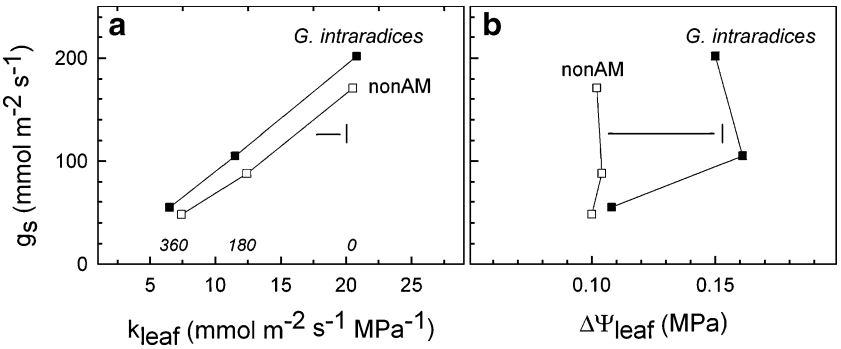


**Fig. 2** Stomatal conductance as a function of $k_{leaf}$ (**a**) and $\Delta\Psi_{leaf}$ (**b**), with exposure to varying NaCl. *Italicized numbers* within plot **a** refer to the three NaCl treatments: 360 mM NaCl, 180 mM NaCl, and 0 NaCl (absence of NaCl stress). Each *symbol* represents the mean of 15–60 measurements. For unstressed plants, $n$=30 for $k_{leaf}$ and $n$=60 for $g_s$. For plants exposed to NaCl, $n$=15 for $k_{leaf}$ and $n$=30 for $g_s$. *Closed symbols*, plants colonized by *G. intraradices*; *open symbols*, nonmycorrhizal plants. *Vertical* and *horizontal bars* represent two times the pooled SE of the means (**a**, 19 mmol $m^{-2}$ $s^{-1}$ and 2.2 mmol $m^{-2}$ $s^{-1}$ $MPa^{-1}$; **b**, 19 mmol $m^{-2}$ $s^{-1}$ and 0.04 MPa)

over mycorrhizal treatments, $\Delta\Psi_{leaf}$ was 0.13 MPa in the absence of NaCl, 0.11 MPa in plants exposed to 360 mM NaCl, and 0.13 MPa in plants exposed to 180 mM NaCl after the water rinse.

When viewed across the entire population of plants in this study, $g_s$ and $k_{leaf}$ were significantly, positively correlated ($P$=0.0005), indicating that $k_{leaf}$ did tend to change in synchrony with $g_s$. Figure 2a depicts the close relationship between mean $g_s$ and mean $k_{leaf}$. Therefore, with respect to changes in $g_s$ and $k_{leaf}$ caused by exposure to NaCl stress, hypothesis 4 was proved. Leaf hydraulic conductance declined 66% with exposure to the 360-mM NaCl treatment and declined 41% with exposure to the 180-mM NaCl treatment. These declines relative to $k_{leaf}$ before exposure to NaCl are similar to the 72% and 48% declines in $g_s$ with exposure to NaCl. The slightly higher $g_s$ in AM plants was not consistently linked to a slightly higher $k_{leaf}$ in AM plants, relative to non-AM plants (Table 1). Therefore, hypothesis 4 was not true with respect to the mild promotion of $g_s$ by *G. intraradices* in the absence of NaCl. Changes in mean $g_s$ did closely track changes in $k_{leaf}$ in both AM and non-AM plants across the range of transpiration rates, with similar slopes in AM and non-AM leaves (Fig. 2a).

We measured some shoot, root, and fungal characteristics often affected by AM symbiosis that might potentially also affect $g_s$ and $k_{leaf}$ (Table 2). Area of leaves sampled for $k_{leaf}$ did not differ between AM and non-AM treatments (Table 2). Root length density was similar in AM and non-AM plants, at about 40 cm $g^{-2}$ dry soil. Root colonization (arbuscular and vesicular colonization) and soil colonization (soil hyphal density) by AM fungi was predictably much higher in AM than in non-AM plants, with non-AM root systems and soils remaining uncolonized. Phosphorus concentrations were quite similar in leaves of AM and non-AM plants, each averaging 4.3 mg $g^{-1}$ dry weight (Table 2). Among those elements assayed, only calcium concentrations differed in AM and non-AM leaves, slightly lower in non-AM leaves. Calcium is integrally involved in the physiology of stomatal opening and closing (Mansfield et al. 1990) and may play a role in tolerance to water stress (Ruiz-Lozano and Azcón 1997). Correlation analysis was conducted for each of the hydraulic parameters with each of the leaf elements, for unstressed conditions. Neither $g_s$, $k_{leaf}$, or $\Delta\Psi_{leaf}$ were significantly correlated with the concentration of phosphorus or calcium in the leaf. There were negative correlations of $g_s$ with magnesium ($P$=0.001) and zinc ($P$=0.0001), and $k_{leaf}$ was positively correlated with leaf C/N ratio ($P$=0.05).

