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Plant responses to arbuscular mycorrhizae under elevated temperature and drought

Phyllis H. Pischl[1,*] *and Nicholas A. Barber*[1,2]

[1] *Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115, USA*
[2] *Institute for the Study of the Environment, Sustainability, and Energy, Northern Illinois University, DeKalb, IL 60115, USA*
*Correspondence address. Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115, USA. Tel: +815-753-4215; Fax: +815-753-0461; E-mail: ppischl@niu.edu

Abstract

Aims
Climate change is predicted to cause both increased temperatures and changes in precipitation, leading to more severe droughts in some areas. How these changes will affect plant growth may depend in part on biotic context. Most vascular plants form symbiotic relationships with arbuscular mycorrhizal fungi (AMF), root symbionts that provide soil nutrients to plants in exchange for carbohydrates, which may reduce the effects of environmental stresses on plants. We investigated if AMF modified temperature and drought effects on plant growth, fitness and defenses against herbivory.

Methods
We manipulated AMF presence, temperature and water availability on bell pepper plants (*Capsicum annuum* L.) in a field setting to measure plant growth and fitness responses. In a growth chamber experiment, we also investigated if AMF influenced insect herbivores feeding on plants at elevated temperatures.

Important findings
Drought consistently reduced plant growth, and AMF did not change drought impacts. However, with sufficient water, AMF tended to benefit plant growth and flower production (but not fruit production) compared to non-mycorrhizal plants. In the growth chamber, temperature and AMF influenced plant protein and phosphorus contents, but not defensive chemistry or herbivore performance. Thus, AMF may ameliorate the effects of temperature stress due to climate change on plants by increasing growth and nutrient content, but these effects do not extend to the constitutive herbivory defenses examined here.

Keywords: aboveground-belowground, arbuscular mycorrhizal fungi, climate change, drought, herbivore, indirect effects

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INTRODUCTION

Anthropogenic climate change will have widespread effects on the abiotic conditions that organisms experience, including increased atmospheric CO_2, drought and elevated temperatures (IPCC 2014). Although annual rainfall may actually increase in some regions, timing of precipitation is predicted to change, leading to more frequent droughts during the growing season (Kling *et al.* 2003). Combined with increased moisture loss under higher temperatures from greater evaporation of soil and surface water and transpiration (Wang *et al.* 2011), these changes represent significant stresses for many plants. Although higher CO_2 concentrations can increase photosynthesis, this benefit is reduced in high temperatures as plants close stomata to conserve water (Ruiz-Vera 2013). However, biotic interactions may modify the effects of abiotic stresses. The symbiotic relationship between arbuscular mycorrhizal fungi (AMF) and plants may ameliorate the stress effects of climate change on plant growth and fitness (Kivlin *et al.* 2013).

AMF form symbiotic relationships with vascular plants' roots and extend the area from which plants obtain soil resources. AMF provide water and minerals (particularly P and N) in exchange for carbohydrates produced photosynthetically (Smith and Read 2008). Root colonization by AMF can benefit plants under drought conditions, increasing plant biomass and root:shoot ratio (Compant *et al.* 2010; Davies *et al.* 2002; Ortiz *et al.* 2015), and a meta-analysis found that AMF consistently reduce the negative growth effects of drought on plants (Worchel *et al.* 2013). A meta-analysis of fungal symbiont effects found that AMF effects on average do not influence plant biomass responses to temperature stress

(Kivlin *et al.* 2013). But the influence of AMF on temperature stress in plants varies among species. For example, Bunn *et al.* (2009) showed a growth benefit of AMF under elevated temperatures, but only for a thermophilic plant species. Different AMF species and isolates may also respond differently to temperature and precipitation variation (Davies *et al.* 2002; Ortiz *et al.* 2015).

Mycorrhizae may also affect the interactions of their host plants with insect herbivores (Barber *et al.* 2013; Kempel *et al.* 2010; Koricheva *et al.* 2009) through at least two mechanisms. First, AMF may increase tolerance by providing nutrients to host plants that mitigate herbivory losses (Bennett *et al.* 2009). Second, AMF can enhance resistance traits such as defensive chemistry (Kempel *et al.* 2010; Pozo and Azcon-Aguilar 2007; Vannette and Rasmann 2012). We focus on this second mechanism by measuring two chemical defense traits and herbivore growth.

The goal of this project was to investigate the effects of AMF on plants under predicted stresses of climate change. A field study investigated the interactions of AMF, drought and elevated temperature on plant growth and fitness. A growth chamber study examined AMF effects on plant resistance under ambient and elevated temperatures. We predicted that drought and elevated temperature would negatively affect plant performance and that plants under elevated temperatures would have reduced resistance, but AMF would mitigate these stress effects.

