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Mycorrhizal promotion of host stomatal conductance in relation to irradiance and temperature

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Abstract Colonization of roots and soil by arbuscular mycorrhizal (AM) fungi sometimes promotes stomatal conductance (g_s) of the host plant, but scientists have had difficulty predicting or manipulating the response. Our objective was to test whether the magnitude of AM influence on g_s is related to environmental conditions: irradiance, air temperature or leaf temperature. Stomatal conductances of two groups of uncolonized sorghum plants were compared to g_s of plants colonized by *Glomus intraradices* (*Gi*) or *Gigaspora margarita* (*Gm*) in 31 morning and afternoon periods under naturally varying greenhouse conditions. Stomatal conductance of *Gi* and *Gm* plants was often markedly higher than g_s of similarly sized nonAM plants. AM promotion of g_s was minimal at the lowest irradiances and lowest air and leaf temperatures, but was substantial at intermediate irradiance and temperatures. AM promotion was again low or absent at the highest irradiances and temperatures. Magnitude of AM promotion of g_s was not a function of absolute g_s. Promotion of g_s by *Gi* and *Gm* was remarkably similar. Differing phosphorus fertilization did not affect g_s.

Keywords Arbuscular mycorrhizal symbiosis · *Gigaspora margarita* · *Glomus intraradices* · *Sorghum bicolor* · Water relations

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Introduction

Arbuscular mycorrhizal (AM) symbiosis often affects the stomatal behavior of host plants (Augé 2000). AM promotion of stomatal conductance to water vapor (g_s), a measure of bulk stomatal openness, has been reported for a number of host and fungal taxa growing in a wide variety of conditions. Experimental results, however, have been sporadic and no clear pattern emerges from the literature of when AM promotion of g_s will occur or how large the effect will be. Mycorrhizal improvement of nutrient uptake has sometimes resulted in increased g_s or transpiration, in that larger or better-nourished plants can show g_s different from those of smaller, nutrient-limited plants (Graham and Syvertsen 1984; Koide 1985; Fitter 1988). Yet AM plants have also shown higher gas exchange than nonAM plants of similar size and foliar P concentrations (Augé et al. 1986; Brown and Bethlenfalvay 1987; Davies et al. 1993). Several potential mechanisms have been investigated but none yet identified that adequately explain the oft-observed AM influence on g_s that occurs independently of host nutrition (Augé 2001).

Environmental variables have considerable influence on g_s (Salisbury and Ross 1985). AM symbiosis can moderate host response to the environment (Sylvia and Williams 1992; Sanchez-Díaz and Honrubia 1994; Shafer and Schoeneberger 1994), but mycorrhizal impact on g_s has rarely been examined as a function of most environmental variables. Stomata respond particularly rapidly to changes in light, temperature and soil moisture. AM influence on g_s has been examined frequently in relation to soil moisture but seldom in relation to light or temperature (Augé 2000). Since AM symbiosis is generally viewed as offering the host plant some measure of resilience to environmental extremes, we hypothesized that AM symbiosis would differentially affect g_s across a range of light and temperature, having the greatest effect at the low and/or high ends of the range. Further, since the AM fungal symbiont relies on host-derived photoassimilates, we speculated that AM promotion of g_s might be relatively higher at low irradiance. Rather than impose

controlled, stressful light and temperature conditions, we examined AM effects on stomatal behavior across a wide range of naturally occurring irradiance and temperature.

The purpose of this study was to gather information that may lead to a better understanding of AM influence on stomatal behavior of host plants, and to increase our ability to predict when AM effects on g_s are most likely to occur. We tested four predictions related to AM promotion of g_s: (1) AM promotion of g_s is higher under high and low light than under intermediate light, (2) AM promotion of g_s is higher at high air temperature than at low air temperature, (3) AM promotion of g_s is higher at high leaf temperature than at low leaf temperature, (4) AM promotion of g_s differs in the morning and afternoon. Predictions generally associated with mycorrhizal experiments of plant water relations were also tested: (5) g_s differs in AM and nonAM plants of similar size; (6) g_s differs in plants colonized by *Glomus intraradices* and *Gigaspora margarita*, (7) g_s differs in nonAM plants given low and high P. We examined plants under ample watered conditions to factor out the confounding influence of drying soils on g_s and focus on the influence of irradiance and temperature.

