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Mycorrhizal roles in broomsedge plants under phosphorus limitation and aluminum toxicity

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Mycorrhizal Roles in Broomsedge Plants Under Phosphorus Limitation and Aluminum Toxicity

Jianchang Ning

**Dissertation submitted to the
Eberly College of Arts and Sciences
at West Virginia University
in partial fulfillment of the requirements
for the degree of**

**Doctor of Philosophy
in
Biology**

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**Morgantown, West Virginia, USA
2000**

Keywords: Aluminum, Broomsedge, Mycorrhizal, Phosphorus

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ABSTRACT

Mycorrhizal Roles in Broomsedge Plants Under Phosphorus Limitation and Aluminum Toxicity

Jianchang Ning

This dissertation examined roles of arbuscular mycorrhizal fungi, of which *Glomus clarum* is the primary species, in broomsedge (*Andropogon virginicus*) plants under limited Pi and elevated Al conditions in four studies. The inoculum originated from an acidic coal-mining site in Morgantown, WV. The inoculum of *Glomus clarum* from INVAM collection, which originated from an alkaline coal-mining site in WV, was also used in two of the studies. Plants were grown in an acid-washed sand culture system with a modified Hoagland's nutrient solution in a growth chamber.

Mycorrhizal fungal colonization pronouncedly enhanced growth of broomsedge plants under limited Pi and/or elevated Al conditions. Under Pi limiting conditions, mycorrhizal broomsedge plants made more investment on roots, drew down solution Pi concentrations to lower level, all of which leads to increasing exploited Pi pool, and increased phosphorus use efficiency. Moreover, mycorrhizal plants balanced accumulation of nutrients under varying Pi availability. All these brought about by mycorrhizal symbioses benefit broomsedge plants overcoming Pi deficiency and supporting optimal growth of host plants under limited Pi availability.

Broomsedge plants do not have an inherent mechanism to block Al influx and tolerate Al toxicity. Mycorrhizal association with AM fungi significantly conferred Al resistance in broomsedge plants by facilitating Pi uptake in the early stages, reducing Al influx, suppressing Al translocation within plants, altering profiles of organic acids, releasing a great amount of organic acids, and improving nutrition of host plants. However, different fungal isolates had differential effects on Pi acquisition, Al influx and translocation, nutrient uptake, and exudation

of organic acids as Al concentrations changed. These differences may be associated with the differential adaptations of these two AM fungal strains to their original habitats.

Moreover, two AM fungal isolates differentially altered physiology of host plants under different Pi and Al levels, such as stomatal conductance, photosynthesis, leaf respiration, root protein, and root acid phosphatase examined in this study. These adjustments of physiological traits might be related to other changes of plants that support sustainable growth of host broomsedge plants.

Therefore, broomsedge plants rely on AM fungi to grow and establish in adverse habitats.

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GENERAL INTRODUCTION

Acidic soils occupy over 50% of the total arable land worldwide (von Uexkull and Mutert 1995). These soils present several challenges to plants, since they are characterized by limited phosphorus (Pi) availability and elevated aluminum (Al) levels. Acidification of soils may be the result of natural weathering processes in areas dominated by granitic parent material and high levels of precipitation. Soil acidification is additionally exacerbated by increased nitrogen (N) inputs, and is a problem in regions receiving high N in deposition or through monotonous agricultural practice. Further, local perturbations, such as coal extraction, often leave behind acidic overburdens that may limit plant growth. Thus, increasing attention has been attracted to sustainable agricultural systems and environmental quality due to the limited Pi availability and elevated Al resulting from the soil acidification.

Phosphorus and Aluminum Chemistry in Soils

Phosphorus is an essential mineral nutrient to a plant, comprising about 0.2% of a plant's dry weight. It is a key substrate in energy metabolism and biosynthesis of nucleic acids and membranes. It is also involved in photosynthesis, respiration, and regulation of a number of enzymes. Phosphorus is one of the three most frequently limiting macro-nutrients for plant growth (two others are N and K) (Schachtman et al. 1998; Raghothma 1999). Soluble Pi exists in soil solutions as H_2PO_4^- or HPO_4^{2-} , and primarily comes from three sources: native minerals, organic materials, and commercial fertilizers. Native phosphorus minerals, such as apatites $\text{Ca}_{10}(\text{X})_2(\text{PO}_4)_6$, where X is Cl, F, OH, or CO_3^{2-} , release Pi into the soil solution through natural weathering processes. Phosphorus is released from organic materials as it is mineralized by microorganisms (Snoeyink and Jenkins 1980). Commercial fertilizers are the primary Pi source in the crop fields. Alterations in rhizosphere properties mediated by plant roots and rhizosphere microbes may change the availability of Pi for plant acquisition and utilization. This available Pi is solely delivered to root/fungal hyphal surfaces by a diffusion process (Marschner 1995).

Aluminum is the most abundant metal and the third most abundant element in the Earth's crust, making up about 7% of its mass, but it is not required by plants for normal growth. The mineral forms of Al exist in soils as hydrous oxides, alumino-silicates, sulphates, and

phosphates. The major factor influencing the rates of dissolution or precipitation of Al minerals is proton (H^+) activity. Soluble Al released from Al minerals is toxic to plants (Haug 1984; Ritchie 1995). Exchangeable Al in soils is a poor indicator of potential Al toxicity to plants due to the complicated chemistry of Al (Andersson 1988; Delhaize and Ryan 1995). When the soil solution pH is less than 5.0, soluble Al exists mainly as octahedral hexahydrate, $Al(H_2O)_6^{3+}$, for convenience, called Al^{3+} . As the pH increases, $Al(OH)^{2+}$ and $Al(OH)_2^+$ are formed. At neutral pH, Al is precipitated as $Al(OH)_3$ (gibbsite). When the pH increases to the value commonly found in the cytoplasm (about 7.4), $Al(OH)_4^-$ dominates Al speciation. In the process of pH alteration, triskaidekalumium, $AlO_4Al_{12}(OH)_{24}(H_2O)_{12}^{7+}$, often referred as Al_{13} , may be unexpectedly formed (Kinraide 1991; Kochian 1995). Soluble Al complexes with organic acids, phosphate, sulphate, and macromolecules (e.g. proteins, DNA) whenever these compounds are present. Root exudates and microbial activities may alter Al speciation in the rhizosphere.

Phosphorus fixation mainly by Al and Fe and their hydroxides is recognized as an important factor contributing to the low available Pi in acid soils, whereas precipitation of Pi and Ca becomes important at higher pH (Figure 0.1). Through precipitation and/or adsorption of $AlPO_4$, bioavailable Pi is thus greatly reduced. Therefore, Al toxicity and Pi deficiency, often co-occur and are the primary factors limiting plant growth and production in acidic soils (Snoeyink and Jenkins 1980, de Miranda and Rowell 1989).

Plant Responses to Limited Pi Availability and Elevated Al

Plant adaptation to a particular environment is driven by natural selection (Antonovics et al. 1971; Humphreys and Bradshaw 1976; Chapin 1980). In response to environmental stress, plants are able to adjust their biochemical, physiological, and phenological characters in the short term, which is called acclimation, and alter genetic composition in the long term, which is called adaptation.

Under limited Pi availability, reduction of growth rate is a typical adaptive mechanism at the plant level (Chapin 1980, 1983), leading to low Pi demand and slow tissue turnover and high Pi retention (Aerts and Chapin 2000). These plant species have high nutrient use efficiency (Aerts and Chapin 2000), but may experience Pi luxury consumption, resulting in low Pi use efficiency (Chapin 1980) when Pi is readily available. Root growth is favored, resulting in a

Figure 0.1. Phosphorus (Pi) precipitation and availability in a soil solution is changed as the soil solution pH changes.

(After the Soil Fertility course by Dr. Louis McDonald at West Virginia University)

large ratio of root to shoot (Chapin 1980; Marschner 1995). High specific root length (root length per unit root mass) is another trait characteristic of plants growing in infertile soils, leading to a large root absorption area (Hajabbasi and Schumacher 1994; Aerts and Chapin 2000). Root distribution may be uneven in the soil. The root system exploits a large volume of soil and tends to proliferate in local Pi rich patches temporally and/or spatially (Caldwell 1989; Lambers et al. 1998). Under deficient Pi condition, roots typically form symbiotic associations with mycorrhizal fungi. Low Pi availability is more detrimental to aboveground tissues than belowground tissues. For example, shoot growth is retarded, reproduction is delayed, and flowers, fruits, and seeds are reduced, all of which may be related to reduced photosynthetic efficiency resulting from carbon limitation (Chapin 1980; Marschner 1995).

At the cell and molecular level, plants may adjust activities of enzymes and enzymatic kinetic parameters, such as H^+ -ATPase, K_m , and V_{max} , up-regulate expression of specific genes, such as Pi transporters, and use an alternative respiration pathway (Clarkson 1985; Theodorou and Plaxton 1993; Mimura 1995; Lambers et al. 1998; Schachtman et al. 1998; Raghothama 1999). Plants are also capable of actively mining Pi from limited sources by altering rhizosphere properties by exuding compounds (e.g. organic acids, acid phosphatases), releasing protons (H^+), and favoring activities of some microbes (Clarkson 1985; Marschner 1991; Barrett-Lennard et al. 1993; Ae and Otani 1997; Lambers et al. 1998; Schachtman et al. 1998; Raghothama 1999). In addition, Pi availability affects acquisition, translocation, and metabolism of other nutrients by plants, resulting in changes of nutrient composition in plants (Pacovsky 1986).

The toxic effects of Al on plants are mainly exerted through the high affinity of Al for many compounds and macromolecules in plants. The most easily recognized symptom of Al toxicity is the inhibition of root elongation (Kinraide et al. 1985; Andersson 1988). Therefore, most studies focus on root responses to elevated Al. When exposed to elevated Al, plants may experience stubby, brittle, and brown roots, reduced initiation of lateral roots, and retardation of root growth (Foy 1983; Andersson 1988; Roy et al. 1988; Taylor 1988). The root apex is believed to be the primary target of Al lesion (Andersson 1988; Kochian 1995). Root tips are swollen and discolored under elevated Al conditions and their structure is often damaged (Andersson 1988; Kochian 1995). The cells of root tips reduce or stop division and expansion, become vacuolated, the cell wall is rigid, their organelles are disordered, and integration and fluidity of the membrane is damaged (Taylor 1988; Kochian 1995). At the biochemical level, DNA synthesis in root tips is inhibited, root respiration is reduced, activity of some enzymes and

uptake of nutrients, such as Pi and Ca, are disrupted (Foy 1983; Roy et al. 1988; Taylor 1988; Kochian 1995). Thus, plants accumulate Al, have low nutrient concentrations, and are susceptible to water stress under elevated Al conditions (Foy 1983; Andersson 1988; Taylor 1988; Kochian 1995; Marschner 1995). After a long-term exposure to Al, plants show Al toxicity symptoms aboveground, for example, slowed shoot growth, Pi deficiency symptoms, and reduced leaf chlorophyll and photosynthesis (Andersson 1988; Roy et al. 1988; Taylor 1988). Al-tolerant plants limit Al translocation to aboveground tissues, produce organic acids to detoxify Al internally and/or externally, change rhizosphere properties to affect Al speciation, influence microbial activities in the rhizosphere to reduce Al availability, form symbiotic association with mycorrhizal fungi to protect the root system, up-regulate gene expression of resistant proteins to reduce Al entry, and enhance nutrient uptake (Marschner 1991, 1995; Delhaize and Ryan 1995; Kochian 1995; Clark 1997). Some other plants like tea (*Camellia sinensis*) accumulate much Al in leaves and tolerate it (Rengel 1996), but this tolerance mechanism is not completely understood. On the other hand, low Al levels (usually < 5 ppm) stimulate plant growth. Although this beneficial effect of Al on plants is not understood, it may be related to Al altering the distribution of growth regulators in roots and serving as a fungicide (Foy 1983; Roy et al. 1988).

Arbuscular Mycorrhizas

Arbuscular mycorrhizas (AM) are the most common mycorrhizal symbioses, which are formed between various plants, from fern to higher plants, and obligately symbiotic fungi in the Glomales, including *Acaulospora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Scutellospora*, and *Sclerocystis*. These AM fungi are mainly characterized by asexual reproduction, aseptate and multi-nucleate mycelium, and intraradical structures. They can not live alone, and must rely on host plants for carbohydrates as energy sources. Their spores can germinate, but produce little mycelium in absence of plant roots. Soluble exudates or extracts from roots of host plants, mainly flavonoids, stimulate hyphal growth and branching. When the hyphae from propagules of three inoculum sources (spores, infected root fragments, and hyphae) contact roots, colonization of roots is initiated by the formation of swollen appressoria at hyphal tips. After a short recognition reaction, the hyphae penetrate the root epidermis and cell walls via biochemical and mechanical processes. The intraradical hyphal branches pass into and grow longitudinally in

the intercellular spaces of the middle and inner cortex of roots. These branches give rise to arbuscules, highly branched structures that form invaginations into cortical cells. Sometimes intercellular hyphae produce a storage structure, called vesicles. Once the fungus is established in roots, external mycelia grow extensively in soils and extremely extend root zones, finally produce spores (Smith and Read 1997).

Intracellular arbuscules are relatively short-lived (about a week), and these are believed to be major site for transfer of mineral elements (especially Pi). The plasma membrane of root cells around the arbuscules, called periarbuscular membrane (PAM), is greatly expanded, thus the membrane surface of the root cells increases considerably, and its properties and functions may be altered. For example, its H^+ -ATPase activity is enhanced (Gianinazzi-Pearson et al. 1991). These changes would favor Pi transfer across the interface. In the processes of Pi and carbon transfers, the proton motive force (PMF) generated by H^+ -ATPase may play an important role. Carbohydrate transfer is suggested to occur between the interface of the intercellular hypha and root cell membrane (Figure 0.2A). However, there is a suggestion of bi-directional transfers of Pi and carbon occurring on the arbuscules simultaneously (Figure 0.2B), because active ATPases are present on both root and fungal membranes in the arbuscular interface. In either case, passive efflux along concentration gradients from the donor, Pi from fungus and carbon (possible sucrose) from root cell, into the interfacial apoplast is followed by active uptake by receiver, Pi by root cell and hexose by fungus. The sucrose effluxed from root cells might be hydrolyzed by an acid invertase from the root cells then converted into hexoses, which are absorbed by the fungus. For the uptake of Pi by fungus from soil, the fungus forms polyphosphate in the hyphae (Bucking and Heyser 1999), which may establish and maintain the driving force for Pi uptake into the hyphae (Smith and Read 1997).

An AM fungus can infect a variety of plant species from lower to higher vascular plant species. An individual plant can be colonized by multiple AM fungi simultaneously. Extensive external mycelia extend from one plant to other plants, forming a hyphal net among plants, which consequently increases colonization of root systems by fungi from the standpoint of the plant community. The hyphal links may play important roles in retaining stability and integration of the plant community by sharing nutrient resources and saving carbon investment

Figure 0.2. Schematic representation of the speculative spatial distribution of H^+ -ATPase and associated transfer processes in AM interfaces.

- A.** Transfer of Pi from fungus to plant across an arbuscular interface, and transfer of sucrose from plant to fungus across the interface between cortical parenchyma cells and intercellular hypha.
- B.** Bi-directional transfer of sucrose and Pi across the same arbuscular interface.

PAM: peri-arbusculaar membrane; FPM: fungal plasma membrane

(After Smith and Read 1997)

A. Spatial Separation of Transfer of Carbon and Pi

B. Bidirectional Transfer of Carbon and Pi Across the Same Interface

on nutrient acquisition structures. These roles undoubtedly aid rapid establishment of seedlings, and help overcome localized stress (Smith and Read 1997).

Benefits Provided by AM Fungal Colonization

Arbuscular mycorrhizas are the most common underground symbioses and have existed for several hundred million years. There exists a wide range of habitat preference by AM fungi, even within the same fungal species. These fungi differentially affect performance of the host plant species. Some fungi may facilitate nutrient uptake and stimulate plant growth, while others may suppress plant growth or have no effect on plant nutrient uptake or/and growth, but provide other benefits to plants. There is no clear relationship between percentage root colonization and plant growth (Clark 1997; Johnson et al. 1997; Smith and Read 1997). Apparently, the functions of AM symbioses go beyond the simple capture of mineral nutrients. So there must be some selection advantage(s) for their wide-spread occurrence.

Several advantages brought about by AM fungal colonization enhance both AM fungal and plant fitness. Experiments have demonstrated that AM fungal colonization ameliorated the effects of adverse edaphic factors on host plants, increased seedling survival, and improved plant growth (Danielson 1985). The most documented benefit provided by AM fungi is increasing acquisition of diffusion-limited mineral nutrients, such as Pi, Cu, Zn, and NH_4^+ . It is reported that AM fungal colonization also improved water relations of host plants in the western USA (Allen et al. 1981) and other parts of the world (Kothari et al. 1990). Moreover, AM fungi conferred metal resistance to the host plants, e.g., Zn and Cd (Gildon and Tinker 1981). Finally, AM fungi aided host plants against invasion and infection of other microorganisms. On the other hand, AM fungi may suppress growth of non-mycorrhizal plants, thus indirectly aiding the establishment of mycorrhizal plants (Francis and Read 1994). All of these factors may increase host plant fitness. Of course, improved plant growth would provide more available carbon for the fungi to utilize, consequently increasing AM fungal fitness (see below) (Newsham et al. 1995; Smith and Read 1997).

Figure 0.3. Effects of changing parameter values on simulated Pi uptake by roots.

k: rate of root elongation; **C_{ii}**: initial Pi concentration; **r_o**: root diameter; **b**: soil buffering power;
D_e: Pi diffusion coefficient; **I_{max}**: maximum Pi inflow rate; **v_o**: transpiration rate; **r_i**: root density;
C_{min}: Pi concentration of no Pi influx; **K_m**: Pi concentration at where Pi uptake rate is I_{max}/2.

(After Clarkson 1985)

Relationships between Root Systems and Nutrient Acquisition

The delivery rate of a nutrient to the root surface (F , mol m⁻² s⁻¹) by diffusion and mass flow is given by:

$$F = -D_e * \frac{dC}{dr} + v * C \quad (1)$$

and diffusion coefficient of the nutrient (D_e , m² s⁻¹) and nutrient uptake rate (dC) are separately determined by:

$$D_e = \theta * f * D_0 \quad (2)$$

$$I_n = \frac{I_{\max} (C - C_{\min})}{K_m + (C - C_{\min})} \quad (3)$$

where C is the nutrient concentration (mol m⁻³), r is root radius (m), v is water flux rate (m³ m⁻² s⁻¹), θ is water status, f is diffusion path (tortuosity factor), D_0 is diffusion coefficient of the nutrient in free solution (m² s⁻¹), I_n is net influx rate of the nutrient (mol m⁻² s⁻¹), $I_{\max}$ is maximal influx rate of the nutrient (mol m⁻² s⁻¹), K_m is the nutrient concentration at one-half of $I_{\max}$ (mol m⁻³), and $C_{\min}$ is the minimum nutrient concentration at which no influx occurs (mol m⁻³). For any given nutrient, according to the above equations (1), (2) and (3), its delivery rate to the root surface is determined by root uptake system ($I_{\max}$, K_m , and $C_{\min}$), root geometry (r), water status (v , θ), the nutrient property (D_0) and its concentration (C). As to those nutrients delivered solely by diffusion, such as Pi, the term $v * C$ is omitted from the equation (1), as mass flow does not contribute. Thus, the concentration, the nutrient uptake system, and root extension into unexploited soil regions are of great importance in acquisition of diffusion-limited nutrients (Clarkson 1985, Lambers et al. 1998). Mathematical models predict that root geometry is more important than the uptake kinetic parameters (Figure 0.3) (Clarkson 1985). Thus, mycorrhizal colonization can improve the exploratory geometry of the root system through the extension of external fungal hyphae into the soil far beyond regions the root system reaches and greatly increase the root surface area:volume ratio (Berta et al. 1993, Smith and Read 1997). Furthermore, these external hyphae may alter the properties of the surrounding soil, which affects availability of nutrients and Al.

In acidic soils, low pH, low Pi, and high Al may directly interfere with acquisition and metabolism of mineral nutrients, limiting plant growth. AM fungal colonization may facilitate

Figure 0.4. Schematic model of relationships among acidic edaphic factors (e.g. limited Pi and elevated Al), nutrient acquisition by plants, mycorrhizas, and plant growth.

Low available Pi and high Al negatively affect nutrient acquisition, and mycorrhizas facilitate nutrient acquisition and may alter plant physiology, consequently affecting plant growth; improved nutrition status enhances plant growth; carbon availability of plant affects mycorrhizal symbiosis.

Solid arrows indicate positive associations, whereas open arrows reflect negative relationships.