METHODS

Study system

Capsicum annuum L. is an annual plant native to the Americas, and its various cultivars are important agricultural crops (USDA NASS 2013). Here, we focus on an heirloom bell pepper cultivar ("California Wonder") that commonly forms mycorrhizal associations across the broad range in which it is planted (Bosland 1996; Votava and Bosland 2002). In both experiments described below, *C. annuum* seeds (W. Atlee Burpee & Co., Warminster, PA, USA) sterilized in 5% bleach were germinated in a 1:1 v/v mix of sterilized sand and soil (Fafard 3B, Sun Gro Horticulture Canada Ltd, Vancouver, Canada).

Field study

To examine AMF effects on plant performance under the stress factors of temperature and drought, we used a 2 × 2 × 3 split-plot design manipulating temperature (elevated vs. ambient, plot-level factor), drought (restricted water vs. sufficient water) and AMF (single vs. mixed vs. control), with 10 plants per treatment combination for a total of 120 plants. Single AMF treatment plants received ~60 spores of *Rhizophagus irregularis* on a perlite carrier (Myke from Premier Tech Biotechnologies, Québec, Canada), and mixed treatment plants were inoculated with ~60 spores of an AMF mix (*R. irregularis, Glomus clarum, Glomus aggregatum, Glomus etunicatum, Glomus deserticola, Glomus monosporus, Glomus mosseae, Gigaspora margarita* and *Paraglomus brasilianum*; Myke plus Endomycorrhizal Inoculant, from BioOrganics, New Hope, PA, USA). Non-mycorrhizal control plants had a sterilized inoculum made by autoclaving the AMF mix. We mixed inocula into the top 5 cm of 1:1 v/v sterilized sand/soil (Fafard 3B, Sun Gro Horticulture Canada Ltd) in 1.84 L pots. Fafard 3B has an initial nutrient content 0.4% total nitrogen, 29 ppm ammonia–nitrogen, 58 ppm nitrate–nitrogen and 2 ppm phosphorus (Danaher *et al.* 2013). Seeds were added and covered with 0.6 cm of soil mix. All plants were inoculated with a fungal-free microbial filtrate to add back a standardized bacterial community, prepared from a slurry of the soil, water and AMF single and mixed inocula that were passed through a 25-micron filter.

Pots were arranged in blocks corresponding to enclosures (see below) on greenhouse benches and rotated weekly for eight weeks, after which all plants were moved to the field site, an open yard (St. Charles, IL, USA) on 14 June 2014. They remained at this site until 9 August 2014. This approximates typical transplanting dates for agricultural or gardening purposes at this latitude. In the field setting, 6 plants (3 AMF treatments × 2 drought treatments) were placed in each of 20 open-top wire enclosures (0.9 × 1.5 m, 1.2 m tall, spaced 1.5 m apart) to prevent mammalian herbivory; these enclosures were also the temperature-manipulation plots, with 10 ambient temperature enclosures surrounded only by wire and 10 elevated temperature enclosures with sides wrapped in plastic sheeting. The soil of each pot was covered with an inverted styrofoam plate with a hole for the plant stem to keep rainwater out. Plants were rotated within each enclosure weekly. To mimic drought conditions, "sufficient" plants received enough water to prevent wilt, and "restricted" treatment plants received half the amount (Staddon *et al.* 2004). These amounts were adjusted during the experimental period to reflect water use in the pots as plants grew: for the first five weeks, sufficient plants received 100 ml and restricted plants received 50 ml of water, and for the remainder of the study, the plants received 200 ml and 100 ml, respectively. All plants were fertilized on July 7 with 5 ml of Osmocote 22-3-8 (Everris NA Inc., Dublin, OH, USA). To monitor temperature conditions in the experiment, a datalogger (ibutton, Maxim Integrated, San Jose, CA, USA) was randomly placed in one pot per enclosure to measure the soil temperature and in four enclosures (two ambient, two elevated), to record air temperature. Soil moisture content in all AMF-free plants was measured in each enclosure on 15 dates (between 26 June and 9 Aug) prior to watering using a soil moisture probe.

During the eight weeks plants were in the field, we counted flowers on each plant on seven dates and estimated wilt prior to watering on twelve dates using a 0–2 scale (0 = no wilt, 1 = leaves bent <90° from petiole, 2 = leaves bent >90° from petiole, following Davies *et al.* (2002). On the last day of the study, we counted fruits produced, where any developing fruit was counted regardless of size. From each plant,

we collected fresh 0.1 g samples of leaf tissue that were frozen at −80°C for N analysis and a small sample of fine roots to quantify AMF colonization. All plants were dried at 60°C for 48 h to determine total plant biomass and root:shoot ratio. Twelve enclosures (six ambient, six elevated) were randomly selected for leaf chemical analyses: N was measured as digestible protein content using the Bradford assay (Bradford 1976; Bio-Rad, Hercules, CA, USA), and P content was measured using the Murphy–Riley procedure (Murphy and Riley 1962). AMF colonization in these same plants was measured in root samples stained with trypan blue using the gridline-intersect method (Phillips and Hayman 1970; McGonigle *et al.* 1990).

