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Variation in aluminum resistance among arbuscular mycorrhizal fungi

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Abstract Arbuscular mycorrhizal (AM) fungi mediate interactions between plants and soils, and are important where nutrient or metal concentrations limit plant growth. Variation in fungal response to edaphic conditions may influence the effectiveness of the plant-mycorrhizal association in some soil environments. *Andropogon virginicus* (broomsedge) colonizes disturbed sites in the eastern United States, including acidic mine soils where aluminum (Al) is phytotoxic, and Al resistance in broomsedge has been associated with colonization by the AM fungus *Glomus clarum*. In the present study, inter- and intra-specific variation to confer Al resistance to broomsedge was assessed among selected species of AM fungi. Broomsedge seeds were grown in sand culture inoculated with one of five isolates of three species of fungi (*G. clarum*, *Acaulospora morrowiae*, and *Scutellospora heterogama*). Plants were exposed to 0 or 400 μM Al in nutrient solution and harvested after 4 or 9 weeks of growth. Mean infection percentage, plant biomass, and plant tissue Al and phosphorus (P) concentrations were measured. *G. clarum* conferred the greatest Al resistance to broomsedge, with the lowest variability among isolates for colonization and growth inhibition by Al [tolerance indices (TI) between 22.4 and 92.7%]. Broomsedge plants colonized by *A. morrowiae* were consistently the most sensitive to Al, with little variation among isolates (TI between 1.6 and 12.1%). Al resistance by *S. heterogama* isolates was intermediate and wide-ranging (TI between 3.9 and 40.0%). Across all AM fungal isolates, resistance was associated with high rates of colonization and low tissue Al concentrations of broomsedge plants. The functional diversity in Al resistance displayed by these AM fungi reflect variation in acclimation mechanisms operating in the mycorrhizal symbiosis under environmental stress.

Keywords Aluminum · AM fungi · Functional diversity · Intraspecific variation

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Introduction

Soil aluminum (Al) toxicity is a problem on 30–40% of the world's arable land, second only to drought as an impediment to maximizing crop yields (Barinaga 1997). Highly weathered soils, or those that developed on acidic parent material, exhibit low pH, limited base cation availability, and elevated levels of available Al. Soil Al also may be enhanced by fertilizer additions, chronic nitrogen deposition, or from disturbance associated with mining (Joslin et al. 1992; McBride 1994; Johnson and Skousen 1995; Aber et al. 1998). The impacts of Al on plants include inhibition of root growth and alteration of nutrient acquisition (Kochian 1995; Ma 2000; Ma et al. 2001). Thus, Al reduces productivity, increases susceptibility to drought, and alters nutritional quality of plants growing on acid soils (Taylor 1988; Kochian 1995).

The association of arbuscular mycorrhizal (AM) fungi (AMF) with plant roots alters plant-soil interactions and enhances plant growth under stressful edaphic conditions (Smith and Read 1997). These changes may result from alterations in carbon allocation and nutrient physiology of the host plant resulting from colonization, or new physiological capacity brought to the symbiosis by AMF. Plant nutrient uptake is increased by mycorrhizal fungi through the expansion of the surface area of the root for absorption, as well as through the mobilization of nutrients from otherwise unavailable sources (O'Keefe and Sylvia 1991; Mendoza and Borie 1998). This benefit is most significant for the acquisition of phosphorous (P) (Lambert et al. 1979; Borie

and Rubio 1999; Del Val et al. 1999; Bever et al. 2001). However, genetic variation among and within AMF species is substantial (Bever and Morton 1999) and may provide variation in the benefits conferred to host plants growing on soils where nutrients are limiting or toxic metals are present. Such functional diversity is exemplified in variation in P uptake and the expression of related genes in *Medicago trunculata* associated with different AMF species (Burleigh et al. 2002).

In soils containing elevated concentrations of toxic metals, mycorrhizae often increase the metal resistance of host plants (Gildon and Tinker 1983; Weissenhorn et al. 1995; Lux and Cumming 2001; Tonin et al. 2001). The mechanisms conferring metal resistance, however, are unclear and will most likely differ among metals and perhaps fungi. Stress resistance of host plants under metal exposure may be related to increased P uptake (Sylvia and Williams 1992; Lux and Cumming 2001). Metal uptake and translocation to plant shoots may be reduced by metal chelation in the mycorrhizosphere, metal binding to hyphal cell walls, or by intracellular sequestration in fungal tissues (Shetty et al. 1994; Jentschke and Godbold 2000; Tonin et al. 2001; Hall 2002).