Materials and methods

Plant materials and culture

Ninety-six 1-l plastic pots were seeded with *Sorghum bicolor* L. cv DeKalb DK40Y on 23 April 2001 with about 70 seeds per pot. The potting medium was composed of 2 parts autoclaved silica sand/1 part soil (Sequatchie, fine-loamy, siliceous, thermic Humic Hapudults, pH 7.5). Twenty-four pots received pot culture colonized by *Glomus intraradices* Schenck and Smith INVAM isolate UT143 (*Gi*), 24 pots received pot culture colonized by *Gigaspora margarita* Gerdemann & Trappe INVAM isolate 215 (*Gm*), 24 pots received nonAM pot culture given weekly applications of P as 0.8 mM KH_2PO_4 (NL), and 24 pots received nonAM pot culture given weekly applications of P as 1.6 mM KH_2PO_4 (NH). *Gm* pot culture was roots and soil of 3-month-old *Zea mays* L. plants grown in a sandy, low-P soil, harvested and stored at 4°C for 6 months prior to inoculation. The *Gi*, NL and NH pot cultures were roots and soil from 11-month-old *S. bicolor* plants grown on the sand/soil medium described above. The nonAM pot cultures, grown in the same greenhouse as *Gi* pot cultures, were used to maintain similar soil water retention properties and encourage similar soil microflora among treatments. To equalize soil composition among treatments, medium for each experimental pot was composed of 240 ml fresh, sterile medium mixed with 60 ml of one of the live AM or nonAM pot cultures, plus 60 ml of autoclaved pot culture of each of the other three types of inoculum. A 150-ml layer of sterile medium was placed at both the top and the bottom of the pots to retard cross-contamination. Eighty-one days after planting (13 July 2001), plants were transplanted into 5.8-l pots using fresh, sterile medium of the composition described above (2 soil:1 sand, v:v).

With each watering, plants received a liquid macro- and micronutrient fertilizer at 10.7 mM N (Champion 15 N-0P-15K Alkaline Plus, Chilean Nitrate Co., Norfolk, Va., USA). Phosphorus was applied weekly during the experiment as 0.8 mM KH_2PO_4 to *Gi*, *Gm* and NL plants. Phosphorus was applied weekly as 1.6 mM KH_2PO_4 to NH plants. Variable rates of phosphorus were given to nonAM plants in an attempt to produce a group of plants similar in size to AM plants, and to characterize the influence of P fertilization on g_s. Plants were sheared back to crowns on 20 August 2001 and allowed to regrow. Plants were adequately watered throughout the experiment, which was conducted in a greenhouse in Knoxville, Tenn., USA.

Data collection

Fifteen replicates were selected from each treatment for study, based on visual appraisal of plant uniformity and vigor. Stomatal conductance, leaf temperature, photon irradiance and relative humidity were measured with a diffusion porometer (AP4, Delta-T Devices, Cambridge, UK) between 1100 and 1230 hours (morning measurements) and between 1400 and 1530 hours (afternoon measurements). Preliminary tests comparing mid-leaf, leaf tip and midway between tip and mid-leaf positions indicated that g_s was highest near leaf tips. During the experiment, g_s of three of the largest leaves of each plant were measured near leaf tips. To minimize effects of diurnal changes on treatment averages, one replicate of each of the four treatments was measured, then the second replicate of each treatment, until all measurements were completed. Ambient irradiance (photosynthetically active radiation, 400–700 nm) on leaves was measured with each measurement of g_s using the porometer's quantum sensor (accuracy confirmed with a recently calibrated quantum sensor prior to starting measurements; LI-190SA, LiCor, Lincoln, Neb., USA). Air temperature was measured at 1-s intervals and averages recorded each minute, with six shaded thermocouples spaced evenly throughout the canopy and connected to a datalogger (CR10x, Campbell Scientific, Logan, Utah, USA). Morning and afternoon measurements of g_s, irradiance, and air and leaf temperatures were conducted several times per week between 10 September and 4 October 2001.

In the first week of g_s measurements, one recently matured leaf was excised from each replicate of each treatment for measurement of phosphorus concentration [P] by spectrophotometry using the vanadate-molybdate-yellow method on samples dry-ashed with magnesium nitrate at 700°C for 2 h and digested in nitric acid (Chapman and Pratt 1961). Following the final measurements of g_s, the number of tillers was counted for each plant, and plants were sheared for measurement of shoot dry weight.

Hyphal, arbuscular and vesicular colonization of roots was determined on 100 microscope fields over several 1-cm root pieces randomly obtained from the root system (McGonigle et al. 1990), after clearing with 10% KOH in an autoclave at 121°C for 15 min, staining with Trypan blue for 1 h, and destaining. Soil hyphal density was measured on a 10-g soil sample from each replicate as described before (Bethlenfalvay and Ames 1987; Miller et al. 1995).

