Effect of soil moisture and temperature during fallow on survival of contrasting isolates of arbuscular mycorrhizal fungi

Y. Lekberg and R.T. Koide

Abstract: Many arbuscular mycorrhizal (AM) fungal species have worldwide distributions. However, it is not clear whether such species have adapted to local conditions. We compared the responses of mesic temperate and semi-arid tropical isolates of *Glomus mosseae* and *Glomus etunicatum* to extremes of temperature and moisture in a pot experiment. Treatments (warm–moist, warm–dry, freeze/thaw–moist, freeze/thaw–dry) were applied to whole soil mycorrhizal inoculum, and their effects were evaluated as both the change in viability of extraradical hyphae and mycorrhizal colonization of bait plants. Moist soil decreased hyphal viability compared with dry soil, irrespective of temperature, but mycorrhizal colonization of bait plants was lower in moist soil only when warm. Frost-heave could have physically ruptured hyphae in the freezing–moist soil without an effect on spores, but parasitism and (or) respiratory depletion of carbon reserves may have reduced survival of all propagules in the warm–moist soil. Hyphae of semi-arid tropical isolates survived all treatments better than hyphae of mesic temperate isolates, but these differences were not reflected in mycorrhizal colonization of bait plants. We found no evidence that these isolates have adapted to local conditions of moisture and temperature. Instead, wide environmental tolerances seem to be present within both populations of these AM fungal species.

Key words: arbuscular mycorrhizal fungi, adaptation, temperature, moisture, fallow, survival.

Résumé : Plusieurs espèces de champignons mycorhiziens arbusculaires (AM) montrent des distributions mondiales. Cependant, on ne sait pas si de telles espèces se sont adaptées aux conditions locales. Les auteurs ont comparé, en pots, les réactions d'isolats de régions mésiques tempérées et semi-aride tropicales du *Glomus mosseae* et du *Glomus etunicatum,* à des températures et des humidités extrêmes. Ils ont appliqué les traitements (chaud–humide, chaud–sec, gel/dégel humide, gel/dégel–sec) à des inoculums mycorhiziens en sol et en ont évalué les effets, à la fois en terme de viabilité des hyphes extra racinaires et de la colonisation de plantes pièges. Les sols humides diminuent la viabilité des hyphes comparativement aux sols secs, indépendamment de la température, mais la colonisation des plants pièges ne diminue qu'en conditions chaudes. Le bris par le gel pourrait avoir rompu physiquement les hyphes dans le sol gelé-humide sans avoir d'effet sur les spores, mais le parasitisme ou l'épuisement respiratoire des réserves de carbone pourraient avoir réduit la survie de toutes les propagules dans le sol chaud humide. Les hyphes des isolats d'origine semi-aride tropicale ont mieux survécu à tous les traitements que les hyphes des isolats d'origine mésique tempérée, mais ces différences ne se traduisent pas dans la colonisation des plantes pièges. Les auteurs n'ont trouvé aucune preuve montrant que ces isolats se seraient adaptés aux conditions locales d'humidité et de température. On observe au contraire une large tolérance environnementale dans les deux populations de ces espèces fongiques AM.

Mots-clés : champignons mycorhiziens arbusculaires, adaptation, température, humidité, jachère, survie.

Introduction

Arbuscular mycorrhizal (AM) fungi are present in a majority of terrestrial ecosystems on earth, and many species appear to have worldwide distributions. For example, *Glomus intraradices* has been found on all five continents (Stahl and Christensen 1990) and in such diverse environments as Swiss alpine meadows (Sykorova et al. 2007), British woodlands (Helgason et al. 1998), Panamanian rainforests (Husband et al. 2002), Zimbabwean maize fields (Lekberg et al. 2007), and geothermal areas in Yellowstone National Park, Wyoming (Appoloni et al. 2008). The factors that permit such broad distributions of species are poorly understood.

Previous research has shown that some isolates of AM fungi perform best at their temperatures of origin (Shenk 1975; Siqueira et al. 1985; Grey 1991), and that isolates from heavy metal contaminated soils can tolerate higher concentrations of heavy metals than those isolated from non-contaminated soils (Weissenhorn et al. 1993; del Val et al. 1999). These phenotypic responses to environmental conditions may indicate that some species are able to adapt to local conditions, resulting in genetically distinct ecotypes with similar morphologies. Alternatively, species may possess general purpose genotypes (GPG), a term coined by Baker (1965), in which single genotypes are able to tolerate a

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Y. Lekberg[1,2] and R. Koide. Department of Horticulture, Pennsylvania State University, University Park, PA 16802, USA.

[1]Corresponding author (e-mail: ylva.lekberg@gmail.com).
[2]Present address: Department of Biology, University of Copenhagen, Øster Farimagsgade 2D, 1353 Copenhagen, Denmark.

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Table 1. Origin and history of isolates used in the study.

Species	Climate	Origin	Year of isolation
Glomus mosseae (MD209)	MT	Maryland, USA	1997
Glomus mosseae	SAT	Southwest Zimbabwe	2001
Glomus etunicatum (PA150)	MT	Pennsylvania, USA	1998
Glomus etunicatum	SAT	Southwest Zimbabwe	2001

Note: Isolates were from a mesic temperate (MT) or semi-arid tropical (SAT) climate. The two MT isolates were obtained from INVAM and the SAT isolates were isolated by the authors from subsistence farmers fields in Zimbabwe.

wide range of environments. A GPG is identified based on a species' distribution in nature, and a species that has a GPG can also be said to possess a broad realized niche. It has been suggested that organisms that are ubiquitous, abundant, and reproduce asexually (van Doninck et al. 2002), such as AM fungi, are likely candidates to harbor GPG. Indeed, the sequence variation within one spore of *Glomus mosseae* may be as great as the sequence variation between geographically distant populations (Lloyd-MacGilp et al. 1996), suggesting a great deal of genetic stability across space.