We verified that temperature and watering treatments were effective by analyzing mean air temperature and soil temperature differences between ambient and elevated exclosures with *t*-tests, and soil moisture using repeated-measured analysis of variance (ANOVA). We analyzed plant response variables (AMF colonization, wilt score, P content, protein content, total biomass, root:shoot, total flower production and fruit production) with split-plot ANOVA treating temperature as the plot-level variable and drought, AMF treatments and their interactions as within-plot variables. *F*-statistics for AMF, drought and interactions were calculated using interaction mean squares in the denominator (Zar 2010). All analyses were carried out in R version 3.0.3 (R Development Core Team 2014).

Herbivory study

To determine how AMF influence plant resistance to herbivory at elevated temperatures, we used a 2 × 3 factorial design manipulating temperature (elevated vs. ambient) and AMF (single vs. mixed vs. control), with 159 plants total ($n = 53$ per AMF treatment combination). We germinated plants as above in 473 ml pots and grew them in the greenhouse for eight weeks. Pots were then relocated to growth chambers at 27°C (ambient) or 30°C (elevated) with a 16:08 h light:dark schedule. An increase of 3°C is a realistic and conservative choice, as predicted temperature increases in the Midwest are 5–10°C by 2100 (Wuebbles and Hayhoe 2004) and comparable to the daytime effect of warming chambers in the field experiment. Pots were rotated within each chamber weekly and watered twice each week. The experiment used three growth chambers, and we performed two trials to account for any chamber effects by switching which chambers were assigned ambient and elevated temperatures. In trial 1, each chamber had 30 plants ($n = 10$ per AMF treatment), and in trial 2, each chamber had 21 or 24 plants ($n = 7$ or 8 per AMF treatment).

After six weeks in the growth chambers, we excised leaves from each plant for herbivore bioassays using sharp scissors to cut the petiole and minimize potential induction of plant defense chemicals (Karban and Baldwin 1997). We fed excised leaves to *Manduca sexta* (Lepidoptera: Sphingidae), a Solanaceae specialist. Eggs of *M. sexta* (Carolina Biological Supply Co., Burlington, NC, USA) were hatched at 27°C, and caterpillars were fed leaves from extra AMF-free *C. annuum* plants. Single neonate caterpillars at the same growth stage were allowed to feed on leaves removed from one test plant in a sealed petri dish for 72 h. Caterpillars were given as many leaves from the assigned plant as they could consume and were then starved for 8 h to clear their digestive tracts and massed to determine growth. We interpret growth as a measure of both plant resistance and food quality, as greater growth can be a result of both low plant defenses and high nutrient content. Four plants from each AMF treatment per growth chamber, per trial, were selected for chemical analyses. Fresh 0.1 g samples of leaf tissue were frozen at −80°C to measure N and activity of two chemical defenses, peroxidase (POD) and protease inhibitors (PI) following Thaler *et al.* (1996) and Moore *et al.* (2003). Samples of fine roots were collected to quantify AMF colonization, and remaining leaf tissue was dried and ground for P analysis.

Caterpillar mass, protein content, P content, PI activity and POD activity were analyzed with ANOVA, treating temperature, AMF treatment and their interactions as fixed factors. *F*-statistics were calculated using chamber mean squares as the denominator for temperature and the chamber × AMF interaction mean squares in the denominator for AMF and the temperature × AMF interaction (Zar 2010). All analyses were carried out in R version 3.0.3 (R Development Core Team 2014).

RESULTS

Field study

The experimental manipulations we established for air and soil temperature, drought and AMF treatments successfully manipulated the conditions of the plants. Air temperature inside chambers was elevated by 2–4°C during the day but showed little difference at night (mean difference ± 1 SE = 1.37 ± 0.06°C, $t_{1356} = 24.38$, $P < 0.001$; see online supplementary Fig. S1A). Similarly, soil temperature differences fluctuated through the day but were consistently warmer in elevated chambers (0.45 ± 0.02°C, $t_{1606} = 29.67$, $P < 0.001$; see online supplementary Fig. S1B). Drought treatment reduced soil moisture by 5–10% across the experiment in both ambient and elevated enclosures ($F_{1581} = 337.4$, $P < 0.001$; see online supplementary Fig. S1C), and temperature treatment had no effect on soil moisture ($F_{1581} = 0.31$, $P = 0.576$). Temperature and drought treatments were sufficient to cause plant stress, as indicated by significant increase in wilt score with elevated temperature and drought (Table 1). Roots of control plants showed essentially no colonization (mean% root length colonized ± 1 SE, 0.3% ± 0.2%), but AMF were evident in both single (33.7% ± 2.0%) and mixed (30.6% ± 3.0%) treatments. No factors other than AMF treatment influenced AMF colonization (Table 1; see online supplementary Fig. S1D).