In addition to their direct effects on plants, metals in the environment may directly alter mycorrhizal communities and, thus, the ability of the fungi to provide benefits and protection from metal toxicity to the host plant (Leyval et al. 1997). Del Val et al. (1999) reported that increasing concentrations of metals decreased AMF population diversity and abundance. Selection due to local edaphic conditions may lead to the development of ecotypic variation within species of fungi resulting in AMF adapted to metalliferous environments (Griffioen et al. 1994). For example, an AM fungus isolated from the metallophytic plant *Viola calaminaria* provided more protection to maize in a contaminated soil than the same fungal species isolated elsewhere (Hildebrandt et al. 1999; Kaldorf et al. 1999). Aluminum effects on spore germination and hyphal growth varied within and among species (Barkdoll 1987; Bartolome-Esteban and Schenck 1994), although the effect that these divergent fungal responses to Al would have on host plant resistance was not examined.

The present study investigates variation of AMF genotypes under Al exposure as a preliminary step towards identifying the mechanism(s) operating in fungi that confer Al resistance to host plants. Fungal isolates of different edaphic origins were selected from the species *Glomus clarum*, *Acaulospora morrowiae*, and *Scutellospora heterogama*. Variation in Al resistance was measured by colonization rates in *Andropogon virginicus* (broomsedge) roots, biomass production of the host plant, and Al and P uptake and translocation by mycorrhizal plants exposed to two Al levels.

Materials and methods

Methods

Variation in Al resistance was measured in 15 fungal isolates, 5 each of *G. clarum* Nicol. and Schenck, *A. morrowiae* Spain and Schenck, and *S. heterogama* (Nicol. and Gerdemann) Walker and Sanders. These species were chosen because they are widely distributed in diverse habitats (J.B. Morton, unpublished) and they exhibited variation in Al resistance in previous studies (K.R. Klugh, J.B. Morton and J.R. Cumming, unpublished data). Isolates were chosen based on soil properties of the originating site, with emphasis on pH as an indicator of available Al concentrations (Table 1).

Isolates from both acidic and neutral soils were maintained in uniform pot cultures in the INVAM Culture Collection (Morton et al. 1993). *A. virginicus* (broomsedge) was used as the host plant, as it is an early successional grass commonly found on acidic soils and unreclaimed mine sites in the eastern United States. It grows where soil

Table 1 Fungal isolates used and soil properties at sites of isolation. Numbers following the dashes in the INVAM reference indicate the number of generations each isolate has been in culture. Information given in as much detail as is known of the originating site

Species	Isolate number	INVAM reference	Soil properties	Location of isolation
Glomus clarum	1	CL156-1	Tropical/acidic	Colombia
	2	BR152B-11	Tropical/acidic	Brazil
	3	MA402B-2	Sand dune/neutral pH	Massachusetts
	4	SC706-3	Wetland/acidic; pH 4.1	South Carolina
	5	WV310-1	Neutral pH	West Virginia
Acaulospora morrowiae	6	BR147C-4	Commercial inoculum/neutral pH	Brazil
	7	CL735-6	Tropical/acidic	Colombia
	8	EC123-4	Tropical/acidic	Ecuador
	9	KS202-3	Konza prairie/neutral pH	Kansas
	10	WV107-5	Minesite; with *Andropogon*; pH 3.7	West Virginia
Scutellospora heterogama	11	WV108-7	Minesite; with *Andropogon* pH 3.7	West Virginia
	12	NY320-8	With clover/apple; pH 6.6	New York
	13	SN722-9	With *Macaranga triloba*; pH 3.3	Singapore
	14	CL157-1	Tropical/acidic	Colombia
	15	IL203A-4	Farmland/neutral pH	Illinois

acidity and metal availability limit the growth of most plant species (Nellesen and Ungar 1993).

Culture

Fungi were propagated on sudan grass (*Sorghum sudanense*) according to Morton et al. (1993). Spores and hyphae of each isolate were collected by wet-sieving (500, 150, and 38 μm nested sieves). This inoculum was added to a 3:1 mixture of fine and coarse acid-washed sand (0.1 *N* HCl for 12 h) (1:10 volume inoculum:sand) in 38×205 cm (width×-height) containers (Cone-tainers; Stuewe and Sons, Corvallis, Ore.), each containing 200 cm^3 inoculum:sand mixture. Control plants (no fungi) were also included, in which pot culture contents of non-mycorrhizal sudan grass were sieved and added to sand in the same proportions. This treatment served as a bacterial control for plants otherwise receiving AM fungal spores and hyphae. Stratified (4 months at 4°C) *A. virginicus* seeds were placed into the cones and, following germination, maintained by adding 0.1-strength Johnson's nutrient solution (Lux and Cumming 2001) with KH_2PO_4 adjusted to 50 μM and either 0 or 400 μM Al added as $Al_2(SO_4)_3$. All solutions were adjusted to pH 4.0 prior to delivery. Plants received 20 ml solution four times daily from an automated delivery system and were propagated in a growth chamber at 28°/21°C with a 14 h/10 h light/dark cycle. Light level within the chamber averaged 260 μmol m^{-2} s^{-2} from mixed incandescent and fluorescent lighting. Plants were exposed to 0 and 400 μM Al treatments for 4 or 9 weeks. The short-term exposure was designed to access fungal Al resistance response while the longer exposure assessed integrated plant-fungal symbioses response to Al.