Temperature and moisture regimes differ drastically among climatic regions of the world. While these factors affect AM fungal growth and symbiotic functioning (e.g., Grey 1991; Auge 2004; Heinemeyer and Fitter 2004; Gavito et al. 2005), they may also influence AM fungal survival. This effect on survival can be indirect when low temperature and (or) drought prevent plant growth seasonally, which interrupts the carbon supply to the fungus. Temperature and moisture, however, may also directly affect fungal survival. For example, freezing temperatures may increase AM fungal mortality (Addy et al. 1997; Klironomos et al. 2001; but see McGonigle and Miller 1999), and one-third of all metabolically active hyphae may die during the wet winter months in Canada (Kabir et al. 1997). On the other hand, viability of hyphae may remain high for long periods under dry conditions (Tommerup and Abbott 1981; Brundrett et al. 1996*a*; Pattinson and McGee 1997), but decline drastically when the soil is wetted periodically (Braunberger et al. 1996; Pattinson and McGee 1997). It is obvious from these studies that temperature and moisture exert strong selection pressures on AM fungal survival, which could lead to adaptations to local climatic conditions. Differences in tolerance *between* AM fungal species have been recorded and may generate seasonal patterns of AM fungal community compositions (Klironomos et al. 2001), but virtually nothing is known about potential differences *within* AM fungal species from disparate environments (Fitter et al. 2004). In this study, we addressed whether contrasting isolates (from mesic temperate and semi-arid tropical climates) of two globally distributed AM fungal species, *G. mosseae* and *G. etunicatum* (Lloyd-MacGilp et al. 1996; Stutz et al. 2000) have adapted to their climates of origin. For example, local adaptation would be consistent with moist, freezing conditions being better tolerated by mesic temperate isolates, which experience moist, freezing soils, than by semi-arid tropical isolates, which do not.

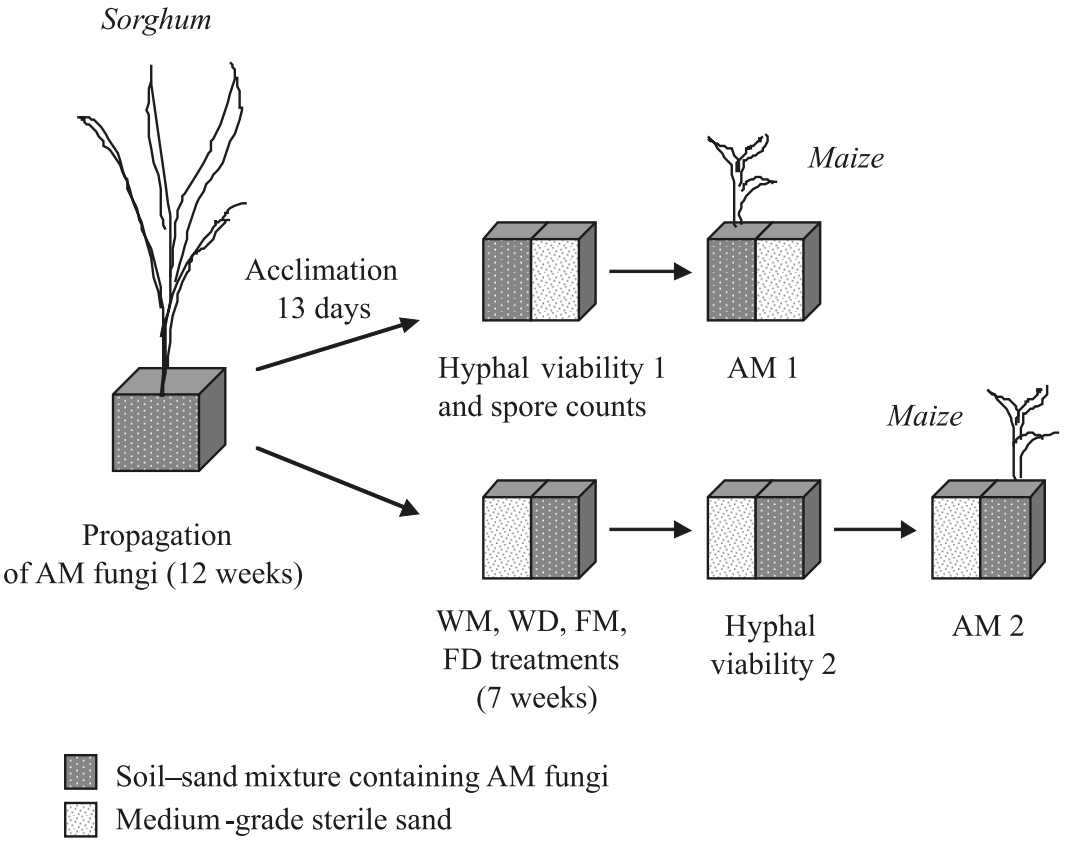
Materials and methods

Direct effects of environmental conditions on AM fungi are difficult to measure because they are obligate symbionts; any fungal response could be indirectly mediated by changes in the host plant. To avoid such confounding effects, we assessed fungal survival in the absence of plants during a fallow period in which the conditions were continuous warm–moist (WM), continuous warm–dry (WD), freeze/thaw–moist (FM), or freeze/thaw–dry (FD) for 7 weeks. We propagated in the greenhouse isolates of *G. mosseae* and *G. etunicatum* from both Zimbabwe (semi-arid tropical isolates, SAT) and the Mid-Atlantic United States (mesic temperate isolates, MT), for a total of four isolates. Hyphal viability and mycorrhizal colonization of roots of bait plants were assessed before and after imposition of the fallow period treatments. We used the difference between before and after assessment as a measure of the effects of the treatment combination. Details of the AM fungal isolates are given in Table 1. The experimental steps are outlined in Fig. 1 and described in detail below. Each treatment combination was replicated six times, resulting in 96 total experimental units.