Experimental design and statistical analysis

Plants were arranged in a completely randomized block design with 15 replicates for each of four treatments, with one replicate of each treatment per block. ANOVA was performed using the MIXED procedure with repeated measures and linear contrasts, with means separated by Fisher's Protected LSD. Multiple correlations were performed using the REG procedure and correlation analyses summarized by Pearson correlation coefficients (r; SAS, Cary, N.C., USA).

Results

Plant and fungus attributes

AM and nonAM plants were similar in size, having similar shoot dry weights and numbers of tillers (Table 1). Fungal species did not affect plant size; *Gi* and *Gm* plants had similar shoot dry weights and numbers of tillers. Differing P fertilization did not affect size of nonAM

plants. NL plants had lower leaf [P] than NH, *Gi* and *Gm* plants.

Gi and *Gm* plants each developed substantial fungal colonization of roots (Table 1). Roots of *Gi* plants had more hyphae, more arbuscules and more vesicles than roots of *Gm* plants (*Gigaspora* does not develop vesicles). NonAM treatments remained nonmycorrhizal. Soil hyphal density was about 5.5-fold higher in AM than in nonAM soils (Table 1). Soil hyphal density was similar in soils colonized by *Glomus intraradices* and *Gigaspora margarita*, and soil hyphal density was similar in the two nonAM soils.

Environmental conditions

The experiment was conducted in a greenhouse to expose plants to a typical and fairly broad range of environmental conditions. During g_s measurements of individual leaves, irradiance ranged from 5 to 2,000 μmol m^{-2} s^{-1}, air temperature from 14.3 to 34.2°C and leaf temperature from 17.5 to 38.6°C. Leaf-air temperature differences for each measurement, which ranged from −3.1 to 12.8°C, were calculated as an additional means of characterizing foliar conditions and comparing responses of AM and nonAM foliage. Treatment averages for morning and afternoon irradiances and temperatures during the experiment are depicted in Fig. 1. Environmental conditions were similar between AM and nonAM treatments for all measurement periods (ANOVA results not shown). Relative humidity during porometry ranged from 40 to 70%.

Stomatal conductance

Varying P fertilization of nonAM plants had almost no effect on g_s (Fig. 2). Stomatal conductance was slightly higher in NH than in NL plants on the morning of day 261 and again on the afternoons of days 256 and 267. Stomatal conductance was higher in NL than in NH plants on the afternoons of days 276 and 277. Mean g_s of NH and NL plants for all measurement periods were 120 and

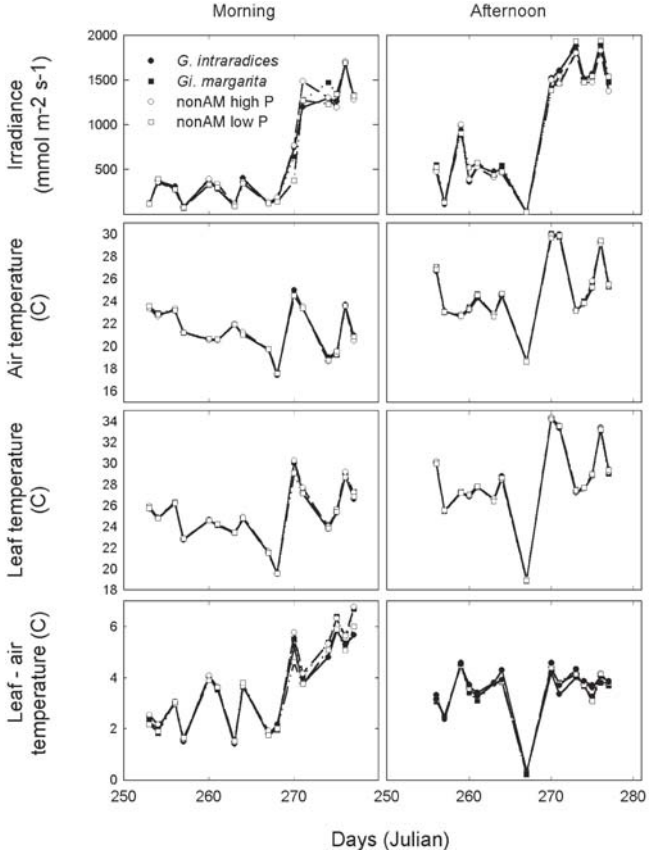

Fig. 1 Environmental conditions during measurement of g_s in the morning and afternoon periods: irradiance, air temperature, leaf temperature and leaf-air temperature difference. Symbols represent means of 15 replicates per treatment, three leaves per replicate (n=45). Day 250 was on 7 September 2001. Pooled standard errors of the means were smaller than the height of symbols for each of the eight panels. The AM versus nonAM and *Gi* versus *Gm* linear contrasts were not significant for any time period for any environmental variable. The NH versus NL contrast was not significant for any time period for any variable except for irradiance on the morning of day 270 and the afternoon of day 260 (*Gi* plants colonized by *Glomus intraradices*, *Gm* plants colonized by *Gigaspora margarita*, *NH* nonAM plants given high phosphorus, *NL* nonAM plants given low phosphorus)

