Arbuscular mycorrhizal fungi enhance aluminium resistance of broomsedge (*Andropogon virginicus* L.)

Jonathan R. Cumming¹ and Jianchang Ning²

Department of Biology, West Virginia University, Morgantown, WV 26506 USA

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Abstract

In the eastern United States, broomsedge (*Andropogon virginicus* L.) is found growing on abandoned coal-fired lands that have extremely acidic soils with high residual aluminium (Al) concentrations. Broomsedge may be inherently metal-resistant and nutrient-efficient or may rely on the arbuscular mycorrhizal (AM) fungal association to overcome limitations on such sites. Broomsedge plants were grown with and without an acidic ecotype AM fungal consortium and exposed to controlled levels of Al in two experiments. The AM fungal consortium conferred Al resistance to broomsedge. Arbuscular mycorrhizal fungi reduced Al uptake and translocation in host plants, potentially reflecting measured reductions in inorganic Al availability in the rhizosphere of mycorrhizal plants. Mycorrhizal plants exhibited lower shoot P concentrations, higher phosphorus use efficiency, and lower root acid phosphatase rates than non-mycorrhizal plants. Aluminium significantly reduced calcium (Ca) and magnesium (Mg) tissue concentrations in both mycorrhizal and non-mycorrhizal plants. However, plant response to any change in nutrient acquisition was substantially less pronounced in mycorrhizal plants. The exclusion of Al and greater stability of tissue biomass accretion–tissue nutrient relationships in mycorrhizal broomsedge plants exposed to Al may be important mechanisms that allow broomsedge to grow on unfavourable acidic soils.

Key words: Acidic soils, calcium, magnesium, nutrient homeostasis, phosphorus.

Introduction

Broomsedge (*Andropogon virginicus* L.) is an early successional species colonizing disturbed sites in the eastern United States (Campbell, 1983). In the coalfields of the Appalachian Mountains, broomsedge dominates abandoned surface strip-mined areas, even after significant periods of time have elapsed since mining (Nellessen and Ungar, 1993). Depending upon the underlying geology, residual soils on such sites may be extremely acidic, nutrients may be limiting, and soil chemistry may be dominated by high concentrations of aluminium (Al) and iron (Fe) (Zeleznik and Skousen, 1996; Fernandez-Marcos et al., 1998). The early and persistent colonization of such sites by broomsedge suggests that this species may be inherently nutrient-efficient and metal-resistant. In a previous study, however, Ning and Cumming (2001) found that broomsedge was highly dependent on the symbiotic association formed with arbuscular mycorrhizal (AM) fungi, and this association played a critical role in increasing phosphorus use efficiency and nutrient homeostasis of broomsedge plants under phosphorus (P) limitation.

Aluminium (Al) present in acidic soils is toxic to plants (Haug, 1984; Andersson, 1988; Kochian, 1995; Ritchie, 1995). Aluminium reduces root growth, alters nutrient availability in the rhizosphere, and impacts nutrient uptake and translocation by plants (Roy et al., 1988; Taylor, 1988; Nichol et al., 1993; Matsumoto, 2000). For example, Al reduces inorganic phosphorus (Pi) availability by forming Al–Pi precipitates in the rhizosphere and restricts P translocation within plants (Randall and Vose, 1963; Clarkson, 1966; Cumming et al., 1986; de Miranda and Rowell, 1989; Macklon et al., 1994). In addition, Al interferes with Ca and Mg uptake and translocation in plants (Haug and Caldwell, 1985; Keltjens and Tan, 1993;
Ryan and Kochian, 1993; Delhaize and Ryan, 1995; Rengel et al., 1995; Lux and Cumming, 2001). These effects result in nutrient imbalances in plants, consequently reducing plant growth.

Some plant species and genotypes within species exhibit resistance to Al exposure. Aluminum resistance in plants may be associated with an alteration in rhizosphere pH, release of organic acids, or $PO_3^{2-}$ efflux from the roots (Delhaize and Ryan, 1995; Kochian, 1995; Matsumoto, 2000). Many of these responses reduce $Al^{3+}$ availability in the rhizosphere, consequently reducing Al accumulation in tissues (Delhaize et al., 1993; Yang et al., 2000). Some plants also develop specific mechanisms to detoxify Al internally by increasing the production of compounds that chelate Al intracellularly (Ma et al., 1998).