Propagation of AM fungal isolates

Three one-week-old sorghum (*Sorghum bicolor* L.) seedlings were transplanted into 10 cm (diameter) pots on 9 October 2003, containing a soil and sand mixture (1:4 *v*/*v*), the soil having been autoclaved for 2 h on each of two consecutive days to destroy all mycorrhizal fungi. The soil was a Hagerstown silty clay loam with a bicarbonate-extractable P concentration of approximately 12 μg $P \cdot g^{-1}$ soil, and the sand was a medium grade silica sand. Each pot was inoculated with >200 spores of one of the four isolates except for *G. mosseae*$_{SAT}$, for which 100 spores were handpicked from the original inoculum source and added to each pot, owing to the presence of contaminating AM fungi. Four pots without AM fungi served as nonmycorrhizal controls (one for each of the four moisture and temperature treatment combinations described below). These pots were interspersed with the other pots throughout the experiment to confirm that no cross-contamination occurred. All pots were placed in the greenhouse and plants were fertilized weekly with 100 mL of a half-strength Hoagland solution (Machlis and Torrey 1956) containing 6 mg $P \cdot L^{-1}$, and watered as needed with tap water between fertilizations. The average air temperature was 21 °C, and high intensity discharge lamps (1000 W, low pressure sodium), provided 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ additional photosynthetically active radiation for 16 $h \cdot d^{-1}$. On 9 January 2004, a core (8 mm diameter and approximately 5 mL in volume) was taken from randomly chosen pots to determine whether spores had formed by the four isolates. Spores from the 106 μm sieve were centrifuged for 1 min in a 60% aqueous sucrose solution (Brundrett et al. 1996*b*), collected on the 106 μm sieve, and assessed for maturity and purity under a micro-

Fig. 1. Outline of experimental steps. All four isolates were propagated on sorghum for 12 weeks followed by a split of the pot where mycorrhizal colonization was measured on plants growing in half of the volume prior to a fallow treatment (AM 1) of either freeze/thaw–dry (FD), freeze/thaw–moist (FM), warm–dry (WD), or warm–moist (WM), which was compared with mycorrhizal colonization growing in the other half of the volume (AM 2) following the fallow treatments.



scope. Plants were fertilized for the last time on 10 January and were watered for the last time on 31 January. Watering intensity had been gradually reduced during the two weeks prior to 31 January. After the plants had completely dried down, shoots were cut off at the soil level.

Cold acclimation

On 12 February, all pots in the high moisture treatments (WM and FM) were re-wetted. FM and FD pots were transferred to a growth chamber for cold acclimation set at 10 °C for 5 d followed by 5 °C for 8 d. Cold acclimation was shown previously to affect fungal survival (Addy et al. 1998). FM pots were kept moist throughout the acclimation period. All pots in the warm treatments (WM and WD) were left in the greenhouse at 21 °C where the WM pots were watered until saturated. On 25 February the soil in all pots were split into two halves and one half was transferred to a new pot, making the total number of pots 192. The resulting empty halves of each pot were filled with AM fungal-free, medium grade silica sand. The splitting of the pots allowed us to compare the change in fungal viability in the same replicate before and after the various treatments, which essentially removed any variability that may have been present among replicates within each treatment.

Initial spore number, initial length of viable hyphae and initial mycorrhizal colonization (AM 1)

On 25 February, half of the pots in all treatments (96) were transferred to a growth chamber set at 25 °C (day) and 20 °C (night) with 310 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation provided by very high output (VHO) fluorescent lamps for 16 $h \cdot d^{-1}$. All pots were watered to saturation on 25 February. On 26 February, a core (8 mm diameter and approximately 5 mL in volume) of soil was taken from the location within the pot containing the soil–sand mix to evaluate the number and purity of spores and to assess viable hyphal length. Owing to the length of time required to process samples, only three randomly chosen replicates per treatment combination were analyzed. The soil–sand was suspended in water and poured through a set of sieves (500, 106, and 46 μm). Hyphae were collected from the 46 μm sieve, and viability was assessed after staining in a fluorescein diacetate solution (FDA) as in Addy et al. (1997). The stained hyphae were observed immediately using an Olympus fluorescence microscope equipped with a 420–490 nm excitation filter and a 500 nm barrier filter. We counted the number of bright green fluorescing hyphae intersecting 25 randomly selected lines on the nitrocellulose filter (representing a length of approximately 78 mm). Only hyphae that had the irregular shape and dichotomous branching pattern typical of AM fungi were counted (Nicolson 1959). Spores were isolated as before.

Maize seeds (*Zea mays* cultivar 'Bodacious') were planted in all pots in the growth chamber on 26 February on the side of the pot containing soil–sand and thinned to two seedlings

Table 2. Mean (±SE) of initial spore number, viable hyphal length 1, and mycorrhizal colonization (AM 1) of the mesic temperate (MT) and semi-arid tropical (SAT) isolates prior to the moisture and temperature treatments.