Table 1 Shoot size, leaf phosphorus concentration [P], mycorrhizal colonization of roots (%) and soil (m per g dry soil). Values represent means of 15 replicates for each parameter (*Gi* plants infected with *Glomus intraradices*, *Gm* plants infected with *Gigaspora margarita*, *nonAM* nonmycorrhizal)

Treatment	Shoot dry weight g	Tillers per pot	[P] mg g^{-1}	Mycorrhizal colonization			
				Root hyphae	Arbuscules	Vesicles	Soil hyphae
Gi	9.1	62	3.4	61	18	9	1.35
Gm	8.0	65	3.3	52	8	0	1.32
NonAM high P	9.4	66	3.7	2	0	0	0.28
NonAM low P	7.8	70	2.2	1	0	0	0.21
Linear contrasts							
AM versus nonAM	NS	NS	*	**	**	**	**
Gi versus *Gm*	NS	NS	NS	*	*	*	NS
NonAM low versus high P	NS	NS	**	NS	NS	NS	NS

*P 0.05, **P 0.01, NS not significant

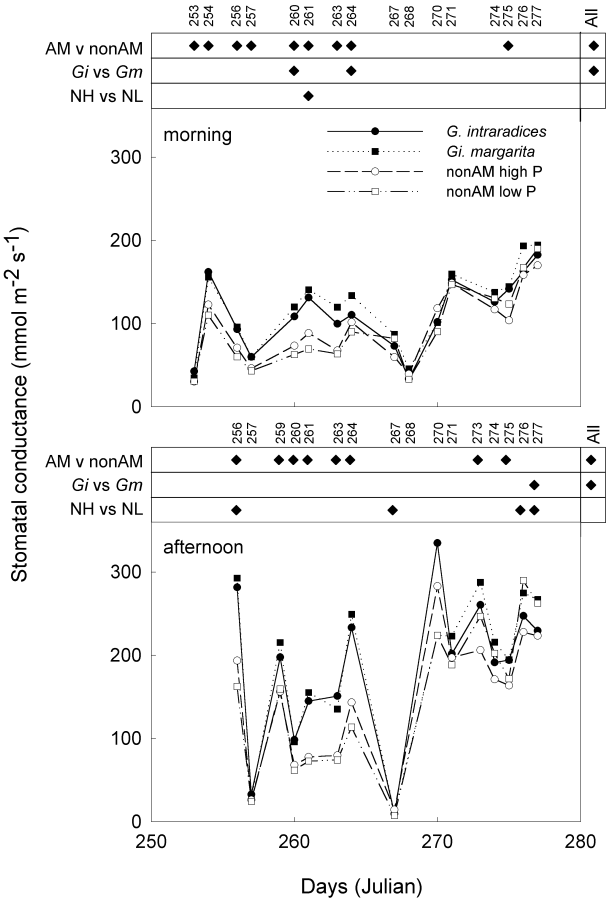

Fig. 2 Stomatal conductance of mycorrhizal and nonmycorrhizal plants in the mornings (*top panel*) and afternoons (*bottom panel*). Symbols represent means of 15 replicates per treatment, three leaves per replicate (n=45). Day 250 was on 7 September 2001. The table at the top of each panel shows significant treatment differences; ◆ indicates linear contrast was significant (P = 0.05) for the morning or afternoon measurement period of a particular day. Numbers along the top of each table identify measurement days (Julian days), and ◆ at *All* denotes that contrast was significant across all days. Pooled standard errors of the means were 2.7 and 5.0 mmol m^{-2} s^{-1} for the morning and afternoon panels, respectively (smaller than height of symbols)