Both AMF treatments significantly reduced protein content in plants, but other treatments had no effect (Table 1,

Table 1: split-plot analyses of variance for the field experiment

	Protein		Phosphorus		Total mass		Root:shoot	
Source	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
AMF	23.81	**<0.001**	0.36	0.705	3.44	**0.043**	0.62	0.542
Temperature	0.48	0.505	0.49	0.498	0.16	0.696	0.12	0.738
AMF × temperature	0.27	0.766	0.40	0.673	1.11	0.341	0.08	0.922
Drought	0.85	0.365	4.02	0.054	70.69	**<0.001**	9.84	**0.003**
AMF × drought	1.14	0.332	0.83	0.445	1.95	0.152	0.57	0.571
Drought × temperature	0.17	0.685	2.42	0.131	0.22	0.640	3.18	0.080
AMF × drought × temperature	0.55	0.583	2.09	0.141	6.97	**0.002**	0.47	0.482
	Wilt		Flowers		Fruits		Colonization	
Source	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
AMF	1.54	0.228	2.09	0.138	0.66	0.524	89.82	**<0.001**
Temperature	8.85	**0.008**	0.75	0.399	4.50	**0.048**	0.59	0.460
AMF × temperature	0.50	0.610	1.40	0.260	1.05	0.362	0.26	0.775
Drought	139.34	**<0.001**	2.20	0.144	3.25	0.077	0.24	0.629
AMF × drought	0.34	0.710	3.86	**0.027**	3.18	**0.049**	0.52	0.598
Drought × temperature	0.19	0.665	0.36	0.554	0.15	0.701	0.11	0.742
AMF × drought × temperature	0.28	0.761	4.44	**0.017**	0.16	0.851	0.21	0.814

F-statistics for AMF and AMF × temperature were calculated with the enclosure × AMF mean square in the denominator; temperature used the enclosure mean square; all other *F*-statistics used the enclosure × AMF × drought mean square. *P* values <0.05 in bold.

Fig. 1A). P content was unaffected by treatments. There was a significant interaction effect of AMF, drought and temperature on total plant biomass (Table 1, Fig. 1C). Drought tended to reduce plant biomass, but this effect varied with AMF treatment under different temperatures. For example, single-AMF plants only exhibited a growth benefit of sufficient water under elevated temperatures, while AMF-free plants showed a stronger growth benefit of sufficient water under ambient temperatures. Drought alone affected root:shoot biomass ratio, with greater relative root growth under the drought treatment (Table 1, Fig. 1B).

There was a three-way interaction effect on flower production such that AMF and temperature had little effect under drought conditions, but different effects with sufficient water (Table 1, Fig. 2A). Elevated temperature decreased flower production for AMF-free plants but increased it for single-AMF plants while mixed-AMF plants were relatively unaffected. Temperature had a weak but significant effect on fruit production, and there was an interaction between AMF and drought such that the non-mycorrhizal plants responded much more positively to an increase in water at ambient temperature than AMF-treated plants (Table 1, Fig. 2B).

Herbivory study

Roots of control plants were uncolonized (all 0), but AMF were evident in both single (47.9% ± 2.2%) and mixed (39.2% ± 2.1%) treatments. Colonization did not differ with temperature (Table 2). AMF affected both protein and P content, with single-AMF plants having the highest content, and non-mycorrhizal plants the lowest, for both variables (Table 2, Fig. 3A and B). All plants also had significantly higher protein content under elevated temperature. AMF and temperature treatments had no effect on PI activity, POD activity or herbivore biomass (Table 2 and online supplementary Table S1).

DISCUSSION

Abiotic changes such as increased temperature and more frequent or more severe droughts are predicted effects of climate change (IPCC 2014) that may cause stress for plants. These environmental changes may increase plant reliance on symbiotic relationships to maintain growth and fitness (Kivlin *et al.* 2013). AMF represent one group of symbionts that may reduce the effects of abiotic stress on plants (Davies *et al.* 2002). Further, AMF may influence biotic stressors such as herbivory (Pozo and Azcon-Aguilar 2007). In this investigation, AMF interacted with abiotic conditions to affect plant performance, but not plant defense against insect herbivory.