AM fungal species	Isolate	No. of spores (mL^{-1} soil)	Viable hyphal length 1 ($cm \cdot mL^{-1}$ soil)	AM 1 (% of root length)
Glomus etunicatum	MT	49 (9.1)a	18.2 (2.68)a	69.5 (1.57)a
Glomus etunicatum	SAT	34 (5.8)a	19.8 (2.84)a	22.7 (1.90)d
Glomus mosseae	MT	14 (3.1)b	14.9 (2.03)a	46.5 (1.74)b
Glomus mosseae	SAT	2.4 (1.1)c	20.1 (4.70)a	35.0 (3.83)c

Note: Different superscripts indicate a significant ($p \leq 0.05$) difference within column means.

per pot after emergence. Plants were watered as needed with distilled water and fertilized with approximately 100 mL of a quarter-strength Hoagland solution without phosphate 2 weeks after planting and weekly thereafter. One maize plant was harvested 4 weeks after planting. The second plant was harvested 7 weeks after planting to be used in case mycorrhizal colonization was low 4 weeks after planting. Percent mycorrhizal colonization was determined on roots growing in the soil–sand mix, not the sand, using the intersect method on roots that had been cleared and stained with trypan blue (Brundrett et al. 1996*b*). Because mycorrhizal colonization after 4 weeks was deemed sufficiently high to evaluate treatment effects during the fallow (see averages in Table 2), no roots from the 7-week harvest were used and all analyses are based on roots harvested after 4 weeks.

Moisture and temperature treatments during the fallow period

All pots in the warm treatments (WM and WD) were kept at 21 °C in the greenhouse. WM pots were watered daily with tap water and the WD pots were unwatered. This resulted in a moisture level of 0.56 g $H_2O \cdot 100\ g^{-1}$ dry soil (±0.02, SE) in the dry pots and 12.4 g $H_2O \cdot 100\ g^{-1}$ dry soil (±1.3, SE) in the moist pots. All pots in the freeze–thaw treatments (FM and FD) were placed in a growth chamber set at –5 °C, where the FM pots were watered prior to being frozen and kept in plastic bags to avoid sublimation. The FD pots were kept dry. We conducted three freeze–thaw cycles during the 7 week fallow period to simulate the conditions commonly experienced by soils in the Mid-Atlantic states. The first thaw cycle occurred after 12 d, the second after 33 d, and the third and final thaw cycle was conducted at the end of the fallow period, 49 d after initiating the fallow. In all thaw cycles the temperature was raised to +5 °C. For the first two thaw cycles, the temperature was kept at +5 °C for two days followed by a lowering of the temperature back to –5 °C. For the third thaw cycle, the temperature was raised to +5 °C for three days, followed by a transfer of all the pots to the growth chamber to determine the treatment effects on hyphal viability and mycorrhizal colonization.

Final length of viable hyphae and final mycorrhizal colonization (AM 2)

All pots were watered to saturation on 17 April and maize seeds (*Z. mays* 'Bodacious') were planted on 18 April in the side of the pot containing the soil–sand mixture. On 18 April, a 5 mL core was taken from the side of the pot containing the soil–sand mixture for assessment of viable hyphal length as above. Again, one maize plant was harvested 4 weeks after planting and as before, mycorrhizal colonization was measured on roots growing in the soil–sand mix, not the sand. The conditions in the growth chambers were the same as described previously.

Calculation and statistical analysis

The effect of fallow treatment on mycorrhizal colonization was characterized with the ratio of mycorrhizal colonization after the treatments to mycorrhizal colonization before the treatments (AM 2 : AM 1). The effect of fallow treatment on the survival of hyphae was calculated in the same fashion (viable hyphal length 2 : viable hyphal length 1). All ratios were analyzed as a four-factor ANOVA in SAS (SAS Institute Inc., Cary, N.C.) and were transformed when necessary to fulfill the requirements of the ANOVA. Owing to the failure of some *G. mosseae*$_{SAT}$ and one *G. etunicatum*$_{SAT}$, the PROC GLM procedure was used in SAS to analyze treatment effects due to the unbalanced nature of the analysis. Mean separations were performed using the least significant difference method and were considered significant when $p \leq 0.05$.

