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Chris A. Martin · Jean C. Stutz

Interactive effects of temperature and arbuscular mycorrhizal fungi on growth, P uptake and root respiration of *Capsicum annuum* L.

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Abstract *Capsicum annuum* (pepper) plants were inoculated with the arbuscular mycorrhizal (AM) fungi *Glomus intraradices* Smith and Schenck, an undescribed *Glomus* sp. (AZ 112) or a mixture of these isolates. Control plants were non-mycorrhizal. Plants were grown for 8 weeks at moderate (20.7–25.4°C) or high (32.1–38°C) temperatures. Colonization of pepper roots by *G. intraradices* or the *Glomus* isolate mixture was lower at high than at moderate temperatures, but colonization by *Glomus* AZ112 was somewhat increased at high temperatures. Pepper shoot and root dry weights and leaf P levels were affected by an interaction between temperature and AM fungal treatments. At moderate temperatures, shoot dry weights of plants colonized by the *Glomus* isolate mixture or non-AM plants were highest, while root dry weights were highest for non-AM plants. At high temperatures, plants colonized by *Glomus* AZ112 or the non-AM plants had the lowest shoot and root dry weights. AM plants had generally higher leaf P levels at moderate temperatures and lower P levels at high temperatures than non-AM plants. AM plants also had generally higher specific soil respiration than non-AM plants regardless of temperature treatment. At moderate temperatures, P uptake by all AM plants was enhanced relative to non-AM plants but there was no corresponding enhancement of growth, possibly because less carbon was invested in root growth or root respiratory costs increased. At high temperatures, pepper growth with the *G. intraradices* isolate and the *Glomus* isolate mixture was enhanced relative to non-AM controls, despite reduced levels of AM colonization and, therefore, apparently less fungal P transfer to the plant.

C. A. Martin (✉) · J. C. Stutz
Department of Applied Biological Sciences/East,
Arizona State University,
Wanner Hall, 7001 E. Williams Field Road, Mesa,
AZ 85212-0180, USA
e-mail: chris.martin@asu.edu
Tel.: +1-480-7271247
Fax: +1-480-7271011

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Introduction

Pepper is one of many horticultural crops grown primarily in mid-latitudes and it is sensitive to high temperatures (Wheeler et al. 2000). The reported optimal range for vegetative and reproductive development of pepper plants is 21–33°C (Rylski and Spigelman 1982). Biologically supraoptimal temperatures above 34°C have been shown to cause marked reduction in pepper plant productivity (Erickson and Markhart 2002) that may be related to shifts in carbon allocation caused by increased respiratory costs.

Roots of pepper normally form symbiotic associations with arbuscular mycorrhizal (AM) fungi (Davies et al. 1992). In mycorrhizal associations, plants supply labile photosynthates to fungi, while fungi aid in the uptake of nutrients, especially P. The potential for AM fungi to increase plant growth under conditions of low soil P has been well documented (Augé 2001). However, some *Glomus* isolates have been shown to stimulate plant growth independent of plant P nutrition or when P is non-limiting (Davies et al. 1993; Fidelibus et al. 2001). Inoculum mixtures of two or more *Glomus* isolates might have an additive, intermediate, or negative effect on plant growth compared with single *Glomus* isolates as an inoculum source (Davies et al. 2000).

Warm soil conditions differentially alter AM fungal activity. Root colonization by AM fungi often decreases when the temperature exceeds 30°C (Bowen 1987), and soil temperatures above 40°C are generally lethal to AM fungi (Bendavid-Val R et al. 1997). For example, the germination of spores of *Glomus coralloidea* and *G. heterogama* was found to decrease above 34°C (Schenck et al. 1975). The presence of AM fungal arbuscules in soybean roots was found to decrease above 30°C, while production of external hyphae outside soybean roots was found to decrease above 34°C (Schenck and Schröder

1974). Haugen and Smith (1992) reported that colonization of cashew (*Anacardium occidentale*) roots by *G. intraradices* declined above 30°C and was severely reduced at 38°C.

The effect of supraoptimal temperatures on AM fungal colonization of pepper roots and subsequent pepper plant growth are unknown. Our objective was to investigate effects of two *Glomus* isolates, applied either singly or as a mixture, on growth of pepper at two temperature regimens (moderate and high) under non-limiting P conditions. Based on the reports cited above, we predicted that supraoptimal high temperatures would have a negative effect on *Glomus* colonization of pepper roots and plant growth. Furthermore, we provided additional P to the soil of non-AM control plants in order to examine changes in carbon allocation at high temperatures of AM and non-AM pepper plants that were independent of P nutrition (Davies et al. 1993).