Field study

Warming enclosures and watering treatments significantly altered air and soil temperature and soil moisture, respectively, and these differences resulted in increased plant stress, as indicated by striking effects on plant wilt, as plants experienced significantly greater wilt under drought and increased temperature conditions. Our enclosures consistently warmed air temperatures during the day, but not at night, a known

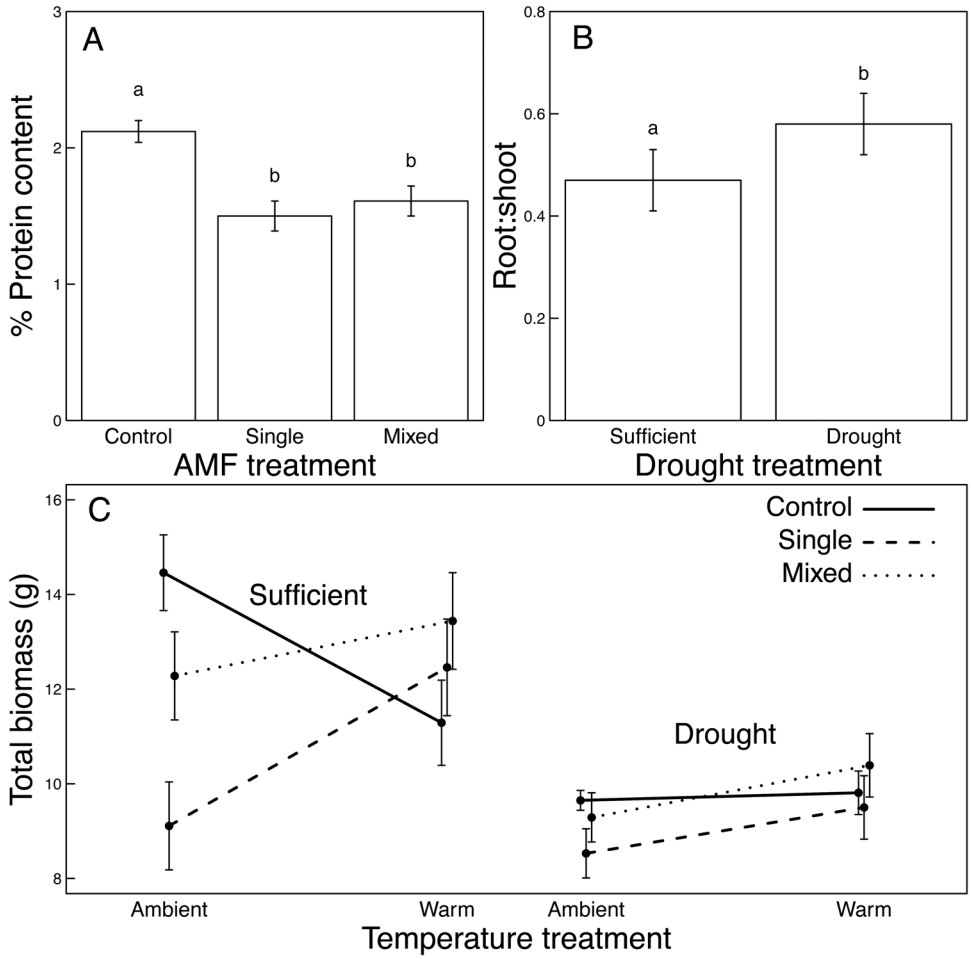

Figure 1: effects of treatments in field study on (**A**) leaf protein content (n = 24 per treatment, total n = 72), (**B**) root:shoot biomass (n = 60 per treatment, total n = 120), and (**C**) total plant biomass (n = 10 per treatment combination, total n = 120). Total plant biomass is separated by drought treatment to ease interpretation. Values in all panels are least square means ± 1 SE, and lower case letters in A and B indicate differences at P <0.05 based on Tukey *post-hoc* tests.

limitation of open-top chambers that has been attributed to evaporative cooling within chambers (Danby and Hik 2007; Munier *et al.* 2010). Nonetheless, the mean temperature within warming chambers was greater, increasing the severity of plant wilt. Although Davies *et al.* (2002) found that AMF can affect susceptibility to drought-induced wilt, decreasing wilt with one AMF species but increasing with another, we found no influence of mycorrhizae on plant wilt severity.

Mycorrhizae did influence plant nutrient status. Both single and mixed-AMF treatments reduced leaf protein content but had no effect on leaf P. Drought and temperature treatments did not influence leaf nutrients, indicating that the AMF effects seen were not context dependent. These results contrast in part with general patterns observed in other AMF–drought studies, which have found P content changes that vary among plant species (Augé 2001). Yet this same review documented little AMF–drought interaction on N, consistent with our protein results. Fertilization of plants may mask AMF effects by eliminating the nutrient uptake benefits provided by fungal mutualists (Johnson *et al.* 2010; Kivlin *et al.* 2013). Although we used a low-P fertilizer, this nutrient subsidy might have compensated for other plant stresses. Nonetheless, these leaf nutrient results suggest that fungal symbiont effects on leaf nutrients may be robust to shifts in abiotic conditions under climate change.

However, drought and temperature treatments interacted with AMF to influence plant growth and fitness. Under drought conditions, there was generally little variation in total biomass, flower production or fruits, and these responses were usually low compared to other treatment combinations. Drought plants allocated more biomass to root growth relative to plants with sufficient water as expected, although AMF had no effect in contrast to work in other *C. annuum* varieties where AMF (but not drought) altered plant allocation (Davies *et al.* 2002). AMF did not alleviate drought stress effects on growth, as seen in other plants (Saia *et al.* 2014). Alleviation of drought stress effects on growth by AMF seems to vary greatly among plant and AMF species (Augé 2001).