.

acquisition and metabolism of mineral nutrients, in turn stimulating plant growth on acidic soils. Additionally, AM fungi may directly alter the physiology and biochemistry of host plants. For example, AM fungal colonization changed hydraulic conductance (Allen et al. 1981), photosynthesis and respiration (Allen et al. 1981; Eissenstat et al. 1993; Peng et al. 1993; Aguilera-Gomez et al. 1998), hormone production (Allen et al. 1980; McArthur and Knowles 1992), root protein production (Gianinazzi-Pearson et al. 1991; McArthur and Knowles 1993; Bago et al. 1997; Benabdellah et al. 1999), and gene transcription and expression in host plants (Harrison 1999). These alternations undoubtedly affect plant performance. Improved plant growth further stimulates nutrient acquisition and metabolism, and produces more carbohydrates that are available for AM fungi to utilize, leading to increased AM fungal activities (Figure 0.4).

OBJECTIVES

During the initiation of this project, I was surprised by the consistent occurrence of broomsedge (*Andropogon virginicus* L.) on most of the abandoned coal mining sites in the area. This plant species is also found under other adverse edaphic habitats (Chapman and Jones 1975; Gibson and Risser 1982; Campbell 1983; Nellessen and Ungar 1993). What makes this plant species have such a distribution pattern? After analysis of the soil and the roots from an abandoned coal mining site in Field Crest, Morgantown, WV, I found that the soil is very acidic, pH from 3.0 to 3.3, and contains very low Melich-extractable Pi (3.76 mg kg^{-1}) and high Al ($363.12 \text{ mg kg}^{-1}$). The plant roots were heavily colonized by AM fungi. A bioassay using wheat (*Triticum aestivum* L.) demonstrated that these soils are very poor (Figure 0.5). Therefore, the questions arose: do the AM fungi aid the plants by allowing them to overcome the Pi limitation and Al toxicity under these conditions? How would this be achieved?

In this project, I used broomsedge plants and AM fungi isolated from the Field Crest coal-mining site near Morgantown, WV. The primary fungal species was *Glomus clarum* Nicolson and Schenck. Also I used some fungal propagules of the same fungal species from the INVAM collection, which originated from a coal mining site in Mingo County, WV, where the soil contains NaHCO_3 -extractable Pi of 19 mg kg^{-1} with pH of 6.6, as a contrast in the last two experiments. This study has four main goals:

Figure 0.5. Bioassay of abandoned coal-mining soils from Field Crest in Morgantown, WV, using wheat (*Triticum aestivum* L.).

The bioassay was conducted in the greenhouse of Biology Department, West Virginia. Pots were filled with the mining soil (SM), neighbor outcrop soil (SH), and potting soil (RF), and 10 seeds of each of two wheat cultivars, one is Al-resistant and another is Al-sensitive, were sown in each of the pots. After a month, these wheat plants were harvested, and their dry weight from each pot was determined after being dried in an oven at 62°C overnight. *P* values for each treatment are: soil < 0.001, genotype = 0.0429, and soil x genotype interaction = 0.595.

- (1) Determine the roles of mycorrhizas under a range of Pi regimes, from low to high availability;
- (2) Investigate if mycorrhizas confer Al resistance in plants exposed to elevated Al;
- (3) Investigate whether different fungal isolates differentially affect the performance of plants exposed to Al;
- (4) Examine what physiological changes would be brought about in the plants by AM fungal colonization under different Pi and Al treatments, and if there are differences of physiology between these two types of mycorrhizal plants exposed to different applications of Pi and Al.

In order to address these goals, a series of experiments were designed and conducted in the growth chambers:

- (i) **Phosphorus Study.** This study included two experiments. The first was a plant growth response experiment with pre-colonized broomsedge plants by the native AM fungi in a growth chamber with a completely randomized block factorial design. Plant growth and nutrition status were analyzed to address differential responses of mycorrhizal and non-mycorrhizal plants in growth and nutrition to varying Pi levels; The second experiment was a Pi depletion experiment with the same growth conditions as in the above experiment to determine enzymatic kinetic parameters of Pi uptake system in the plants.
- (ii) **First Aluminum Study.** This study used pre-colonized broomsedge plants by the native AM fungi, including two experiments. The first was a multiple harvest dose response experiment with a 2 x 5 factorial design. Three harvests were conducted in the week 4, 6, and 8, respectively. Rhizosphere pH and Al concentrations were monitored over the experimental period, and plant growth, root acid phosphatase activity, and tissue Al concentrations were analyzed at each harvest, to investigate the interactions between mycorrhizal and Al treatment along the development of broomsedge plants; The second experiment was an extended dose response experiment with a 2 x 4 factorial design. Plant growth, tissue Al concentration, and nutrition were determined at the final harvest to examine the roles of mycorrhizas in Al resistance of broomsedge plants.
- (iii) **Second Aluminum Study.** This study used pre-colonized broomsedge plants separately by each of the two isolates of *Glomus clarum* from the two mining sites

using a 3 x 4 factorial design. Rhizosphere organic acids were extracted at the final harvest time and analyzed with Dionex. Plant growth, tissue Al concentration, and nutrition status were also determined at the final harvest. This study was to address the differential effects of the two AM fungal isolates on the exudation of organic acids, plant growth, and nutrition, and the roles of organic acids in Al resistance of mycorrhizal and non-mycorrhizal broomsedge plants.

- (iv) **Physiology Study.** This study used pre-colonized broomsedge plants separately by each of the two isolates of *Glomus clarum* from the two mining sites using a 3 x 2 x 2 factorial design. Leaf photosynthetic rate, leaf respiratory rate, chlorophyll concentration, nitrogen concentration, root acid phosphatase activity, and root protein production, and also plant growth and tissue Al and macronutrient concentrations were determined to examine interactive effects of Pi and Al on physiology and growth of mycorrhizal and non-mycorrhizal plants.

CHAPTER I

Effects of Mycorrhizal Fungal Colonization on Responses of Broomsedge Plant to Varying Pi Availability

Introduction

The benefits accrued to plants by their mycorrhizal symbionts are often ascribed to enhanced phosphorus (Pi) nutrition (Smith and Read 1997). Increased phosphorus acquisition by plants as a result of arbuscular mycorrhizal (AM) fungal colonization may result from: (1) the exploration of a larger soil volume by extraradical hyphae; (2) a greater movement of Pi into mycorrhizal fungal hyphae due to high efficiency of Pi absorption systems, more effective competition for Pi with soil microorganisms, and formation of polyphosphate inside hyphae; and (3) the solubilization of poorly available phosphorus sources by modification of the rhizosphere (Hayman 1983; Bolan 1991; Smith and Read 1997). However, AM fungal enhancement of plant growth may not be fully explained by the facilitation of Pi acquisition by AM fungi (Ross 1971; Plenchette et al. 1983; Pacovsky et al. 1986). Mycorrhizal fungi also enhance the uptake of other nutrient elements, including copper (Cu) and zinc (Zn) (Ross 1971; Pacovsky 1986; Leake and Read 1989). Thus, fostering nutrient balance in the host plant may be more critical than the acquisition of any one nutrient element.

The regulation of nutrient uptake in roots leads to the internal nutrient balance of the plant (Clarkson 1985; Marschner 1995; references therein). This balance in nutrient acquisition by the plant under edaphic stress, as modulated by mycorrhizal fungi, may be critical to its growth and survival. For example, the amelioration of Al toxicity by mycorrhizal fungi in *Liriodendron tulipifera* is associated not only with the maintenance of P acquisition, but also with higher and more stable concentrations of Ca, Cu, and Zn in the roots or shoots (Lux and Cumming 1999). Similarly, Cumming and Weinstein (1990) found that Pi limitation led to the loss of Na exclusion from shoots of non-mycorrhizal *Pinus rigida* seedlings, whereas this effect was not noted in seedlings colonized by the ectomycorrhizal fungus *Pisolithus tinctorius*. In addition, both AM and ectomycorrhizal fungi may suppress the uptake of other elements, such as

Al, Fe, and Mn, that may be present at toxic levels in some soils (Pacovsky 1986; Cumming and Weinstein 1990; Kothari et al. 1991). Thus, mycorrhizal fungi play a role in whole-plant nutrient balance by aiding in the uptake of limiting nutrients, maintaining nutrient uptake, or both under edaphic stress.

Broomsedge (*Andropogon virginicus* L.) is a dominant bunch-grass found colonizing extreme edaphic environments in the eastern United States (Chapman and Jones 1975; Gibson and Risser 1982; Campbell 1982, 1983, Morton 1986; Nellessen and Ungar 1993). Improvement of soil conditions, such as through the application of nitrogen fertilizers, can exclude this species from a plant community. Preliminary studies demonstrated that broomsedge is a highly mycotrophic species (Ning, unpublished data). These patterns suggest that broomsedge is adapted to infertile habitats and may rely on AM fungi to maintain nutrient balance under both nutrient-limiting and stressful edaphic conditions.

The goals of this study were to assess the capacity of broomsedge to grow under varying solution Pi levels and to investigate the role AM fungi play in aiding growth and nutrient acquisition of this plant species. I hypothesize that broomsedge plants rely on AM fungi to overcome the Pi limitation through changes in Pi acquisition and/or utilization. Further, this enhancement of Pi acquisition would enable broomsedge plants to maintain a more balanced accumulation of other nutrients under variable Pi environments.

Materials and Methods

Source of AM Fungal Inoculum

AM fungal inoculum was generated from broomsedge plants collected from the Field Crest abandoned coal mine in Morgantown, West Virginia, USA. Although the site has been abandoned for about 50 years, much surface soil remains devoid of vegetation. The vegetated surface consists of a broomsedge sward surrounding stunted red maple (*Acer rubrum*) and big-toothed aspen (*Populus grandidentata*) trees. Soil pH ranged from 3.0 to 3.3 and extractable Pi by Melich III was $3.76 \pm 0.54 \text{ mg kg}^{-1}$.

To produce the inoculum of AM fungi, 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 a month, the pot contents became the source of infective inoculum for the experiment.

Preparation of Plants

Broomsedge seeds were sown around the transplants in nursery pots (15 cm diameter). Broomsedge seeds also were sown in pots containing sterile-germinated sudan-grass and the mixture of autoclaved mine-soil and sand (1:3 v/v) with the bacteria extracted from the mine-soil. Seedlings from these pots served as non-mycorrhizal controls. After four weeks growth, roots of a small subset of seedlings (*ca.* 10) were examined to determine mycorrhizal status. Tissue P status was analyzed after wet digestion (Parkinson and Allen 1975) by the molybdate blue method (Olsen and Sommers 1982). Tissue phosphorus concentrations and tissue dry weights between mycorrhizal and non-mycorrhizal plants did not differ significantly.

Nutrient Solution and Growth Conditions

Mycorrhizal and non-mycorrhizal broomsedge seedlings were transplanted and grown in D16 Deepots (5 cm diameter x 18 cm height) (Stuewe and Sons, Inc., Corvallis, Oregon, USA) pre-filled with 220 cm³ of a 3:1 (v/v) mixture of coarse:fine acid-washed sand. Deepots were placed into a growth chamber with 14 hours of light at 28°C, 60% RH, and 10 hours of darkness at 21°C, 50% RH. Average light intensity at pot height in the chamber was 260 $\mu\text{mol m}^{-2} \text{s}^{-1}$ from mixed fluorescent and incandescent sources. Plants received a baseline nutrient solution containing Ca (0.675 mM), K (0.79 mM), Mg (0.25 mM), NO₃ (1.5 mM), NH₄ (0.5 mM), SO₄ (0.25 mM), B (23.14 μM), Fe (25 μM), Mn (4.57 μM), Zn (0.38 μM), Cu (0.16 μM), and Mo (0.06 μM). Pi as the treatment was added separately. All solutions were adjusted to pH 4.0 before application. Solutions (approximately 15 ml) were automatically delivered to the plants three times each day for eight weeks.

Growth Response Experiment

Treatments consisted of five Pi concentrations (10, 20, 40, 60, and 100 μM as NaH₂PO₄), which were delivered in the nutrient solutions outlined above. After eight weeks, sand was removed from roots under running de-ionized water, and shoots were rinsed in distilled water. Roots were excised, and shoots and roots were dried at 62°C and weighed. Percentage mycorrhizal colonization of roots was assessed on a 5% root sub-sample by the gridline intersect

method (Giovannetti and Mosse 1980). Roots and shoots from the same block were pooled, ground, and digested (Parkinson and Allen 1975). Phosphorus concentrations of digest solutions were determined spectrophotometrically (Olsen and Sommers 1982). All other mineral elements (Fe, Mn, Mg, Ca, Zn, Cu, Na, and K) of the digests were analyzed by ICP (Leeman PS 950) by the National Research Center for Coal and Energy Analytical Laboratory at West Virginia University.

Pi Depletion Experiment

Plants were grown for eight weeks as noted above and treated with 20, 40, 60, and 100 μM Pi as NaH_2PO_4 in the base solution. Plants were removed from sand, roots were gently washed in running distilled water, and plants were transferred into 200 ml glass tubes containing the appropriate treatment solution. Solutions were aerated and changed every 12 hours for 48 hours. Following the last solution change, each tube was sampled at 5, 10, 15, 30, 60, 90, 120, 150, 180, 240, 300, and 360 minutes. Phosphate concentrations of these samples were determined by the molybdate blue method (Olsen and Sommers, 1982) when Pi concentration was higher than 5 μM and the malachite green method (Motomizu et al. 1983) when Pi concentration was lower than 5 μM . Roots and shoots were dried and weighed.

Experiment Design and Data Analysis

Within the growth chamber, treatments were arranged in a randomized complete block factorial (Pi-by-mycorrhizal fungal treatment) design, with three blocks accounting for environmental variation within the chamber. Each treatment had 3 or 4 replicates within a block.

Phosphorus use efficiency (PUE) (Chapin 1980; Baon et al. 1993) was calculated as:

$$(\text{plant dry weight}) / (\text{plant P amount}).$$

For the Pi depletion experiment, the Pi instantaneous uptake rate and the minimum solution Pi concentration ($C_{\min}$) at which net Pi influx is zero for each treatment were determined by fitting a 2nd-order polynomial equation ($Y = aX^2 + bX + c$) to the experimental data (Claassen and Barber 1974), where Y is Pi concentrations in solutions and X is time (minute).

Taking the derivative yields:

$$Y' = 2aX + b$$

where Y' is the change in solution Pi concentrations with the time X .

According to the derivative, the Pi instantaneous uptake rate at the time $X = 0$ is equal to the coefficient b .

To find C_{min} , firstly, solve the above derivative equation to obtain the time at when the C_{min} is reached, then the value of time was placed back into the 2nd-order polynomial equation, after re-arranging, the following equation was derived:

$$C_{min} = c - (b^2 / (4a)).$$

Data were analyzed by analysis of covariance with solution Pi concentration treated as a covariate and regression analysis using the statistical package JMP (SAS Institute, Cary, North Carolina, USA).

Results

Mean percentage mycorrhizal colonization in broomsedge plants was about 70% across the treatments (data not presented). Phosphate treatment had no significant effect on percentage colonization. None of the non-mycorrhizal plants were contaminated by the mycorrhizal fungi. Mycorrhizal and non-mycorrhizal plants differed significantly in their patterns of growth in response to Pi availability (Figure 1.1). Mycorrhizal fungal colonization significantly enhanced plant growth at 40 μ M Pi and below, where non-mycorrhizal plants exhibited extremely limited growth. At 60 μ M Pi, Pi-deficiency symptoms, such as purple leaf margins, in non-mycorrhizal plants disappeared, but the pronounced difference in growth still remained between mycorrhizal

Figure 1.1. Influence of Pi treatments on shoot (**circle**) and root (**triangle**) dry weights (**A**), and cumulative height (**circle**) and number of tillers (**triangle**) (**B**) of mycorrhizal (**solid**) and non-mycorrhizal (**open**) broomsedge (*A. virginicus*) plants.

P values from ANCOVA:

A. Shoot dry weight:

Myc: < 0.001, Pi: < 0.001, and Myc * Pi: < 0.001;

Root dry weight:

Myc: < 0.001, Pi: < 0.001, and Myc * Pi: < 0.001.

B. Cumulative height:

Myc: < 0.001, Pi: < 0.001, and Myc * Pi: < 0.001;

Number of tillers:

Myc: < 0.001, Pi: < 0.001, and Myc * Pi: 0.020.

Table 1.1. Shoot and root P concentrations and P transfer percentage to shoot in mycorrhizal and non-mycorrhizal broomsedge (*A. virginicus*) plants as influenced by Pi treatment.

Pi Treatment (μM)	Myc	Shoot P Concentration (mg g^{-1})	Root P Concentration (mg g^{-1})	P Transfer (%)
10	+	0.872 (0.083)	0.444 (0.011)	73.79 (0.068)
	-	1.303 (0.204)	0.541 (0.095)	79.32 (2.328)
20	+	0.694 (0.016)	0.506 (0.095)	69.57 (4.013)
	-	1.112 (0.137)	0.663 (0.061)	75.59 (3.820)
40	+	1.054 (0.245)	0.604 (0.104)	75.76 (1.949)
	-	1.840 (0.306)	0.925 (0.050)	79.29 (1.285)
60	+	1.296 (0.205)	0.704 (0.110)	74.97 (4.110)
	-	1.368 (0.203)	0.811 (0.110)	79.24 (1.323)
100	+	2.155 (0.281)	1.081 (0.074)	79.67 (2.409)
	-	1.760 (0.291)	0.889 (0.185)	76.18 (2.994)
P values	Myc	0.007	0.019	0.013
	Pi	< 0.001	< 0.001	0.168
	Myc * Pi	0.028	0.092	0.060

Figure 1.2. Influence of Pi treatment on P use efficiency (PUE) of mycorrhizal (**solid**) and non-mycorrhizal (**open**) broomsedge (*A. virginicus*) plants.

Slopes and *P* values from the regression:

Mycorrhizal:

Slope: -0.012, and *P* value: < 0.001;

Non-mycorrhizal:

Slope: -0.004, and *P* value: 0.046.

Table 1.2. *P* values from ANCOVA for nutrient concentrations of shoots and roots in broomsedge (*A. virginicus*) plants as influenced by mycorrhizal (**Myc**) and Pi treatments.

Element	Shoot			Root		
	Myc	Pi	Myc * Pi	Myc	Pi	Myc * Pi
K	< 0.001	0.015	0.006	ns	ns	ns
Ca	ns [†]	< 0.001	ns	0.020	< 0.001	ns
Mg	0.028	0.002	ns	< 0.001	0.003	0.003
Fe	0.007	< 0.001	0.026	ns	ns	ns
Mn	< 0.001	0.001	0.001	< 0.001	< 0.001	< 0.001
Cu	< 0.001	0.040	ns	< 0.001	< 0.001	ns
Na	< 0.001	< 0.001	0.002	ns	ns	ns

[†] $\alpha=0.05$, the same hereafter.

Figure 1.3. Influence of Pi treatment on shoot K (**A**), Fe (**B**), Mn (**C**), and Na (**D**) concentrations, and root Mg (**E**) and Mn (**F**) concentrations of mycorrhizal (**solid**) and non-mycorrhizal (**open**) broomsedge (*A. virginicus*) plants.

Slopes and *P* values from the regression:

A. Shoot K:

Mycorrhizal:

Slope: 0.02, and *P* value: ns;

Non-mycorrhizal:

Slope: 0.473, and *P* value: 0.007.

B. Shoot Fe:

Mycorrhizal:

Slope: -0.271, and *P* value: < 0.001;

Non-mycorrhizal:

Slopes: -1.446 (linear) and 0.009 (quadratic), and *P* value: 0.004 (linear) and 0.033 (quadratic).

C. Shoot Mn:

Mycorrhizal:

Slope: -0.271, and *P* value: < 0.001;

Non-mycorrhizal:

Slope: -1.689, and *P* value: 0.002.

D. Shoot Na:

Mycorrhizal:

Slope: -0.332, and *P* value: ns;

Non-mycorrhizal:

Slope: -13.884 (linear) and 0.090 (quadratic), and *P* value: 0.003 (linear) and 0.021 (quadratic).

E. Root Mg:

Mycorrhizal:

Slope: 0.007, and *P* value: < 0.001;

Non-mycorrhizal:

Slope: > -0.001, and *P* value: ns.

F. Root Mn:

Mycorrhizal:

Slope: -0.267, and *P* value: ns;

Non-mycorrhizal:

Slope: -2.118, and *P* value: < 0.001.

and non-mycorrhizal plants. At 100 μM Pi, all growth traits (shoot and root mass, cumulative height, and number of tillers) were similar between mycorrhizal and non-mycorrhizal plants. Mycorrhizal plants allocated more biomass to roots at all Pi concentrations except 100 μM (Figure 1.1).

Differential effects of mycorrhizal fungal colonization as a function of Pi concentrations were observed for shoot P concentrations (Table 1.1). At low Pi availability, shoot P concentrations of mycorrhizal plants were actually less than those of non-mycorrhizal plants. For root P concentration (Table 1.1), however, mycorrhizal and non-mycorrhizal plants had similar response patterns to Pi treatment, and non-mycorrhizal plants had significantly higher root P concentrations than mycorrhizal plants. Furthermore, mycorrhizal plants retained more P in roots than non-mycorrhizal plants (Table 1.1), reflecting a change in P allocation resulting from mycorrhizal fungal colonization.