Mycorrhizal colonization

The impact of Al on fungal colonization of roots was assessed through a mean infection percentage (MIP) assay (Moorman and Reeves 1979). Plants were harvested following 4 weeks of Al exposure and roots were stained following the method of Koske and Gemma (1989). Roots were destained in deionized water and then scored for percentage colonization using the gridline-intersect method (Giovanetti and Mosse 1980; Newman 1966). Mycorrhizal colonization was also estimated in plants exposed to Al for 9 weeks by the same staining method using a small (0.25 g) sample of fine roots from the center of the root system.

Plant growth responses

Following each harvest, plants were dried at 60°C and root and shoot biomass was measured. Root and shoot tissues were ground and digested in HNO_3/H_2O_2 (Jones and Case 1990). The digests were analyzed for Al by graphite furnace atomic absorption spectroscopy (Varian; Mulgrave, Victoria, Australia). P concentrations of digested shoot and root tissues were determined spectrophotometrically following the methods of Tauskey and Shorr (1953).

Statistical analyses

Treatments were replicated eight times, with five replicates harvested for the short term study (n=5; N=180) and three replicates for the long-term experiment (n=3; N=96). Data were analyzed by a two-way nested ANOVA (species, isolate within species, Al level) to determine species-level variation and as three 5×2 (isolate×Al level) factorial designs to assess isolate-level variation. Biomass and tissue Al concentrations were analyzed after log-transformation of the data. Colonization data was analyzed after arcsine transformation. Means were separated by the Tukey-Kramer HSD protocol. A tolerance index (TI) denoting Al resistance was defined as:

$$\mathrm{TI} = \frac{\text{mean plant biomass at 400 } \mu\text{M Al}}{\text{mean plant biomass at 0 } \mu\text{M Al}} \times 100 \qquad (1)$$

This index was used to correlate mycorrhizal plant responses to pH (acid or neutral) of the soil from which isolates were collected. The TI was analyzed as a two-way ANOVA (species×soil type). Means presented in figures and tables are non-transformed values. All statistical analyses were executed using JMP statistical software (SAS Institute, Cary, N.C.).

Results

Mycorrhizal colonization

Fungal colonization of broomsedge roots after 4 weeks growth varied with species (P<0.001). Across Al treatments, mean colonization of *G. clarum*, *A. morrowiae*, and *S. heterogama* was 78%, 28%, and 38%, respectively. Significant intraspecific variation in infectivity was detected for all species at 4 weeks under control conditions of 0 μM Al (*G. clarum*, P=0.043; *S. heterogama*, P<0.001; *A. morrowiae*, P<0.001) (Fig. 1a–c). Isolates of *A. morrowiae* and *S. heterogama* exhibited the most variation in colonization, whereas those of *G. clarum* had similar colonization. Colonization varied by isolate within *A. morrowiae* (P<0.001 for isolate×Al interaction) at 400 μM Al and it was inhibited to less than 20% across all isolates. In contrast, Al increased colonization for all *G. clarum* isolates (P<0.001), and did not significantly alter mycorrhizal development among *S. heterogama* isolates (P=0.221).

Mycorrhizal colonization following 9 weeks growth varied among isolates of *A. morrowiae* (P=0.002) and *S. heterogama* (P=0.001), but not among those of *G. clarum* (P=0.782). These differences were not related to Al ex-