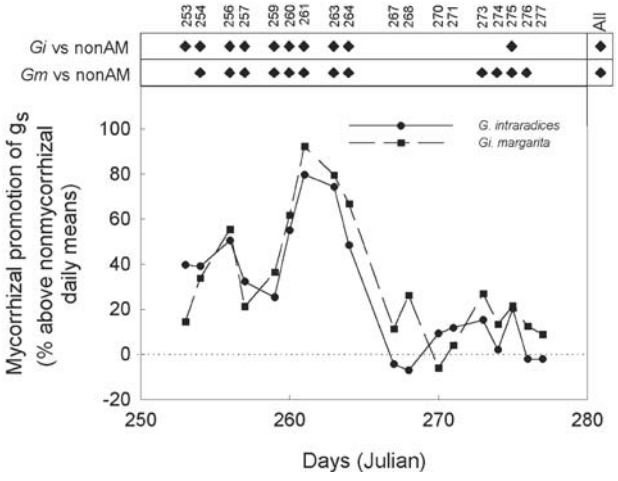

Fig. 3 Promotion of g_s by *Glomus intraradices* and *Gigaspora margarita* relative to nonAM plants, with morning and afternoon data sets averaged. Mycorrhizal promotion of g_s was calculated as a percent from data portrayed in Fig. 2: (absolute g_s of *Gi* or *Gm* plants − mean g_s of nonAM plants)/mean g_s of nonAM plants × 100. A value of 0% promotion denotes that daily average g_s of *Gi* or *Gm* plants was similar to that of nonAM plants. For 14 of the days, symbols represent means of 30 replicates per treatment, three leaves per replicate, n=90 (see Fig. 2 for days on which g_s was measured both morning and afternoon); n=45 for the other 4 days. ANOVA table at the top of the panel as in Fig. 2. Pooled standard errors of the means was 1.8% (smaller than height of symbols)

122 mmol m^{-2} s^{-1}, respectively. Because g_s was similar in NH and NL plants throughout the experiment, the stomatal behavior of AM plants is compared below to the average of nonAM (NH + NL) plants.

Mycorrhizal colonization by either fungus markedly increased g_s of host plants during much of the experiment (Fig. 2 and Fig. 3). Stomatal conductance was significantly higher in AM than in nonAM plants for 9 of the 16 morning measurement periods and in 8 of the 15 afternoon measurement periods (Fig. 2). NonAM plants never exhibited higher average g_s than AM plants during the experiment. *Gi* and *Gm* plants had mostly similar g_s, with g_s slightly higher in *Gm* than in *Gi* plants on two mornings and one afternoon. Mean g_s of *Gi* and *Gm* plants for all measurement periods were 147 and 156 mmol m^{-2} s^{-1}, respectively. Average morning and afternoon g_s across all treatments were 107 and 167 mmol m^{-2} s^{-1}, respectively.

Promotion of g_s by *Glomus intraradices* and *Gigaspora margarita*, relative to nonAM plants (average of NH and NL plants), was quite similar in the mornings and afternoons and is portrayed in Fig. 3 as the average of the morning and afternoon measurement periods. AM promotion of g_s was substantial during roughly the first quarter of the experiment (36%, averaged for *Gi* and *Gm* over days 253–257), quite marked during the second quarter (AM average of 70% over days 260–264), lacking during the third quarter (AM average of 5% over days 267–271 with no significant *Gi* or *Gm* promotion on any of those days), and moderate during the final quarter (AM average of 11% over days 273–277). Viewed in terms of daily averages, the less pronounced AM promotion of g_s in the latter half of the study coincided with higher light and temperature levels. The difference in AM promotion of g_s in the first compared with the second quarter of the experiment was not associated with differences in mean daily irradiance, air or leaf temperatures.

Gi promoted g_s more during the first week of the experiment than during the last week, whereas *Gm* promoted g_s on four of the first five measurement days as well as on four of the last five measurement days. Comparing promotion of g_s over the course of the experiment using repeated measures analysis, *Gm* had a greater overall influence than *Gi*: 34% versus 27% (P=0.003), respectively. Overall, however, there was a striking coherence between *Gi* and *Gm* plants; promotion