Phosphorus use efficiencies (PUE) of mycorrhizal and non-mycorrhizal broomsedge plants depended on Pi availability (Figure 1.2) (ANCOVA: Myc, $P < 0.001$; Pi, $P < 0.001$; and Myc*Pi, $P = 0.006$). Phosphorus use efficiency in mycorrhizal plants was higher than non-mycorrhizal plants at the low Pi treatments, but mycorrhizal and non-mycorrhizal plants had similar PUE at the high Pi treatment (Figure 1.2).

The different patterns of growth, shoot P concentrations, and PUE between mycorrhizal and non-mycorrhizal plants suggested that mycorrhizal fungal colonization may have altered some nutrient relationship other than P. Mycorrhizal plants contained significantly higher shoot concentrations of Mg (Myc: 2.164 mg g^{-1} and non-myc: 1.838 mg g^{-1}) and Cu (Myc: $14.0 \mu\text{g g}^{-1}$ and non-myc: $9.1 \mu\text{g g}^{-1}$) and root concentrations of Ca (Myc: 2.608 mg g^{-1} and non-myc: 2.025 mg g^{-1}) and Cu (Myc: $22.7 \mu\text{g g}^{-1}$ and non-myc: $9.1 \mu\text{g g}^{-1}$), but significantly lower shoot Na (Myc: 0.161 mg g^{-1} and non-myc: 0.299 mg g^{-1}) concentration than non-mycorrhizal plants (Table 1.2). The mycorrhizal and Pi treatments interacted in influencing shoot K, Fe, Mn, and Na concentrations and root Mg and Mn concentrations (Figure 1.3). Consistently, the response of nutrients to the Pi treatments were less in mycorrhizal plants than in non-mycorrhizal plants, reflecting greater stability in shoot and root nutrient relations of mycorrhizal plants, such as shoot K, Fe, Mn, and Na concentrations, and root Mn concentrations (Figure 1.3).

In addition to measuring plant tissue P concentrations and within plant allocation, the Pi depletion technique was used to measure Pi uptake rates by root systems of mycorrhizal and non-

Table 1.3. Pi instantaneous uptake rates and minimum solution Pi concentrations ($C_{\min}$) for Pi influx in mycorrhizal and non-mycorrhizal broomsedge (*A. virginicus*) plants as influenced by Pi treatment.

Pi (μM)	Myc	Uptake Rate ($\mu\text{mol g}^{-1} \text{min}^{-1}$)	$C_{\min}$ ($\mu\text{mol L}^{-1}$)
20	+	0.082	0.359
	-	0.158	5.018
40	+	0.074	1.670
	-	0.118	6.980
60	+	0.069	11.923
	-	0.121	22.532
100	+	0.076	50.085
	-	0.119	48.636
LSD[†]		0.030	5.491
P	Myc	0.003	0.010
Values	Pi	ns	< 0.001
	Myc x Pi	ns	ns

[†]Tukey's least significant difference at 0.05 level with $n = 10$.

mycorrhizal broomsedge plants. Mycorrhizal plants had significantly lower Pi uptake rate than non-mycorrhizal counterparts under the same Pi level (Table 1.3). Pi uptake rates of mycorrhizal roots averaged $0.075 \mu\text{mol g}^{-1} \text{min}^{-1}$ and were independent of Pi treatment. In contrast, Pi uptake rates by non-mycorrhizal roots increased when Pi became severely limited ($20 \mu\text{M Pi}$) (Table 1.3). In addition, the uptake data indicated a significant divergence in the minimum Pi concentration (C_{min}) to which mycorrhizal and non-mycorrhizal plants could draw down Pi in the nutrient solution (Table 1.3). Mycorrhizal root systems drew Pi down to lower concentrations than non-mycorrhizal plants at 20, 40, and $60 \mu\text{M Pi}$ treatments.

Discussion

Arbuscular mycorrhizal fungi play important roles in enhancing host plant growth under adverse soil conditions (Daft and Nicolson 1974; Daft and Hacskeylo 1976; Lindsey et al. 1977; Allen and Allen 1980; Lambert and Cole 1980; Danielson 1985). The results of the present study are consistent with these previous reports. When Pi availability was low, mycorrhizal fungal colonization significantly increased number of tillers and their heights, consequently leading to greater biomass accumulation in mycorrhizal than non-mycorrhizal plants (Figure 1.1). When Pi was readily available, the AM mycorrhizal fungi no longer exerted a beneficial effect on host plant growth. Thus, the AM fungi may switch from mutualistic symbiosis under Pi-limited conditions to a mildly parasitic association when Pi is readily available (Johnson et al. 1997; Smith and Read 1997). In addition, mycorrhizal fungal colonization altered biomass allocation (Figure 1.1). More biomass was allocated to roots in mycorrhizal plants and non-mycorrhizal plants when Pi availability is low (Figure 1.1), which undoubtedly increases the acquisition of limited resources (Chapin 1980, 1991).

Differences in nutrient concentrations between mycorrhizal and non-mycorrhizal plants can be related to differences in acquisition, transport, and/or utilization within the plant (Marschner 1995). In non-mycorrhizal broomsedge shoots and roots, biomass production was modulated to maintain tissue P concentrations within narrow bounds (Table 1.1), which may reflect the general regulation of P homeostasis observed in higher plants (Mimura 1995). In contrast, mycorrhizal plants had lower shoot Pi concentrations when Pi is limiting and accumulated P to high levels when Pi is readily available, which appears to indicate that the P homeostasis of the plants was altered by AM fungal colonization.

Phosphorus translocation to the shoot of mycorrhizal plants was less than non-mycorrhizal plants under limited Pi availability, but increased as Pi levels increased (Table 1.1). This tendency of mycorrhizal plants to limit P translocation from roots under low Pi availability reflects an increased investment of a limiting resource (Pi) into belowground biomass production (roots/hyphae) (Figure 1.1), which would serve to increase the acquisition of this limiting resource (Chapin 1980, 1991). When Pi was readily available, more P was transported to shoots, which leads to P luxury consumption (Chapin 1980) or Pi storage that would be used in the future to support long-term growth (Aerts and Chapin 2000). In support of this observation, the change observed in PUE as available Pi changed and the differences in this response between mycorrhizal and non-mycorrhizal plants suggest that mycorrhizal fungal colonization alters within-plant Pi utilization to overcome Pi limitation under low Pi availability and to support sustainable growth under variable Pi environments (Chapin 1980; Aerts and Chapin 2000).

The rates of Pi uptake measured by the Pi depletion technique in the present study reflect the difference in the overall P status and P demand of the mycorrhizal versus non-mycorrhizal plants. The Pi uptake rates were less in mycorrhizal than non-mycorrhizal plants for any given Pi treatment. Since Pi uptake rates increase in plants under Pi limitation (Clarkson and Scattergood 1982; Clarkson and Luttge 1991), it would appear that mycorrhizal broomsedge plants were under less Pi limitation than their non-mycorrhizal counterparts. In the sand culture system, mycorrhizal plants dominated the root zone to a greater extent than non-mycorrhizal plants. Greater root biomass and associated mycorrhizal hyphae would more effectively exploit the pool of Pi available in the root zone. In addition, mycorrhizal plants could draw down solution Pi concentrations to lower levels (Table 1.3), further increasing the total Pi pool exploited and increasing the diffusive gradients to mycorrhizal plant roots. Thus, mycorrhizal plants, with higher PUE (Figure 2), greater belowground exploitation (Figure 1.1), and lower C_{min} (Table 1.3), more effectively acquired Pi and were under less Pi stress at the lower Pi treatments. As a consequence, mycorrhizal broomsedge plants, overall, did not exhibit any increase in root Pi uptake rates (Table 1.3). These findings are contrary to those of Cress et al. (1979), where mycorrhizal tomato plants were shown to have greater Pi uptake rates. However, taken together with the patterns of growth and P utilization within broomsedge plants, our findings suggest that mycorrhizal plants adapt to Pi limitation not by increasing the amount of Pi transport systems, but primarily by allocating more effort to root zone exploitation, which is consistent with model

predictions (Clarkson 1985). This allocation change would be, in the long term, an effective mechanism of overcoming Pi limitation (Chapin 1980, Lambers et al. 1998).

Other nutrient responses suggest that AM fungi effectively improve the nutrient status of the host plants (Table 1.2, Figure 1.3). Copper concentrations of both shoots and roots in mycorrhizal plants were significantly elevated by AM fungi. A similar pattern has been observed in mycorrhizal soybean (Pacovsky 1986). Copper is an important component of many enzymes involving redox reactions (Marschner 1995). Copper deficiency affects electron transfer in PS I (Marschner 1995), often results in decreases in the activities of anti-oxidative enzymes (Yu et al. 1998), and limits the growth of plants (Cook et al. 1997). Enhanced Cu uptake by the mycorrhizal symbiosis in broomsedge could also contribute to the observed increases in the plant growth and may be involved in altered PUE exhibited in mycorrhizal plants. For example, if mycorrhizal plants were more effective at energy capture and carbohydrate transformations as a result of enhanced Cu nutrition, then these plants would be able to capture more carbon given a restricted supply of Pi. The lower concentrations of foliar P, K, Mn and Fe observed for mycorrhizal plants at low Pi availability are consistent with growth dilution effects resulting from increased Cu acquisition.

Sodium concentrations of shoots in non-mycorrhizal plants were elevated when Pi became limited, and decreased as P status was improved with increased Pi availability (Table 1.2, Figure 1.3). This may reflect a loss of membrane selectivity under Pi-limitation and the subsequent loss of Na exclusion. Cumming and Weinstein (1990) also noted Na accumulation in non-mycorrhizal pine under Pi limitation. Furthermore, mycorrhizal broomsedge plants maintained relatively constant foliar K, Fe, Mn, and Na concentrations across Pi treatments, which would be important to support the normal metabolism and optimal growth of broomsedge plants under varying Pi environments (Mooney and Winner 1991). These patterns all indicate that AM fungi are important in balancing the nutritional status of plants under Pi stress.

One possible factor leading to the observed convergence in biomass of mycorrhizal and non-mycorrhizal plants at 100 μM Pi is the constraint resulting from limited root volume (Thomas and Strain 1991). If mycorrhizal plants at high Pi no longer could add biomass due to such limitation, non-mycorrhizal plant biomass would approach that of mycorrhizal plants. However, I feel this may not be the case due to the significantly different slopes exhibited by mycorrhizal and non-mycorrhizal plants between 60 and 100 μM Pi.

The association of mycorrhizal fungi with the roots of broomsedge plants influences the nutrition of this species in several ways, all of which increase plant growth. Enhanced capture of Pi through greater biomass allocation to root systems and effective Pi drawdown leads to better plant growth under Pi limiting conditions. Higher PUE of mycorrhizal plants at low Pi availability increases biomass production when Pi limits growth of non-mycorrhizal plants. The strategies of resource allocation, lower C_{min} , and higher PUE in mycorrhizal plants under low Pi availability undoubtedly facilitate the acquisition of other nutrient resources. Enhanced acquisition of Cu may play a role in the observed growth dilution by increasing PUE when Pi is not optimal. In viewing broad patterns of nutrition in this experiment, one striking feature is the more stable elemental concentrations, but less constant P concentrations, of tissues of mycorrhizal than non-mycorrhizal plants grown over the range of Pi studied. These patterns reflect a well-regulated nutrient homeostasis in mycorrhizal broomsedge under varying Pi availability that is not evident in non-mycorrhizal plants.

CHAPTER II

Mycorrhizal Roles in Al Resistance of Broomsedge

Introduction

Aluminum (Al) present in acidic soils can be toxic to plants (Haug 1984; Ritchie 1995). In addition to reducing root growth, Al affects nutrient availability in soils and the uptake and translocation of nutrients by plants (Roy et al. 1988; Taylor 1988; Rengel and Robinson 1989; Nichol et al. 1993). For example, Al reduces inorganic phosphorus (Pi) availability by forming Al-Pi precipitates in the rhizosphere and restricts P translocation within a plant (Randall and Vose 1963; Clarkson 1966; de Miranda and Rowell 1989; Macklon et al. 1994), and Al additionally 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). These effects undoubtedly result in the imbalance of nutrients in plants, consequently reducing plant growth.

Some plant species/cultivars exhibit persistent growth under Al exposure. The Al resistance in plants may be associated with alteration in rhizosphere pH, release of organic acids, and Al efflux from the roots (Delhaize and Ryan 1995; Kochian 1995; Marschner 1995), which affects Al availability in soil solutions, consequently leading to lower tissue Al concentrations. Some plants develop specific mechanisms to detoxify Al internally by increasing production of compounds to chelate Al (Ma et al. 1998). Moreover, a mycorrhizal role is emphasized in protection of roots from Al toxicity in plants where Pi deficiency-induced root responses are impaired by Al (Marschner 1995).

Arbuscular mycorrhizal (AM) fungi are widely established in acidic soils (Clark 1997). AM fungal colonization improves seedling survival and enhances plant growth (Danielson 1985). One of the best described roles of AM fungi is the facilitation of acquisition of mineral nutrients by the host plants as a result of extensive external fungal hyphae exploiting a larger volume of soil and mining scarce resources that are otherwise unavailable for roots (Smith and Read 1997). Phosphorus is the most documented nutrient improved by AM symbiosis (Bolan 1991; Smith and Read 1997; Chapter I). Moreover, AM fungal colonization enhances the uptake

of copper (Cu) and zinc (Zn) (Ross 1971; Pacovsky 1986; Smith and Read 1997; Chapter I). In addition, AM fungi may reduce the accumulation of some other elements, such as Mn and Fe (Pacovsky 1986; Kothari et al. 1991; Chapter I).

Given these roles of AM fungi in mycorrhizal symbioses, AM fungal colonization may confer Al resistance to the host plants by facilitating nutrient acquisition for the host plants under Al exposure. A limited number of experiments have demonstrated that AM fungal colonization ameliorates Al effects on plant growth and nutrition (Koslowsky and Boerner 1989; Medeiros et al. 1994; Mendoza and Borie 1998; Lux and Cumming 1999). However, it is not clear how this amelioration is achieved. Furthermore, AM fungi differentially affect plant nutrition in different developmental stages (Pacovsky 1986), and plant growth, nutrition, and root enzymes differentially respond to Al at different stages (Rengel and Robinson 1989; Widell et al. 1994). Thus, it may be important to elucidate different times in the process of plant development where there are differential effects of mycorrhizal presence as a function of Al treatments.

The present study aimed to investigate the response of broomsedge (*Andropogon virginicus*) plants to different levels of Al, to examine AM fungal roles in the nutrition and growth of plants exposed to Al, and to determine how mycorrhizal plants differ from non-mycorrhizal plants in Al resistance. I hypothesized that 1) mycorrhizal and non-mycorrhizal plants would differ in their response patterns to varying Al concentrations in root zones at different stages of plant development; 2) mycorrhizal plants and non-mycorrhizal plants also would differ in the ways of achieving Al resistance. I used broomsedge (*Andropogon virginicus* L.), a dominant grass species in many adverse edaphic habitats, and native AM fungi isolated from an abandoned acidic coal mining soil to test these hypotheses in two experiments.

Materials and Methods

Preparation of AM Fungal Inoculum

AM fungal inoculum was generated from broomsedge plants collected from the Field Crest abandoned coal mine in Morgantown, West Virginia, USA, in which *Glomus clarum* is the main fungus with *Gigaspora gigantea* also present. Although the site has been abandoned for about 50 years, much surface soil remains devoid of vegetation. The vegetated surface consists of a broomsedge sward surrounding stunted red maple (*Acer rubrum*) and big-toothed aspen

(*Populus grandidentata*) trees. Soil from the site has a pH from 3.0 to 3.3 (soil-water paste) and contains Melich-extractable Pi of 3.76 mg kg⁻¹ and Al of 363.1 mg kg⁻¹ soil.

To produce the inoculum of AM fungi, 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 a month, the pot contents became the source of infective inoculum for the experiment.

Preparation of Plants

Broomsedge seeds were sown around the transplants in nursery pots (15 cm diameter). Broomsedge seeds also were sown in pots containing sterile-germinated broomsedge and the mixture of autoclaved mine-soil and sand (1:3 v/v) with the bacteria extracted from the mine-soil. Seedlings from these pots served as a non-mycorrhizal control. After four weeks growth, roots of a small subset of seedlings (*ca.* 10) were examined to determine mycorrhizal status. Tissue P status was analyzed after wet digestion (Parkinson and Allen 1975) by the molybdate blue method (Olsen and Sommers 1982). Tissue phosphorus concentrations and tissue dry weights between mycorrhizal and non-mycorrhizal plants did not differ significantly.

Sand Culture and Growth Conditions

Mycorrhizal and non-mycorrhizal broomsedge seedlings were transplanted and grown in D16 Deepots (5 cm diameter x 18 cm height) (Stuewe and Sons, Inc., Corvallis, Oregon, USA) pre-filled with 220 cm³ of a 3:1 (v/v) mixture of coarse:fine acid-washed sand. Deepots were placed into a growth chamber with 14 hours of light at 28°C, 60% RH, and 10 hours of darkness at 21°C, 50% RH. Average light intensity at pot height in the chamber was 260 $\mu\text{mol m}^{-2} \text{s}^{-1}$ from mixed fluorescent and incandescent sources. Plants received a baseline nutrient solution containing Pi (40 μM) of NaH₂PO₄, Ca (0.675 mM), K (0.79 mM), Mg (0.25 mM), NO₃ (1.5 mM), NH₄ (0.5 mM), SO₄ (0.25 mM), B (23.14 μM), Fe (25 μM), Mn (4.57 μM), Zn (0.38 μM), Cu (0.16 μM), and Mo (0.06 μM). All solutions were adjusted to pH 4.0 after Al treatment was added and before application. Solutions (approximately 15 ml) were automatically delivered to the plants three times each day for eight weeks.

Multiple Harvest Dose Response

Treatments were 0, 10, 50, 100, and 200 μM Al in a form of AlCl_3 . Simulation analysis of these solution by the program GEOCHEM (Parker et al. 1993) indicated that Al^{3+} concentrations were 0, 5.72, 29.91, 62.75, and 133.90 μM in the solutions, respectively. For data analysis hereafter, delivered Al concentrations were used. A mycorrhizal-by-Al factorial design was employed with 10 replicates for each treatment combination. The influence of plants on solution pH was monitored every week by collecting excess solution flowing the Deepots (leachate) for an hour after delivery of solution. The first harvest was conducted after week 4, the second after week 6, and the final harvest after week 8. Three, three, and four plants from each combination were randomly chosen for the first, second, and third harvest, respectively. Plants were gently removed from Deepots and roots were rinsed free of sand with running de-ionized H_2O . Roots were excised. To determine acid phosphatase (APase) activity, 5-8 cm root tips were cut off, washed in de-ionized water, chopped into 1 cm pieces, and transferred into centrifuge tubes containing 4.5 ml of the appropriate treatment solution. Then, 0.5 ml of 1 mM nitrophenylphosphate (NPP) was added to each tube and tubes were incubated for 1 hour 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 at 410 nm wavelength (Tabatabai and Bremner 1969).

The remainder of the root system as well as the shoot were rinsed in running de-ionized H_2O and dried. After they were dried at 60°C for at least 24 hours, roots and shoots were weighed and ground in a Wiley mill. Ground roots and shoots were digested in concentrated H_2SO_4 / 30% hydrogen peroxide (Parkinson and Allen 1975). Phosphorus and Al concentrations of the digests were determined spectrophotometrically using the molybdate blue method (Olsen and Sommers 1982) and the eriochrome cyanine method (Anonymous 1985), respectively.

Extended Dose Response

Treatments were 0, 200, 400, and 1000 μM Al in a form of AlCl_3 . Simulation analysis of these solutions by the program GEOCHEM (Parker et al. 1993) indicated that Al^{3+} concentrations were 0, 133.9, 379.4, and 945.8 μM in the solutions, respectively. The treatments were arranged in a mycorrhizal-by-Al factorial design in the growth chamber with 10 replicates for each treatment combination. At the termination of the experiment following eight weeks of treatment, tiller number of each plant was counted and cumulative length of all these tillers was recorded.

Sand was removed from roots under running de-ionized water, and shoots were rinsed in de-ionized water. Roots then were excised. Percentage mycorrhizal colonization of roots was assessed on a 5% root sub-sample by the gridline intersect method (Giovannetti and Mosse 1980). Shoots and roots were dried at 60°C, weighed, and ground. Tissue samples were digested as noted above. The concentration of P in the digests was determined according to Taussky and Shoor (1953). All other mineral elements (Al, Fe, Mn, Mg, Ca, Zn, Cu, Na, and K) of the digests were analyzed by inductively coupled plasma (ICP) emission spectrophotometry (Leeman PS 950) by the National Research Center for Coal and Energy Analytical Laboratory at West Virginia University.