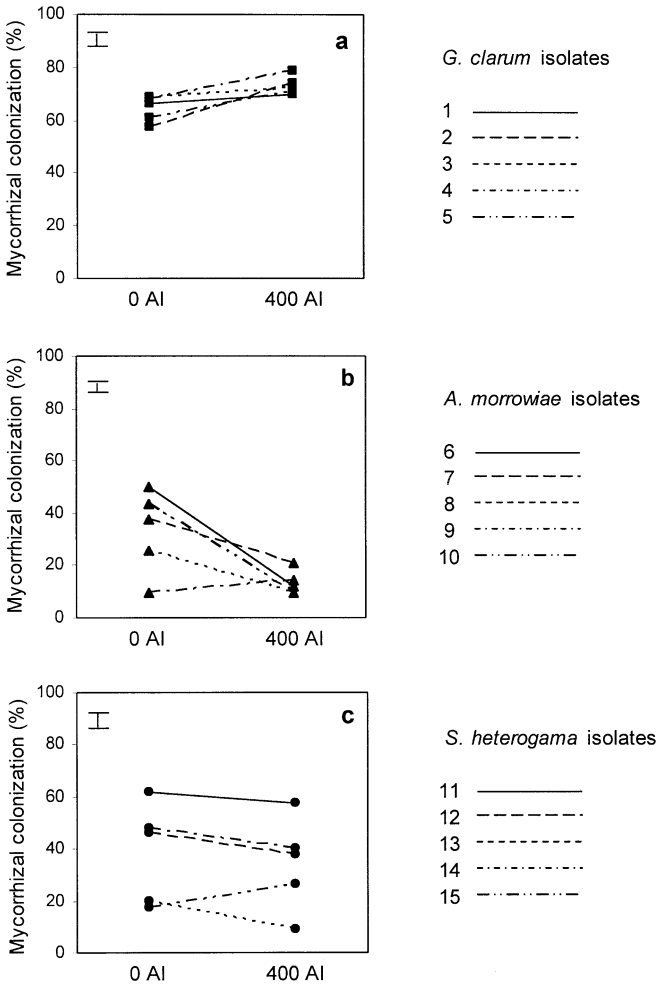

Fig. 1 Mean infection percentage (MIP) of roots of broomsedge plants at 0 and 400 μM aluminum (Al) exposure after 4 weeks of growth by isolates within **a** *Glomus clarum*, **b** *Acaulospora morrowiae*, and **c** *Scutellospora heterogama*. *Error bars* Standard errors of the means (n=5). Isolates listed within each species refer to isolates as labeled in Table 1

posure (P>0.05 for isolate×Al interaction for each species; data not presented), but more likely reflected heritable differences in aggressiveness among isolates of these species.

Plant growth

Under 0 μM Al treatment, mycorrhizal broomsedge plants, regardless of fungal species, were approximately 100% larger than nonmycorrhizal plants, reflecting the highly mycotrophic nature of broomsedge (Table 2). Species-level differences in total plant biomass were notable in plants grown for both 4 (P=0.001) and 9 weeks (P=0.003) (Figs. 2 and 3, respectively).

Following 4 weeks of growth, most of the variation detected in biomass measurements among isolates of each fungus was due to differences in growth of plants under 0 μM Al exposure. Under 0 μM Al conditions, plant biomass varied by 36% among *G. clarum* isolates, 53% among *A. morrowiae* isolates, and 61% among *S. heterogama* isolates (Fig. 2a–c). Al exposure decreased total plant biomass for all isolates of *G. clarum* (P=0.001), *A. morrowiae* (P<0.001), and *S. heterogama* (P<0.001), although the extent of inhibition by Al varied with species. At 400 μM Al, broomsedge plants colonized by *G. clarum* exhibited the least reduction in growth, with variation among isolates similar to that at 0 μM Al (Fig. 2a). In contrast, broomsedge plants colonized by *A. morrowiae* were extremely sensitive to Al and this sensitivity was uniform among all isolates (Fig. 2b). Plants colonized by *S. heterogama* were intermediate in their response, with isolate-level discrimination in sensitivity to Al (Fig. 2c).

In plants grown for 9 weeks, total plant biomass responses exhibited similar patterns to those plants harvested for MIP assessments. Growth was inhibited by Al within each fungal treatment (*G. clarum*, P=0.012; *A. morrowiae*, P<0.001; *S. heterogama*, P<0.001) (Fig. 3a–c). Intraspecific variation was detected within *A. morrowiae* (P=0.009) and *S. heterogama* (P=0.055) species, although differences were primarily expressed at 0 μM Al (P>0.05 for Al×isolate interaction) (Fig. 3a–c). Variation among isolates of all three fungal species was high under 0 μM Al conditions. With exposure to Al, however, growth was affected least in plants colonized by *G. clarum* and isolate-level variability was relatively high (Fig. 3a). Plants colonized by *A. morrowiae* exhibited the greatest sensitivity to Al, with little or no variation among isolates (Fig. 3b). Plants colonized by *S. heterogama* exhibited intermediate responses (Fig. 3c).

Table 2 Total plant biomass and aluminum (Al) and phosphorus (P) concentrations of shoots and roots of broomsedge plants as influenced by fungal species and exposure to Al for 9 weeks. Means followed by the same letters are not significantly different

Fungal species	Al treatment (μM)	Total biomass (mg)	Shoot Al (μg/g)	Root Al (μg/g)	Shoot P (mg/g)	Root P (mg/g)
G. clarum	0	853.6±190 a	10.9±1.2 d	341.8±35 b	1.42±0.09 a	0.95±0.08
	400	388.1±60 b	43.2±3.1 c	1,867.9±104 a	0.97±0.08 b	0.89±0.05
A. morrowiae	0	721.1±190 a	9.9±0.9 d	415.9±50 b	1.43±0.13 ab	1.04±0.10
	400	29.1±7 cd	165.2±27.5 ab	3,089.3±295 a	1.38±0.10 ab	0.98±0.19
S. heterogama	0	804.6±151 a	12.5±1.9 d	334.4±42 b	1.38±0.11 ab	0.84±0.08
	400	161.7±57 c	93.3±17.7 b	2,494.2±200 a	1.57±0.19 ab	1.16±0.35
Control	0	370.5±57 b	15.0±5.7 d	583.3±155 b	1.42±0.13 ab	1.21±0.12
	400	30.1±6 cd	225.4±106.5 a	2,720.5±305 a	2.02±0.34 a	0.64±0.14