Arbuscular mycorrhizal (AM) fungi may play a role in the protection of roots from Al toxicity by mediating interactions between Al, Pi, and plant roots (Marschner, 1995). Arbuscular mycorrhizal fungi are widely established in acidic soils (Clark, 1997), and colonization of plant roots by AM fungi often improves seedling survival and enhances plant growth on such soils (Danielson, 1985). The role of AM fungi in mediating Al toxicity may be especially pronounced where Al-induced P-deficiency dominates plant response to Al (Lux and Cumming, 2001). The extensive external fungal hyphae exploit a large volume of soil and mine scarce nutrient resources that are otherwise unavailable for uptake by roots (Smith and Read, 1997). This effect is most pronounced for Pi (Bolan, 1991; Smith and Read, 1997; Ning and Cumming, 2001), although AM fungal colonization may enhance the uptake of copper (Cu) and zinc (Zn) as well (Ross, 1971; Pacovsky, 1986; Smith and Read, 1997; Lux and Cumming, 2001; Ning and Cumming, 2001). In addition, AM fungi may reduce the accumulation of other elements, such as Al, Fe, and manganese (Mn), which are problematic in acidic soils (Pacovsky, 1986; Kothari et al., 1991; Lux and Cumming, 2001).

Colonization of roots by AM fungi may confer Al resistance to broomsedge plants colonizing acidic mine spoils by facilitating nutrient acquisition for the host plants under Al exposure and/or by altering the availability/speciation of Al in the rhizosphere. A limited number of experiments have demonstrated that AM fungal colonization ameliorates Al toxicity in plants (Koslosky and Boerner, 1989; Medeiros et al., 1994; Mendoza and Borie, 1998; Lux and Cumming, 2001), however, it is not clear how this amelioration is achieved. The present study investigated the response of broomsedge plants to Al and the influence of AM fungi on the growth and nutrition of plants exposed to Al. A community of AM fungi, including Glomus clarum (Nicolson & Schenck) and Gigaspora gigantea ((Nicol. & Gerd.) Gerd. & Trappe), isolated from an abandoned strip mine was used as the inoculum source. Two experiments were undertaken. The first focused on the influence of AM fungi and Al on Pi relations of broomsedge plants at low to moderate Al exposures. The second experiment spanned a greater concentration range of Al and focused on mycorrhizal mediation of Al-induced nutrient perturbations in broomsedge. It was hypothesized that mycorrhizal and non-mycorrhizal plants would differ in their responses to Al in the rhizosphere and that such differences would result from reduced Al–Pi interactions and the maintenance of overall nutrient acquisition mediated by AM fungal colonization.

## Materials and methods

### Preparation of AM fungal inoculum

Arbuscular mycorrhizal fungal inoculum was generated from broomsedge plants collected from an abandoned coal mine in Field Crest, near Morgantown, West Virginia, USA. Glomus clarum was the primary fungal species, with Gigaspora gigantea also present. Although the site has been abandoned for approximately 50 years, much of the surface soil remains devoid of vegetation due to the harsh chemical conditions. The vegetated surface consists of a broomsedge sward surrounding stunted red maple (Acer rubrum L.) and big-toothed aspen (Populus grandidentata Michx.) trees. Soil from the site has a pH of 3.0–3.3 (soil–water paste) and contained Mehlich-extractable Al of 363 mg kg$^{-1}$ soil. The inorganic monomeric Al concentration in surface water at the site was c. 600 μM, as measured by the eriochrome cyanine method (Anonymous, 1985; Lux and Cumming, 2001).

To produce AM fungal inoculum, broomsedge plants with intact mycorrhizal roots and adhering soil from the mine site were transplanted into 15 cm diameter pots containing a mixture of autoclaved mine-soil and sand (1:3 v/v). After 1 month, the pot contents became the source of inoculum for the experiments. These pots are termed ‘nursery pots’ hereafter. A set of axenically germinated broomsedge plants grown in the same soil–sand mixture with a bacterial extract from roots of field-collected plants served as a source of inoculum for non-mycorrhizal treatments.

### Preparation of plants

Broomsedge seeds were sown around the perimeter of both mycorrhizal and non-mycorrhizal nursery pots. After 4 weeks growth, roots of a small subset of seedlings (c. 10) were examined to determine mycorrhizal status. Seedlings were well colonized (72±7%). Tissue P status was analysed after wet digestion in concentrated $H_2SO_4$ (50% $H_2O_2$ (Parkinson and Allen, 1975) by the molybdate blue method (Olsen and Sommers, 1982). Tissue phosphorus concentrations and tissue dry weights did not differ significantly between mycorrhizal and non-mycorrhizal plants (Ning and Cumming, 2001).