Materials and methods

Pepper seedlings were potted into 3-l containers filled with an autoclaved substrate mixture of river sand, coarse silica sand (particle diameter $\leq$4.1 mm) and Gilman clay loam (pH 7.3, EC 0.25 dS/m, 11.3 g P per kg soil, 13.2 g organic matter per kg soil) (3:2:1 v/v/v). At potting, seedling plants were inoculated with either an isolate (63D) of *G. intraradices* from Santa Teresa, N. M., USA (elevation 1,200 m, mean daily maximum air temperature July 33°C/January 11°C), an isolate (25A) of an undescribed *Glomus* species designated AZ 112 from Wittmann, Ariz., USA (elevation 425 m, mean daily maximum air temperature July 40°C/January18°C), a mixture of these two *Glomus* isolates, or a non-AM control. For each isolate, AM fungal cultures were established using *Sorghum sudanense* (Sudan grass) as a host plant, and inoculum potentials were determined using the most probable numbers assay (Alexander 1982) prior to the experiment. AM fungal inocula, which consisted of roots and the soil/sand mix of the culture, were diluted to provide equal inoculum densities (approximately 1.7×10^5 propagules per pot). Control pots received 100 ml of an inoculum washing that had been passed through a 45-μm sieve.

Plants were then grown for 8 weeks in a walk-in growth chamber calibrated to provide a moderate (20.7°C min/dark period to 25.4°C max/light period) or high (32.1°C min/dark period to 38°C max/light period) temperature environment. Each temperature treatment was ramped from minimum and maximum set points in a 24-h sinusoidal wave pattern. Air and soil temperatures were monitored and found to be similar. The growth chamber was illuminated at 400 μmol/m^2/s for 16 of 24 h by an equal number of mercury vapor and high pressure sodium lamps. Relative humidity was regulated at near 30%. All plants received 8.3 g of isobutylidene diurea controlled-release fertilizer (20 N, 0 P, 16.6 K, 2 Fe, 1.4 Mn). AM and non-AM plants also received a 200-ml solution of P at 22 and 44 μl/l, respectively, on a weekly basis (Davies et al. 1993). All plants were watered with de-ionized water acclimated to growth-room temperatures when soil water content reached a management-allowed-deficit value of 20% (Welsh and Zajicek 1993); thus, plants were not subject to water stress.

Prior to harvest, root respiratory efflux of CO_2 gas from the surface of each pot was measured during a 4-h interval in the middle of the light period, when growth chamber temperatures were highest, using a LI 6000-09 soil respiration chamber (LI-COR Inc., Lincoln, Neb., USA) attached to a LI-6200 portable photosynthesis system. Root respiration per pot was derived from the mean of four CO_2 flux measurements. Specific root respiration (Rsp) was calculated as total CO_2 efflux from the pot substrate surface divided by root dry weight and expressed as μmol CO_2/s/g root tissue dry weight.

At harvest, root systems were washed and 1-cm root segments were sampled and stained in acid fuchsin (Kormanik and McGraw 1982). AM fungal colonization was assessed using the magnified intersections method (McGonigle et al. 1990). Plants were separated into roots and shoots, oven dried and weighed. Phosphorus concentrations in dry, pulverized leaf tissue were determined by the ascorbic method (Watanabe and Olson 1965).

A split-plot experimental design was replicated five times with two temperature treatments as the main plots and four AM fungal treatments as subplots. An analysis of variance of treatment main effects and interactions was calculated for all data using general linear models procedures (SAS version 6.03, Cary, N.C., USA). Percent AM fungal total colonization and arbuscule and vesicle formation data were arc-sin transformed to approximate a normal distribution for analysis of treatment effects. Actual percentage means were reported.

Results and Discussion

Total colonization and arbuscule and vesicle formation in pepper roots were affected by an interaction of temperature with AM fungal treatments (Table 1). Values for these parameters in roots colonized by *G. intraradices* and the *Glomus* mixture were lower at high temperatures than at moderate temperatures. These findings support an earlier report by Udaiyan et al. (1996) that root colonization of *Acacia farnesiana* by *G. fasciculatum* and *G. geosporum* was negatively correlated to increasing air temperature. In contrast, we found that total colonization and arbuscule formation in roots colonized by *Glomus* AZ112, an isolate from a warmer climate than the *G. intraradices* isolate, appeared to be slightly increased by high temperatures. We observed no vesicles in roots colonized by the *Glomus* AZ112 isolate at either temperature treatment. All non-AM control plants remained non-mycorrhizal.

Mycorrhizal fungi can enhance P uptake by pepper (Davis et al. 2000). Accordingly, to be able to detect non-P related AM fungal effects on plant growth, we gave all non-AM plants twice as much P fertilizer in an attempt to compensate for an expected AM enhancement of plant P uptake. Despite this attempt at compensation, pepper leaf P levels were affected by an interaction of temperature and AM fungal treatments (Table 1). At moderate temperatures, leaf P levels of mycorrhizal plants were about 1.4 times higher than those of non-AM plants, but were about 20% lower than non-AM plants at high temperatures. Leaf P of non-AM control plants at moderate and high temperatures was similar.

