THE EFFECT OF VA MYCORRHIZAE ON SHOOT BIOMASS AND P UPTAKE OF GRASSES USED TO REVEGETATE COARSE TACONITE IRON ORE TAILING[1]

by

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Abstract. A greenhouse study was conducted to determine the effect of the indigenous VAM fungus *Glomus claroideum* on shoot biomass and uptake of phosphorus (P) of two warm-season grasses, big bluestem (*Andropogon gerardi*) and little bluestem (*Schizachyrium scoparium*), and the cool-season grass Canada wild rye (*Elymus canadensis*) in coarse taconite iron ore tailing.

Plants were grown at seven levels of P availability (Olsen extractant) for 60 days with and without *G. claroideum* inoculation in a randomized complete block design. Each treatment was replicated three times. Low P levels were 0.6, 1.7, and 2.7 mg kg^{-1}, moderate P levels were 5.0 and 7.0 mg kg^{-1}, and high P levels were 14 and 25 mg kg^{-1}. At harvest, plants in each pot were combined and measured. Roots were stained and analyzed for the presence and percentage of *G. claroideum* colonization.

Big bluestem responded positively to *G. claroideum* formation at low and moderate levels of P. Canada wild rye responded positively at low P but responded negatively at moderate P. Little bluestem responded with increased shoot biomass at moderate and high P whereas only P uptake was increased at low to moderate P.

In general, warm-season prairie grasses derived the greatest increase in shoot biomass and P uptake with *G. claroideum* at low to moderate levels of available P. The cool-season grass only benefitted at low P. At P levels above that considered adequate for most crops in agricultural soils (14 mg kg^{-1}), VAM fungi did not confer this benefit. The addition of *G. claroideum* to coarse taconite iron ore tailings may reduce the amount of P fertilization necessary to establish adequate plant cover.

Canada wild rye harbored the greatest amounts of *G. claroideum* colonization and may be suitable to increase the VAM infectivity potential of the tailing to favor more competitive long-lived grass species.

Key words: mycorrhizal fungi, phosphorus response, prairie grasses, mineland reclamation

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Introduction

In 1980 the Minnesota Department of Natural Resources instituted reclamation rules that require the establishment of a 90% cover of vegetation on tailing deposits within three years, and the establishment of stable, self-sustaining plant communities within ten years (Department of Natural Resources 1980). Successful revegetation of coarse taconite tailing has proved to be difficult because it possesses several adverse characteristics that severely limit establishment of vegetation. These include an alkaline pH, exceedingly low levels of organic matter, available nitrogen (N) and phosphorus (P), and low water-holding capacity (Table 1).

Table 1. Chemical and physical characteristics of coarse taconite iron ore tailing sampled at USX Corp., Mountain Iron, Minnesota.

pH	8.2
Organic matter (%)	0
Nitrate-N (mg kg^{-1})	0.8
Phosphorus (Olsen)(mg kg^{-1})	1.0
Potassium (mg kg^{-1})	225
Calcium (mg kg^{-1})	560
Magnesium (mg kg^{-1})	78
Zinc (mg kg^{-1})	0.17
Iron (mg kg^{-1})	96
Manganese (mg kg^{-1})	36
Copper (mg kg^{-1})	0.29
Bulk density (g/cm^{-3})	1.70
Particle distribution (%):	
>2mm	35
Sand	63
Silt	1
Clay	1
Water retention (% by weight):	
15 bar (approx. field capacity)	2
1/10 bar (approx. perm. wilt. pt.)	1

Selection of plants that can tolerate these stressful conditions is an important aspect of our revegetation strategy. Perennial tallgrass prairie species such as big bluestem (*Andropogon gerardi* Vitm.), little bluestem (*Schizachyrium scoparium* (Michx.) Nash.), and Canada wild rye (*Elymus canadensis* L.) grow in soils that are alkaline and infertile and tolerate adverse conditions such as drought and wide temperature extremes (Risser *et al.* 1981). Therefore they may be particularly well-suited to grow in a stressful coarse tailing environment. In a preliminary field study, these three species had high germination rates and successfully overwintered in coarse tailing (Noyd *et al.* 1992).

Successful introduction of prairie plants into coarse tailing requires an understanding of their nutrient requirements, environmental tolerances, and ecological interactions. In temperate grasslands, prairie grasses commonly form associations with soilborne fungi called vesicular-arbuscular mycorrhizae (VAM) (Hetrick and Bloom 1983, Liberta and Anderson 1986). This interaction is an important aspect of restoration ecology because VAM fungi influence plant nutrient requirements and thus contribute to competitive ability and fitness (Fitter 1977). Enhanced growth of mycorrhizal plants has been attributed to increased uptake and transport of P (Hetrick 1988, Smith 1988, Bolan 1991), drought tolerance, and improved soil structure through encapsulation of organic matter by extramatrical hyphae. Thus, the interaction between a plant species and mycorrhizal fungi ultimately impacts their chances of successfully colonizing the tailing substrate.