## Discussion

AM plants often have higher $g_s$ than non-AM plants (Augé 2000). High $g_s$ translates into higher rates of transpiration. Increased $g_s$ or gas exchange rates in AM plants have been recorded under amply watered conditions (e.g., Bryla and Duniway 1997; Augé et al. 2004a), during drought (e.g., Allen 1982; Augé et al. 2004b), and after exposure to NaCl stress (Ruiz-Lozano et al. 1996; Cho et al. 2006). The size of the AM-induced increase in unstressed plants is often about 15% to 50%, depending on host species and experimental conditions (Augé et al. 2001). Our objective was to determine if this increase is enough to cause a discernable change in hydraulic conductance or transpiration-induced water potential gradients in leaves of AM plants.

As has been reported before for associations with *G. intraradices* in squash (Augé et al. 2007) and other plant species under conditions of ample water (e.g., Augé et al. 2004a, b), association with *G. intraradices* consistently resulted in higher $g_s$ in these squash plants over the course of the experiment. Care was taken to produce AM and non-AM plants of similar size and leaf phosphorus nutrition, to obviate the possibility that an AM-induced increase in $g_s$

**Table 2** Shoot, root, and fungal characteristics of non-AM plants and plants colonized by *G. intraradices*

| | *G. intraradices* | non-AM | *P* |
|---|---|---|---|
| Shoot dry weight (g) | 5.2±0.4 | 5.6±0.3 | 0.10 |
| Leaf area, unstressed leaves ($cm^2$) | 14.9±1.5 | 13.0±0.6 | 0.25 |
| Leaf area, NaCl leaves ($cm^2$) | 11.2±1.9 | 13.7±1.2 | 0.25 |
| Root length density (cm $g^{-1}$ dry soil) | 36±3.3 | 43±2.6 | 0.07 |
| Arbuscular root colonization (%) | 59±2.7 | 0±0 | <0.0001 |
| Vesicular root colonization (%) | 28±1.9 | 0±0 | <0.0001 |
| Soil hyphal density (cm $g^{-1}$ dry soil) | 94.0±9 | 0.4±0.2 | <0.0001 |
| Leaf [P] (mg $g^{-1}$) | 4.3±0.1 | 4.3±0.2 | 0.83 |
| Leaf [K] (mg $g^{-1}$) | 3.0±0.1 | 2.9±0.1 | 0.26 |
| Leaf [N] (%) | 3.3±0.1 | 3.1±0.2 | 0.33 |
| Leaf [C] (%) | 39±0.4 | 40±0.2 | 0.17 |
| Leaf C/N ratio | 12.4±0.5 | 13.2±0.6 | 0.10 |
| Leaf [Ca] (mg $g^{-1}$) | 6.0±0.5 | 5.3±0.4 | 0.05 |
| Leaf [Mg] (mg $g^{-1}$) | 3.1±0.1 | 3.1±0.2 | 0.69 |
| Leaf [Mn] (μg $g^{-1}$) | 25±1 | 26±2 | 0.68 |
| Leaf [Cu] (μg $g^{-1}$) | 35±13 | 41±11 | 0.26 |
| Leaf [Zn] (μg $g^{-1}$) | 90±6 | 86±5 | 0.50 |

Plants and soils were assayed on the day that each plant's hydraulic parameters were measured. Leaf area refers to average area of the leaf sampled for hydraulic measurements, not total leaf area of shoots. Elemental analysis was performed on leaves used for hydraulic measurements of plants exposed to NaCl. $n$=15, except leaf area where $n$=30. $P$ is the probability that AM and non-AM means were not significantly different.

was simply a matter of "big plant little plant" dynamics or related to differences in phosphorus concentrations (which can affect $g_s$, e.g., Radin 1984). It is also conceivable that $k_{leaf}$ or $\Delta\Psi_{leaf}$ might be affected, if only slightly, by how far water has to travel across leaves to replenish transpirational losses, in larger and smaller leaves having similar $g_s$. The criteria for leaf sampling for hydraulic measurements resulted in leaves of similar size in AM and non-AM treatments, precluding a possibly confounding effect of size differences among treatments. Further, correlation analysis between leaf area and $g_s$ (correlation coefficient=0.06, $P$=0.39), leaf area and $k_{leaf}$ (correlation coefficient=0.04, $P$=0.57), and leaf area and $\Delta\Psi_{leaf}$ (correlation coefficient=−0.02, $P$=0.74) revealed that leaf size was not linked to these hydraulic parameters.