### Sand culture and growth conditions

Mycorrhizal and non-mycorrhizal broomsedge seedlings were transplanted into and grown in D16 Deepots (5 cm diameter × 18 cm height) (Stuewe and Sons, Inc., Corvallis, Oregon, USA) prefilled with 220 cm$^3$ of a 3:1 (v/v) mixture of coarse: fine acid-washed sand. Deepots were placed into a growth chamber with 14 h of light at 28 °C, 60% RH, and 10 h of darkness at 21 °C, 50% RH. Average light intensity at pot height in the chamber was 260 μmol m$^{-2}$ s$^{-1}$ from mixed fluorescent and incandescent sources. Plants received a baseline nutrient solution containing $NO_3$ (1.5 mM), $NH_4$ (0.5 mM),...
K (0.79 mM), Ca (0.675 mM), Mg (0.25 mM), SO$_4$ (0.25 mM), H$_2$PO$_4$ (40 µM), Fe (25 µM), B (23.14 µM), Mn (4.57 µM), Zn (0.38 µM), Cu (0.16 µM), and Mo (0.06 µM). The pH of this formulation was c. 6.5 prior to adjustment to 4.0. For Al exposures, this solution was modified as indicated by the addition of Al as AlCl$_3$. All solutions were adjusted to pH 4.0 after Al was added and before application. Aluminium concentrations in the delivered solutions were within 10% of target concentrations as measured by the eriochrome cyanine method for inorganic monomeric Al. Solutions (approximately 15 ml) were automatically delivered to the plants three times each day for 8 weeks.

**Experiment 1: impacts of Al on Pi relationships of mycorrhizal broomsedge**

Aluminium was delivered to broomsedge plants at concentrations of 0, 10, 50, 100, and 200 µM Al. Chemical speciation analysis of these solutions by the program GEOCHEM (Parker et al., 1995) indicated that Al$^{3+}$ concentrations were 0, 5.72, 29.91, 62.75, and 133.90 µM in the treatment solutions, respectively. For data analysis hereafter, delivered Al concentrations are presented.

Root zone pH and Al concentration were measured at weeks 4, 6, and 8 by collecting leachate flowing from the deepots following delivery of nutrient solutions. Solution Al was determined using the eriochrome cyanine method. Plants were harvested following 8 weeks exposure to Al. At harvest, plants were gently removed from deepots and roots were excised from shoots. Roots were rinsed in deionized H$_2$O. To determine acid phosphatase (APase) activity, five 8 cm root subsamples were removed from the root system, washed in deionized water, cut into 1 cm pieces, and transferred into centrifuge tubes containing 4.5 ml of the appropriate nutrient solution containing Al. Then, 0.5 ml of 1 mM p-nitrophenylphosphate (NPP) was added to each tube and tubes were incubated for 1 h at room temperature. After the incubation period, 1 ml of 0.5 N NaOH was added to each tube. Acid phosphatase activity (nitrophenol produced) was spectrophotometrically determined (Tabatabai and Bremner, 1969).

Percentage mycorrhizal colonization of roots was assessed on a 5% fresh mass sample from the youngest portion of the root system. This region was sampled in order to assess the potential impacts of Al on mycorrhizal colonization. Mycorrhizal colonization was determined on a 5% fresh mass root subsample as noted above. Shoots and remaining roots were dried at 60 °C, weighed, ground, and digested as noted above. The concentration of P in the digests was determined as noted above.

Other mineral elements (Al, K, Ca, Mg, Fe, Mn, Zn, Cu) in the digests were analysed by inductively coupled plasma (ICP) emission spectrophotometry by the National Research Center for Coal and Energy Analytical Laboratory at West Virginia University.