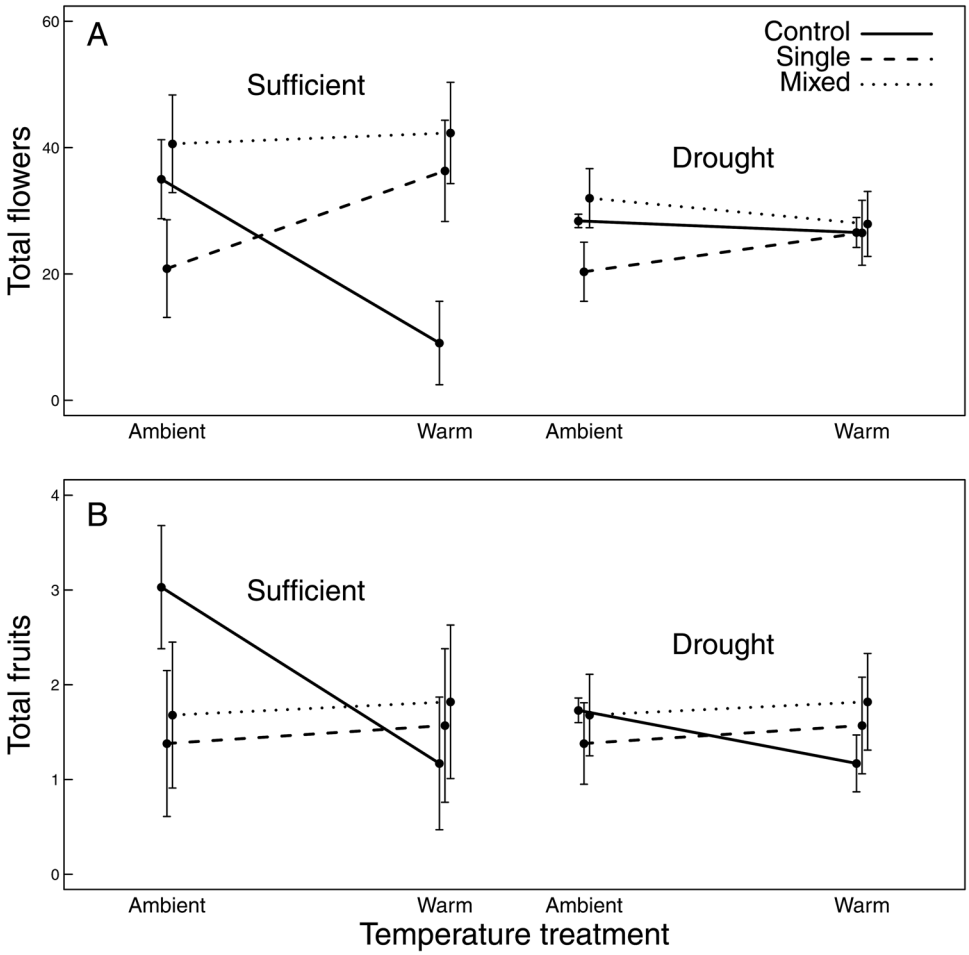

Figure 2: effects of treatments in field study on (**A**) total flower production and (**B**) total fruit production, separated by drought treatment to ease interpretation. In both panels, $n = 10$ per treatment combination and total $n = 120$. Values in all panels are least square means ± 1 SE.

AMF may improve water relations in plants through several possible mechanisms. These may include indirect effects resulting from improved nutrition and plant size or the direct effects of lower resistance water transport and higher transpiration rates (Smith and Read 2008) and may occur during insufficient or sufficient water conditions. Changes in soil characteristics which improve water relations with plants have also been shown to be due to the colonization of the soil by external mycelium (Augé 2004). With sufficient water, AMF plants can have increased stomatal conductivity and transpiration rates (Huang *et al.* 1985). These stomatal changes may also be the result of hormonal changes in the plants that occur during AMF colonization (Drüge and Schonbeck 1993).