Across taxa that vary in $g_s$, the higher rates of transpirational water loss from leaves that accompany higher $g_s$ appear to be compensated for by an increase in $k_{leaf}$: increased capacity to move water across a leaf to replace that being lost through transpiration (Franks 2006). If $k_{leaf}$ did not increase with $g_s$, then water potential gradients across the leaf would increase. This could be potentially harmful to leaf physiology if the gradients became large enough.

Leaf hydraulic conductance did not increase in AM plants to compensate for the higher $g_s$ of unstressed AM plants relative to non-AM plants. Consequently, $\Delta\Psi_{leaf}$ did tend to be higher in AM leaves. The squash $\Delta\Psi_{leaf}$ was near the low end of the range observed by Franks in his examination of ten plant species. At near 0.1 MPa, $\Delta\Psi_{leaf}$ was also below the threshold that Franks notes could potentially impair plasmodesmatal function. Passage through the plasmodesmata can be blocked if the pressure differential between cells exceeds about 0.2 MPa (e.g., Oparka and Prior 1992), and average $\Delta\Psi_{leaf}$ was below this for both AM and non-AM plants. Therefore, there appears to be no concern that the AM-induced elevation in $g_s$ and accompanying increase in $\Delta\Psi_{leaf}$ had a potentially negative impact on the leaf.

Transpiration-induced water potential gradients across leaves tend to be relatively stable with changes in $g_s$. For instance, across an almost 30-fold increase in $g_s$ observed over ten plant species, increases in $\Delta\Psi_{leaf}$ were just slightly more than twofold (Franks 2006). We also observed this stability in $\Delta\Psi_{leaf}$ in squash plants in relation to $g_s$ declines with NaCl exposure. Over all treatments, NaCl caused $g_s$ to decline by about 60%, whereas $\Delta\Psi_{leaf}$ was essentially unchanged, 0.126 MPa for unstressed plants and 0.119 MPa with NaCl exposure (a 5% difference). Leaf hydraulic conductance of our squash plants given ample water (about 21 mmol $m^{-2}$ $s^{-1}$ $MPa^{-1}$) was higher than that observed previously for two other crop species, *Triticum aestivum* (~15 mmol $m^{-2}$ $s^{-1}$ $MPa^{-1}$) and *Vicia faba* (~11 mmol $m^{-2}$ $s^{-1}$ $MPa^{-1}$; Franks 2006), and near or below those values after exposure to NaCl.

The higher $\Delta\Psi_{leaf}$ observed in leaves of AM plants has interesting implications. While evolution in plants appears to have favored steady-state $\Delta\Psi_{leaf}$, there has also been an overall trend toward slightly higher $\Delta\Psi_{leaf}$ in more recent plant taxa having higher productivity. Franks (2006) suggests that tolerance of larger $\Delta\Psi_{leaf}$ —more capacity for hydrodynamic water potential drawdown—would allow $g_s$ to increase more quickly than $k_{leaf}$, which would facilitate higher rates of leaf gas exchange. A higher $\Delta\Psi_{leaf}$ in leaves of mycorrhizal plants would therefore be consistent with the higher rates of gas exchange that often accompany mycorrhizal symbiosis and that are presumed to be necessary to supply the carbon needs of the fungal symbiont (Augé 2001).

**Acknowledgments** This work was supported by the Agricultural Experiment Station at the University of Tennessee. We are grateful to Casey Barrickman for nutrient analyses.