Data Calculation and Analysis

Percentage inhibition of nutrient uptake and plant resistance index were separately calculated as follow (Rengel and Robinson 1989):

$$\text{inhibition} = \frac{M_0 - M_j}{M_0} \times 100$$

$$\text{resistance index} = W_{\text{Al}} / W_{\text{control}}$$

where M_0 and M_j are separately total content of a nutrient in a plant at 0 and Al level j and W_{Al} and W_{control} are plant dry weights with Al treatment and without Al treatment, respectively.

Data were analyzed by analysis of covariance with solution Al concentrations treated as a covariate, and regression analysis using the statistical package JMP (SAS Institute, Cary, North Carolina, USA).

Results

Multiple Harvest Dose Response

Differential effects of mycorrhizal fungal colonization as a function of Al concentrations were observed on leachate pH in the early period of the experiment (Table 2.1). Mycorrhizal plants were more sensitive in the leachate pH to changes of the solution Al concentrations than

Table 2.1. *P* values from ANCOVA for leachate pH in broomsedge (*A. virginicus*) plants as influenced by mycorrhizal (**Myc**) and Al treatments at different time (week).

Source	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Myc	< 0.001	0.035	ns [†]	0.001	ns	ns
Al	0.034	0.044	ns	ns	ns	0.032
Myc * Al	< 0.001	0.050	ns	0.020	ns	ns

[†] $\alpha = 0.05$, the same hereafter.

Figure 2.1. Influence of Al treatment on leachate pH of mycorrhizal (**solid**) and non-mycorrhizal (**open**) broomsedge (*A. virginicus*) plants.

Slopes and *P* values from the regression:

A. Week 3

Mycorrhizal:

Slope: -0.00247, and *P* value: < 0.001;

Non-mycorrhizal:

Slope: 0.00422 (linear) and -0.00002 (quadratic), and *P* value: 0.028 (linear) and 0.059 (quadratic).

B. Week 4

Mycorrhizal:

Slope: -0.00224, and *P* value: 0.004;

Non-mycorrhizal:

Slope: -0.00003, and *P* value: ns.

C. Week 6

Mycorrhizal:

Slope: 0.00592 (linear) and -0.00003 (quadratic), and *P* value: 0.006 (linear) and < 0.001 (quadratic);

Non-mycorrhizal:

Slope: 0.00037, and *P* value: ns;

Figure 2.2. Influence of Al treatment on plant mass of mycorrhizal (**solid**) and non-mycorrhizal (**open**) broomsedge (*A virginicus*).

P values from ANCOVA:

A. The first harvest:

Myc: ns, Al: 0.040, and Myc*Al: 0.028.

B. The second harvest:

Myc: ns, Al: ns, and Myc*Al: ns.

C. The third harvest:

Myc: < 0.001 , Al: 0.008, and Myc*Al: ns.

Figure 2.3. Influence of Al treatment on root acid phosphatase (APase) activity of mycorrhizal (**solid**) and non-mycorrhizal (**open**) broomsedge (*A. virginicus*) plants.

P values from ANCOVA:

A. The first harvest:

Myc: < 0.001 , Al: 0.054, and Myc * Al: ns.

B. The second harvest:

Myc: 0.027, Al: ns, and Myc * Al: ns.

C. The third harvest:

Myc: < 0.001 , Al: ns, and Myc * Al: ns.

non-mycorrhizal plants (Figure 2.1). The leachate pH of all other plants, except ones exposed to 10 μM Al, which had leachate pH higher than 4.8, was below 4.5 (data not presented), suggesting that Al existed primarily as Al^{3+} in the root zones of these plants.

There were differential effects of mycorrhizal fungal colonization on plant mass only in the first harvest under different Al solution concentrations (Figure 2.2). As the Al concentrations increased, dry weights of non-mycorrhizal plants decreased, whereas Al below 100 μM stimulated mycorrhizal plant growth and even 200 μM Al had no effect on mycorrhizal plants (Figure 2.2) compared to the control plants. When plants were exposed to Al for longer periods of time (8 weeks), mycorrhizal benefit became more pronounced (Figure 2.2) (mycorrhizal plants with mean dry weight of 1.268 g over all the Al treatments and 0.690 g for non-mycorrhizal plants).

Acid phosphatase (APase) activity is a marker for Pi limitation (e.g. Fries et al. 1998) and was used as an indicator of Al effects on Pi acquisition by broomsedge. The APase activity of mycorrhizal plants was consistently lower than that of non-mycorrhizal plants across all Al treatments and at all harvests, and the APase of both mycorrhizal and non-mycorrhizal plants decreased over time (Figure 2.3). The exception was for mycorrhizal plants exposed to 50 μM Al at the second harvest. Taken together with growth data (Figure 2.2), it appears as if this treatment was anomalous.

Mycorrhizal fungal colonization significantly reduced Al influx into host plants and limited Al translocation within host plants at 100 and 200 μM Al during the first six weeks (Table 2.2). During the last two weeks of this experiment, mycorrhizal plants had marginally higher shoot and root Al concentrations than non-mycorrhizal plants under 200 μM Al exposure. When plants were exposed to 50 μM Al and below in the first and second harvests or even 100 μM Al in the third harvest, there was no significant difference in shoot and root Al concentrations between mycorrhizal and non-mycorrhizal plants (Table 2.2).

Mycorrhizal and non-mycorrhizal plants significantly differed in shoot and root P in the first four weeks as Al concentrations changed (Table 2.3). Shoot and root P concentrations of mycorrhizal plants were significantly higher than those of non-mycorrhizal plants in the first harvest at 50 μM Al and above, but there was no significant difference in tissue P concentration between them at 0 and 10 μM Al treatment. For the second harvest, mycorrhizal plants generally had higher shoot and root P concentrations than non-mycorrhizal plants. But shoot P

Table 2.2. Shoot and root Al concentrations (mg g⁻¹) of mycorrhizal and non-mycorrhizal broomsedge (*A. virginicus*) plants as influenced by Al treatments.

Al (μ M)	Myc	1 st Harvest		2 nd Harvest		3 rd Harvest	
		Shoot	Root	Shoot	Root	Shoot	Root
0	+	0.231	0.507	0.159	0.229	0.048	0.076
	-	0.251	0.261	0.247	0.459	0.156	0.055
10	+	0.274	0.395	0.111	0.198	0.147	0.060
	-	0.220	0.398	0.191	0.136	0.198	0.074
50	+	0.256	0.495	0.254	0.35	0.162	0.053
	-	0.286	0.556	0.147	0.349	0.118	0.070
100	+	0.294	0.509	0.188	0.171	0.412	0.502
	-	1.109	1.695	0.602	1.213	0.408	0.430
200	+	0.699	1.675	0.381	0.506	0.926	1.004
	-	1.143	1.610	1.304	1.678	0.788	0.874
Tukey's LSD[†]		0.512	0.693	0.289	0.493	0.176	0.212
P values	Myc	ns	ns	ns	ns	ns	ns
	Al	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Myc*Al	0.058	ns	< 0.001	< 0.001	0.060	ns

[†] Least significant difference at $\alpha = 0.05$ with $n = 3$.

Table 2.3. Shoot and root P concentrations (mg g^{-1}) of mycorrhizal and non-mycorrhizal broomsedge (*A. virginicus*) plants as influenced by Al treatments.

Al (μM)	Myc	1 st Harvest		2 nd Harvest		3 rd Harvest	
		Shoot	Root	Shoot	Root	Shoot	Root
0	+	1.544	0.910	1.778	0.836	0.859	0.962
	-	1.074	0.622	1.468	0.801	1.357	0.635
10	+	1.204	1.004	1.677	1.168	1.165	0.841
	-	1.282	0.828	1.587	0.751	1.273	0.834
50	+	2.183	1.361	2.004	1.263	1.213	0.701
	-	1.305	0.488	1.232	0.630	1.379	0.572
100	+	1.922	1.272	1.855	0.982	1.164	0.737
	-	1.201	0.286	1.412	0.558	1.691	0.891
200	+	2.256	1.503	1.702	1.252	1.613	1.385
	-	0.998	0.518	1.860	0.716	1.763	0.806
Tukey's LSD[†]		0.603	0.418	0.695	0.333	0.476	0.532
P values	Myc	ns	0.011	0.052	0.007	0.042	ns
	Al	ns	ns	ns	ns	0.001	ns
	Myc*Al	0.012	0.006	ns	ns	ns	ns

[†] Least significant difference at $\alpha = 0.05$ with $n = 3$.

concentrations of mycorrhizal plants from the third harvest were generally higher than those of non-mycorrhizal plants (Table 2.3).

Extended 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 . The patterns of growth responses to Al concentrations in the solutions significantly differed between mycorrhizal and non-mycorrhizal plants (Figure 2.4). Mycorrhizal plants had no significant reduction in shoot mass and cumulative height under exposure to Al up to 400 μM (Figure 2.4). However, non-mycorrhizal plants even at 200 μM Al exhibited Al toxicity symptoms, such as brown root tips (data not presented), and showed significant reduction in shoot and root mass (Figure 2.4). At 1000 μM Al, shoot and root of mycorrhizal plants were 22 and 18 fold larger than those of non-mycorrhizal plants, and mycorrhizal plants were 15 fold higher and 4.5 fold more tillers than non-mycorrhizal plants (Figure 2.4). In addition, mycorrhizal plant allocated more biomass to roots than non-mycorrhizal plants (Figure 2.4).

Mycorrhizal colonization was significantly affected by solution Al concentrations (Figure 2.5). Below 400 μM Al, mycorrhizal colonization of broomsedge plants increased as Al concentrations increased. When solution Al concentration reached 1000 μM , mycorrhizal colonization percentage decreased (Figure 2.5).

Effects of mycorrhizal fungal colonization on shoot and root Al concentrations depended on the solution Al concentrations (Figure 2.6). Mycorrhizal plants maintained significantly lower shoot and root Al concentrations than non-mycorrhizal plants at 400 and 1000 μM Al levels, where non-mycorrhizal plants 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 concentrations, there was no significant difference in shoot and root Al concentrations between mycorrhizal and non-mycorrhizal plants (Figure 2.6).

Different patterns of growth and shoot and root Al concentrations indicated that mycorrhizal fungal colonization may have altered other nutrient composition of shoots and roots. Differential effects of mycorrhizal fungal colonization as a function of the solution Al

Figure 2.4. Influence of Al treatment on shoot (**circle**) and root (**triangle**) dry weights (**A**), cumulative height (**circle**) and number of tillers (**triangle**) (**B**) of mycorrhizal (**solid**) and non-mycorrhizal (**open**) broomsedge (*A. virginicus*) plants.

P values from ANCOVA:

A. Shoot mass:

Myc: < 0.001, Al: < 0.001, and Myc * Al: 0.013;

Root mass:

Myc: < 0.001, Al: < 0.001, and Myc * Al: 0.029.

B. Cumulative height:

Myc: < 0.001, Al: < 0.001, and Myc * Al: < 0.001;

Number of tillers:

Myc: ns, Al: < 0.001, and Myc * Al: < 0.001.

Figure 2.5. Mycorrhizal colonization of broomsedge (*A. virginicus*) plants as influenced by Al treatment.

Slope: 0.086 (linear) and -0.000066 (quadratic), and *P* value: < 0.001 (linear) and 0.003 (quadratic).

Figure 2.6. Influence of Al treatment on shoot (**circle**) and root (**triangle**) Al concentrations of mycorrhizal (**solid**) and non-mycorrhizal (**open**) broomsedge (*A. virginicus*) plants.

P values from ANCOVA:

Shoot Al concentration:

Myc: ns, Al: < 0.001 , and Myc * Al: < 0.001 ;

Root Al concentration:

Myc: 0.020, Al: < 0.001 , and Myc * Al: < 0.001 .

Table 2.4. *P* values from ANCOVA for shoot and root nutrients of broomsedge (*A. virginicus*) plants as influenced by mycorrhizal (**Myc**) and Al treatments.

	Source	P	K	Ca	Mg	Fe	Mn	Cu	Zn	Na
Shoot	Myc	< 0.001	0.012	ns	ns	ns	0.001	ns	ns	ns
	Al	< 0.001	ns	< 0.001	< 0.001	0.030	ns	0.028	0.001	0.039
	Myc * Al	< 0.001	0.004	ns	0.015	0.002	ns	0.002	0.008	ns
Root	Myc	ns	0.027	0.034	0.006	ns	ns	< 0.001	ns	ns
	Al	0.001	ns	< 0.001	< 0.001	< 0.001	ns	ns	ns	< 0.001
	Myc * Al	< 0.001	ns	ns	ns	< 0.001	0.017	< 0.001	< 0.001	ns

Figure 2.7. Influence of Al treatment on shoot nutrient concentrations of mycorrhizal (**solid**) and non-mycorrhizal (**open**) broomsedge (*A. virginicus*) plants.

Slopes and *P* values from the regression:

A. Shoot P concentrations:

Mycorrhizal:

N/A;

Non-mycorrhizal:

Slope: 0.00242 (linear) and -0.000003 (quadratic), and *P* value: < 0.001 (linear and quadratic).

B. Shoot K concentrations:

Mycorrhizal:

Slope: 0.00248, and *P* value: 0.039;

Non-mycorrhizal:

Slope: 0.01941 (linear) and -0.00002 (quadratic), and *P* value: 0.003 (linear) and < 0.001 (quadratic).

C. Shoot Mg concentrations:

Mycorrhizal:

Slope: -0.00161, and *P* value: < 0.001;

Non-mycorrhizal:

Slope: -0.00296, and *P* value: < 0.001.

D. Shoot Fe concentrations:

Mycorrhizal:

Slope: -0.65437 (linear) and 0.00054 (quadratic), and *P* value: 0.019 (linear) and 0.030 (quadratic);

Non-mycorrhizal:

Slope: 0.35987, and *P* value: 0.005.

E. Shoot Cu concentrations:

Mycorrhizal:

N/A;

Non-mycorrhizal:

Slope: 0.02277, and *P* value: 0.010.

F. Shoot Zn concentrations;

Mycorrhizal:

N/A;

Non-mycorrhizal:

Slope: 0.10410, and *P* value: < 0.001.

Figure 2.8. Influence of Al treatment on root nutrient concentrations of mycorrhizal (**solid**) and non-mycorrhizal (**open**) broomsedge (*A. virginicus*) plants.

Slopes and *P* values from the regression:

A. Root P concentrations:

Mycorrhizal:

Slope: 0.00054 (linear) and -0.000005 (quadratic), and *P* value: < 0.001 (linear and quadratic);

Non-mycorrhizal:

Slope: 0.00074 (linear) and -0.000001 (quadratic), and *P* value: 0.016 (linear) and < 0.001 (quadratic).

B. Root Fe concentrations:

Mycorrhizal:

N/A;

Non-mycorrhizal:

Slope: 2.30579, and *P* value: 0.002.

C. Root Mn concentrations:

Mycorrhizal:

Slope: 1.83537 (linear) and -0.00189 (quadratic), and *P* value: < 0.001 (linear and quadratic);

Non-mycorrhizal:

Slope: -0.41900, and *P* value: 0.004.

D. Root Cu concentrations:

Mycorrhizal:

Slope: -0.03638, and *P* value: 0.005;

Non-mycorrhizal:

N/A.

E. Root Zn concentrations;

Mycorrhizal:

Slope: -0.10398, and *P* value: 0.002;

Non-mycorrhizal:

Slope: 0.12970, and *P* value: < 0.001.

Figure 2.9. Relationships between resistance index and percentage inhibition of nutrient uptake in mycorrhizal (**solid**) and non-mycorrhizal (**open**) broomsedge (*A. virginicus*) plants.

Slopes and R^2 from the regression:

A. Phosphorus		
Mycorrhizal:	Slope:	-0.0056, and R^2 : 0.50;
Non-mycorrhizal:	Slope:	-0.0091, and R^2 : 0.89.
B. Potassium		
Mycorrhizal:	Slope:	-0.0038, and R^2 : 0.30;
Non-mycorrhizal:	Slope:	-0.0089, and R^2 : 0.85.
C. Calcium		
Mycorrhizal:	Slope:	-0.0040, and R^2 : 0.77;
Non-mycorrhizal:	Slope:	-0.0089, and R^2 : 0.92.
D. Magnesium		
Mycorrhizal:	Slope:	-0.0048, and R^2 : 0.81;
Non-mycorrhizal:	Slope:	-0.0092, and R^2 : 0.94.
E. Iron		
Mycorrhizal:	Slope:	-0.0026, and R^2 : 0.39;
Non-mycorrhizal:	Slope:	-0.0099, and R^2 : 0.80.
F. Manganese		
Mycorrhizal:	Slope:	-0.00215, and R^2 : 0.31;
Non-mycorrhizal:	Slope:	-0.0094, and R^2 : 0.86.
G. Copper		
Mycorrhizal:	Slope:	-0.0034, and R^2 : 0.66;
Non-mycorrhizal:	Slope:	-0.0100, and R^2 : 0.95.
H. Zinc		
Mycorrhizal:	Slope:	-0.0044, and R^2 : 0.69;
Non-mycorrhizal:	Slope:	-0.0089, and R^2 : 0.85.
I. Sodium		
Mycorrhizal:	Slope:	0.0001, and R^2 : < 0.01;
Non-mycorrhizal:	Slope:	-0.0096, and R^2 : 0.85.

concentrations were pronounced for shoot P, K, Mg, Fe, Cu, and Zn concentrations, and root P, Fe, Mn, Cu, and Zn concentrations (Table 2.4). Under Al exposure (200, 400, and 1000 μM Al), mycorrhizal plants maintained narrow boundaries of shoot P, K, Fe, and Cu concentrations across the Al concentrations (Figure 2.7), reflecting that mycorrhizal plants maintained uptake of these nutrients under Al exposure. In non-mycorrhizal plants, however, shoot P, K, Mg, Fe, Cu, and Zn concentrations were pronouncedly interfered by 1000 μM Al concentration (Figure 2.7). Of interesting was shoot P concentration reduction in non-mycorrhizal plants by 1000 μM Al, which was 13.7 fold lower than that of mycorrhizal plants, reflecting a general effect of Al on P acquisition by plants.

Effect of high Al concentrations on P concentrations in non-mycorrhizal plants was also observed for root P concentrations (Figure 2.8). Variations of root P, Cu, and Zn concentrations in mycorrhizal plants may be associated with prior balancing these nutrient concentrations in shoots (Figure 2.7 and 2.8). Mycorrhizal plants also had stable root Fe concentrations across Al levels, but root Fe concentrations of non-mycorrhizal plants increased at 400 and 1000 μM Al levels (Figure 2.8). At 1000 μM Al, there was significant difference in root Mn concentrations between mycorrhizal and non-mycorrhizal plants (Figure 2.8).

Different patterns of shoot and root mass and nutrient concentrations in response to Al concentrations suggested that mycorrhizal fungal colonization may have ameliorated Al detrimental effects on nutrient acquisition. Balanced accumulation of nutrients in mycorrhizal plants exhibited less Al inhibition of nutrient uptake, consequently resulting in a loose relationship between resistance index and Al inhibition of P, K, Ca, Mg, Fe, Mn, Cu, Zn and Na uptake (Figure 2.9). On the other hand, adverse effects of Al on nutrient acquisition observed in higher plants were operative in non-mycorrhizal broomsedge plants as well (Table 2.2, Figure 2.7 and 2.8), so, Al resistance index decreased as Al inhibited worse uptake of P, K, Ca, Mg, Fe, Cu, Zn, and Na (Figure 2.9).

Discussion

Broomsedge is found colonizing acidic soils in the mid-Atlantic United States (Campbell 1983) and is the dominant herbaceous species colonizing extremely edaphic sites (Chapman and Jones 1975; Gibson and Risser 1982), such as abandoned coal mines (Nellssen and Ungar 1993). The chemistry of such soils is dominated by phytotoxic Al. The present study indicates that

broomsedge is not inherently an Al tolerant, with significant reductions in growth occurring at and above 100 μM Al (Figure 2.2 and 2.4).

The present study shows that AM fungi provide a great benefit to broomsedge plants in terms of plant growth (Figure 2.2 and 2.4) as demonstrated previously (Medeiros et al. 1994, Mendoza and Borie 1998). Furthermore, mycorrhizal plants did not exhibit Al toxic symptoms of growth at 200 μM Al and below, even showed stimulated growth under lower than 100 μM Al concentrations (Figure 2.2 and 2.4). When the Al treatment was over 400 μM , which is approximately the Al concentration of the soil solution in the coal-mining site from where the inoculum originated, mycorrhizal broomsedge plants exhibited some growth reduction, but were still much larger than non-mycorrhizal plants. This indicates that this native AM fungal consortium has adapted to acidic soils and Al stress. In addition, mycorrhizal broomsedge plants allocated more biomass to roots, which would enhance acquisition of limited resources imposed by Al presence, such as phosphorus.

Under low Al levels, such as 10 μM , the leachate pH of both mycorrhizal and non-mycorrhizal plants initially rose up to 5.0 after they established, which would significantly affect Al speciation in the solution (Figure 2.1). But the high Al concentrations (over 50 μM) in the nutrient solutions limited the capability of plants to alter leachate pH (Figure 2.1), which may be related to high Al buffering capacity under high Al concentrations. These results are consistent with the previous reports (Rengel and Robinson 1989), suggesting that the rhizosphere pH change under 50 μM and higher concentrations plays a minor role in Al resistance of plants (Miyasaka et al. 1989, Rengel and Robinson 1989).