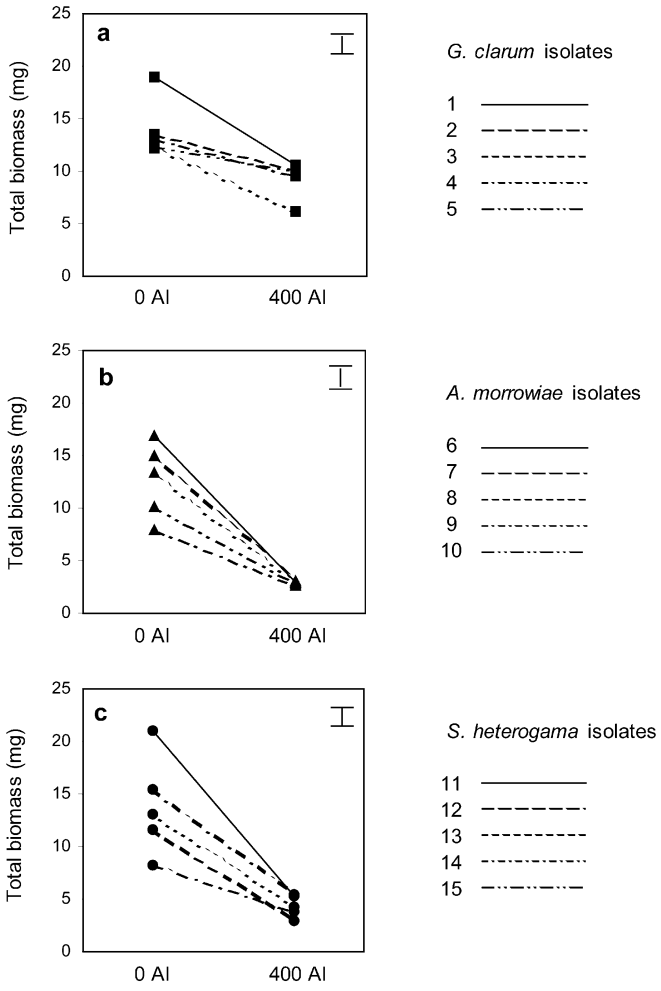

Fig. 2 Total biomass of mycorrhizal broomsedge plants at 0 and 400 μM Al exposure after 4 weeks of growth by isolates within **a** *G. clarum*, **b** *A. morrowiae*, **c** *S. heterogama*. *Error bars* Standard errors of the means (n=5). Isolates listed within each species refer to isolates as labeled in Table 1

Tissue Al and P concentrations

Intraspecific variation in Al and P concentrations was not detected in root or shoot tissues of any fungal treatments (P>0.05 for all isolate×Al interactions) (Table 2). Shoot Al concentrations varied by species (P<0.001) and concentrations were significantly higher in plants exposed to Al (P<0.001). Concentrations of Al in foliage of nonmycorrhizal broomsedge plants were as much as 10-fold higher than that of mycorrhizal plants (Table 2). Plants grown with *G. clarum* isolates translocated the least amount of Al to shoots, while *A. morrowiae*-associated plants yielded the greatest concentration of shoot Al of the mycorrhizal treatments. Root Al concentrations differed between species of fungi (P=0.033), and were significantly higher in plants treated with Al (P<0.001).

Shoot P concentrations were 33% lower in *G. clarum*-associated plants with Al exposure (P<0.001). Nonmycorrhizal plants exhibited a nearly 2-fold greater concentration of shoot P at 400 μM Al (2.03 μg/g) compared to mycorrhizal plants (0.97–1.57 μg/g, depending on fungal species) (Table 2). Root P concentrations decreased under Al exposure (P=0.006), whereas fungal colonization and fungal species did not alter P concentrations in the roots (P>0.05).