When provided sufficient water, there were strong temperature-dependent AMF effects for all three measures of plant performance. Non-mycorrhizal plants showed a consistent pattern of reduced growth, flower and fruit production under elevated temperatures. This is particularly striking because these plants had the highest growth and fruit production of all treatment combinations. Under sufficient water, plants colonized by AMF exhibited very different temperature responses. Single-species inoculum showed a distinct increase in growth and flowers at higher temperatures, opposite the pattern of non-AMF plants. These plants also had the lowest growth and fewest flowers at ambient temperatures. A mix of AMF species resulted in intermediate values of growth and little response to temperature. Thus, under non-stressed conditions (sufficient water, ambient temperature) the AMF treatments used in this study may have incurred a cost that resulted in reduced growth and fitness relative to uncolonized plants. A mixture of AMF may buffer the effects of warmer temperatures on plant growth (Bunn *et al.* 2009) and avoid potential negative effects of costly individual AMF species, similar to findings in soybean where a mixture of AMF neither increased nor decreased growth more than any of the individual component species (Grümberg *et al.* 2015). We used AMF treatments that are commercially available for agriculture, gardening and conservation restorations. The single species, *R. irregularis*, has been isolated, is commercially produced worldwide (Wahbi *et al.* 2015), and is widely used in mycorrhizal research. Examining how *R. irregularis* differs from a mixture of other species may help illuminate how well laboratory and other

Table 2: analyses of variance for the herbivory experiment

	Protein		Phosphorus		Caterpillar mass	
Source	*F*	*P*	*F*	*P*	*F*	*P*
AMF	8.97	**0.009**	104.44	**<0.001**	1.26	0.336
Temperature	66.54	**0.001**	0.28	0.627	7.68	0.22
AMF × temperature	2.44	0.149	1.50	0.281	0.29	0.76
	PI		POD		Colonization	
	F	*P*	*F*	*P*	*F*	*P*
AMF	1.18	0.357	3.57	0.078	362.23	**<0.001**
Temperature	1.19	0.337	0.70	0.449	3.82	0.122
AMF × temperature	0.89	0.449	2.45	0.148	3.40	0.085

F-statistics for AMF and AMF × temperature were calculated with the chamber × AMF mean square in the denominator; temperature used the chamber mean square. *P* values <0.05 in bold.

controlled studies can be extended to field conditions, where plants likely interact with multiple mycorrhizae species.

Flower production patterns mirrored total biomass, with both AMF treatments producing more flowers under warmer temperatures. However, this increase in flowers did not translate into increased fruit production. In fact, mycorrhizal plants fruit production did not vary across temperature or drought treatments, and significant effects of treatments seem to be driven only by higher fruit production in non-mycorrhizal plants under ambient temperatures and sufficient water. This may represent an additional cost of the AMF symbiosis. While plant biomass increases are likely to benefit AMF because increased photosynthetic area may increase the supply of photosynthates, a shift in resources to fruit production (at the potential expense of leaf growth) could reduce benefits to fungi.

Herbivory study

In the herbivory study, mycorrhizal treatments affected leaf characteristics, but in different ways than in a field setting. Additionally, elevated temperature altered one leaf characteristic, protein content, unlike in the field study where temperature manipulation had no influence on leaf traits. For *C. annuum* plants grown in growth chambers at constant temperature, AMF colonization (and single-species colonization in particular) increased both protein and P contents of leaves, although the protein pattern was weaker under warm temperatures. This differed from the field experiment where AMF treatments did not change percent P and had the opposite effect on protein. Although chamber temperatures were comparable to daytime field temperatures, the invariant abiotic environment may have altered AMF behavior and provisioning to plants. Field study plants were also fertilized once, and this nutrient addition may be responsible for the differences in protein content (1.5–2% in the field, <1% in growth chambers) and effects on leaf P. Additional nutrients can negate AMF benefits (Johnson *et al.* 2010), and this may be a particular reason why no AMF effect on P was detected in the field study.

The two defensive traits we measured, POD and PI activities, were not affected by temperature or AMF treatments. Although AMF tended to increase protein content in leaves, this was apparently not invested in these defensive proteins. It is important to note that we measured constitutive levels of these defenses. Inducible defense traits expressed following herbivore attack (or a chemical or mechanical simulated attack) may be expressed very differently. For example, polyphenol oxidase may be induced by herbivore wounding or methyl jasmonate application, while PI as measured here can be induced by methyl jasmonate (Tan *et al.* 2011). Within Solanaceae, inducibility of defenses is associated with self-compatibility (Campbell and Kessler 2013), so differences in POD, PI and other herbivore resistance among treatment levels here might be detectable only following induction, as *C. annuum* is self-compatible (Votava and Bosland 2002).

Although our treatments led to differences in P and protein content of leaves, there were no effects on the growth of caterpillars fed these leaves, in contrast to some previous studies where AMF alone influenced herbivore performance (Koricheva *et al.* 2009; Kempel *et al.* 2010). The increases in protein and P may not have been large enough to change the nutritional quality of the foliage for *M. sexta* in a meaningful way. However, leaves fed to caterpillars presumably only expressed constitutive levels of defenses, as discussed above, and herbivore effects could be different if our study had included an induction treatment (Barber 2013; Bennett *et al.* 2009; Kempel *et al.* 2010). An important next step would be to test herbivore performance on plants under varying environmental conditions (AMF, temperature, or drought) but allow herbivore attack to proceed in a more realistic way, including induction followed by later herbivore feeding or bioassays (Kiers *et al.* 2010; Thaler *et al.* 1996).