Shoot and root Al and P concentrations (Table 2.2 and 2.3) indicated that in the early stage of the development, AM fungi facilitated P acquisition in broomsedge plants exposed to 50 μM Al and above, which undoubtedly supports long-term growth of host plants under Al exposure. The capability of mycorrhizal fungi mining P from limited P sources under conditions of Al existence was observed in other fungi and plants (Cumming and Weinstein 1990). Meanwhile, AM fungi limited Al influx into host plants, further improving the growth of host broomsedge plants. These two roles of AM fungi in broomsedge plants under Al toxicity are important for this plant species to establish in acidic soils, where phosphorus limitation and Al toxicity are the primary factors limiting plant growth. For a long-term Al exposure, mycorrhizal plants maintained lower shoot and root Al concentration than non-mycorrhizal plants when Al concentrations in root zones were higher than 400 μM (Figure 2.6). The reduction of shoot and

root Al concentrations in host plants by AM fungi were also found in other plant species (Koslowsky and Boerner 1989; Medeiros et al. 1994; Mendoza and Borie 1998). Disruption of P availability and acquisition by Al is believed to be one of Al toxic mechanisms in plants (Randall and Vose 1963; Clarkson 1966; Foy 1983; Roy et al. 1988; Andersson 1988; de Miranda and Rowell 1989; Taylor 1988; Tan and Keltjens 1990; Macklon and Sim 1992). The present study showed that Al exposure adversely affected P uptake by non-mycorrhizal broomsedge plants, especially in the early stages of plant development (Table 2.3). Data of acid phosphatase activity (Figure 2.3) also suggest that mycorrhizal plants were under less P stress than non-mycorrhizal plants. For a long-term Al exposure, high (1000 μM) Al extremely reduced shoot and root P concentrations of non-mycorrhizal plants (Figure 2.7 and 2.8). However, mycorrhizal plants maintained stable foliar P concentrations across the Al levels (Figure 2.7).

Numerous experiments have demonstrated that Al interferes with the acquisition of cations in plants (Rengel and Robinson 1989, Huang et al. 1992, Nichol et al. 1993, Wheeler and Dodd 1995, Lindberg and Strid 1997), although there are reports that root growth cessation and Al-induced alterations in divalent cation flux are not correlated (Ryan and Kochian 1993, Ryan et al. 1993, Jones et al. 1998). These all are short-term experiments and suggest that Al toxicity and resistance in plants may or may not be related to uptake and translocation of divalent cations from the rhizosphere. In long-term Al studies, the disruption of Ca and Mg nutrition is frequently observed (Rengel and Robinson 1989, Tan et al. 1993). The similar detrimental effects of Al on shoot and root Ca and Mg concentrations were noted for both mycorrhizal and non-mycorrhizal broomsedge plants in the present study (Table 2.4, Figure 2.7 and 2.8), suggesting that AM fungi have no direct effect on acquisition of these two cations (Smith and Read 1997).

Differences in shoot and root nutrient concentrations between mycorrhizal and non-mycorrhizal plants exposed to Al can be related to differential effects of Al on nutrient availability to and acquisition by plants (Foy 1983; Roy et al. 1988; Marschner 1995). Aluminum inhibits root elongation, which reduces root zones, and affects membrane permeability of root cells, consequently changing elemental influx into roots (Foy 1983; Andersson 1988; Taylor 1988; Kochian 1995). Therefore, non-mycorrhizal broomsedge plants exhibited disrupted status of shoot and root nutrition (Figure 2.7 and 2.8). Arbuscular mycorrhizal fungi greatly enhanced root growth (Figure 2.4), mined scarce resources (Table 2.2 and 2.3, Figure 2.7 and 2.8), and reduced Al impacts (Figure 2.2, 2.4, and 2.9). All these

benefits of AM fungi to host plants fostered the balanced accumulation of shoot K, Fe, Cu, and Zn, concentrations and root Fe concentrations in broomsedge plants (Table 2.4, Figure 2.7 and 2.8).

The differences in relationships of Al resistance index and Al inhibition of nutrient uptake between mycorrhizal and non-mycorrhizal plants suggest that detrimental effects of Al on nutrient acquisition in non-mycorrhizal plants may be one of primary Al toxic mechanisms (Foy 1983; Taylor 1988; Kochian 1995). In mycorrhizal broomsedge plants, AM fungi limited Al influence (Table 2.2, Figure 2.6) and improved nutrition of host plants (Table 2.4, Figure 2.7 and 2.8), so that the host plants were no longer under nutrient stress as much as non-mycorrhizal plants were. Given these conditions, Al resistance in mycorrhizal plants is less dependent on reduction of Al inhibition of nutrient uptake as indicated by Figure 2.9. This relationship between Al resistance and Al inhibition of nutrient uptake also implies that balanced accumulation of nutrients maybe become more important than facilitation and/or reduction of nutrient uptake in mycorrhizal plants exposed to Al.

In conclusion, broomsedge appears not to have an inherent mechanism to block Al influx and resist Al in the rhizosphere. Although broomsedge plants are capable of adjusting the rhizosphere pH, this capability is very limited, especially at high Al concentrations. Aluminum significantly reduced broomsedge plant growth possibly by mainly interfering availability, acquisition, and translocation of nutrients in non-mycorrhizal plants, as evidenced by close relationships between Al resistance and Al inhibition of nutrient uptake in non-mycorrhizal plants. The acid ecotypic AM fungi used in this experiment conferred Al resistance to broomsedge. Arbuscular mycorrhizal fungi enhanced P acquisition by and reduced Al influx to host plants in the early stage of development. For a long-term high Al exposure, reduction of Al influx into host plants, consequently leading to less Al impacts on the host plants, and regulation of P uptake by AM fungi may be an important mechanism for plants to survive and establish in acidic soils. The impacts of Al on nutrient acquisition in mycorrhizal plants were less pronounced than in non-mycorrhizal plants. The balanced accumulation of nutrients in mycorrhizal plants exposed to Al may be another important mechanism involved in the Al resistance. Thus, the symbiosis between AM fungi and broomsedge plants plays critical roles in Al resistance of this plant species, which allows this plant species establish and grow in acidic soils.

CHAPTER III

Differential Responses of Different Mycorrhizal and Non-Mycorrhizal Broomsedge Plants to Aluminum

Introduction

Plant roots release organic acids into the rhizosphere in response to some adverse environmental factors (Curl and Truelove 1986; Kochian 1995; Marschner 1995; Strom 1997). Aluminum is one of the primary edaphic factors limiting plant growth on acidic soils. However, there is a wide range of Al sensitivity among and within plant species (Andersson 1988; Blamey et al. 1992; Bona et al. 1994; Wheeler and Dodd 1995). For example, there is differential Al sensitivity of two co-existing woodland plant species that may be associated with Al disruption of macronutrients in plants (Andersson 1992; Andersson and Brunet 1993). Different responses of 10 selected crops to Al were observed, which is not related to root cation-exchange capacity (Blamey et al. 1992). With 34 species and 143 genotypes of temperate legume exposed to Al, large different responses were observed in terms of growth and tissue nutrients (Wheeler and Dodd 1995). One proposed mechanism for Al resistance in plants is the chelation of Al in the rhizosphere by organic acids exuded from root systems (Delhaize and Ryan 1995; Kochian 1995; Ma 2000). Different plant species excrete different organic acids in response to Al treatments. Quantity of organic acids is also different with different plant species and cultivars and Al levels. For example, snapbean (*Phaseolus vulgaris*) produces citrate, but the tolerant cultivar (Dade) exudes 50 times more citric acid with Al exposure compared to without Al exposure, and 10 times more than the sensitive cultivar (Romano) (Miyasaka et al. 1991). The Al-tolerant cultivar ET3 of wheat (*Triticum aestivum*) releases 5- to 10-fold more malic acid than Al-sensitive cultivar ES3 and the effect is more pronounced with longer Al exposure (Delhaize et al. 1993). More oxalic acid is secreted by buckwheat (*Fagopyrum esculentum*) roots in response to increased Al concentrations in the bathing solution and longer Al exposure (Ma et al. 1997). Different organic acids differentially ameliorate Al toxicity in plants. Citrate, oxalate, malate, tartrate, succinate, lactate, formate, and acetate, in order of importance, detoxify Al (Taylor 1988; Miyasaka et al. 1991; Strom 1997).

A role of mycorrhizal fungi in Al resistance of plants has been recognized (Koslowsky and Boerner 1989; Shaw and Read 1989; Cumming and Weinstein 1990; Medeiros et al. 1994; Yang and Goulart 1997; Mendoza and Borie 1998; Chapter II). One of the important benefits provided by mycorrhizas to plants exposed to Al is the facilitation of nutrient acquisition, especially Pi, and reduction of Al uptake (Koslowsky and Boerner 1989; Medeiros et al. 1994; Smith and Read 1997; Mendoza and Borie 1998; Chapter II). In the previous study with broomsedge (*Andropogon virginicus*) (Chapter II), arbuscular mycorrhizal (AM) fungi collected from an acidic minesoil significantly reduced the impacts of Al on growth and nutrient relations.

Furthermore, different mycorrhizal fungi, even strains of the same fungal species, differentially contribute to acidic resistance and metal resistance in host plants (Goldon and Tinker 1981; Koslowsky and Boerner 1989; Medeiros et al. 1994; Clark 1997). These differential effects of different mycorrhizal fungi may be associated with their roles in the original habitats. For example, switchgrass plants colonized by an AM fungal isolate from a high Al site had significantly lower tissue Al concentrations, but higher shoot mass, and more tillers than plants colonized by an isolate from a low Al site (Koslowksy and Boerner 1989).

Because root exudation of organic acids plays an important role in Al detoxification, I hypothesized that AM fungi may confer Al resistance by altering the profile of root exudates which may protect roots from the detrimental effects of Al on growth of host plants. An early review by Curl and Truelove (1986) highlighted the influence of microorganisms present in the rhizosphere on root exudation. A study of cucumber (*Cucumis sativus*) by Pinior et al. (1999) suggest that mycorrhizal fungal colonization alters the profile of the root exudates. Furthermore, there are no investigations of how this alteration of root exudation affects Al resistance in plants.

The present study examined the profiles of organic acids from the rhizosphere of broomsedge plants exposed to different Al levels, investigated mycorrhizal effects on rhizosphere organic acids and its relationship with Al resistance in the plants, and assessed the effects of different AM fungal isolates on plant nutritional status and their relationships with the root exudation of organic acids. I hypothesize that 1) different AM fungi differentially affect exudation of organic acids from root systems; 2) the differences in the exudation of organic acids may be involved in differential Al resistance among different mycorrhizal and non-mycorrhizal plants.

Materials and Methods

Preparation of AM Fungal Inocula

AM fungal inocula were used in this study, separately originated from two abandoned coal mining sites in West Virginia. One site is in Mingo County, having soil pH of 6.6 and containing NaHCO_3 -extractable Pi of 19 mg per kg of soil. At this pH, Al is not toxic to plants. Soil from the other site in Monongalia County has a pH from 3.0 to 3.3, containing Melich-extractable Pi of 3.76 mg per kg of soil and Al of 363.12 mg per kg of soil.

To produce the inocula of *Glomus clarum*, inocula (WV233-1 and WV219A-5) from the INVAM collection at West Virginia University, which originated separately from the Monongalia site and the Mingo site, were used to inoculated sterilely germinated broomsedge plants, respectively. These inoculated broomsedge plants were planted into 15 cm diameter pots containing a mixture of autoclaved mine-soil from the Monongalia site and sand (1:3 v/v). After a month, the pot contents became the sources of infective LM and HM inoculum for the experiment, respectively, where LM and HM separately represent mycorrhizal fungi from low pH site at Monongalia abandoned coal mine and high pH site at Mingo abandoned coal mine.

Preparation of Plants

Broomsedge seeds were sown around the transplants in nursery pots (15cm diameter). Broomsedge seeds also were sown in pots containing sterile-germinated broomsedge and the mixture of autoclaved mine-soil and sand (1:3 v/v). Seedlings from these pots served as a non-mycorrhizal control. After four weeks growth, roots of a small subset of seedlings (*ca.* 10 from each pot) were examined to determine mycorrhizal status. Tissue P status was analyzed after wet digestion (Parkinson and Allen 1975) by the molybdate blue method (Olsen and Sommers 1982). Tissue phosphorus concentrations and tissue dry weights between mycorrhizal and non-mycorrhizal plants did not differ significantly at the time of transplanting.

Nutrient Solution and Growth Conditions

Mycorrhizal and non-mycorrhizal broomsedge seedlings were transplanted and grown in D16 Deepots (5 cm diameter x 18 cm height) (Stuewe and Sons, Inc., Corvallis, Oregon, USA) pre-filled with 220 cm³ of a 3:1 (v/v) mixture of coarse:fine acid-washed sand. Deepots were placed into a growth chamber with 14 hours of light at 28°C, 60% RH, and 10 hours of darkness

at 21°C, 50% RH. Average light intensity at pot height in the chamber was $260 \mu\text{mol m}^{-2} \text{s}^{-1}$ from mixed fluorescent and incandescent sources. Plants received a baseline nutrient solution containing PO_4 (40 μM), Ca (0.675 mM), K (0.79 mM), Mg (0.25 mM), NO_3 (1.5 mM), NH_4 (0.5 mM), SO_4 (0.25 mM), B (23.14 μM), Fe (25 μM), Mn (4.57 μM), Zn (0.38 μM), Cu (0.16 μM), and Mo (0.06 μM). All solutions were adjusted to pH 4.0 after Al treatment was added and before application. Solutions (approximately 15 ml) were automatically delivered to the plants three times each day for eight weeks.

Plant Growth

Following establishment (a week), seedlings were exposed to 0, 200, 400, or 1000 μM Al in a form of AlCl_3 . Simulation analysis of these solutions by the program GEOCHEM (Parker et al. 1993) indicated that Al^{3+} concentrations were 0, 133.9, 379.4, and 945.8 μM in the solutions, respectively. For data analysis, delivered Al concentrations were used. The treatments were arranged in a Al-by-mycorrhizal factorial design in a growth chamber with 10 replicates for each treatment combination. Solution draining (leachate) from four Deepots per treatment was collected for an hour following one solution delivery weekly. The leachate pH was measured.

Plants were harvested following eight weeks of treatment. Plants were gently removed from the pots. After extraction of organic acids, roots were excised. Shoots and roots were rinsed in running de-ionized water, dried at 60°C, weighed, and ground. Tissue digestion and determination of tissue elements were the same as in previous experiments (Chapter II).

Extraction and Analysis of Rhizosphere Organic Acids

Extraction The root system with intact sand was dipped into de-ionized water in a volumetric container. The water volume used (V_{water}) depended on the root system size. The change in the volume was recorded and represented the total rhizosphere volume plus the root system and adhering sand volumes (V_{total}). The root system was shaken gently for 5 minutes, removed, dried by folding it in tissue paper, and submerged into another volumetric container containing a known volume of water. The change in the volume was the root volume (V_{root}). The extraction solution in the first container was transferred into 15 ml centrifuge tubes. The sand remaining in the first container was dried in an oven and its volume was measured (V_{sand}).

Preparation for the Ion Chromatography Analysis Ten mM of $\text{Na}_2\text{-EDTA}$ was added into each of the centrifuge tubes containing the extraction solution to a final concentration of

10% (v/v). The pH was adjusted to neutral and samples were concentrated using a SpeedVac (Savant corp., NY). The residual salt in each tube was dissolved in 1 ml of de-ionized water, which was passed through a C18 column (Dionex Corp, Sunnyvale, CA).

Ion chromatography Analysis The ion chromatography analysis was performed using a Dionex DX-300 Ion Chromatograph, an IonPac ICE-AS6 column, with detection by conductivity (Dionex Corp., Sunnyvale, CA). The operating conditions were after the suggestion by the company with a little of modifications: 1.6 μM heptafluorobutyric acid as the eluent at 1 ml min^{-1} flow rate and 0.4 μM tetrabutyl-ammonium hydroxide as the suppressant. A mixture of 20 μM , 50 μM , or 100 μM standard organic acids (acetate, citrate, formate, fumarate, glycolate, malate, succinate, tartrate), which was prepared following the same procedure as the samples, was analyzed with the samples in the same run. Data were acquired and calibrated with the standard organic acids using Dionex AI-450 Chromatography Software Program (release 3.33). The final amount (μmol) of organic acid was calculated as:

$$C \cdot V_{\text{water}}$$

where C was concentration of an organic acid from Dionex, and concentration of an organic acid (μM) was calculated using following equation:

$$(C \cdot V_{\text{water}}) / (V_{\text{total}} - V_{\text{root}} - V_{\text{sand}})$$

Data Analysis

Data were analyzed by analyses of covariance with Al concentrations treated as a covariate and regression analysis using the statistical package JMP (SAS Institute, Cary, North Carolina, USA).

Results

Through the experiment, leachate pH with Al treatments was never higher than 4.20 (data not presented), indicating that the Al primarily existed as Al^{3+} in the solutions (Snoeyink and Jenkins 1980).

Non-mycorrhizal plants exhibited Al toxicity symptoms even at 200 μM Al level, such as stunted roots (data not presented). At higher Al levels, non-mycorrhizal plants grew little (Figure 3.1). Colonization by both AM fungal isolates significantly enhanced shoot and root dry weights at 400 μM Al level and below, at which Al levels there was no significant difference in shoot and root mass between LM and HM mycorrhizal plants (Figure 3.1). At 1000 μM Al level, HM mycorrhizal plants were still significantly larger than non-mycorrhizal plants, but LM mycorrhizal and non-mycorrhizal plants did not significantly differ in shoot and root mass (Figure 3.1), reflecting that different AM fungal isolates differentially affected growth of the same host plant species under Al exposure.

Al treatment did not significantly affect on mycorrhizal colonization (Myc $P = 0.951$, Al $P = 0.307$, and Myc*Al $P = 0.603$). LM and HM colonization over all the Al treatments was 36.16% and 33.93%, respectively.

Shoot and root Al concentrations of mycorrhizal and non-mycorrhizal plants depended on the solution Al concentrations (Figure 3.2). Shoot Al concentrations of both mycorrhizal plants colonized by LM and HM isolates were significantly lower than those of non-mycorrhizal plants at all Al levels except at 200 μM Al, where LM mycorrhizal and non-mycorrhizal plants did not significantly differ (Figure 3.2). For root Al concentrations, non-mycorrhizal plants had significantly higher values at all Al levels than LM mycorrhizal plants, but did not significantly differ from HM mycorrhizal plants except at 200 μM Al level, where root Al concentration of HM mycorrhizal plants was significantly lower than that of non-mycorrhizal plants (Figure 3.2).

Figure 3.1. Influence of Al treatment on shoot (**A**) and root (**B**) mass of LM mycorrhizal (**solid circle**), HM mycorrhizal (**solid triangle**), and non-mycorrhizal (**open circle**) broomsedge (*A. virginicus*) plants.

P values from ANCOVA:

A. Shoot mass

Myc: < 0.001 , Al: < 0.001 , and Myc * Al: ns[†];

B. Root mass

Myc: < 0.001 , Al: < 0.001 , and Myc * Al: 0.032.

[†] $\alpha = 0.05$, the same hereafter.

Figure 3.2. Influence of Al treatment on shoot (**A**) and root (**B**) Al concentrations of LM mycorrhizal (**solid circle**), HM mycorrhizal (**solid triangle**), and non-mycorrhizal (**open circle**) broomsedge (*A. virginicus*) plants.

P values from ANCOVA:

A. Shoot Al concentrations

Myc: 0.035, Al: < 0001, and Myc * Al: < 0.001.

B. Root Al concentrations

Myc: 0.054, Al: < 0.001, and Myc * Al: 0.043.

Table 3.1. Influence of Al treatment on productions of organic acids ($\mu\text{mol g}^{-1}$ of root mass) extracted from the rhizosphere of LM mycorrhizal, HM mycorrhizal, and non-mycorrhizal broomsedge (*A. virginicus*) plants.

Al (μM)	Myc	Formate	Acetate	Glycolate	Lactate	Malate	Tartrate	Citrate	unknown [†]
0	LM	2.734	1.721	2.301	6.524	2.116	0.274	1.427	15.110
	HM	4.133	1.625	7.578	4.418	3.966	0.276	1.791	27.860
	NM	3.452	3.896	0	5.063	1.523	0	0.707	0
200	LM	1.854	3.259	0	7.010	0.972	0.244	1.511	11.502
	HM	3.618	1.636	0	4.509	3.319	0.179	2.495	20.543
	NM	1.974	1.683	0	8.398	3.579	0	1.532	0
400	LM	2.110	3.418	0	7.166	0	0	2.291	0
	HM	7.475	6.301	0	7.838	3.954	0	2.718	0
	NM	11.935	4.127	0	19.590	3.041	0	2.361	0
1000	LM	6.402	5.038	0	21.032	0	0	2.665	0
	HM	3.336	4.128	0	4.724	0	0	1.977	0
	NM	6.573	7.104	0	8.438	2.366	0	5.218	0
LSD^f		3.732	2.063	2.071	3.767	1.899	0.022	0.730	5.333
P	M	ns [*]	ns	< 0.001	ns	0.003	< 0.001	0.002	< 0.001
values	Al	0.041	< 0.001	0.005	< 0.001	0.021	< 0.001	< 0.001	< 0.001
	M*Al	ns	ns	0.010	0.002	ns	< 0.001	< 0.001	< 0.001

[†] Unidentified organic acid at *ca* 26.0 minute of retention time, and its unit is area per gram of root mass.