**Data calculation and analysis**

Phosphorus use efficiency (PUE) (Boan et al., 1993) was calculated as:

\[
\text{PUE} = \frac{\text{plant dry weight}}{\text{plant P content}}
\]

The Al-Pi experiment was a 2×5 (mycorrhizal-by-Al) factorial design with five replicate plants for each treatment combination. Plant mass data were not normally distributed according to the Shapiro–Wilk W Test and were natural-log transformed prior to analysis of variance (ANOVA). Other measures (leachate pH and Al concentration, APase, PUE) were analysed by ANOVA. The high Al concentration dose response experiment was a blocked 2×4 (mycorrhizal-by-Al) factorial design with 10 replicate plants for each treatment combination. Blocks accounted for environmental variation within the growth chamber environment. Plant growth data over this wider Al concentration range were normally distributed, as was tissue nutrient data. Treatment means within mycorrhizal treatments were compared using the Tukey–Kramer HSD procedure. Analysis of covariance (ANCOVA) was used to investigate the relationships between growth and tissue element concentrations. Data were analysed using the statistical package JMP (SAS Institute, Cary, North Carolina, USA).

**Results**

**Impacts of Al on Pi relationships of mycorrhizal broomsedge**

The leachate pH of solutions from mycorrhizal and non-mycorrhizal plants was higher under low Al exposures, and pH from mycorrhizal plants was higher than non-mycorrhizal plants at higher Al concentrations (P=0.006 for the mycorrhiza-by-Al interaction) (Fig. 1A). The leachate pH of most treatments was below 4.5 and, at this pH, Al would exist primarily as Al$^{3+}$ in the root zones of both mycorrhizal and non-mycorrhizal broomsedge plants (Snoeyink and Jenkins, 1980). However, the analysis of leachate Al concentrations indicated that inorganic monomeric Al in the rhizosphere of mycorrhizal broomsedge plants was consistently less than that measured for non-mycorrhizal plants (P <0.001) (Fig. 1B).

The mean percentage colonization of inoculated broomsedge plants was 60% across all Al treatments and the rate of colonization was unaffected by Al (P=0.711) (data not presented). None of the non-mycorrhizal plants were colonized by mycorrhizal fungi.

Mycorrhizal and non-mycorrhizal plants diverged in their growth responses to Al in solution (Fig. 2). Following 8 weeks of Al exposure, shoot and root growth of mycorrhizal plants were stimulated by low Al concentrations up to 50 µM and were unaffected by higher concentrations of Al in solution. In non-mycorrhizal plants shoot and root mass were significantly reduced by Al above 50 µM, with reductions of up to 47% and 57% at 200 µM Al, respectively (P=0.031 and 0.033 for the mycor-
rhiza-by-Al interactions) (Fig. 2). Over the range of Al concentrations tested in this experiment, there was slight divergence between mycorrhizal treatments in the accumulation of Al in shoots (Table 1). The concentration of Al in non-mycorrhizal plant shoots increased above that of control plants in the 100 μM Al treatment; shoot Al increased at 200 μM Al in mycorrhizal plants (Table 1). In roots, similar patterns were noted, with Al increasing significantly above controls at and above 100 μM Al in non-mycorrhizal plants, but only at 200 μM Al in mycorrhizal plants (Table 1). Mycorrhizal colonization significantly reduced the accumulation of Al in broomsedge roots, notably at 100 and 200 μM Al (Table 1).

Mycorrhizal and non-mycorrhizal plants differed significantly in shoot and root P concentrations in response to Al (Fig. 3A). Shoot P concentrations of mycorrhizal plants were significantly lower than those of non-mycorrhizal plants (P < 0.014). Shoot P concentrations were unaffected by Al exposure (P = 0.360) (Fig. 3A). Root P concentrations of mycorrhizal and non-mycorrhizal plants did not differ (P = 0.125) and were not affected by Al (P = 0.181) (Fig. 3A). Phosphorus use efficiency (PUE) depended on both mycorrhizal inoculation and exposure to Al (Fig. 3B). Across all Al treatments, PUE for mycorrhizal plants was 17% greater for mycorrhizal than for non-mycorrhizal plants. However, this difference was more pronounced with higher Al concentrations (P < 0.001 for the mycor-
rhiza-by-Al interaction) (Fig. 3B). Acid phosphatase (APase) activity, considered a marker for Pi limitation (Fries et al. 1998), was used as an indicator of the Al effects on Pi acquisition by broomsedge in the present study. The APase activity of mycorrhizal plants was consistently lower than that of non-mycorrhizal plants across all Al treatments ($P<0.001$) (Fig. 3C). Neither Al treatment alone ($P=0.718$) nor in interaction with mycorrhizal treatment ($P=0.598$) affected root APase activity of broomsedge plants (Fig. 3C).