Results

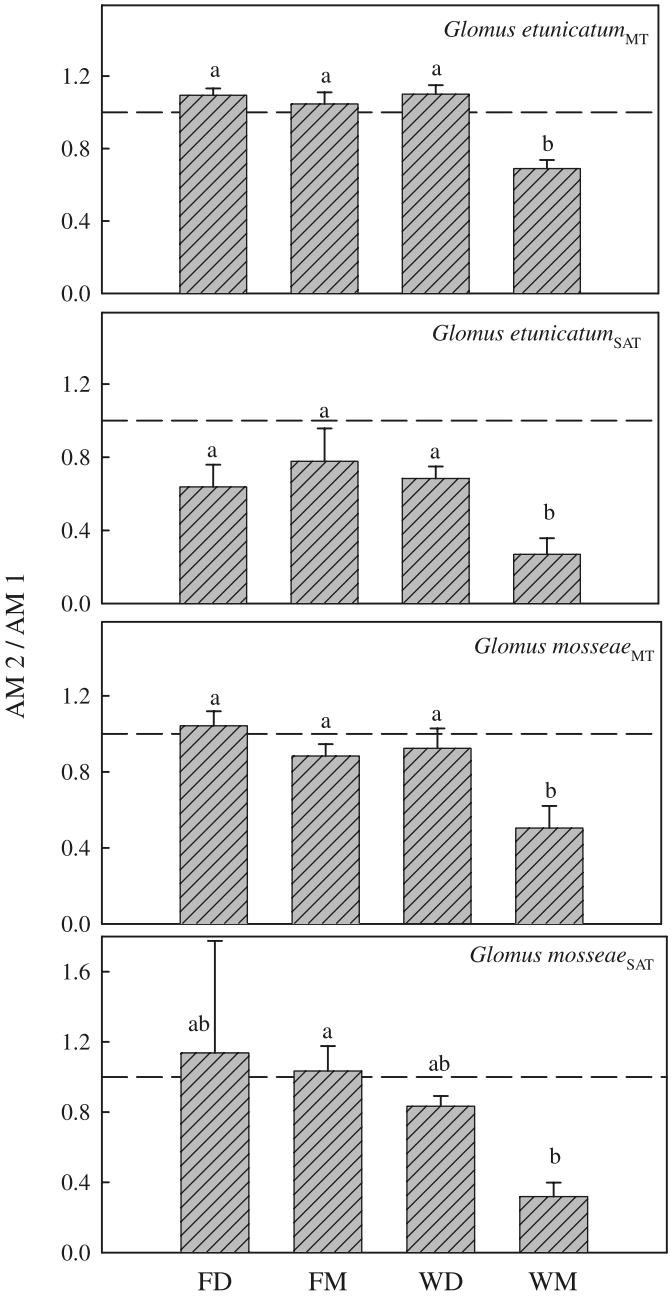
Mycorrhizal colonization

The control plants remained nonmycorrhizal throughout the experiment, indicating that no cross contamination occurred during the course of the experiment. We detected very few viable hyphae in the nonmycorrhizal control plants, suggesting that by employing the morphological criteria of Nicolson (1959), the majority of the hyphae counted in the experimental pots were those of AM fungi. The initial spore numbers, viable extraradical hyphal lengths, and mycorrhizal colonization prior to the imposition of temperature and moisture treatments are given in Table 2. The AM 2 : AM 1, which is one measure of AM fungal survival, showed a significant moisture × temperature × isolate origin interaction ($F_{[1,72]} = 6.42$, $p = 0.013$). There was also an isolate origin × AM fungal species interaction ($F_{[1,72]} = 6.84$, $p = 0.011$), stemming from the fact that the AM 2 : AM 1 of *G. etunicatum*$_{SAT}$ was lower than for the other three isolates. Owing to these interactions, the means for all treatment combinations are plotted in Fig. 2. In general, the WM treatment significantly reduced mycorrhizal colonization compared with the other treatments. There were no other significant three- and four-way interactions.

Hyphal viability

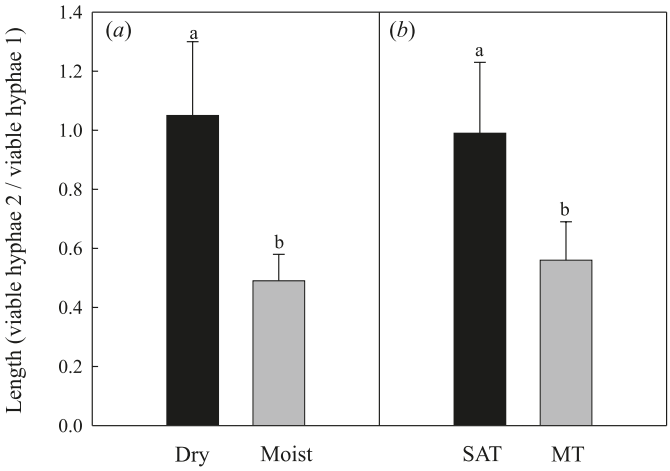
The survival of hyphae (expressed as the ratio of the length of viable hyphae 2 to the length of viable hyphae 1) was significantly affected by moisture level ($F_{[1,31]} = 8.08$,

Fig. 2. Means (±SE) of the ratio of mycorrhizal colonization before and after a 7-week fallow in either freeze/thaw–dry (FD), freeze/thaw–moist (FM), warm–dry (WD), or warm–moist (WM) conditions. Within each AM fungal isolate, different letters indicate a significant difference at the 0.05 probability level, $n = 6$ except for *Glomus mosseae*$_{SAT}$ where $n = 3$ and 4 in the warm and freezing treatments, respectively. The reference line of 1 indicates where AM 2 = AM 1 (i.e., no treatment effect).



$p = 0.008$), such that it did not decline under dry conditions (i.e., length of viable hyphae 2 = length of viable hyphae 1), but was reduced to half in the moist treatments (Fig. 3*a*). Furthermore, survival of hyphae of semi-arid tropical isolates was significantly ($F_{[1,31]} = 5.41$, $p = 0.03$) less affected by moisture and temperature than survival of hyphae from the mesic temperate isolates (Fig. 3*b*). There were no significant two-way, three-way, or four-way interactions.

Fig. 3. Main factor means (±SE) of the ratio of length of viable hyphae before and after a 7-week fallow as (*a*) a result of moisture and (*b*) isolate origin. A value of 1 indicates where length of viable hyphae 2 = length of viable hyphae 1 (i.e., no treatment effect), and different letters indicate a significant difference at the 0.05 probability level.