^f Tukey's Least Significant Difference at 0.05 level with N of from 5 to 12.

^{*} $\alpha = 0.05$, the same hereafter.

Figure 3.3. Influence of Al treatment on citrate (**A**) and lactate (**B**) production of LM mycorrhizal (**solid circle**), HM mycorrhizal (**solid triangle**), and non-mycorrhizal (**open circle**) broomsedge (*A. virginicus*) plants.

Slopes and *P* values from the regression:

A. Citrate

LM mycorrhizal: slope = 0.00129 and *P* value = 0.015;

HM mycorrhizal: slope = -0.00036 and *P* value: ns;

Non-mycorrhizal: slope = 0.00457 and *P* value < 0.001.

B. Lactate

LM mycorrhizal: slope = 0.01358 and *P* value < 0.001;

HM mycorrhizal: slope = 0.00040 and *P* value: ns;

Non-mycorrhizal: slope = 0.04190 (linear) and -0.00004 (quadratic), and *P* value = 0.003 (linear) and 0.006 (quadratic).

Figure 3.4. Influence of Al treatment on total amount (μmol), total production ($\mu\text{mol g}^{-1}$), and total concentration (mM) of organic acids (R-COOH) in rhizosphere of LM mycorrhizal (**solid circle**), HM mycorrhizal (**solid triangle**), and non-mycorrhizal (**open circle**) broomsedge (*A. virginicus*) plants.

P values from ANCOVA:

A. Total amount of organic acids (μmol):

Myc: < 0.001 ;

Al: < 0.001 ;

Myc * Al: ns.

B. Total production of organic acids ($\mu\text{mol g}^{-1}$):

Myc: < 0.001 ;

Al: 0.008;

Myc * Al: < 0.001 .

C. Total concentration of organic acids (mM):

Myc: < 0.001 ;

Al: 0.026;

Myc * Al: ns.

Patterns of exudation of organic acids depended on both Al and mycorrhizal treatments (Table 3.1). Glycolate was released only by LM and HM mycorrhizal plants under a condition of no Al exposure. Tartrate and an unidentified organic acid were also exuded only by both mycorrhizal plants at 0 and 200 μM Al. Exudation of lactate and citrate by LM mycorrhizal, HM mycorrhizal, and non-mycorrhizal plants depended on the solution Al concentrations (Table 3.1). Release of citrate from non-mycorrhizal roots was extremely stimulated by increased Al concentrations (Figure 3.3). For both mycorrhizal plants, low Al concentrations enhanced exudation of citrate, but 1000 μM Al did not stimulate excretion of citrate, even reduced its production in HM mycorrhizal plants (Figure 3.3). Lactate exudation from non-mycorrhizal roots was stimulated by 200 and 400 μM Al concentrations, but 1000 μM Al remarkably reduced lactate production in non-mycorrhizal plants (Figure 3.3). Al exposure had no significant effect on lactate exudation in both LM and HM mycorrhizal plants except at 1000 μM Al, where LM mycorrhizal plants exuded a large amount of lactate. Mycorrhizal fungal colonization had no significant effect on formate and acetate production (Table 3.1).

When all organic acids released from root systems were taken into consideration together, exudation of organic acids from root systems of either mycorrhizal or non-mycorrhizal plants was significantly reduced by Al exposure (Figure 3.4A). However, mycorrhizal plants exuded more organic acids than non-mycorrhizal plants, especially HM mycorrhizal plants released significantly a greater amount of organic acids than non-mycorrhizal plants (Figure 3.4A). Production of organic acids based on root mass reflects capability of plants to exude organic acids in response to Al treatments. LM mycorrhizal, HM mycorrhizal, and non-mycorrhizal plants differed in production of total organic acids as Al concentrations changed (Figure 3.4B). Both LM and HM mycorrhizal plants produced significantly higher organic acids than non-mycorrhizal plants when no Al was applied. At 1000 μM Al, HM mycorrhizal plants released less organic acids per gram of root mass than LM mycorrhizal and non-mycorrhizal plants. Total concentration of organic acids in rhizosphere indirectly reflects Al accumulation and availability in rhizosphere. Mycorrhizal plants had higher concentrations of organic acids in rhizosphere than non-mycorrhizal plants at 200 μM Al or when no Al was applied (Figure 3.4C). At 1000 μM Al, organic acid concentration in rhizosphere of HM mycorrhizal plants was higher than that of LM mycorrhizal and non-mycorrhizal plants.

Table 3.2. *P* values from ANCOVA for the shoot and root nutrient concentrations of LM mycorrhizal, HM mycorrhizal, and non-mycorrhizal broomsedge (*A. virginicus*) plants.

	Source	P	K	Ca	Mg	Fe	Mn	Cu	Zn	Na
Shoot	Myc	0.014	ns	ns	ns	ns	0.020	0.001	0.031	ns
	Al	< 0.001	ns	< 0.001	< 0.001	ns	ns	ns	ns	0.050
	Myc*Al	< 0.001	0.004	ns	0.004	0.025	ns	ns	ns	ns
Root	Myc	ns	< 0.001	ns	ns	ns	< 0.001	< 0.001	< 0.001	ns
	Al	< 0.001	Ns	ns	< 0.001	< 0.001	ns	Ns	ns	0.004
	Myc*Al	ns	< 0.001	ns	ns	< 0.001	ns	ns	ns	ns

Figure 3.5. Influence of Al treatment on shoot and root nutrient concentrations of LM mycorrhizal (**solid circle**), HM mycorrhizal (**solid triangle**), and non-mycorrhizal (**open circle**) broomsedge (*A. virginicus*) plants.

Slopes and *P* values from the regression:

A. Shoot P concentrations

LM mycorrhizal: slope = -0.00094 and *P* value = 0.001;

HM mycorrhizal: slope = 0.00029 and *P* value: ns;

Non-mycorrhizal: slope = -0.00136 and *P* value < 0.001.

B. Shoot K concentrations

LM mycorrhizal: slope = 0.00181 and *P* value: ns;

HM mycorrhizal: slope = 0.00188 and *P* value: ns;

Non-mycorrhizal: slope = -0.00873 and *P* value = 0.011.

C. Root K concentrations

LM mycorrhizal: slope = 0.00708 and *P* value = 0.008;

HM mycorrhizal: slope = 0.02561 (linear) and -0.00002 (quadratic), and *P* value = 0.003 (linear) and 0.025 (quadratic);

Non-mycorrhizal: slope = -0.01368 and *P* value = 0.007.

D. Shoot Mg concentrations

LM mycorrhizal: -0.000091 and *P* value: ns;

HM mycorrhizal: slope = -0.00520 (linear) and 0.000004 (quadratic), and *P* value = 0.041 (linear) and 0.083 (quadratic);

Non-mycorrhizal: slope = -0.00364 and *P* value < 0.001.

E. Shoot Fe concentrations

LM mycorrhizal: slope = 0.11763 and *P* value: ns;

HM mycorrhizal: slope = -2.11247 (linear) and 0.00182 (quadratic), and *P* value = 0.003 (linear) and 0.005 (quadratic);

Non-mycorrhizal: slope = 0.56728 and *P* value = 0.003.

F. Root Fe concentrations

LM mycorrhizal: slope = -2.48287 (linear) and 0.00277 (quadratic), *P* values = ns (linear) and 0.039 (quadratic);

HM mycorrhizal: N/A;

Non-mycorrhizal: slope = 3.47617 and *P* value = 0.002.

Different patterns of growth, shoot and root Al concentrations, and excreted organic acids indicated that mycorrhizal fungal colonization may have altered other nutrient composition of shoots and roots. Colonization of both AM fungal isolates significantly reduced shoot and root Mn and Zn concentrations across all Al levels, but enhanced shoot and root Cu concentrations under Al exposure (Table 3.2). Shoot P, K, Mg, and Fe concentrations and root K and Fe concentrations of LM mycorrhizal, HM mycorrhizal, and non-mycorrhizal plants depended on the solution Al concentrations (Table 3.2). Shoot P concentrations of non-mycorrhizal plants were extremely disrupted by Al presence (Figure 3.5). Both mycorrhizal plants under Al exposure had much higher shoot P concentrations relative to that of non-mycorrhizal plants except at 1000 μM Al, where shoot P concentration of LM mycorrhizal plants was greatly reduced (Figure 3.5).

Shoot K and Mg concentrations and root K concentrations of non-mycorrhizal plants were significantly reduced by 400 and 1000 μM Al concentrations, where, however, both LM and HM mycorrhizal plants still remained high concentrations of these nutrients (Figure 3.5). Non-mycorrhizal plants increased shoot and root Fe concentrations as the solution Al concentrations increased, but LM and HM mycorrhizal plants in shoot Fe concentrations divergently responded to increased Al concentrations (Figure 3.5). Root Fe concentrations of both mycorrhizal plants were low and relatively stable across the Al levels (Figure 3.5).

Discussion

In the previous Al study (Chapter II), multiple AM fungi functioning together, provided a great benefit to broomsedge plants, and protected host plants from Al toxicity through reduction of Al influx and translocation and amelioration of Al effects on nutrient acquisition and translocation. These results suggested that AM fungi were important to this plant species establishing in acidic soils with elevated Al. However, different fungal species, even different strains of the same fungal species, differentially enhance growth of host plants (Medeiros et al. 1994; Clark 1997). The present study shows that two fungal isolates differentially benefited broomsedge plants under severe Al toxicity, but there was no significant difference in this aspect between these two fungal isolates when they were exposed to Al at 400 μM and below (Figure 3.1). LM plant growth was extremely reduced by 1000 μM Al treatment as were non-mycorrhizal plants (Figure 3.1). This pattern might imply that the symbiotic LM plants are only

adapted to mild Al toxicity present in their original habitats (about 360 mg Al per kg of soil), and very high Al would be toxic to LM plants as well as non-mycorrhizal plants. Superiority of HM fungus to host plants exposed to 1000 μ M Al may be associated with its capability to mining scarce phosphorus, as evidenced that P concentrations of HM mycorrhizal plants were not significantly affected by Al (Figure 3.5). This role of HM fungus may be originated from its original habitats where phosphorus limitation is the primary factor for plants to establish. These results indicate that local adaptation of AM fungi to edaphic stress is important to host plants to establish in such habitats.

Reduction of Al influx into shoot and root by AM fungi may be one of the important mechanisms in Al resistance of higher plants (Medeiros et al. 1994; Mendoza and Borie 1998; Chapter II). The present study revealed that both LM and HM fungi significantly reduced Al influx into host plants (Figure 3.2). But different fungal isolates took different ways to limit Al influx. LM fungus limited Al influx into both shoots and roots of host plants, whereas HM fungus primarily reduced Al translocation into shoots of broomsedge plants (Figure 3.2). This observation is contrary to the previous report on *Panicum virgatum* (Koslowsky and Boerner 1989).

In response to deficient Pi and excess Al, plant roots release organic acids (Curl and Truelove 1986; Kochian 1995; Strom 1997). The roles of these organic acids in overcoming Pi limitation and Al toxicity have been experimentally demonstrated (Miyasaka et al. 1991; Delhaize et al. 1993; Jones and Darrah 1994; Delhaize and Ryan 1995; Ma et al. 1997; Zhang et al. 1997). Organic acids chelate metals, reducing their precipitation reaction with Pi and reducing the activity of toxic free metal ions in the rhizosphere. The present study showed that release of citrate by non-mycorrhizal root systems was stimulated by Al exposure, and non-mycorrhizal plants exuded more citrate as the solution Al concentrations increased (Table 3.1, Figure 3.3). This pattern of citrate responses in non-mycorrhizal broomsedge plants to increased Al concentration is consistent with reports on Al-resistant plants (Miyasaka et al. 1991; Delhaize et al. 1993; Ryan et al. 1995; Pellet et al. 1996; Ma et al. 1997), which reflects the general response of Al-tolerant plants to increasing Al.

In both LM and HM mycorrhizal plants, however, there were no such responsive patterns of organic acids to increasing Al concentrations that was observed in non-mycorrhizal plants (Table 3.1, Figure 3.3 and 3.4), which suggests a difference in root characters between mycorrhizal and non-mycorrhizal plants (Berta et al. 1993; Miller et al. 1997) and mycorrhizal

fungus colonization may pose carbon constraints on host plants under stressful conditions (Po and Cumming 1997; Smith and Read 1997). Because mycorrhizal roots were bigger than non-mycorrhizal roots (Figure 3.1), mycorrhizal plants exuded a greater amount of organic acids than non-mycorrhizal plants (Figure 3.4A). However, chelation of Al in rhizosphere and releasing phosphorus from bound complexes are not determined by the production of organic acids from the root systems, but by concentrations of organic acids presented in rhizospheres. Mycorrhizal plants had higher concentrations of organic acids in rhizosphere than non-mycorrhizal plants at 200 μM Al or no Al applied. Especially HM mycorrhizal plants had higher organic acids in rhizosphere than non-mycorrhizal plants (Figure 3.4C). So that more Al was chelated and more P was released from bound complexes by organic acids in the rhizosphere of mycorrhizal plants under these conditions than that of non-mycorrhizal plants, which undoubtedly contributes Al resistance observed in mycorrhizal plants (Figure 3.1). Furthermore, mycorrhizal plants reduced Al influx into shoots and roots (Figure 3.2), and balanced accumulation of nutrients (Table 3.2, Figure 3.5), which all would contribute to Al resistance in host plants. However, different mycorrhizal plants were not completely the same in amelioration of Al detrimental effects on host plants. The difference in plant mass between LM and HM mycorrhizal plants may be at least partially related to the difference in the concentrations of organic acids in their rhizosphere, particularly at 1000 μM Al (Figure 3.1 and 3.4C). LM and HM mycorrhizal plants considerably differed in the extents of limiting Al influx into shoots and roots (Figure 3.2), and improvement of P nutrition (Figure 3.5), which also contribute to the difference in Al resistance between LM and HM mycorrhizal plants.

Differences in nutrient concentrations among different mycorrhizal and non-mycorrhizal plants exposed to Al can be related to colonization of different mycorrhizal fungal strains, Al influence, and exudation of organic acids (Rengel and Robinson 1989; Nichol et al. 1993; Jones and Darrah 1994; Delhaize and Ryan 1995; Marschner 1995; Smith and Read 1997; Strom 1997). Mycorrhizal fungal colonization reduced Al concentrations (Figure 3.2), and altered profiles and concentrations of organic acids in rhizosphere (Table 3.1, Figure 3.3 and 3.4), which undoubtedly affects plant nutrition. Nutrition of non-mycorrhizal broomsedge plants was disrupted by Al (Figure 3.5). Phosphorus was the most sensitive to Al exposure (Figure 3.5). At 200 μM Al, shoot P concentration of non-mycorrhizal plants was reduced 10 times compared to the control (Figure 3.5). Moreover, Al reduced shoot K and Mg and root K concentrations, but increased shoot and root Fe concentrations of non-mycorrhizal plants (Figure 3.5). Calcium

concentrations of all plants, no matter whether they were mycorrhizal or non-mycorrhizal, were significantly reduced by Al, which reflects the general detrimental effect of Al on Ca uptake by plants (Huang et al. 1992), and AM fungi have no effect on Ca uptake (Smith and Read 1997). Results from previous experiments (Koslowsky and Boerner 1989, Medeiros et al. 1994) demonstrated that different fungal isolates differentially affect nutrient concentrations of shoots and roots of host plants. The present study similarly revealed differential influences on nutrient concentrations by different AM fungal isolates, especially on shoot P, Fe, and Mn concentrations (Table 3.2, Figure 3.5), which suggests differential roles of different AM fungi in plant nutrition (Medeiros et al. 1994; Clark 1997; Smith and Read 1997).

Release of organic acids by root systems may affect mineral nutrient availability to and acquisition by plants (Jones and Darrah 1994; Strom 1997). Mycorrhizal and non-mycorrhizal plants significantly differed in tissue Mn, Cu, and Zn, independent of Al concentrations in the root zones (Table 3.2). In addition, the differences in tissue P, K, Mg, and Fe between mycorrhizal and non-mycorrhizal plants changed as Al concentrations in the root zones changed (Table 3.2, Figure 3.5). These differences in tissue nutrients between mycorrhizal and non-mycorrhizal plants exposed to varying Al concentrations may be associated with the differences in the rhizosphere organic acids (Figure 3.4). Organic acids have a high affinity with Al so that nutrients could be freed from Al-bound complexes by organic acids (Taylor 1988; Miyasaka et al. 1991; Jones and Darrah 1994; Strom 1997). The differences in tissue nutrients between mycorrhizal and non-mycorrhizal plants brought about by organic acids might be related to anion channels, co-transport, and/or anti-transport processes suggested in other studies (Schwenke and Wagner 1992; Ryan et al. 1997; Strom 1997; Parker and Pedler 1998). Additionally, the higher shoot P concentration of HM mycorrhizal than LM mycorrhizal plants exposed to 1000 μM Al (Figure 3.5) may be ascribed to the higher concentration of organic acids in their rhizosphere than that of LM mycorrhizal plants (Figure 3.4).

Mycorrhizal fungal colonization not only quantitatively but also qualitatively altered profiles and concentrations of organic acids exuded from root systems (Table 3.1, Figure 3.3 and 3.5), which implies that the physiology of host broomsedge plants was altered by colonization of either AM fungal isolate, as has been previously reported (McArthur and Knowles 1992, 1993; Benabdellah et al. 2000), but different fungal isolates differentially affected the profiles and concentrations. The differences in the exudation of organic acids observed among LM

mycorrhizal, HM mycorrhizal, and non-mycorrhizal plants in the present study might be associated with the changes in physiology.

From this study, I would have expected that broomsedge plants colonized by the LM isolate of *Glomus clarum* would have been higher Al resistance than the plants colonized by the HM isolate under Al exposure. In contrast, both isolates were equally effective at fostering growth of host plant exposed to up to 400 μM Al, and also root colonization by these fungi was not affected by Al exposure. One reason for this lack of difference in Al resistance between isolates from divergent habitats may be the cultural history of these isolates. Both isolates were obtained from the INVAM collection at West Virginia University (Morton et al. 1993), in which isolates were maintained in a common soil (pH 6.2) for numerous generations (5 generations for HM and one generation for LM isolate). Due to the multinucleate nature of AM fungi, selection pressure in the INVAM collection may be different from those present in the fields, consequently leading to differential roles from the ones in the fields and exhibiting similar responses under common conditions (Morton 1990; Stutz and Morton 1996).

The present study demonstrated that LM mycorrhizal, HM mycorrhizal, and non-mycorrhizal broomsedge plants differentially responded in tissue Al concentrations, tissue nutrient concentrations, and profiles and concentrations of organic acids exuded from root systems to the solution Al concentrations, consequently resulting in differential patterns of growth in response to Al treatments. Mycorrhizal fungal colonization increased the amount of organic acids released into rhizosphere by root systems, pronouncedly reduced Al influx into roots and translocation into shoots, which undoubtedly reduces adverse effects of Al on host plants, and facilitated P uptake by host plants. But LM and HM mycorrhizal plants differed in the amount and concentrations of organic acids in the rhizosphere, the extents of limiting Al into shoots and roots, and tissue P concentrations. Citrate exuded from non-mycorrhizal root systems increased as Al concentrations increased, reflecting a general response of Al-tolerant plants to increasing Al concentrations in root zones. In both LM and HM mycorrhizal plants, no such pattern was observed, but there were a greater amount and concentrations of organic acids in their rhizosphere, which would contribute to Al resistance in mycorrhizal broomsedge plants. Mycorrhizal fungal colonization ameliorated detrimental effects of Al on nutrition of host plants. The difference in Al resistance between LM and HM mycorrhizal plants under severe Al toxicity (1000 μM) may be ascribed to the difference in the concentrations of organic acids in the rhizosphere and the improvement of P status of host plants. The differences in reduction of Al

influx into shoots and roots and improvement of P nutrition of host plants between LM and HM fungal isolates may be originated from their original habitats, respectively. The changes in profiles and amount of organic acids released from root systems of host plants brought about by different AM fungal isolates suggest changes in the physiology of broomsedge plants as a result of colonization by AM fungi, which might be associated with other alterations in host plants, such as activation of anion channels, co-transport, and/or anti-transport processes between cations and anions. Therefore, different AM fungal isolates differentially ameliorate adverse influences of Al on host plants through different ways, which all enhances growth of host plants under Al toxicity and other environmental stresses.