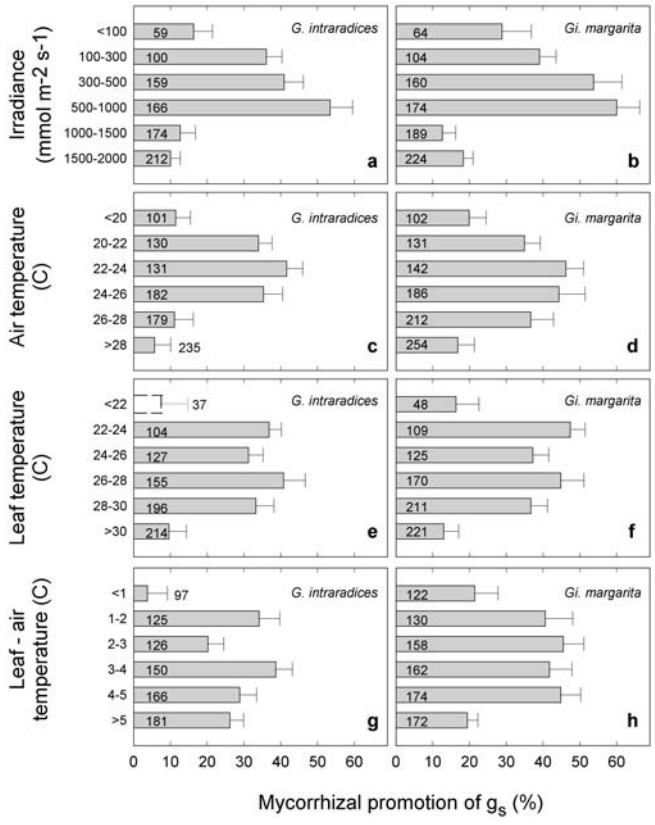

Fig. 4 Mycorrhizal promotion of stomatal conductance (g_s) as a function of irradiance and temperature. All measurements (all leaves, both diurnal time periods of all days of experiment) were ordered by irradiance and depicted at six irradiance levels (**a**, **b**). *Bars* represent means (+SE) of each irradiance range, n=105–336. All measurements were similarly re-ordered for the subsequent three pairs of panels; ordered by air temperature (**c**, **d**; n=101–309 for the six sub-ranges of air temperature), ordered by leaf temperature (**e**, **f**; n=126–293 for the six sub-ranges of leaf temperature), and ordered by leaf-air temperature difference (**g**, **h**; n=130–336 for the six sub-ranges of leaf-air temperature). Mycorrhizal promotion of g_s was calculated as described in the text. Numbers within histogram bars are the means of absolute g_s for each sub-range. Dotted line in panel e enclosing bar for <22 sub-range of leaf temperature signifies negative number (mycorrhizal promotion of g_s = −7%)

of g_s by the two fungal genera was mostly similar both in timing and degree.

The results in Fig. 4 illustrate AM promotion of g_s within particular irradiance and temperature ranges (sub-ranges within the broader, entire range for an environmental parameter). For each panel, measurements of all leaves for all mornings and afternoons of the experiment were ordered by the parameter represented on the y axis of that panel, and means calculated and depicted for six sub-ranges of the parameter. Generally, AM colonization promoted g_s less at either end of the entire range of an environmental parameter. For instance, for irradiance below 100 and above 1000 μmol m^{-2} s^{-1}, *Gi* promotion of g_s was 16% or less, while between 100 and 1000 μmol m^{-2} s^{-1}, *Gi* promotion of g_s ranged from 36 to 53% (Fig. 4a). The same trend was evident for *Gm* plants (Fig. 4b), although *Gm* promotion of g_s was still fairly strong below 100 μmol m^{-2} s^{-1}. As irradiance increased in increments to 1000 μmol m^{-2} s^{-1}, *Gm* promotion also increased, from 29% below 100 μmol m^{-2} s^{-1}, to 39% at 100–300 μmol m^{-2} s^{-1}, to 54% at 300–500 μmol m^{-2} s^{-1} and to 60% at 500–1000 μmol m^{-2} s^{-1}. *Gm* promotion of g_s then dropped to 18% or less in the 1000–1500 and 1500–2,000 μmol m^{-2} s^{-1} sub-ranges.

Similar trends were observed when viewing AM promotion of g_s as a function of both air and leaf temperatures (Fig. 4c–f). At air temperatures below 20 and above 26°C, *Gi* promotion of g_s was modest (11% or below). But in the three intermediate air temperature sub-ranges, *Gi* had a much greater influence, promoting g_s by 34–42%. Again, the pattern was similar in *Gm* plants. The lowest promotion of g_s occurred at each end of the range and the highest promotion at more moderate air temperatures. For both *Gi* and *Gm* plants, promotion of g_s was minor or absent at the most extreme leaf temperature sub-ranges, below 22 and above 30°C. But in the intermediate leaf temperature sub-ranges, *Gi* and *Gm* each had a much greater influence, promoting g_s by 31–47%. The AM effect on g_s was also mostly diminished at either edge of the range of leaf-air temperature difference, especially in *Gm* plants (Fig. 4g, h). At leaf-air temperature differences of <1 or more than 5°C, *Gm* promoted g_s by about 20% or less. When leaf-air temperature difference was within the range of 1–5°C, *Gm* promoted g_s by about 40% or more.