Relationships between variables

Under Al exposure, total biomass of broomsedge plants was positively correlated with mycorrhizal colonization across all fungal species-isolate combinations (r=0.783) (Fig. 4a). The extent of mycorrhizal colonization also influenced the accumulation of Al in broomsedge foliage, with low shoot Al occurring in plants with high colonization (r=−0.728) (Fig. 4b). Total plant biomass was correlated with shoot Al concentrations (r=−0.733), with smaller plants exhibiting the highest concentrations of Al in the shoots (Fig. 4c). In each of these relationships, isolates within species tended to group together, reflecting interspecific differences in Al resistance (Fig. 4a–c). The largest intraspecific variation

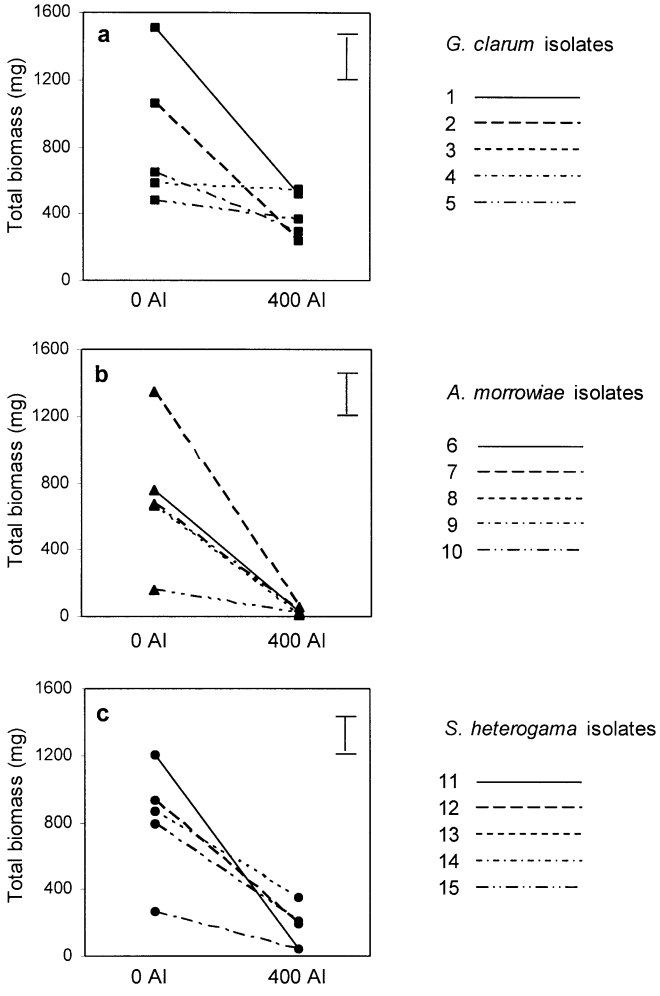

Fig. 3 Total biomass of mycorrhizal broomsedge plants at 0 and 400 μM Al exposure after 9 weeks of growth by isolates within **a** *G. clarum*, **b** *A. morrowiae*, **c** *S. heterogama*. *Error bars* Standard errors of the means (n=3). Isolates listed within each species refer to isolates as labeled in Table 1

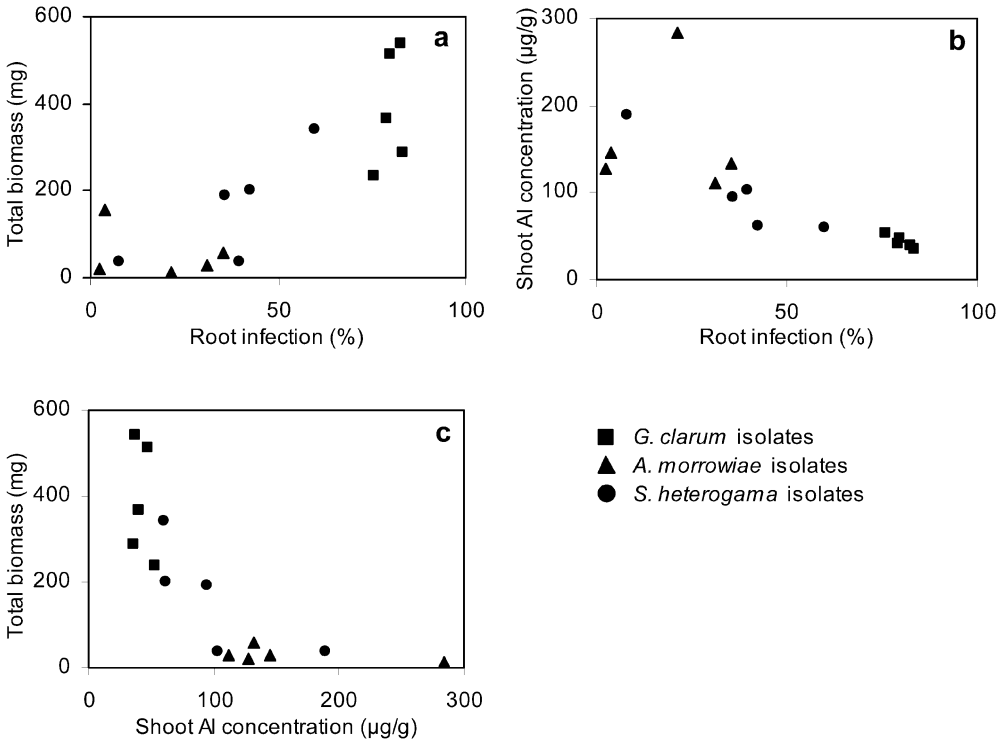

Fig. 4 Relationships between responses of broomsedge plants exposed to 400 µM Al for 9 weeks with respect to **a** plant biomass and colonization, **b** shoot Al and colonization, and **c** plant biomass and shoot Al. The average response of each isolate is shown (*n*=3)