**High Al concentration dose response**

To gain insight into the extent to which the observed mycorrhizal benefit could function at higher Al levels, mycorrhizal and non-mycorrhizal broomsedge plants were exposed to Al concentrations up to 1000 μM. Mycorrhizal colonization increased with exposure to Al, although this increase was only significant at 400 μM Al (Table 2). Analysis of covariance indicated that variation in the level of colonization did not influence broomsedge plant growth within Al treatments ($P=0.271$).

Mycorrhizal colonization increased the resistance of broomsedge across this concentration gradient (Fig. 4). Mycorrhizal plants exhibited stable shoot and root mass, tiller production, and total height up to Al up to 400 μM (Fig. 4). By contrast, non-mycorrhizal plants at Al concentrations as low as 200 μM had significantly less shoot and root mass, tiller production, and total height than 0 μM Al treatments or corresponding mycorrhizal plants exposed to Al (Fig. 4). At 1000 μM Al, shoots and roots of mycorrhizal plants were 22- and 18-fold larger than those of non-mycorrhizal plants, and mycorrhizal plants had 4.5-fold more tillers and 15-fold greater total height than non-mycorrhizal plants (Fig. 4).
Aluminium resistance in mycorrhizal broomsedge plants was associated with the exclusion of Al from shoot and root tissues, especially at high solution Al concentrations (Fig. 5). By comparison with mycorrhizal plants, non-mycorrhizal broomsedge exposed to 400 and 1000 µM Al had 1.8- and 5.6-fold higher shoot Al concentrations and 4.2- and 7.2-fold higher root Al concentrations, respectively. At 0 and 200 µM Al, shoot and root Al concentrations between mycorrhizal and non-mycorrhizal plants were of similar magnitude (Fig. 5). In analyses of covariance between whole plant mass and tissue Al concentrations, it was evident that values for non-mycorrhizal plants exposed to 1000 µM Al diverged from patterns exhibited by all other treatments. Thus, although points are presented in Fig. 6, these data were omitted from statistical analysis. Mycorrhizal plants were larger than non-mycorrhizal plants at any given tissue Al concentration (P < 0.001 for the mycorrhiza effect for both shoots and roots) (Fig. 6). Taken together with Fig. 5, this pattern suggests that mycorrhizal fungi confer to broomsedge plants a significant capacity for Al exclusion. Relationships between plant mass and the concentration of Al in shoot and root tissues did not differ between mycorrhizal and non-mycorrhizal plants (Fig. 6A, B) (P=0.999 and 0.551 for the mycorrhiza-by-Al interaction for shoots and roots, respectively). Thus, tissue Al accumulation in broomsedge was equally toxic to mycorrhizal and non-mycorrhizal plants, but, over the range of Al exposures used, higher solution Al concentrations were required to effect the same Al concentration in mycorrhizal by comparison with non-mycorrhizal plants.

Aluminium in solution altered the concentrations of K, Ca, Mg, and P in broomsedge shoots and roots, and these responses were modified by mycorrhizal colonization (Table 3). Inoculation reduced the effects of Al on shoot and root K concentrations (Table 3). By contrast, reductions in shoot and root Ca concentrations resulting from Al exposure were larger for mycorrhizal compared to non-mycorrhizal plants (Table 3). Mycorrhizal plants had higher shoot Mg concentrations, yet lower root Mg concentrations than non-mycorrhizal plants (Table 3). Under Al exposure, mycorrhizal plants exhibited more consistent shoot and root Mg concentrations, except at 1000 µM Al (Table 3). Shoot P concentrations of mycorrhizal plants were lower than non-mycorrhizal plants and were not affected by Al. Shoot P concentrations of non-mycorrhizal plants were extremely limited at 1000 µM Al (Table 3). Root P concentrations of mycorrhizal and non-mycorrhizal plants were similar, except at 1000 µM Al, where Al significantly reduced the accumulation of P in non-mycorrhizal roots (Table 3).

The concentration of micronutrients in shoots and roots of broomsedge plants were significantly affected by mycorrhizal inoculation, but not affected by Al. As such, means for mycorrhizal treatments across Al treatments are presented in Table 4. Mycorrhizal broomsedge plants
contained lower concentrations of all micronutrients, except root Cu, which was greater than non-mycorrhizal plants.