Discussion

Adaptations to local conditions

The world-wide distribution of many AM fungal species is the result of either locally adapted ecotypes or global general purpose genotypes (GPGs). While the potential for ecotypic variation in plants has been known since Turesson's pioneering work (Turesson 1922), this topic has rarely been addressed for AM fungi. In fact, the underlying assumption in much AM fungal research and inoculation programs is that these organisms possess broad tolerances, or GPGs, because isolate origins are seldom considered. Stahl and Christensen (1991) investigated the degree of plasticity in *G. mosseae* isolates from dissimilar habitats and came to the conclusion that plasticity alone could not explain the wide distribution of this fungus, but that ecotypes of AM fungi may exist. We observed a significant moisture × temperature × isolate origin interaction in the ratio of mycorrhizal colonization after the treatments to mycorrhizal colonization before the treatments (AM 2 : AM 1), indicating that the behavior of the isolate depended on the conditions during the fallow. However, the responses were not consistent with those expected as a result of adaptation to climate because the MT isolates did not tolerate freezing, moist conditions any better than the SAT isolates (Fig. 2), which do not regularly experience moist conditions and never experience freezing conditions during fallow. Because increased drought tolerance may also confer an increased tolerance to cold (Siminovitch and Cloutier 1983; but see Klironomos et al. 2001), we cannot exclude the possibility that adaptive responses would have been observed had we chosen to study other environmental factors. Indeed, ecotypic differentiation has been suggested in response to salinity (Carvalho et al. 2004), as well as to metal contaminations (Weissenhorn et al. 1993; del Val et al. 1999), indicating that AM fungi may adapt to certain environmental factors.

Nonetheless, we found it to be quite remarkable that the viability of the SAT isolates remained high (based on both hyphal survival and mycorrhizal colonization) after a 7-week fallow in –5 °C with three freeze–thaw cycles. Kuszala et al. (2001) showed that other tropical isolates survive long storage (>8 months) in –18 °C conditions and suggested freezing temperatures as a means for long-term storage of AM fungal germplasm. Furthermore, AM fungal isolates transplanted from a warmer area may persist for several growing seasons in a cooler area (Weinbaum et al. 1996), albeit with less extreme temperature differences between the two habitats than the ones employed by Kuszala et al. (2001) or in the current study. The transplant approach is in many ways superior to the approach taken here, in that repeated subculturing of isolates could result in adaptations to greenhouse conditions. Unfortunately, the transplant approach could not be used in this study because our desire was to compare responses of the same morphospecies from different climatic regions, which forced us to utilized isolates in culture. However, owing to their relatively recent isolation (Table 1), it is not likely that substantial genetic change would have occurred. We use the term morphospecies in favour of species to describe our isolates, because until we have conducted careful genetic analyses of isolates from disparate climates, we do not know whether our morphologically recognized AM fungal species actually comprise a range of cryptic species.

The detrimental impact of warm-moist soil

Warm–moist soil conditions had the greatest detrimental impact on overall mycorrhizal colonization in our study (as indicated by significantly lower AM 2 : AM 1 values for the WM treatment for all isolates except *G. mosseae*$_{SAT}$ in Fig. 2). Pattinson and McGee (1997) also showed that AM fungal survival may decline drastically when the soil is wetted, but remain high for extended periods in dry soils. Furthermore, time of storage may be an important predictor of subsequent mycorrhizal colonization in moist, but not dry soils (Miller et al. 1985). The underlying mechanism for this decline is unknown but could be due either to significant mycorrhizal fungal respiration in moist soils during a time when there is no photosynthesis and thus to depletion of carbon reserves, or to parasitism by other fungi or bacteria (Daniels and Menge 1980). The relative importance of respiratory carbon depletion and parasitism is unknown. Braunberger et al. (1996) showed that the infectivity of extraradical hyphae may be eliminated by wet–dry cycles, whereas spores appear to be less affected. Thus, treatments are unlikely to affect all propagules equally. We saw a decline in both mycorrhizal colonization of bait plants (Fig. 2) and hyphal viability in the warm–moist soil, but whether or not the decline in mycorrhizal colonization was the result of hyphae alone cannot be deduced.

Whereas overall mycorrhizal colonization of bait plants showed the greatest decline in the warm–moist conditions, survival of hyphae decreased in all moist soils, irrespective of temperature (as indicated by the significant effect of moisture in Fig. 3*a*). The decline in hyphal viability in moist freezing soil could have been caused by the physical disruption due to frost-heave, which is similar to the disturbance caused by tillage (Kabir et al. 1997), although it is debatable whether or not this disturbance occurs at a scale sufficiently fine to damage AM hyphae (McGonigle and Miller 1999). The fact that mycorrhizal colonization was reduced only in the warm–moist treatment, while hyphal survival was reduced in both moist treatments, is consistent with the hypothesis that colonized root pieces and spores, which are not expected to be affected by frost-heave, were important sources of inoculum in this study.

Survival of SAT hyphae

That hyphae of the SAT isolates survived the fallow better than the MT isolates is intriguing (Fig. 3*b*). In 9 out of 36 samples, the ratio of viable hyphal length 2 : viable hyphal length 1 was greater than one, and six of those were found in the tropical isolates. A ratio greater than one could have been caused either by hyphae of AM fungi (or something resembling AM fungal hyphae) growing during the fallow period, or by a clumped distribution of hyphae in the pot. AM fungal hyphae are not expected to grow during the fallow because they are presumably biotrophic, and we find it unlikely that non-AM fungal hyphae were counted because they might have been expected to grow best in the warm and moist condition, yet in only one out of the nine samples did we record a ratio greater than one in that treatment. It is possible that the hyphae of tropical isolates were more spatially clumped within the pot and that clumping resulted in insufficient sampling, but a *t* test of the standard deviations generated from each treatment combination of the topical and temperate isolates revealed no statistically significant difference either before ($p = 0.36$) or after ($p = 0.33$) the fallow, suggesting that the hyphal distribution of tropical isolates were not significantly more clumped than the temperate isolates. Finally, when all nine replicates with a ratio greater than one were removed from the analyses, the hyphal viability of the SAT isolates was still greater than that of the MT isolates ($p = 0.01$). The only possible explanation we can offer for this is that erratic rainfall in the semi-arid tropical regions may have resulted in hyphae that are able to survive for long periods without a host plant, irrespective of the soil conditions. However, owing to the poorer survival of *G. etunicatum*$_{SAT}$ as assessed by mycorrhizal colonization (Fig. 2), it appears as if this may not be a trait that extends to all fungal structures. Clearly, this is an area that would benefit from more research.