Irradiance was significantly but not closely correlated with air or leaf temperatures (correlation coefficients of 0.39 and 0.58, respectively), and the curvilinear AM promotion of g_s occurred independently among the environmental variables. For example, looking at just g_s measurements made at irradiances of 100–1000 μmol m^{-2} s^{-1}, an irradiance range across which promotion increased (Fig. 4a, b), both *Gi* and *Gm* promotion of g_s across the air temperature range was still lowest at each edge of the range and highest in the middle of the range (plot not shown).

Numbers within bars in Fig. 4 show mean absolute g_s within each sub-range. The curvilinear AM effect over the ranges of irradiance and temperatures was not associated with extent of stomatal openness; it was not simply a result of diminished ability to detect AM effects due to low g_s. The AM effect, which was less pronounced at the lowest extreme of irradiance, air and leaf temperatures, was also less marked at the higher extremes of irradiance, air and leaf temperatures, despite the fact that absolute values of g_s were at their highest in the upper irradiance and temperature ranges. AM promotion of g_s was not linked directly with absolute g_s (Fig. 5). In some prior studies, AM promotion of g_s relative to nonAM controls occurred only at the highest absolute g_s encountered in an experiment (Ebel et al. 1996; Green et al. 1998), but that was not the case in the current study.

Correlation analyses of all individual leaf data across all treatments confirmed the curvilinear nature of AM promotion of g_s depicted in Fig. 4. There was a significant quadratic correlation of AM promotion of g_s with each of

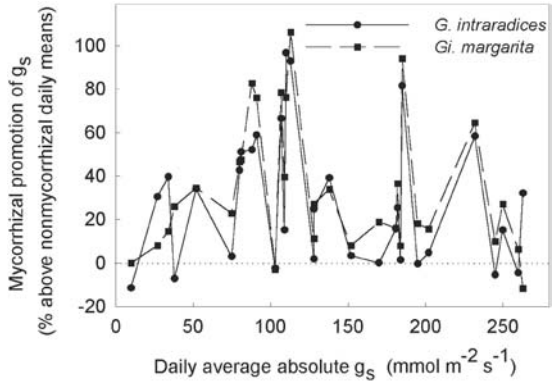

Fig. 5 Mycorrhizal promotion of g_s as a function of absolute g_s (daily average of all g_s measurements of all leaves of all treatments). Symbols represent means of 15 replicates per treatment, three leaves per replicate (n=45)

the four environmental parameters (P = 0.001 for each) although r values were small (0.09–0.18). Absolute g_s was linearly correlated with each environmental parameter (P = 0.001 for each, r = 0.16–0.44). Neither relative g_s (magnitude of AM promotion) or absolute g_s was correlated with leaf [P].

Observations related to the predictions made were as follows: (1) AM promotion of g_s was higher under moderate irradiance than under the lowest or highest irradiances. (2) AM promotion of g_s was higher at moderate air temperatures than at the lowest or highest air temperatures. (3) AM promotion of g_s was higher at moderate leaf temperatures than at the lowest or highest leaf temperatures. (4) AM promotion of g_s was similar in the morning and afternoon periods. (5) g_s differed markedly in AM and nonAM plants of similar size. (6) Promotion of g_s by *Gi* and *Gm* was remarkably similar both in timing and degree, but *Gm* promoted g_s slightly more than *Gi* over the course of the experiment. (7) g_s was similar in nonAM plants given low and high P and having significantly different leaf [P].

Discussion

Mycorrhizal effects on stomatal behavior have been unpredictable, occurring in about 40% of experiments involving amply watered AM and nonAM plants of similar size (Augé 2000). Where AM symbiosis has affected stomatal behavior, it almost always increased g_s or transpiration. Mycorrhizae-induced increases in g_s are often subtle, but g_s of AM plants twofold higher than those of nonAM plants have been recorded (e.g., Allen 1982; Allen and Boosalis 1983), as occurred in the current experiment. Stomatal conductance of sorghum cultivars other than DeKalb DK40Y (used in the current experiment) has previously been affected by colonization by *Glomus mosseae* and *Glomus macrocarpum* (Sieverding 1984, 1986) but not by *Glomus intraradices* (Ibrahim et al. 1990; Ebel et al. 1994; Augé et al. 1995). In other host species, *Gi* plants have had higher g_s than nonAM plants (Augé et al. 1986, 1992; Ebel et al. 1997), as have *Gm* plants (Wang et al. 1989).