CHAPTER IV

Phosphorus and Aluminum Interactive Effects on Different Mycorrhizal and Non-Mycorrhizal Broomsedge Plants

Introduction

The mycorrhizal symbioses between arbuscular mycorrhizal (AM) fungi and higher plants have existed for millions of years and the benefits of this mutualism to host plants have long been recognized (Mosse 1973; Newsham et al. 1995; Allen 1996; Smith and Read 1997). One of the most documented benefits brought about by AM fungi to the host plant is the facilitation of phosphorus (Pi) uptake (Mosse 1973; Hayman 1983; Bolan 1991; Smith et al. 1994). The increased acquisition of Pi is closely related to extensive fungal hyphae exploration of a larger volume of soil and mining scarce resources that are otherwise unavailable for roots (Smith and Read 1997).

The benefits provided by AM fungi to host plants may also be associated with the changes in plant physiology. For example, mycorrhizal fungal colonization alters the distribution of H^+ -ATPase in the plasma membrane of the host root cells (Gianinazzi-Pearson et al. 1991). The increased activity of membrane H^+ -ATPase in root cells might be related to Pi transfer across the fungus-plant interface (Smith and Read 1997). In addition, the alteration of physiological parameters in host plants may play roles in plants overcoming environmental stress (Allen et al. 1981; Hayman 1983; McArthur and Knowles 1993). Furthermore, mycorrhizal fungal colonization induces morphological modifications in the root system of the host plants (Berta et al. 1993). It is therefore unlikely that a root system, which is densely colonized by mycorrhizal fungi, will not be changed physiologically and metabolically.

There are reports that physiological traits of plants are changed upon mycorrhizal fungal colonization. For example, mycorrhizal fungal colonization alters host plant phytohormones (Allen et al. 1980; McArthur and Knowles 1992), phosphatases (Dighton 1983; McArthur and Knowles 1993; Fries et al. 1998), ATPase and other proteins (Gianinazzi-Pearson et al. 1991; McArthur and Knowles 1992, 1993, Bago et al. 1997; Boucher et al. 1999; Benabdellah et al.

1999, 2000), metabolic pathways (Clapperton and Reid 1992; McArthur and Knowles 1992; Harrison and Dixon 1994), gene transcription and expression (Harrison 1999), hydraulic conductance (Allen et al. 1981; Smith and Read 1997), photosynthesis and respiration (Allen et al. 1981; Eissenstat et al. 1993; Peng et al. 1993; Aguilera-Gomez et al. 1998; Boucher et al. 1999), kinetics of nutrient absorption (Cress et al. 1979; Kuhn et al. 2000; Chapter I), and root exudates (Pinior et al. 1999; Chapter III). Allen et al. (1981) found that AM fungal colonization significantly increased photosynthetic rate as a consequence of increased leaf chlorophyll and stomatal conductance. Phosphatase and/or other proteins of mycorrhizal roots may be higher (McArthur and Knowles 1992; Fries et al. 1998; Benabdellah et al. 1999) or lower (Dighton 1983; Cumming 1996) than their non-mycorrhizal counterparts, depending on plant species, fungal species, and environmental conditions. Boucher et al. (1999) demonstrated that different arbuscular mycorrhizal (AM) fungi had differential influences on leaf chlorophyll, proteins, and sugars.

These reports indicate that changes in plant physiology brought about by mycorrhizal fungal colonization are not casual phenomena. However, it is not clear how physiological responses of plants to mycorrhizal fungal colonization change under various edaphic conditions.

Broomsedge (*Andropogon virginicus*) is a mycotrophic species dominating many poor soil habitats and it relies on AM fungi to overcome detrimental edaphic conditions (Chapters I, II and III). Differences in exudation profiles of organic acids between mycorrhizal and non-mycorrhizal plants suggest that the physiology of broomsedge plants is altered by colonization of AM fungi (Chapter III). Previous experiments demonstrated that acid phosphatase, chlorophyll, and photosynthesis of broomsedge plants from a mine site were higher than those from an old field, but there was no difference in transpiration between them (Chapman and Jones 1975; Nellessen and Ungar 1993). These patterns indicate that this plant species may adjust its physiology in response to the adverse edaphic stresses present in the mine soil. However, it is not clear whether the plants in the above experiments were already colonized by mycorrhizal fungi. If these plants were already colonized by AM fungi, are these differences due to plants themselves or interactions between plants and fungi?

Mycorrhizal symbiosis considerably reduced Al influx into host plants and translocation within host plants, and facilitated Pi uptake by host plants (Chapter I, II, and III). These changes might be associated with alterations in physiology of host plants brought about by mycorrhizal symbiosis. Different AM fungi differentially affect Al influx, Pi uptake, and exudation of

organic acids in host broomsedge plants (Chapter III). So, differences in physiology between these different mycorrhizal plants would be also expected. In the present study, I examined interactive effects of Pi, Al, and AM symbioses on biomass, foliar nitrogen, leaf chlorophyll, photosynthesis, leaf respiration, root acid phosphatase, root proteins, and tissue nutrient concentrations of broomsedge (*Andropogon virginicus* L.) plants. Two fungal isolates of the same species, *Glomus clarum* Nicolson and Schenck, were used. They originated from abandoned coal mine soils differing in pH. I hypothesized that there would be differences in physiology and growth among different mycorrhizal and non-mycorrhizal plants under different Pi and Al levels because different mycorrhizal and non-mycorrhizal plants differed in Pi-by-Al interactions as Pi and Al concentrations change as observed in previous studies.

Materials and Methods

Preparation of AM Fungal Inocula

AM fungal inocula were used in this study, separately originated from two abandoned coal mining sites in West Virginia. One site is in Mingo County, having soil pH of 6.6 and containing NaHCO_3 -extractable Pi of 19 mg per kg of soil. At this pH, Al is not toxic to plants. Soil from the other site in Monongalia County has a pH from 3.0 to 3.3, containing Melich-extractable Pi of 3.76 mg per kg of soil and Al of 363.12 mg per kg of soil.

To produce the inoculum of *Glomus clarum*, inocula (WV233-1 and WV219A-5) from the INVAM collection at West Virginia University, which originated separately from the Monongalia site and the Mingo site, were used to inoculate setrilely germinated broomsedge plants, respectively. These inoculated broomsedge plants were planted into 15 cm diameter pots containing a mixture of autoclaved mine-soil from the Monongalia site and sand (1:3 v/v). After two months, the pot contents became the sources of infective LM and HM inoculum for the experiment, respectively, where LM and HM separately represent mycorrhizal fungi from low pH site at Monongalia abandoned coal mine and high pH site at Mingo abandoned coal mine.

Preparation of Plants

Broomsedge seeds were sown around the transplants in nursery pots (15cm diameter). Broomsedge seeds also were sown in pots containing sterile-germinated broomsedge and the

mixture of autoclaved mine-soil and sand (1:3 v/v). Seedlings from these pots served as a non-mycorrhizal control. After four weeks growth, roots of a small subset of seedlings (*ca.* 10 from each pot) were examined to determine mycorrhizal status. Tissue P status was analyzed after wet digestion (Parkinson and Allen 1975) by the molybdate blue method (Olsen and Sommers 1982). Tissue phosphorus concentrations and tissue dry weights between mycorrhizal and non-mycorrhizal plants did not differ significantly at the time of transplanting.

Nutrient Solution and Growth Conditions

Mycorrhizal and non-mycorrhizal broomsedge seedlings were transplanted and grown in D16 Deepots (5 cm diameter x 18 cm height) (Stuewe and Sons, Inc., Corvallis, Oregon, USA) pre-filled with 220 cm³ of a 3:1 (v/v) mixture of coarse:fine acid-washed sand. Deepots were placed into a growth chamber with 14 hours of light at 28°C, 60% RH, and 10 hours of darkness at 21°C, 50% RH. Average light intensity at pot height in the chamber was 260 $\mu\text{mol m}^{-2} \text{s}^{-1}$ from mixed fluorescent and incandescent sources. Plants received a baseline nutrient solution containing Ca (0.675 mM), K (0.79 mM), Mg (0.25 mM), NO₃ (1.5 mM), NH₄ (0.5 mM), SO₄ (0.25 mM), B (23.14 μM), Fe (25 μM), Mn (4.57 μM), Zn (0.38 μM), Cu (0.16 μM), and Mo (0.06 μM). All solutions were adjusted to pH 4.0 before application. Solutions (approximately 15 ml) were automatically delivered to the plants three times each day for eight weeks.

Measurements

Photosynthesis, stomatal conductance, and respiration of leaves were determined on day 50 with a LI-6200 Photosynthesis System (LICOR Inc., Lincoln, NE, USA). Photosynthesis and stomatal conductance were measured on the third fully developed leaf of each plant from 10 to 11 a.m. under the daytime growth conditions, and measurement of respiration was conducted on the same leaf of each plant from 9 to 10 p.m. in the dark. The leaf dimensions within the cuvette were recorded.

Determination of chlorophyll followed a modified method of Porra et al. (1989). On day 51, approximately 5 cm of the same leaves for the measurement of photosynthesis and respiration above were exercised, weighed, and cut into 2 mm long sections. These pieces of leaves were instantly transferred into test tubes on ice. Two-ml of methanol was added to each tube. The tubes were sealed with parafilm and placed in a refrigerator overnight. Chlorophyll

was determined by measuring absorbance at 652 and 665.2 nm with a spectrometer. Chlorophyll concentrations were calculated using the equations of Porra et al. (1989):

$$\text{Chlorophyll (a+b) } (\mu\text{g ml}^{-1}) = 22.12 A^{652} + 2.71 A^{665.2}.$$

On day 55, four plants from each treatment were harvested, roots were washed in running de-ionized water. Acid phosphatase was determined as follows. Root tips (5 to 8 cm) were cut off, washed in de-ionized water, chopped into 1 cm pieces, and transferred to centrifuge tubes containing 4.5 ml of the appropriate treatment nutrient solution. Then, 0.5 ml of 1 mM nitrophenylphosphate (NPP) was added to each tube and tubes were incubated for 1 hour 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 at 410 nm (Tabatabai and Bremner 1969).

The remaining roots of each plant above were ground with a mortar and pestle in 20 ml ice-cold buffer (5% (w/v) polyvinylpyrrolidone (PVP) in 0.1 M, pH 7.9 Tris-HCl) on ice, and centrifuged at 12,000 g for 20 minutes at 4°C. The supernatant was kept on ice for a protein assay. Proteins were determined following the Bradford (1976) method using SIGMA Protein Assay Kit (Product # P 5656, Sigma Chemical Co. Ltd., St. Louis, MO, USA).

The aboveground tissues of the plants above were dried at 60°C for 48 hours and ground. Approximately 4.8 µg aliquot of the sample was loaded to a nitrogen analyzer (model NA 1500 Series 2, Henry Schein, Inc, NY, USA) to obtain nitrogen and carbon concentrations.

On day 56, the experiment was terminated, sand was removed from the roots of remaining plants under running de-ionized water, and shoots were rinsed in de-ionized water. Root and shoot dry weights were determined after they dried at 60°C for at least 24 hours. After wet digestion (Parkinson and Allen 1975), phosphorus concentrations of the digest solutions were determined spectrophotometrically (Taussky and Shoor 1953), and other mineral elements of the digests were analyzed using an inductively coupled plasma (ICP) emission spectrophotometer (Leeman PS 950) by the National Research Center for Coal and Energy Analytical Laboratory at West Virginia University.

Experimental Design and Data Analysis

Mycorrhizal treatments were LM, HM, and non-mycorrhizal, Pi treatments were 40 μM and 100 μM NaH_2PO_4 , and Al treatments were 0 and 200 μM AlCl_3 . Simulation analysis of these solutions by the program GEOCHEM (Parker et al. 1993) indicated that Al^{3+} concentrations were 0, 0, 133.9, and 112.4 μM in the solutions of 40 μM Pi, 100 μM Pi, 40 μM Pi + 200 μM Al, and 100 μM Pi + 200 μM Al, respectively. Within a growth chamber, treatments were randomly arranged in a 2 x 2 x 3 factorial (Pi-by-Al-by-mycorrhizal treatments) design. Each treatment had 10 replicates. Data were analyzed by analysis of variance using the statistical package JMP (SAS Institute, Cary, North Carolina, USA).

Results

Effects of mycorrhizal fungal colonization on shoot and root mass depended on Pi and Al concentrations (Figure 4.1). Tissue biomass of non-mycorrhizal plants was enhanced by high (100 μM) Pi relative to low (40 μM) Pi without regard to Al exposure, but reduced by Al exposure without regard to Pi concentrations, reflecting the general beneficial effect of easily available Pi and toxicity of elevated Al to plants. Both LM and HM isolates increased shoot and root mass of host plants at 40 μM Pi or exposed to Al without regard to Pi concentrations, indicating that AM fungi benefit host plants under adverse conditions. At 100 μM Pi and no Al presence, both mycorrhizal plants were smaller than non-mycorrhizal plants, exhibiting mild parasitic effects on host plants by mycorrhizal symbioses. HM isolate was more effective in enhancing host plant growth than LM isolate under all conditions except at 100 μM Pi and no Al presence, where there was no significant difference in tissue biomass between LM and HM mycorrhizal plants (Figure 4.1).

Leaf chlorophyll concentrations were significantly affected by both Pi ($P < 0.001$) and Al ($P = 0.003$), and foliar N concentrations were significantly affected only by Al ($P = 0.011$), but there was no significant interaction on these two traits between any of the main effects.

Figure 4.1. Influence of Pi and Al treatments on shoot (**A**) and root (**B**) mass of LM mycorrhizal (**solid bar**), HM mycorrhizal (**hatched bar**), and non-mycorrhizal (**open bar**) broomsedge (*A. virginicus*) plants.

P values from ANOVA:

Source	df	A. Shoot Mass	B. Root Mass
Myc	2	< 0.001	< 0.001
Pi	1	< 0.001	< 0.001
Al	1	< 0.001	< 0.001
Myc * Pi	2	0.006	< 0.001
Myc * Al	2	< 0.001	< 0.001
Pi * Al	1	0.002	0.006
Myc * Pi * Al	2	0.003	0.002

Stomatal conductance depended on Pi and Al concentration in root zones and mycorrhizal fungal isolates (Table 4.1). Stomatal conductance of non-mycorrhizal plants was reduced by high Pi when no Al was present, but enhanced by Al treatment when high Pi was applied at the same time (Table 4.2). LM isolate decreased stomatal conductance of host plants at 100 μM when no Al was present, but increased stomatal conductance at 100 μM Pi plus 200 μM Al, and had no effect on it under other conditions. HM isolate decreased stomatal conductance at 40 μM Pi without regard to Al exposure, but increased stomatal conductance at 100 μM Pi when Al was present, and had no effect on it at 100 μM Pi and no Al (Table 4.2).

Leaf photosynthetic rate of mycorrhizal and non-mycorrhizal plants depended on Pi concentrations (Table 4.1). Photosynthetic rate of non-mycorrhizal plants was enhanced by Al treatment (Table 4.2). LH isolate had no significant effect on photosynthetic rate except under conditions of 40 μM Pi plus 200 μM Al, where photosynthetic rate of LM mycorrhizal plants was significantly higher than that of non-mycorrhizal plants. HM isolate increased photosynthetic rate of host plants only at 100 μM Pi and no Al presence, and under other conditions, did not significantly affect this trait of host plants.

Leaf respiratory rate of mycorrhizal and non-mycorrhizal plants depended on Al concentrations (Table 4.1). Leaf respiratory rate of non-mycorrhizal plants was enhanced by Al exposure and also by high Pi application when Al was present (Table 4.2). LM isolate increased leaf respiration of host plants at 40 μM Pi and no Al presence, but reduced it under Al exposure without regard to Pi concentrations. HM isolate also reduced leaf respiration of host plants under Al exposure without regard to Pi concentrations. Under conditions of 40 μM Pi and no Al presence or 100 μM Pi plus 200 μM Al, respiration of LM mycorrhizal plants was significantly higher than that of HM mycorrhizal plants.

Root protein of mycorrhizal and non-mycorrhizal plants depended on Pi concentrations (Table 4.1). Root total soluble protein concentrations of non-mycorrhizal plants were reduced by high Pi when no Al was present, but enhanced by Al exposure under a condition of 100 μM Pi (Figure 4.2), which may be a reflection of the repressible Pi transport system and defense response to Al operated in higher plants, respectively. LM isolate significantly reduced the root protein of host plants without regard to Al exposure, but had no significant effect on it at 40 μM Pi. HM isolate significantly increased the root protein of host plants at 40 μM Pi when no Al was present, but reduced the root protein at 100 μM Pi plus 200 μM Al, and had no pronounced

Table 4.1. Probabilities from ANOVA for stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$), photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$), leaf respiratory rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$), root total soluble protein ($\mu\text{g g}^{-1}$), and root acid phosphatase (APase) ($\mu\text{mol g}^{-1} \text{h}^{-1}$) of LM mycorrhizal, HM mycorrhizal, and non-mycorrhizal broomsedge (*A. virginicus*) plants.

Source	Stomatal Conductance	Photosynthetic Rate	Respiratory Rate	Root Protein	APase
Myc	< 0.001	0.0199	ns	ns	ns
Pi	0.001	ns*	< 0.001	0.019	< 0.001
Al	< 0.001	< 0.001	< 0.001	< 0.001	ns
Myc * Pi	< 0.001	0.002	ns	0.016	ns
Myc * Al	< 0.001	ns	0.018	ns	0.021
Pi * Al	< 0.001	0.017	0.001	ns	ns
Myc * Pi * Al	< 0.001	ns	ns	ns	ns

* $\alpha = 0.05$, the same hereafter.

Table 4.2. Stomatal conductance ($\mu\text{mol m}^{-2} \text{s}^{-1}$), photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$), and leaf respiratory rates ($\mu\text{mol m}^{-2} \text{s}^{-1}$) in LM mycorrhizal, HM mycorrhizal, and non-mycorrhizal broomsedge (*A. virginicus*) plants as influenced by Al exposure or Pi concentrations.

	Stomatal Conductance	Photosynthetic Rate	Respiratory Rate
0 μM Al, 40 μM Pi			
LM	0.241	1.953	0.475
HM	0.217	1.613	0.350
NM	0.247	1.822	0.300
0 μM Al, 100 μM Pi			
LM	0.219	2.159	0.328
HM	0.193	2.546	0.362
NM	0.189	1.854	0.313
200 μM Al, 40 μM Pi			
LM	0.253	3.452	0.332
HM	0.236	2.059	0.268
NM	0.254	2.339	0.488
200 μM Al, 100 μM Pi			
LM	0.384	2.411	0.750
HM	0.303	2.469	0.628
NM	0.254	2.358	0.889
LSD[†]	0.014	0.380	0.112

[†] Tukey's Least significant difference at $\alpha = 0.05$ with $n = 12$.

Figure 4.2. Influence of Pi and Al treatments on root total soluble protein concentration (**A**) and root acid phosphatase activity (**B**) of LM mycorrhizal (**solid bar**), HM mycorrhizal (**hatched bar**), and non-mycorrhizal (**open bar**) broomsedge (*A. virginicus*) plants.

effect on this parameter under other two conditions examined in this experiment (Figure 4.2).

Root acid phosphatase (APase) of mycorrhizal and non-mycorrhizal plants depended on Al concentrations (Table 4.1). Root APase activity of non-mycorrhizal plants was reduced by high Pi without regard to Al exposure, and insensitive to Al exposure (Figure 4.2). LM isolate had no significant effect on the APase of host plants when no Al was present, but decreased it under Al exposure. HM isolate had no significant effect on the APase of host plants under any of conditions examined in this study (Figure 4.2).

Shoot and root P concentrations depended on solution Pi and Al concentrations and AM fungal presence (Table 4.3). Shoot and root P concentrations of non-mycorrhizal plants were not affected by Pi treatment when no Al was present (Figure 4.3), reflecting the general regulation of Pi homeostasis in higher plants. Shoot P concentration of non-mycorrhizal plants was reduced by Al exposure when 40 μM Pi was applied, but Al exposure increased root P concentration under the same condition, exhibiting that Al blocked P translocation within plants. At 100 μM Pi, shoot P concentration of non-mycorrhizal plants was enhanced by Al exposure, but Al exposure had no pronounced effect on root P at that Pi level, indicating that easily available Pi ameliorated detrimental effect of Al on P uptake. LM isolate reduced shoot P concentrations of host plants under conditions of 40 μM Pi or 100 μM Pi plus 200 μM Al, but enhanced shoot P concentrations of host plants at 40 μM Pi plus 200 μM Al, and had no pronounced effect on it at 100 μM Pi. HM isolate reduced shoot P concentrations of host plants under conditions of 40 μM Pi or 100 μM Pi plus 200 μM Al, but enhanced it at 100 μM Pi or 40 μM Pi plus 200 μM Al. Tissue Al concentrations depended on Pi concentration, Al concentration and mycorrhizal colonization (Table 4.3). Shoot Al concentrations of non-mycorrhizal plants were enhanced by Al exposure. Under Al exposure, low Pi treatment resulted in higher shoot Al concentrations than high Pi. LM isolate had no significant effect on shoot Al concentration of host plants when no Al was applied without regard to Pi concentration, but reduced shoot Al concentrations under Al exposure. HM isolate significantly reduced shoot Al concentrations under any condition. Non-mycorrhizal root Al concentrations were enhanced by Al exposure at 40 μM Pi, but reduced by high Pi under Al exposure. Both LM and HM isolates reduced root Al concentrations of host plants under any conditions except at 100 μM Pi plus 200 μM Al for LM isolate and 40 μM Pi without Al for HM isolate, at where LM and HM isolates had no pronounced effect root Al concentrations of host plants (Figure 4.4).