In conclusion, warm, moist soils had the greatest negative impact on fungal survival during fallow. However, we saw no indication that the isolates from different climates, tested here, respond in a way that suggests that they are adapted to their local climatic conditions. Instead, it appears that these climatically unadapted morphospecies possess traits that confer tolerances to a broad range of environmental conditions, perhaps due to possession of GPGs, which may enable establishment across climatic regions without the need for local adaptation. In this study, only two morphospecies that are relatively common in disturbed agricultural systems were used. To assess the general applicability of our findings, more isolates need to be tested, including morphospecies originating from other vegetation systems. An increased knowledge in this area will not only improve our understanding of AM fungal ecology, but will also indicate

whether local isolates are necessary for successful inoculation programs.

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References

Addy, H.D., Miller, M.H., and Peterson, R.L. 1997. Infectivity of the propagules associated with extraradical mycelia of two AM fungi following winter freezing. New Phytol. **135**: 745–753. doi:10.1046/j.1469-8137.1997.00707.x.

Addy, H.D., Boswell, E.P., and Koide, R.T. 1998. Low temperature acclimation and freezing resistance of extraradical VA mycorrhizal hyphae. Mycol. Res. **102**: 582–586. doi:10.1017/S0953756297005376.

Appoloni, S., Lekberg, Y., Tercek, M.T., Zabinski, C.A., and Redecker, D. 2008. Molecular community analysis of arbuscular mycorrhizal fungi in roots of geothermal soils in Yellowstone National Park (USA). Microb. Ecol. In press [online]. doi: 10.1007/s00248-008-9384-9. PMID:18449467.

Auge, R.M. 2004. Arbuscular mycorrhizae and soil/plant water relations. Can. J. Soil Sci. **84**: 373–381.

Baker, H.G. 1965. Characteristics and modes of origin of weeds. *In* The genetics of colonizing species. *Edited by* H.G. Baker and G.L. Stebbins. Academic Press, New York, N.Y.

Braunberger, P.G., Abbott, L.K., and Robson, A.D. 1996. Infectivity of arbuscular mycorrhizal fungi after wetting and drying. New Phytol. **134**: 673–684. doi:10.1111/j.1469-8137.1996.tb04933.x.

Brundrett, M.C., Ashwath, N., and Jasper, D.A. 1996*a*. Mycorrhizas in the Kakadu region of tropical Australia. Plant Soil, **184**: 159–171. doi:10.1007/BF00029285.

Brundrett, M., Bougher, N., Dell, B., Grove, T., and Malajczuk, N. 1996*b*. Working with mycorrhizas in forestry and agriculture. ACIAR Monograph 32, Canberra ACT, Australia.

Carvalho, L.M., Correia, P.M., and Martins-Loucao, M.A. 2004. Arbuscular mycorrhizal fungal propagules in a salt marsh. Mycorrhiza, **14**: 165–170. doi:10.1007/s00572-003-0247-4. PMID: 12811643.

Daniels, B.A., and Menge, J.A. 1980. Hyper parasitization of vesicular arbuscular mycorrhizal fungi. Phytopathology, **70**: 584–588.

del Val, C., Barea, J.M., and Azcon-Aguilar, C. 1999. Assessing the tolerance to heavy metals of arbuscular mycorrhizal fungi isolated from sewage sluge-contaminated soils. Appl. Soil Ecol. **11**: 261–269. doi:10.1016/S0929-1393(98)00153-X.

Fitter, A.H., Heinemeyer, A., Husband, R., Olsen, E., Ridgway, K.P., and Staddon, P.L. 2004. Global environmental change and the biology of arbuscular mycorrhizas, gaps and challenges. Can. J. Bot. **82**: 1133–1139. doi:10.1139/b04-045.

Gavito, M.E., Olsson, P.A., Rouhier, H., Medina-Penafield, A., Jakobsen, I., Bago, A., and Azcon-Aguilar, C. 2005. Temperature constraints on the growth and functioning of root organ cultures with arbuscular mycorrhizal fungi. New Phytol. **168**: 179–188. doi:10.1111/j.1469-8137.2005.01481.x. PMID: 16159332.

Grey, W.E. 1991. Influence of temperature of colonization of spring barleys by vesicular arbuscular mycorrhizal fungi. Plant Soil, **137**: 181–190. doi:10.1007/BF00011196.

Heinemeyer, A., and Fitter, A.H. 2004. Impact of temperature on the arbuscular mycorrhizal (AM) symbiosis, growth responses of the host plant and its AM fungal partner. J. Exp. Bot. **55**: 525–534. doi:10.1093/jxb/erh049. PMID:14739273.

Helgason, T., Daniell, T.J., Husband, R., Fitter, A.H., and Young, J.P.W. 1998. Ploughing up the wood-wide web. Nature (London), **394**: 431. doi:10.1038/28764. PMID:9697763.