Pepper shoot and root dry weights were also affected by an interaction of temperature and AM fungal treatments (Table 1). At moderate temperatures, shoot dry weights of plants colonized by the *Glomus* isolate mixture or the non-AM control plants were about 1.3 times higher than of those colonized by *G. intraradices* or *Glomus* AZ112. In contrast, shoot dry weights at high temperatures were highest for plants colonized by the *Glomus* isolate mixture or the *G. intraradices* isolate and lowest for plants colonized by the *Glomus* AZ112 isolate. At moderate temperatures, root dry weights of non-AM

Table 1 Percentages of arbuscules and vesicles, and total colonization of roots, shoot dry weight, root dry weight, specific root respiration (*Rsp*), and leaf phosphorus (*P*) concentration of pepper plants at moderate (20.7–25.4°C) or high (32.1–38.0°C) temperatures inoculated with *Glomus intraradices*, *Glomus* AZ112, or an inoculum mixture containing both AM fungi isolates, or not inoculated as control. Significance is shown as non-significant (ns) or significant at the 5% (*), 1% (**), or 0.1% (***) levels

Treatment	Arbuscules %	Vesicles %	Colonization %	Shoots g per plant	Roots g per plant	Rsp μmol/s/g	Leaf P mg/g
Moderate (20.7–25.4°C)							
Glomus intraradices	29.9	16.1	42.3	2.3	1.0	0.068	2.3
*Glomus*AZ112	13.4	0	15.1	2.3	1.2	0.057	3.5
Mixture	45.0	20.4	54.2	3.0	1.3	0.058	2.8
Control	0	0	0	2.9	1.5	0.035	2.0
High (32.1–38°C)							
G. intraradices	14.9	3.2	20.1	3.4	1.0	0.065	1.8
*Glomus*AZ112	18.9	0	19.7	1.9	0.8	0.054	1.9
Mixture	6.4	2.9	12.3	3.8	1.4	0.057	1.6
Control	0	0	0	2.6	0.9	0.047	2.2
Significance							
Temperature	**	**	***	ns	**	ns	***
AM fungi	ns	**	**	***	**	***	**
Temperature × fungus	**	*	**	***	**	ns	***

plants were 1.2–1.5 times higher than those of AM plants. But at high temperatures, root dry weights were highest for plants colonized by the *Glomus* isolate mixture or the *G. intraradices* isolate and lowest for non-AM plants or those colonized by the *Glomus* AZ112 isolate.

The Rsp of pepper plants was affected by mycorrhizal treatment (Table 1). Specifically, Rsp of AM plants was about 1.5 times higher than that of non-AM control plants. Surprisingly, Rsp was not affected by either the temperature treatments or an interaction of temperature and AM treatment.

It is not unusual for some *Glomus* isolates to suppress host plant growth under non-limiting P conditions (Peng et al. 1993; Augé 2001). At moderate temperatures, we found that all three mycorrhizal treatments tended to suppress pepper root growth compared with non-AM plants. One possible explanation for these results is that the fungus supplied the host plant with sufficient nutrients, reducing the demand for carbon from the host plant to stimulate growth. However, mycorrhizal plants had higher Rsp than non-AM control plants at both temperature treatments. This suggests, alternatively, that the carbon cost/benefit ratio of the AM association to pepper plants was high and/or that fungal activity was high (Graham et al. 1996). This situation may have occurred especially at moderate temperatures for plants colonized by *G. intraradices*, which had higher Rsp and lower root dry weights than non-AM controls.

At high temperatures, *G. intraradices* and the *Glomus* isolate mixture enhanced overall plant growth relative to non-AM control plants. This enhancement of plant growth by *G. intraradices* and the *Glomus* isolate mixture occurred despite the apparent lack of an increase in P uptake. Moreover, there was a synergistic growth enhancement associated with the *Glomus* isolate mixture at high temperatures that was not wholly achieved by inoculation with either *Glomus* isolate alone. One possible explanation for this response is that there was less P transfer to the host plant due to lower colonization levels, causing pepper plants to invest more carbon in roots, as seen by their higher root mass relative to plants with the other AM treatments. Further, *Glomus* AZ112 was not an efficient isolate at high temperatures; it failed to form vesicles and plants colonized by this isolate had lower shoot and root growth than plants colonized by the other AM fungal treatments.

The results obtained support our prediction that high temperatures would reduce AM fungal colonization of pepper roots and reduce P uptake, except in the case of the *Glomus* AZ112 isolate, which was not efficient at either temperature range, despite having originated from relatively warm climatic conditions. In contrast, the *G. intraradices* isolate was more efficient at enhancing shoot growth at high than moderate temperatures, while the *Glomus* isolate mixture was relatively efficient at enhancing shoot growth at both moderate and high temperatures. Further research is needed to elucidate mechanisms for these differential AM effects on growth of pepper plants at high temperatures and their relationship to pepper plant P nutrition and carbon allocation.

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