Table 4.3. *P* values from ANOVA for shoot and root Al and nutrient concentrations of LM mycorrhizal, HM mycorrhizal, and non-mycorrhizal broomsedge (*A. virginicus*) plants.

	Source	Al	P	K	Ca	Mg
Shoot	Myc	< 0.001	< 0.001	0.004	0.022	0.003
	Pi	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Al	< 0.001	< 0.001	0.031	< 0.001	0.015
	Myc*Pi	0.027	0.008	< 0.001	ns	0.030
	Myc*Al	< 0.001	ns	ns	ns	ns
	Pi*Al	ns*	< 0.001	ns	ns	ns
	Myc*Pi*Al	0.018	< 0.001	ns	0.021	< 0.001
Root	Myc	< 0.001	0.004	0.001	ns	ns
	Pi	< 0.001	< 0.001	ns	< 0.001	< 0.001
	Al	ns	< 0.001	0.002	< 0.001	< 0.001
	Myc*Pi	0.042	< 0.001	ns	ns	ns
	Myc*Al	0.007	0.003	0.027	ns	ns
	Pi*Al	0.012	ns	ns	0.046	0.026
	Myc*Pi*Al	< 0.001	< 0.001	ns	0.013	0.028

Figure 4.3. Influence of Pi and Al treatments on shoot (**A**) and root (**B**) P concentrations of LM mycorrhizal (**solid bar**), HM mycorrhizal (**hatched bar**), and non-mycorrhizal (**open bar**) broomsedge (*A. virginicus*) plants.

Figure 4.4. Influence of Pi and Al treatments on shoot (**A**) and root (**B**) Al concentrations of LM mycorrhizal (**solid bar**), HM mycorrhizal (**hatched bar**), and non-mycorrhizal (**open bar**) broomsedge (*A. virginicus*) plants.

Differences in growth (Figure 4.1), physiology (Table 4.1 and 4.2, Figure 4.2), tissue P and Al concentrations (Figure 4.3 and 4.4) among LM mycorrhizal, HM mycorrhizal, and non-mycorrhizal plants suggest that tissue nutrient concentrations of these plants may have been altered by Pi and Al treatments. Shoot K concentrations of mycorrhizal and non-mycorrhizal plants depended on Pi concentration, but their root K concentrations changed as Al concentrations changes (Table 4.3). Shoot K concentrations of non-mycorrhizal plants were reduced by high Pi without regard to Al exposure, but enhanced by Al exposure without regard to Pi concentration (Table 4.4), which may be related to dilution effect of growth enhanced by high Pi and the general regulation of potentials across cell membranes in response to Al exposure. At 40 μM Pi without regard to Al exposure, both LM and HM isolates decreased shoot K concentrations of host plants. But HM isolate increased it at 100 μM Pi when no Al was present, and LM isolate had no effect on it under the same conditions. Root K concentrations of non-mycorrhizal plants were enhanced by high Pi under Al exposure, and also enhanced by Al exposure at 40 μM Pi, but reduced by Al exposure at 100 μM Pi. LM isolate increased root K concentrations of host plants at 40 μM Pi or 100 μM Pi plus 200 μM Al. HM isolate had no significant effect on root K except at 40 μM Pi plus 200 μM Al, where HM mycorrhizal root K concentration was lower than that of non-mycorrhizal (Table 4.4).

Tissue Ca concentrations depended on Pi, Al, and mycorrhizal treatment (Table 4.3). Shoot Ca concentrations of non-mycorrhizal plants were enhanced by high Pi without regard to Al exposure, but reduced by Al exposure without regard to Pi treatment (Table 4.4), suggesting the general response patterns of Ca nutrition to high available Pi and elevated Al in higher plants. LM and HM isolates both increased shoot Ca concentrations of host plants at 40 μM Pi or 100 μM Pi plus 200 μM Al. Under conditions of 100 μM Pi or 40 μM Pi plus 200 μM Al, these two isolates had no significant effect on the shoot Ca (Table 4.4). Root Ca concentrations of non-mycorrhizal plants were enhanced by high Pi when no Al was present, but reduced by Al exposure at 100 μM Pi (Table 4.4). LM isolate increased root Ca concentrations of host plants at 40 μM Pi or 100 μM Pi plus 200 μM Al, but HM isolate decreased root Ca concentration of host plants at 100 μM Pi when no Al was present (Table 4.4).

Shoot and root Mg concentration depended on solution Pi, Al concentrations and AM fungal presence (Table 4.3). Shoot Mg concentrations of non-mycorrhizal plants were not

Table 4.4. Influence of Pi and Al treatments on shoot and root K (mg g⁻¹), Ca (mg g⁻¹), and Mg (mg g⁻¹) concentrations of LM mycorrhizal, HM mycorrhizal, and non-mycorrhizal broomsedge (*A. virginicus*) plants.

	Shoot			Root		
	K	Ca	Mg	K	Ca	Mg
0 μM Al, 40 μM Pi						
LM	18.411	15.445	7.064	16.402	18.257	4.287
HM	17.777	16.126	6.321	11.549	15.692	4.818
NM	20.951	13.504	6.806	11.073	12.992	4.174
0 μM Al, 100 μM Pi						
LM	15.564	21.457	7.102	13.291	21.540	7.978
HM	17.339	19.200	7.945	12.166	20.559	6.045
NM	15.443	21.095	6.691	12.289	24.281	9.268
200 μM Al, 40 μM Pi						
LM	17.188	11.614	6.545	18.046	14.172	3.486
HM	17.751	10.628	6.972	10.942	13.834	2.932
NM	22.881	9.918	5.193	19.789	13.603	3.563
200 μM Al, 100 μM Pi						
LM	17.967	16.012	6.648	16.004	20.127	6.349
HM	18.302	16.788	7.304	13.632	16.133	4.336
NM	17.794	12.030	7.008	15.230	12.964	3.383
LSD [†]	1.523	2.047	0.516	2.609	3.557	1.538

[†] Tukey's Least significant difference at 0.05 level with n = 3.

affected by Pi treatment without regard to Al exposure, but reduced by Al exposure at 40 μM Pi or enhanced by Al exposure at 100 μM Pi (Table 4.4), which exhibited that Pi treatment had no a direct effect on shoot Mg concentrations and effects of Al on shoot Mg depended on Pi concentrations. LM isolate increased shoot Mg of host plants at 40 μM Pi without regard to Al exposure. HM isolate also increased shoot Mg concentrations of host plants at 100 μM Pi without regard to Al exposure and at 40 μM Pi plus 200 μM Al as well. Root Mg concentrations of non-mycorrhizal plants was enhanced by high Pi when no Al was present, but reduced by Al exposure at 100 μM Pi (Table 4.4). LM isolate increased root Mg concentration of host plants at 100 μM Pi plus 200 μM Al, but HM isolate decreased root Mg concentration of host plants at 100 μM Pi when no Al was present (Table 4.4).

Discussion

Plants may adjust physiological activities in response to changes of environmental conditions (Marschner 1995; Lambers et al. 1998). Phosphorus effects on plant physiology are expected because P is an essential component of energy metabolism and nucleic acids and proteins. Phosphorus deficiency may induce APase activity (Caradus and Snaydon 1987; Duff et al. 1994), alter levels of ATP (Muchhal et al. 1997), and hormones (Morgan 1990), and activate the alternative respiratory pathway (Theodorou and Plaxton 1993). Aluminum impacts on plant physiology have been reported and reviewed (Roy et al. 1988; Taylor 1988a, 1988b; Miyasaka et al. 1989, 1991; Cumming and Weinstein 1990; Delhaize and Ryan 1995; Kochian 1995; Slaski et al. 1996). Elevated Al may inhibit ATPase activity (Matsumoto and Yamaya 1986; Lindberg and Griffiths 1993; Widell et al. 1994), reduce APase activity (Shaw and Read 1989), photosynthesis (Roy et al. 1988; Cumming and Weinstein 1990; Schlegel and Godbold 1991), and root respiration (Roy 1988; Cumming et al. 1992), alter levels of other proteins (Caldwell 1989; Slaski et al. 1996; Basu et al. 1997; Blancaflor et al. 1998), pigments (Roy et al. 1988; Schlegel and Godbold 1991; Ayala-Silva and Al-Hamdani 1997), membrane potentials (Miyasaka et al. 1989; Pineros and Tester 1993; Olivetti et al. 1995; Lindberg and Strid 1997), ion flux (Miyasaka et al. 1989; Huang et al. 1992; Nichol et al. 1993; Calba and Jaillard 1997; Lindberg and Strid 1997), and metabolic pathways (Schlegel and Godbold 1991; Zhang et al.

1994; Yamaguchi et al. 1999), and induce production of organic acids (Miyasaka et al. 1991; Delhaize et al. 1993; Ma et al. 1997; Larsen et al. 1998; Chapter III).

In this study, Pi treatment increased or decreased leaf chlorophyll, stomatal conductance, photosynthetic rate, respiratory rate, root protein, and root APase activity of non-mycorrhizal plants, depending on Al presence, and Al exposure changed leaf chlorophyll, stomatal conductance, photosynthetic rate, respiratory rate, root protein, and root APase activity of non-mycorrhizal plants, depending on Pi levels (Table 4.1 and 4.2, Figure 4.2). The results from This study reveal that toxic Al presence in root zones may disrupt P acquisition by a plant and translocation within a plant (Figure 4.3), interfere Ca and Mg uptake (Table 4.4), and affect stomatal behaviors, which may directly or indirectly influence photosynthesis and respiration (Table 4.2). All these consequently result in reduced plant growth (Figure 4.1), which are consistent with previous reports (Roy et al. 1988; Taylor 1988; Miyasaka et al. 1989; Cumming and Weinstein 1990; Delhaize and Ryan 1995; Kochian 1995; Slaski et al. 1996). Plants may induce protein production in response to toxic Al presence (Figure 4.2) as reported (Basu et al. 1997). Easily available Pi may reduce Al influx (Figure 4.4), improve P nutrition (Figure 4.3), support protein induction under Al exposure (Figure 4.2), and ameliorate detrimental effects of Al on Ca and Mg uptake (Table 4.4), consequently leading to enhanced growth (Figure 4.1).

Mycorrhizal symbioses facilitated Pi acquisition under limited Pi availability, but may or may not affect Pi uptake when Pi is easily available (Figure 4.3), and reduced Al influx and translocation within host plants (Figure 4.4). The differences in Pi x Al interactions between mycorrhizal and non-mycorrhizal plants on shoot and root P and Al concentrations may cause or be a result of changes in physiology of host plants under different environmental conditions (Allen et al. 1981; Hayman 1983; Haselwandter 1995; Smith and read 1997; Aguilera-Gomez et al. 1998; Baker et al. 1998; Boucher et al. 1999). The results from the present study showed that mycorrhizal and non-mycorrhizal plants significantly differed or not in stomatal conductance, photosynthetic rate, leaf respiratory rate (Table 4.2), root protein concentration, root APase (Figure 4.2), and tissue macronutrients (Table 4.4), depending on Pi and Al concentrations in root zones. These changes in physiology and tissue P and Al concentrations of broomsedge plants would contribute to the differences in biomass observed in this study (Figure 4.1). However, these changes in physiology of broomsedge plants neither parallel to the differences in tissue P and Al concentrations nor to the differences in biomass of broomsedge plants, which

may suggest complex Pi-by-Al interactions (Clarkson 1966; Snoeyink and Jenkins 1980; Foy 1983; de Miranda and Rowell 1989; Marschner 1995; Ritchie 1995; Yang and Goulart 1997).

HM mycorrhizal plants had higher tissue P concentrations than LM mycorrhizal plants under Al exposure or high available Pi condition (Figure 4.3), but lower tissue Al concentrations and LM mycorrhizal plants (Figure 4.4). LM mycorrhizal plants tolerated higher Al than HM mycorrhizal plants when they were exposed to Al (Figure 4.1 and 4.4). Differences in physiology between LM and HM mycorrhizal plants thus would be expected, and these differences in physiology would be changed as Pi and Al concentrations change (Hayman 1983; Cumming and Weinstein 1990; Koslowsky and Boerner 1989; Zel et al. 1993; Bartolome-Esteban and Schenck 1994; Medeiros et al. 1994; Clark 1997). LM mycorrhizal plants had higher stomatal conductance than HM mycorrhizal plants independent of Pi and Al treatments (Table 4.2). LM and HM mycorrhizal plants significantly differed or not in photosynthesis, leaf respiration (Table 4.2), root protein concentrations, root APase (Figure 4.2), and tissue macronutrients (Table 4.4), depending on Pi and Al concentrations in root zones. These differences may contribute to growth. For example, HM mycorrhizal plants had low leaf respiration (high Pi), high photosynthesis (high Pi) (Table 4.2), low shoot Al (Figure 4.4), high shoot P (high Pi) (Figure 4.3), and high Mg (high Pi) (Table 4.4), consequently leading to large plants under these conditions (Figure 4.1).

LM mycorrhizal plants are able to tolerate Al to certain extent, and HM mycorrhizal plants are capable of mining scarce P resources. These differences in host plants brought about by colonization of different AM fungal isolates may be associated with the roles these fungi play in their original habitats. LM fungus was from a low pH and high Al site, where elevated Al is one of the primary factors limiting growth and establishment of plants and fungi. However, HM fungus originated from a high pH site, where most Pi would be fixed by Ca so that available Pi to plants would be limited. Thus, these AM fungi appear to have adapted to their original environments as revealed through this study and previous study (Chapter III).

This study demonstrated that mycorrhizal fungi play an important role in plants under limited Pi and elevated Al conditions, but different AM fungal isolates differentially affect the host plants under different conditions. With the Al treatment, both fungal isolates significantly enhanced plant growth in comparison to non-mycorrhizal plants. Without the Al treatment, however, the HM fungal isolate performed much better than LM fungal isolate in terms of plant biomass when the plants were supplied with limited Pi (Figure 4.1). The HM fungus may have

refined its function in aiding host plants to cope with limited Pi availability in the high pH soils where most Pi is precipitated with Ca, and the LM fungus may have placed its effort in ameliorating Al toxicity present in the acidic soils. The data from the tissue P and Al concentrations (Figure 4.3 and 4.4) further support the concept of the local adaptation of these two AM fungal isolates. HM mycorrhizal plants were still able to maintain the normal Pi uptake and P translocation and restricted Al influx into plants under Al treatment, consequently leading to better growth. On the other hand, LM mycorrhizal plants maintained the normal physiology and growth even though they had considerable Al inside shoots under 200 μ M Al treatment, which is lower than the Al concentration in the mining soil from where this fungal isolate was originated.

SUMMARY

Limited phosphorus (Pi) availability and elevated aluminum (Al) level associated with soil acidification are a great challenge to the sustainable agricultural systems and environmental quality. Under acidic conditions ($\text{pH} < 5.0$), Al exists mainly as Al^{3+} , which is toxic to plants and also forms precipitates with phosphate. Thus, Al toxicity and Pi deficiency often co-occur and are the primary factors limiting plant growth and production in acidic soils.

The most present-living plants are potentially to form symbiotic association with mycorrhizal fungi. Arbuscular mycorrhizal (AM) symbioses are the most commonly mycorrhizal associations between obligately symbiotic fungi and higher plants. Mycorrhizal fungal colonization increases seedlings survival and establishment, and improves plant growth and competition, through facilitation of acquisition of diffusion-limited mineral nutrients, especially, Pi, improvement of water relations, conferment of metal resistance, defense of invasion and infection of pathogenic microbes, and suppression of non-mycorrhizal plant growth. These benefits may be achieved by extensive exploration of a large volume of soil, improvement of plant nutrition and alteration of plant physiology and biochemistry.

This dissertation project, including four studies, examined roles of an arbuscular mycorrhizal fungus, *Glomus clarum*, played in broomsedge (*Andropogon virginicus*) plants under limited Pi and elevated Al conditions in a series of experiment conducted in a growth chamber. The infective inoculum originated from an abandoned acidic coal-mining site in Morgantown, West Virginia, USA. The inoculum of the same fungal species from INVAM collection a West Virginia University, which originated from an abandoned alkaline coal-mining site in Mingo County, West Virginia, USA, was also used in two of the studies. Broomsedge seedlings were pre-colonized by AM fungi from the above inoculum, then transplanted into an acid-washed sand culture system, and watered with a modified Hoagland's base nutrient solution with an adjusted pH of 4.0 before delivery.

Mycorrhizal symbioses pronouncedly enhanced growth of host broomsedge plants under limited Pi and/or elevated Al conditions. Under Pi limiting conditions, mycorrhizal broomsedge plants made more investment on roots, consequently exploring root zones effectively, drew down the solution Pi concentrations to lower levels, leading to increasing exploited Pi pool, and increased phosphorus use efficiency (PUE). Moreover, mycorrhizal broomsedge plants balanced

accumulation of nutrients under varying Pi availability. All these brought about by mycorrhizal association with the AM fungi benefit broomsedge plants overcoming Pi deficiency and supporting optimal growth of broomsedge plants under limited Pi availability.

Broomsedge plants do not have an inherent mechanism to block Al influx into plants and tolerate Al toxicity. Mycorrhizal association with the AM fungi significantly conferred Al resistance in broomsedge plants by facilitating Pi uptake in the early stages, reducing Al influx into roots, suppressing Al translocation within plants, altering profiles of organic acids, increasing concentrations of organic acids in rhizospheres, improving nutrition of host broomsedge plants. These differences in Pi uptake and utilization, Al influx and translocation, exudation of organic acids, and nutrition between mycorrhizal and non-mycorrhizal broomsedge plants may contribute to the observed differences in growth patterns of mycorrhizal and non-mycorrhizal broomsedge plants in responses to Pi and Al availability and variability, and may also be the primary mechanisms for this plant species to grow and establish in many adverse habitats. However, different fungal isolates had differential effects on Pi acquisition of, Al influx into and translocation within, exudation of organic acids from root systems of, and nutrient uptake by broomsedge plants as Pi and/or Al concentrations in root zones change. These differences may be associated with the differential adaptations of these two AM fungal strains to their original habitats, which may consequently lead to distribution of this plant species in the eastern United States.

The differences in Pi acquisition and utilization, and Al influx and translocation between mycorrhizal and non-mycorrhizal broomsedge plants under Pi and/or Al levels might cause or be a result of changes in physiology of broomsedge plants. Mycorrhizal and non-mycorrhizal plants may or may not significantly differ in stomatal conductance, photosynthetic rate, leaf respiratory rate, root protein concentration, root acid phosphatase, and tissue macronutrients, depending on Pi and Al concentrations in root zones. Different AM fungi differentially altered physiology of host broomsedge plants. For example, mycorrhizal plants colonized by the fungus from low pH site (LM) had higher stomatal conductance than the mycorrhizal plants colonized by the alkaline fungus (JM), independent of Pi and Al treatments. Moreover, compared to LM mycorrhizal plants, HM mycorrhizal plants had low leaf respiration (at low Pi, no Al), high photosynthesis (at high Pi), low shoot Al, high shoot P (at high Pi), and high Mg (at high Pi), consequently leading to large plants under these conditions.

Evidently, this dissertation project revealed that broomsegde plants rely on AM fungi to overcome limited Pi availability and/or elevated Al toxicity. Forming mycorrhizal associations with AM fungi is the main mechanism for broomsedge to grow and establish in various adverse habitats.

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M. S. in Botany, Institute of Botany, Chinese Academy of Sciences, Beijing, China
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Experience

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- Assistant Professor in Botany, from Jan. of 1994 to May of 1995, Institute of Botany, Chinese Academy of Sciences, Beijing, China
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- System Administrator and Supervisor of Computing Facilities, from Jan of 1994 to May of 1995, Institute of Botany, Chinese Academy of Sciences, Beijing, China
- Networking Specialist, from August of 1993 to May of 1995, Institute of Botany, Chinese Academy of Sciences, Beijing, China
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Honors and Activities

- HERF Fellowship 1996-1999, West Virginia University
- Candidate for Excellent Research Award 1994, Institute of Botany, Chinese Academy of Sciences
- Award of Outstanding Research Paper 1991, Institute of botany, Chinese Academy of Sciences
- Honors for Excellent Students 1985-1987, Jilin Agricultural University
- Member of Ecological Society of America 1998
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- Deputy Chair and Secretary-General of Graduate Association 1990, Institute of Botany, Chinese Academy of Sciences