Husband, R., Herre, E.A., and Young, J.P.W. 2002. Temporal variation in the arbuscular mycorrhizal communities colonising seedlings in a tropical forest. FEMS Microbiol. Ecol. **42**: 131–136. doi:10.1111/j.1574-6941.2002.tb01002.x.

Kabir, Z., O'Halloran, I.P., and Hamel, C. 1997. Overwinter survival of arbuscular mycorrhizal hyphae is favored by attachments to roots but diminished by disturbance. Mycorrhiza, **7**: 197–200. doi:10.1007/s005720050181.

Klironomos, J.N., Hart, M.M., Gurney, J.E., and Moutoglis, P. 2001. Interspecific differences in the tolerance of arbuscular mycorrhizal fungi to freezing and drying. Can. J. Bot. **79**: 1161–1166. doi:10.1139/cjb-79-10-1161.

Kuszala, C., Gianinazzi, S., and Gianinazzi-Pearson, V. 2001. Storage conditions for the long-term survival of AM fungal propagules in wet sieved soil fractions. Symbiosis, **30**: 287–299.

Lekberg, Y., Koide, R.T., Rohr, J.R., Aldrich-Wolfe, L., and Morton, J.B. 2007. Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. J. Ecol. **95**: 95–105. doi:10.1111/j.1365-2745.2006.01193.x.

Lloyd-MacGilp, S.A., Chambers, S.M., Dodd, J.C., Fitter, A.H., Walker, C., and Young, J.P.W. 1996. Diversity of the ribosomal internal transcribed spacer within and among isolates of *Glomus mosseae* and related mycorrhizal fungi. New Phytol. **133**: 103–111. doi:10.1111/j.1469-8137.1996.tb04346.x.

Machlis, L., and Torrey, J.G. 1956. Plants in action. Freeman. San Fransisco, Calif.

McGonigle, T.P., and Miller, M.H. 1999. Winter survival of extraradical hyphae and spores of arbuscular mycorrhizal fungi in the field. Appl. Soil Ecol. **12**: 41–50. doi:10.1016/S0929-1393(98)00165-6.

Miller, R.M., Carnes, B.A., and Moorman, T.B. 1985. Factors influencing survival of vesicular-arbuscular mycorrhizal propagules during topsoil storage. J. Appl. Ecol. **22**: 259–266. doi:10.2307/2403343.

Nicolson, T.H. 1959. Mycorrhiza in the Gramineae, I. Vesicular-arbuscular endophytes, with special reference to the external phase. Trans. Br. Mycol. Soc. **42**: 421–438.

Pattinson, G.S., and McGee, P.A. 1997. High densities of arbuscular mycorrhizal fungi maintained during long fallows in soils used to grow cotton except when the soil is wetted periodically. New Phytol. **136**: 571–580. doi:10.1046/j.1469-8137.1997.00783.x.

Shenk, N.C. 1975. Temperature and light effect on contamination and spore germination of vesicular–arbuscular mycorrhizal fungi. Mycologia, **67**: 1189–1192. doi:10.2307/3758840. PMID: 1214835.

Siminovitch, D., and Cloutier, Y. 1983. Drought and freezing tolerance and adaptation in plants, some evidence of near equivalence. Cryobiology, **20**: 487–503. doi:10.1016/0011-2240(83)90037-8. PMID:6617235.

Siqueira, J.O., Sylvia, D.M., Gibson, J., and Hubbel, D.H. 1985. Spores, germination, and germ tubes of vesicular–arbuscular mycorrhizal fungi. Can. J. Microbiol. **31**: 965–972.

Stahl, P.D., and Christensen, M. 1990. Population variation in the mycorrhizal fungus *Glomus mosseae*, uniform garden experiments. Mycol. Res. **94**: 1070–1076.

Stahl, P.D., and Christensen, M. 1991. Population variation in the mycorrhizal fungus *Glomus mosseae*, breadth of environmental tolerance. Mycol. Res. **95**: 300–307.

Stutz, J.C., Copeman, R., Martin, C.A., and Morton, J.B. 2000. Patterns of species composition and distribution of arbuscular mycorrhizal fungi in arid regions of southwestern North America and Namibia, Africa. Can. J. Bot. **78**: 237–245. doi:10.1139/cjb-78-2-237.

Sykorova, Z., Wiemken, A., and Redecker, D. 2007. Cooccurring *Gentiana verna* and *Gentiana acaulis* and their neighboring plants in two Swiss upper montane meadows harbor distinct arbuscular mycorrhizal fungal communities. Appl. Environ. Microbiol. **73**: 5426–5434. doi:10.1128/AEM.00987-07. PMID: 17630317.

Tommerup, I.C., and Abbott, L.K. 1981. Prolonged survival and viability of VA mycorrhizal hyphae after root death. Soil Biol. Biochem. **13**: 431–433. doi:10.1016/0038-0717(81)90090-0.

Turesson, G. 1922. The species and the variety as ecological units. Hereditas, **3**: 100–113.

van Doninck, K., Shön, I., Bruyn, L., and Martens, K. 2002. A general purpose genotype in an ancient asexual. Oecologia (Berl.), **132**: 205–212. doi:10.1007/s00442-002-0939-z.

Weinbaum, B.S., Allen, M.F., and Allen, E.B. 1996. Survival of arbuscular mycorrhizal fungi following reciprocal transplanting across the Great Basin, USA. Ecol. Appl. **6**: 1365–1372. doi:10.2307/2269614.

Weissenhorn, I., Leyval, C., and Berthelin, J. 1993. Cd-tolerant arbuscular mycorrhizal (AM) fungi from heavy-metal polluted soils. Plant Soil, **157**: 247–256. doi:10.1007/BF00011053.