Our goal is to increase ability to forecast mycorrhizal effects on g_s: when promotion of g_s will occur and how large the effect will be. The specific objective of the current experiment was to determine whether AM symbiosis promoted g_s more often or to a greater degree under some environmental conditions than others. To enhance statistical resolving power, we included several replicates, several subsamples, and several measurement periods: g_s measurements of 45 leaves per treatment for each of 31 measurement periods. The hope was to observe differences in g_s between AM plants and similarly sized nonAM plants on at least some occasions, so that we might be able to relate the presence or absence of a mycorrhizal effect to certain light or temperature conditions. We were surprised to observe that both fungi resulted in frequent and quite high promotion of g_s, which enabled us to fine-tune the analysis and relate size of the promotion to particular levels of irradiance and temperature.

Symbiosis by both AM fungal species enhanced stomatal opening of host plants to the highest extent under moderate environmental conditions. AM promotion of g_s was relatively low at the lowest irradiance and temperature levels occurring naturally in this greenhouse experiment. The AM effect was also relatively small at irradiance levels above 1,000 mmol m^{-2} s^{-1} and above leaf temperatures of 30°C. These findings suggest that researchers may be less likely to observe mycorrhizal effects on g_s at either edge of the light and temperature ranges encountered by plants in their particular experimental conditions.

Stomatal conductance was not the focus of an earlier greenhouse study of drought tolerance but was measured in amply watered AM and nonAM plants on 4 days before the drought treatment began (Augé et al. 2001). Over the entire range of irradiance encountered (25–820 μmol m^{-2} s^{-1}), g_s of *Gi* plants relative to nonAM plants was lowest at the lowest irradiance (<100 μmol m^{-2} s^{-1}) for each of three host species, cowpea, bush bean and soybean. This was not a result of absolute g_s being so low that it obscured the ability to resolve AM effects. Absolute g_s (which averaged 262–590 mmol m^{-2} s^{-1} among treatments and species over the 4 days) was similar or only somewhat lower at <100 μmol m^{-2} s^{-1} than in the other irradiance sub-ranges for each of the three host species.

Augé (2000) (Table 1) summarized 33 studies reporting g_s data for amply watered AM and nonAM plants of similar size. These reports provided light and temperature ranges or set points under which plants were grown but did not give values at the time of each g_s measurement. Additionally, prior studies typically did not involve large numbers of measurements. Therefore, it is not possible to scrutinize the prior literature for promotion of g_s by AM symbiosis as a function of light or temperature. But it is possible to look for trends. For instance, AM symbiosis promoted g_s or transpiration in at least one host species in

all of the eight studies conducted in environmentally controlled growth chambers, while AM symbiosis promoted g_s or transpiration in only three of the 22 studies conducted in greenhouses. This may indicate that AM symbiosis is more likely to promote g_s when plants are growing in moderate environments. Irradiance was in the range of 160–500 μmol m^{-2} s^{-1} for each of the eight growth chamber studies. In our greenhouse experiment, irradiance ranged from near 0 to 2,000 μmol m^{-2} s^{-1}, as it did for many of the greenhouse experiments referred to above, but AM promotion of g_s was particularly strong within the moderate irradiance range that occurred in the growth room studies.

NH and NL plants had quite similar g_s in the current experiment, despite the fact that among all treatments, differences in shoot size and especially leaf [P] were largest between these two treatments. This, in conjunction with the strong coherence between fungal genera, increases confidence in the findings of substantial promotion of g_s by AM symbiosis. Whatever caused AM plants to have higher g_s than nonAM plants was evidently invoked in a similar way by *Gi* and *Gm*.

Although the shape of the plots was quite similar, *Gm* promoted g_s to a slightly higher degree than did *Gi* over the course of the experiment, and AM promotion of g_s at the highest and lowest levels of irradiance, air temperature and leaf temperature tended to persist more strongly in *Gm* plants than in *Gi* plants. In a previous work, g_s was substantially higher in *Gm* than in smaller *Gi* plants, both before and after a drought episode (Dixon et al. 1994).

Why would the magnitude of AM promotion of g_s be related to light? One reason may involve the influence of the symbiosis on carbon dynamics of the host. Concentrations of CO_2 in leaves and sink strength each act as physiological regulators of gas exchange (Thorne and Koller 1974; Herold 1980; Mansfield et al. 1981), and AM symbiosis can affect both internal CO_2 concentrations (Sánchez-Díaz et al. 1990) and sink strength (Douds et al. 2000). It is conceivable that at low irradiance, low to negligible net carbon exchange rates may be insufficient to cause internal CO_2 concentrations to differ in AM and nonAM plants, resulting in similar g_s. If the effect of internal CO_2 concentrations or sink strength on g_s is attenuated at high irradiance, perhaps damped out by high photosynthetic rates, this may also limit AM promotion of g_s at high irradiance. These remarks are quite speculative but suggest a direction for further study.

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