The relationships between broomsedge plant growth and shoot tissue element concentrations are presented in Fig. 7. These relationships for non-mycorrhizal plants were skewed by plants exposed to 1000 \( \mu \text{M} \) Al, which diverged in their responses from all other treatment groups (Fig. 7). As such, although these points are presented in Fig. 7, the data were omitted from analysis of covariance. Broomsedge plant biomass was negatively correlated with shoot K concentration (\( P < 0.001 \)), and mycorrhizal and non-mycorrhizal plants exhibited similar relationships between biomass production and shoot K concentration (\( P = 0.335 \) and 0.099 for the mycorrhiza and mycorrhiza-by-slope interaction effects, respectively) (Fig. 7A). Plant biomass was positively correlated with shoot Ca concentration (\( P < 0.001 \)), although these relationships were altered by mycorrhiza inoculation (Fig. 7B). Mycorrhizal plants produced more biomass at any given shoot Ca concentration (\( P < 0.001 \) for the mycorrhiza effect) and biomass production was less affected by a change in Ca in mycorrhizal plants (\( P = 0.030 \) for the slope interaction). Plant growth was positively related to shoot Mg (\( P = 0.049 \)), although differences between mycorrhizal and non-mycorrhizal plants in their relationships between biomass production and shoot Mg concentration could not be resolved due to the high variability in the non-mycorrhizal treatment (\( P = 0.135 \) and 0.389 for the mycorrhiza and mycorrhiza-by-slope interaction effects, respective-
Biomass was negatively correlated to shoot P concentration in both mycorrhizal and non-mycorrhizal plants ($P=0.018$), although mycorrhizal plants produced more biomass at any given P concentration across the range of shoot P measured ($P=0.027$ for the mycorrhiza effect). Inoculation did not alter the relationships between shoot biomass and shoot P ($P=0.145$ for the slope interaction) (Fig. 7D).

The relationships between broomsedge plant growth and root tissue element concentrations are presented in Fig. 8. As with shoots, plant biomass was negatively correlated with root K concentration ($P<0.001$) and inoculation did not alter this relationship ($P=0.806$ and 0.282 for the mycorrhiza and mycorrhiza-by-slope interaction effects, respectively) (Fig. 8A). Plant biomass was positively correlated with root Ca concentration ($P<0.001$). There was trend for greater growth at any given Ca concentration in mycorrhizal plants ($P=0.070$ for the mycorrhiza effect); biomass production was similarly affected by a change in root Ca in both mycorrhizal and non-mycorrhizal plants ($P=0.778$ for the slope interaction) (Fig. 8B). Growth of both mycorrhizal and non-mycorrhizal broomsedge was positively related to root Mg ($P<0.001$), although mycorrhizal plants produced more biomass per unit root Mg ($P=0.006$ for the mycorrhiza effect). Mycorrhizal plants and non-mycorrhizal plants exhibited similar relationships between biomass production and changes in root Mg concentration ($P=0.429$ for the slope interaction) (Fig. 8C). Biomass tended to decline with root P concentration in both mycorrhizal and non-mycorrhizal plants, although these effects were not significant ($P=0.089$, 0.202, and 0.934 for the P, mycorrhiza, and slope interaction effects, respectively) (Fig. 8D).

**Discussion**

Broomsedge is found colonizing acidic soils in the mid-Atlantic United States (Campbell, 1983) and is the dominant herbaceous species growing on extreme edaphic sites, such as abandoned coal mines (Chapman and Jones, 1975; Gibson and Risser, 1982; Nellessen and Ungar, 1993). Depending on the composition of the overburden,
the chemistry of such soils may be dominated by phytotoxic Al. Broomsedge may thus be inherently resistant to Al or may rely on symbiotic AM fungi to overcome edaphic limitations on such sites.

The Al resistance of broomsedge and the influence of AM fungi on the response of broomsedge to Al in the rhizosphere was investigated. Inoculum for this study was collected from an acidic (pH=3.0) coal mine, where Al dominated both soil (360 mg kg\(^{-1}\) soil) and soil water (600 \(\mu M\) Al chemistry. Although broomsedge is the dominant vegetation on the site and many others like it in the region, the present study indicates that broomsedge is not tolerant of Al at concentrations noted in these soils, and exhibited significant reductions in growth above 50 \(\mu M\) Al (Figs 2, 4). However, the colonization of roots by AM fungi conferred Al resistance to broomsedge plants (Figs 2, 4), as has been demonstrated previously for several other plant species (Medeiros et al., 1994; Mendoza and Borie, 1998; Lux and Cumming, 2001). Mycorrhizal broomsedge plants did not exhibit any Al toxicity symptoms up to 200 \(\mu M\) Al, and showed stimulated growth at low Al concentrations (Fig. 2). When solution Al was 400 \(\mu M\) and greater, mycorrhizal broomsedge plants exhibited only slight growth reductions, whereas the growth of non-mycorrhizal plants was severely limited (Fig. 4). This indicates that the native AM fungal consortium used in the present study is