## References

Allen MF (1982) Influence of vesicular-arbuscular mycorrhizae on water movement through *Bouteloua gracilis* (H.B.K.) Lag ex Steud. New Phytol 91:191–196

Allen MF, Smith WK, Moore TS Jr, Christensen M (1981) Comparative water relations and photosynthesis of mycorrhizal and non-mycorrhizal *Bouteloua gracilis* H.B.K. New Phytol 88:683–693

Augé RM (2001) Water relations, drought and VA mycorrhizal symbiosis. Mycorrhiza 11:3–42

Augé RM (2000) Stomatal behavior of arbuscular mycorrhizal plants. In: Kapulnik Y, Douds DD (eds) Arbuscular mycorrhizas: physiology and function. Kluwer, Dordrecht, The Netherlands, pp 201–237

Augé RM, Moore JL, Cho K, Stutz JC, Sylvia DM, Al-Agely AK, Saxton AM (2003) Relating foliar dehydration tolerance of mycorrhizal *Phaseolus vulgaris* to soil and root colonization by hyphae. J Plant Physiol 160:1147–1156

Augé RM, Moore JL, Sylvia DM, Cho K (2004a) Mycorrhizal promotion of host stomatal conductance in relation to irradiance and temperature. Mycorrhiza 14:85–92

Augé RM, Stodola AJW, Tims JE, Saxton AM (2001) Moisture retention properties of a mycorrhizal soil. Plant Soil 230:87–97

Augé RM, Sylvia DM, Park SJ, Buttery BR, Saxton AM, Moore JL, Cho K (2004b) Partitioning mycorrhizal influence on water relations of *Phaseolus vulgaris* into soil and plant components. Can J Bot 82:503–514

Augé RM, Toler HD, Moore JL, Cho K, Saxton AM (2007) Comparing contributions of soil versus root colonization to variations in stomatal behavior and soil drying in mycorrhizal *Sorghum bicolor* and *Cucurbita pepo*. J Plant Physiol 164:1289–1299

Bryla DR, Duniway JM (1997) Growth, phosphorus uptake, and water relations of safflower and wheat infected with an arbuscular mycorrhizal fungus. New Phytol 136:581–590

Cho K, Toler HD, Lee J, Ownley BH, Jean C Stutz JC, Moore JL, Augé RM (2006) Mycorrhizal symbiosis and response of sorghum plants to combined drought and salinity stresses. J Plant Physiol 163:517–528

Cowan IR, Farquhar GD (1997) Stomatal function in relation to leaf metabolism and environment. In: Jennings DH (ed) Integration of activity in the higher plant. Cambridge University Press, Cambridge, UK, pp 471–505

Farquhar GD, Buckley TN, Miller JM (2002) Optimal stomatal control in relation to leaf area and nitrogen content. Silva Fenn 36:625–637

Franks P (2006) Higher rates of leaf gas exchange are associated with higher leaf hydrodynamic pressure gradients. Plant Cell Environ 29:584–592

Khalvati MA, Hu Y, Mozafar A, Schmidhalter U (2005) Quantification of water uptake by arbuscular mycorrhizal hyphae and its significance for leaf growth, water relations, and gas exchange of barley subjected to drought stress. Plant Biol 7:706–712

Mansfield TA, Hetherington AM, Atkinson CJ (1990) Some current aspects of stomatal physiology. Annu Rev Plant Physiol Plant Mol Biol 41:55–75

Radin JW (1984) Stomatal responses to water stress and to abscisic acid in phosphorus-deficient cotton plants. Plant Physiol 76: 392–394

Oparka KJ, Prior DAM (1992) Direct evidence of pressure-generated closure of plasmodesmata. Plant Journal 2:741–750

Querejeta JI, Allen MF, Caravaca F, Roldan A (2006) Differential modulation of host plant delta C-13 and delta O-18 by native and nonnative arbuscular mycorrhizal fungi in a semiarid environment. New Phytol 169:379–387

Ruiz-Lozano JM, Azcón R (1997) Effect of calcium application on the tolerance of mycorrhizal lettuce plants to polyethylene glycol-induced water stress. Symbiosis 23:9–21

Ruiz-Lozano JM, Azcón R, Gomez M (1996) Alleviation of salt stress by arbuscular-mycorrhizal *Glomus* species in *Lactuca sativa* plants. Physiol Plant 98:767–772

Schulte PJ (1993) Tissue properties and water relations of desert shrubs. In: Smith JAC, Griffiths H (eds) Water deficits: plant responses from cell to community. Bios Scientific, Oxford, UK, pp 177–192

Taiz L, Zeiger E (2006) Plant physiology, 4th edn. Sinauer, Sunderland, MA

Tyree MT, Cheung YNS (1977) Resistance to water flow in *Fagus grandifolia* leaves. Can J Bot 55:2591–2599