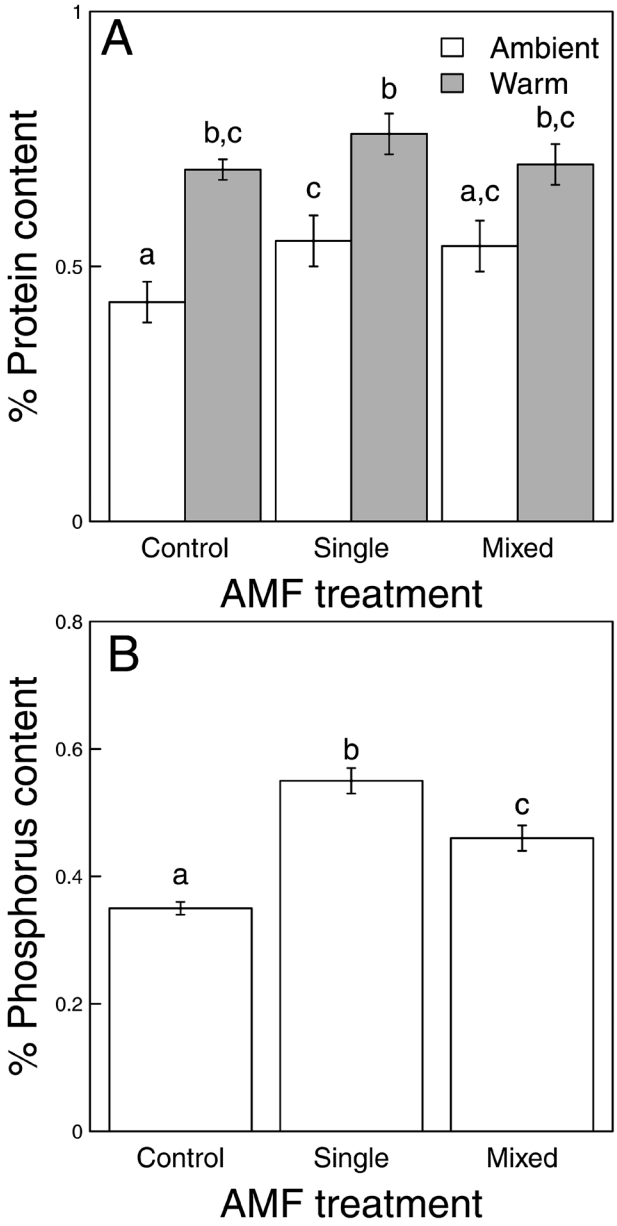

Figure 3: effects of treatments in herbivory study on (**A**) leaf protein (n = 12 per treatment, total n = 72) and (**B**) leaf P content (n = 24 per treatment, total n = 72). Values in all panels are least square means ± 1 SE, and lower case letters in A and B indicate differences at P <0.05 based on Tukey *post-hoc* tests.

Additionally, indirect defenses can play a role in plant resistance. The number of flowers a plant produces has been linked to the attraction of predators of herbivores that reduce damage (Abdala-Roberts *et al.* 2014). Given the changes in flower production we documented, AMF plants in the field study could have a protective advantage by producing more flowers that would not be detected in the growth chamber herbivory experiment, where predators were absent.

Conclusions

These two experiments together demonstrate that, while AMF effects on plants can vary with abiotic environmental conditions, these effects may be highly context dependent, challenging our ability to predict the outcomes of these multispecies interactions in future climates. In particular, AMF–temperature effects varied in strength and even direction for several response variables in both experiments. Nonetheless, the field study demonstrated that AMF may ameliorate temperature stress effects on plant growth and flower production. In neither experiment did temperature or water availability influence percent AMF colonization, despite differences in AMF effects on plants. This suggests that colonization alone is not a strong indicator of the strength of plant–mycorrhizal interactions.

Both studies also demonstrated that a mix of AMF species may have different effects than a single species. Frequently, the mixed treatment resulted in weaker effects than single species, suggesting that a community of AMF may average the effects of the component species (Grümberg *et al.* 2015). Not all AMF provide the same benefits to host plants, and plants may allocate carbohydrates differentially among multiple colonizing fungi (Grman 2012; Kiers *et al.* 2010). The reciprocal costs and benefits between host plants and AMF species likely lead to complex coevolutionary patterns that vary across abiotic and geographic conditions (Johnson *et al.* 2010). Thus in some conditions, one AMF species may contribute to higher plant nutrient content than a mix that may contain several less efficient AMF species. Because of this, finding the best combinations of AMF and plant species has been proposed as an important goal not only in agriculture, where it has the potential to boost yields and reduce fertilizer needs (Tanwar *et al.* 2013), but in plant conservation and ecosystem restoration (Johnson *et al.* 2010), where natural plant populations may face significant abiotic stresses in the coming decades.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Plant Ecology* online.

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