Fig. 7. Relationships between whole plant biomass and shoot potassium (A), calcium (B), magnesium (C), and phosphorus (D) concentrations of mycorrhizal (filled symbols) and non-mycorrhizal (open symbols) broomsedge plants exposed to Al for 8 weeks. Mean values (0, 200, 400 \(\mu M\) Al \((n=4)\), 1000 \(\mu M\) Al \((n=3)\)) of 2–3 pooled plant tissue samples presented with standard errors. Grey symbols represent non-mycorrhizal plants exposed to 1000 \(\mu M\) Al that have been omitted from analysis.
adapted to acidic soils and associated Al stress, and the processes involved in Al resistance in the fungi conferred Al resistance to host plants colonized by these fungi.

Aluminium availability and toxicity in the rhizosphere are influenced by the chemical characteristics of the soil solution bathing the root. Both plants and micro-organisms, including mycorrhizal fungi, influence the chemistry of this solution (Ritchie, 1995). Under exposure to low Al concentrations, the leachate pH of both mycorrhizal and non-mycorrhizal plants was as high as 4.8 (Fig. 1), which would alter Al speciation in the rhizosphere (Snoeyink and Jenkins, 1980). Although mycorrhizal and non-mycorrhizal plants diverged in their capacity to alter rhizosphere pH (Fig. 1), Al concentrations over 50 μM limited the capability of either mycorrhizal or non-mycorrhizal plants to raise leachate pH (Fig. 1). This may be related to the high buffering capacity of solutions containing Al or to a change in root physiology generating OH⁻ (Calba and Jaillard, 1997). These results are consistent with the previous reports (Miyasaka et al., 1989; Rengel and Robinson, 1989) and suggest that rhizosphere pH change plays a minor role in Al resistance of plants colonized by AM fungi.

Aluminium in solution in this experimental system has three fates: loss from the root zone in leachate, chelation with mobile and fixed ligands in the rhizosphere such as Fig. 8. Relationships between whole plant biomass and root potassium (A), calcium (B), magnesium (C), and phosphorus (D) concentrations of mycorrhizal (filled symbols) and non-mycorrhizal (open symbols) broomsedge plants exposed to Al for 8 weeks. Mean values (0, 200, 400 μM Al (n=4), 1000 μM Al (n=3)) of 2–3 pooled plant tissue samples presented with standard errors. Grey symbols represent non-mycorrhizal plants exposed to 1000 μM Al that have been omitted from analysis.
root exudates and cell walls, and uptake by the plant. Over the course of the first experiment, less inorganic monomeric Al was detected in leachate collected from mycorrhizal than non-mycorrhizal plants (Fig. 1). Although the fate of this Al is not known for certain, less Al was detected in mycorrhizal plant tissues and Al may have been chelated in rhizosphere, for example, by organic acids (Kochian, 1995), or bound to fungal cell walls in the sand matrix surrounding mycorrhizal plant roots. A reduction in available Al in the rhizosphere of mycorrhizal plants would contribute to limited Al accumulation in both shoots and roots of mycorrhizal plants observed in both experiments (Table 1; Fig. 5). This suppression of Al uptake and translocation to shoots of mycorrhizal plants is consistent with most previous reports (Koslowsky and Boerner, 1989; Medeiros et al., 1994; Mendoza and Borie, 1998).

Aluminium-induced organic acid exudation has been associated with Al resistance in higher plants (Kochian, 1995; Delhaize and Ryan, 1995; Ma, 2000; Matsumoto, 2000). Aluminium enhances the exudation of malate in wheat (Delhaize et al., 1993), oxalate in buckwheat (Zhang et al., 1998), and citrate in corn (Pellet et al., 1995), snapbean (Miyasaka et al., 1991), and soybean (Silva et al., 2001). Organic acid release into the rhizosphere appears to be a functional Al resistance mechanism that chelates Al extracellularly, reducing Al uptake and subsequent impacts on root physiological processes. In the present study, the substantially lower accumulation of Al in mycorrhizal broomsedge plants (Table 1; Fig. 5) similarly suggests a change in Al speciation and availability in the rhizosphere as a consequence of AM fungal colonization, although the mechanism of such change remains to be elucidated.