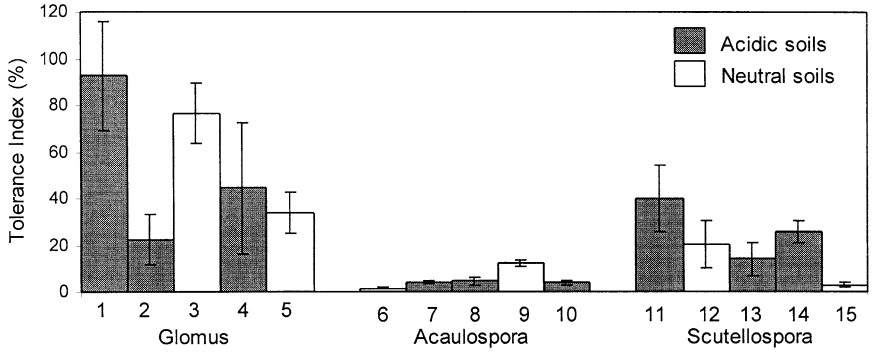

Fig. 5 Tolerance index (TI) of broomsedge plants grown under 400 µM Al for 9 weeks as influenced by fungal species and isolate and the original soil pH of each isolate. Isolates are numbered as in Table 1. *Error bars* Standard error of the means (*n*=3)

was noted for *S. heterogama*, consistent with patterns of growth shown in Figs. 2 and 3.

Effect of original soil pH on tolerance

No consistent relationship was observed between the TI of each isolate and soil pH at the site of fungal origin ($P>0.05$) (Table 2). Isolates from acidic soils generally did not confer a higher degree of protection to host plants compared to the isolates collected from soils of neutral pH (Fig. 5).

Discussion

AM fungi alter plant responses to stressful soil conditions. In addition to their role in nutrient acquisition, AM fungi alter relationships between plants and soil metals such as Al (Leyval et al. 1997; Rufyikiri 2000; Lux and Cumming 2001; Cumming and Ning 2003), to confer varying degrees of resistance. Results of this study document wide functional differences amongst three AMF species but, more importantly, these differences varied considerably amongst isolates, regardless of origin. On the one hand, isolates of *G. clarum* were uniformly the most aggressive, stimulated the strongest plant growth benefit and conferred the highest resistance to Al with a shift of Al translocation away from shoots. In contrast, *S. heterogama* provided more moderate resistance to Al, but variation amongst isolates was considerably greater across measurements of colonization, biomass, and shoot Al. Isolates of *A. morrowiae* were more uniform than those of *S. heterogama* and conferred weak resistance to Al.

Variation in early colonization (MIP) could be a direct result of inhibition of spore germination and/or early hyphal elongation, which Bartolome-Esteban and Schenck (1994) found varied significantly among and within AMF species exposed to Al. This variation occurred at both low and high Al exposure. However, over a longer growth period, intraspecific differences were not as evident, suggesting that perhaps secondary colonization was not as sensitive to Al as primary colonization. Once secondary

colonization occurs, plants gain the benefit of the AM association and differences present at earlier life stages appear to be lost.

Differences in plant response to Al resulting from colonization by various AMF species may reflect different physiological mechanisms that vary with fungal species/isolate. For example, the uptake and translocation of Al by broomsedge plants exhibited different patterns that were fungal-species-specific (Fig. 4). This variation may reflect changes in root zone Al speciation resulting from differences in Al binding to hyphae and/or fungal exudates. The abundant production of hyphae and the deposition of fungal exudates in the mycorrhizosphere surrounding roots alter the chemical speciation of ions in the root zone (Gadd 1993; Galli et al. 1994). Metal adsorption to hyphae or internal sequestration (Kaldorf et al. 1999; Joner et al. 2000; Tonin et al. 2001) and changes in metal speciation and solubility resulting from the production of exudates (Lux and Cumming 2001; Cumming and Ning 2003) all reduce the impacts of soil metal on the host plant.