In a previous study, inoculation of broomsedge plants by this same AM fungal consortium increased phosphorus use efficiency and improved nutrient homeostasis in plants under Pi limitation (Ning and Cumming, 2001). Since the disruption of Pi availability and acquisition by Al may be one toxic mechanism in plants (Clarkson, 1966; Foy, 1983; Cuming et al., 1986; Roy et al., 1988; Tan and Keljens, 1990; Macklon and Sim, 1992; Lux and Cumming, 2001), P physiology was investigated in the present work. Exposure to Al did not consistently affect shoot or root P concentrations in either mycorrhizal or non-mycorrhizal broomsedge plants at concentrations ≤400 μM Al, although tissue P concentrations dropped dramatically in non-mycorrhizal plants exposed to 1000 μM Al (Fig. 3; Table 3). These patterns suggest that Al–Pi interactions did not influence broomsedge growth under Al exposure. Indeed, the relationships between plant mass and tissue P concentrations indicate that P became concentrated in plant tissues as Al inhibited plant growth (Figs 7, 8). Other measures of P physiology similarly reflected the significant influence of AM fungi on P acquisition, with little impact of Al on these processes. Patterns of APase activity (Fig. 3C) indicate that mycorrhizal plants were under less P stress than non-mycorrhizal plants, and this may be the result of the higher PUE of mycorrhizal plants (Fig. 3B). These benefits of AM fungi on P relations have been well documented (Hayman, 1983; Bolan, 1991; Smith and Read, 1997) and support earlier work highlighting increased P use efficiency in mycorrhizal broomsedge under P limitation (Ning and Cumming, 2001). However, these patterns do not support the original hypothesis that interactions between Al and Pi would determine the broomsedge plant response to Al.

Numerous experiments have demonstrated that Al interferes with the acquisition of cations in plants (Rengel and Robinson, 1989; Rengel, 1990; Huang et al., 1992, 1996; Nichol et al., 1993; Tan et al., 1993; Wheeler and Dodd, 1995; Lindberg and Strid, 1997; Lux and Cumming, 1999, 2001). In the present study, Al depressed concentrations of Ca and Mg in both shoots and roots in both mycorrhizal and non-mycorrhizal plants (Table 4). However, mycorrhizal and non-mycorrhizal plants diverged in their mass-tissue nutrient concentration relationships for these elements (Figs 7, 8; Table 4). The growth of mycorrhizal plants was less affected by Al-induced changes in shoot or root Ca or Mg concentrations, suggesting that the colonization of broomsedge plants by AM fungi alters fundamental nutrient homeostasis. Mycorrhizal plants exhibited more stable tissue nutrient–biomass accretion relationships than non-mycorrhizal plants and this stability may increase Al resistance and allow successful colonization of stressful soil environments. In a previous study, a similar phenomenon in broomsedge plants grown under Pi limitation was noted (Ning and Cumming, 2001) and it was proposed that enhanced micronutrient acquisition by mycorrhizal plants altered the nutrient–mass relationships. In the present study, mycorrhizal and non-mycorrhizal plants differed, but concentrations in mycorrhizal plants were typically lower than concentrations in non-mycorrhizal plants (Table 4), which may reflect a growth dilution in mycorrhizal plants. Thus, alternative influences of AM fungi on host physiology, such as altered flux of carbon to the rhizosphere, altered water relations (Allen et al., 1981; Auge et al., 1995; Miller et al., 1997), or phytohormone changes (Allen et al., 1980, 1982), may be involved in the greater growth of mycorrhizal plants at any given tissue nutrient concentration. This capacity would clearly represent an important adaptation for growth on acidic, nutrient-limited edaphic environments.

In conclusion, broomsedge, a species found colonizing extremely acidic soil environments in the eastern United States, appears not to be inherently resistant to Al in the rhizosphere. However, an acidic ecotype AM fungal consortium conferred Al resistance to broomsedge plants, promoting substantial growth at Al concentrations as high as 1000 μM. The exclusion of Al from tissues, a change in
P homeostasis, a reduction in the impacts of Al on cation acquisition, and higher biomass accretion–tissue nutrient relationships in mycorrhizal plants exposed to Al may be important mechanisms involved in Al resistance. Thus, the symbiosis between AM fungi and broomsedge plants plays critical roles in Al resistance of this plant species, which allows broomsedge to colonize and grow in unfavourable acidic edaphic environments.

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