Al resistance in non-mycorrhizal plants is associated with organic acid exudation (Ma 2000; Hall 2002). Organic acids such as citrate, malate, and oxalate form stable complexes with Al that lead to the detoxification of Al in the rhizosphere. In mycorrhizal plants, differences in organic acid exudation may play a similar role, and variation in plant production of metal-binding organic acids may be influenced differentially by different AMF species/isolates. The chelation or sequestration of metals by fungal/hyphae exudates reduce direct toxicity to the host by reducing metal ion availability in the rhizosphere (Mendoza and Borie 1998; Rufyikiri et al. 2000; Cumming and Ning 2003). Klugh and Cumming (2003) reported that 99% of Al was chelated by exudates in the root zone of *Liriodendron tulipifera* plants colonized by *G. clarum*, whereas only 63% was chelated in the root zone of nonmycorrhizal plants. The strong relationships between biomass and shoot Al and shoot Al to colonization (Fig. 4) suggest that fungi that maintain high levels of colonization under Al exposure concurrently limit Al bioavailability. However, the clustering of isolates within each species across these relationships also suggests that fungal genera may possess different physiological capabilities to alter Al availability in the rhizosphere. Indeed, Klugh and Cumming (2003) noted that Al resistance in mycorrhizal *L. tulipifera* was not associated with any unique pattern of increased organic acid exudation, but with the sustained exudation of these compounds under Al exposure. Hyphal development beyond the rhizosphere was not measured in this study, but it could be an important factor in fungal tolerance to metals by altering soil conditions and physiological responses of plants.

The lack of consistent mycorrhizal and Al influences on P acquisition is notable (Table 2), since Al forms insoluble complexes with P in the rhizosphere/root (de Miranda and Rowell 1989; Macklon and Sim 1992) and increased growth responses to AM fungi are commonly linked to an increase in P uptake (Lambert et al. 1979; Borie and Rubio 1999; Del Val et al. 1999; Bever et al. 2001). However, both this study and that of Cumming and Ning (2003) report tissue P values nearly 2-fold higher in nonmycorrhizal plants than in colonized plants under Al exposure. Thus, growth responses were not congruent with tissue P values. This may indicate a greater P-use efficiency (plant biomass/plant P content) in mycorrhizal grasses, rather than an actual increase in useable P resulting from mycorrhization (Ning and Cumming 2001). AM fungi provide the main pathway for plant uptake of P, although growth patterns or P tissue concentrations may not be altered by colonization (Smith et al. 2003).

In the present study, fungal isolates collected from acidic soils were not more tolerant than isolates from non-acidic sites. This lack of ecotypic separation of fungal isolates may have several explanations. First, the culture of fungi under selection-neutral environments, such as exist in large-scale culture collections, may lead to the dilution of genes conferring stress resistance as fungi reproduce through hyphae and spores over time. For example, acidic isolates of *G. clarum* (CL156 and BR152B) were in culture for 1 and 11 generations, respectively. These isolates exhibited vast differences in TI, with the tolerance decreasing with increased time under culture conditions. However, this pattern of culture-induced adaptive change was not observed among isolates of *A. morrowiae* and *S. heterogama* in this experiment. Malcova et al. (2003) measured a similar change in *G. intraradices*, where an isolate collected from a soil with elevated manganese concentrations conferred less metal resistance to maize after 2 years of culture in metal-free soil than the same isolate kept in soil from the sampling site. Together, these results suggest that assessments of AM fungal response to specific edaphic factors requiring the function of specific stress resistance genes may not be reliable if isolates are used after long-term culture in the absence of any selection pressures. Secondly, the contrasting patterns between species tested suggest genetic changes in nuclear populations among species may not be predictable in the presence or absence of selection pressures. Little is known about the mechanisms responsible for maintenance of gene frequencies or rates of change due to drift. Finally, although soil pH is known from all sites of isolate collection, microsite differences and other soil properties, such as the concentrations of organic compounds that complex and detoxify Al, is not known. Therefore, soil pH alone may not be a good indicator of isolate resistance to Al (Thomas and Hargrove 1984).

These patterns of functional diversity in Al resistance suggest that taxonomic diversity of mycorrhizal fungal communities may be important for plant community stability as ecosystems undergo change. Acid deposition, agricultural practices, and mining contribute to soil acidification, which can exacerbate Al toxicity. Host plants can influence AMF community structure through regulation of carbon allocation to roots and in altering soil conditions in the rhizosphere (Eom et al. 2000). Thus, selection of AMF ecotypes may be regulated in part by plant community structure (Van der Heijden 1998), responding to edaphic conditions such as soil pH and available Al. A functionally diverse community of soil microbes capable of adapting to

changing soil conditions will, therefore, directly influence the resulting plant community, yet relatively little is understood of the physiology that drives functional diversity. Future studies to determine the fungal physiological mechanism(s) of conferred Al resistance should focus on expression of genes causally linked to metal tolerance, adaptation to metal-contaminated soils and Al detoxification, uptake, and sequestration by AM fungi.

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