Various growth responses of plants to mycorrhizal colonization have been reported. Effects range from growth suppression (Bethlenfalvay *et al.* 1983, Buchwalda and Goh 1982), no improvement (Pairunan *et al.* 1980, Boerner 1992), to a considerable growth enhancement (Mosse 1973, Powell 1977, Boerner 1992, Hetrick *et al.* 1988a). In general, beneficial responses are greatest in P-deficient soils and decrease with increasing additions of P (Mosse 1973, Abbott and Robson 1977, 1984, Hayman 1983) and vary with the species of mycorrhizae used (Schubert and Hayman 1986). Differences in responsiveness among plant species and VAM fungi are related to morphological and physiological factors such as root architecture (Hetrick et al 1988b), root morphology (Baylis 1970, Reinhardt and Miller 1990) and growth rate (Hall 1975). Since the P status of the host is critical to the way it responds to mycorrhizal formation, a more comprehensive plant response can be obtained by comparing the effects of mycorrhizae on P uptake and growth (shoot biomass) over a gradient of P availabilities (Abbott and Robson 1984).

Knowledge of the mycorrhizal responsiveness of potentially useful plant species in a coarse tailing medium will help determine plant species selection and fertilization rates required to establish vegetation in the field. Our objective was to determine the relative mycorrhizal responsiveness of big bluestem, little bluestem, and Canada wild rye growing in coarse taconite iron ore tailing. In a greenhouse experiment, we determined the effect of *Glomus claroideum* Schenck & Smith, a species of mycorrhizal fungi adapted to the tailing environment, on shoot biomass and P uptake of these grasses over a gradient of P availabilities. In addition, we measured root and shoot biomass, root length, mycorrhizal sporulation and hyphal length. Our emphasis in this paper is shoot biomass as the primary measure of plant responsiveness because of the importance of vegetative cover as the main criterion of reclamation success.

Materials and Methods

Coarse taconite tailings were collected from USX Corporation, Mountain Iron, Minnesota. A randomized complete block design was used, consisting of 3 replications for each of 42 treatments. Coarse iron tailing was steam pasteurized and weighed (5 kg pot^{-1}) into 4 L pots. Seven levels of P, low (0.6, 1.7, and 2.7), moderate (5.0 and 7.0), and high (14 and 25 mg P L^{-1}) solution were mixed as KH_2PO_4. Phosphorus solutions were buffered as necessary to pH 7.0 and a final volume of 400 mL solution was added per pot. To assure that P would be the only limiting nutrient, N was supplied once per week beginning in week 3 as a 100 mL pot^{-1} solution of NH_4NO_3 at a concentration of 10 mg N L^{-1} and micronutrients were added as 100 mL of a 10% Long-Ashton solution (Hewitt 1966) at weeks 3, 5, and 7.

One half of the pots were inoculated with *G. claroideum*, a species of mycorrhizal fungi that was identified (Schenk and Perez 1990) associated with roots of white sweet clover (*Melilotus alba* Desr.) growing in an adjacent fine tailing area with similar edaphic properties as coarse tailing. Infected roots were cut into 5-mm long fragments. One gram of dried infected root tissue was added to each mycorrhizal pot at a depth of 5 cm. Control pots received equal amounts of autoclaved roots. To introduce microflora associated with pot culture inoculum to nonmycorrhizal treatments, 10 mL of a VAM spore-free washing of a fine tailing filtrate, obtained with a 20 um filter, was added to control pots.

Seeds of the native prairie grasses Canada wild rye, big bluestem, and little bluestem, were soaked for 30 minutes in distilled water and sown into pots. After 2 weeks, plants were thinned to 8 plants pot^{-1} to reduce root competition. Plants were sparingly watered daily and pots were brought to field capacity by watering to weight weekly. Plants were grown in a greenhouse under natural light from April 1 to June 1 at temperatures averaging about 20° C.

Plants were harvested 60 days after planting. The eight plants in each pot were combined for all measurements. Shoots were oven dried (60°C), weighed, and ground. Tissue P concentrations of the dried shoots were determined colormetrically using molybdovanadate method (Jackson 1958). At harvest, available P in the tailing was estimated by $NaHCO_3$ extractant (Watanabe and Olsen 1965). Treatment means of shoot biomass and P uptake were compared using a paired two-tailed t-test at the 90% confidence level ($P < 0.1$).

Roots were air dried, weighed, and a 0.30-g subsample was stained in trypan blue to assess percent root length colonized by VAM by the gridline intersect method (Kormanic and McGraw 1982).

Results

Big Bluestem

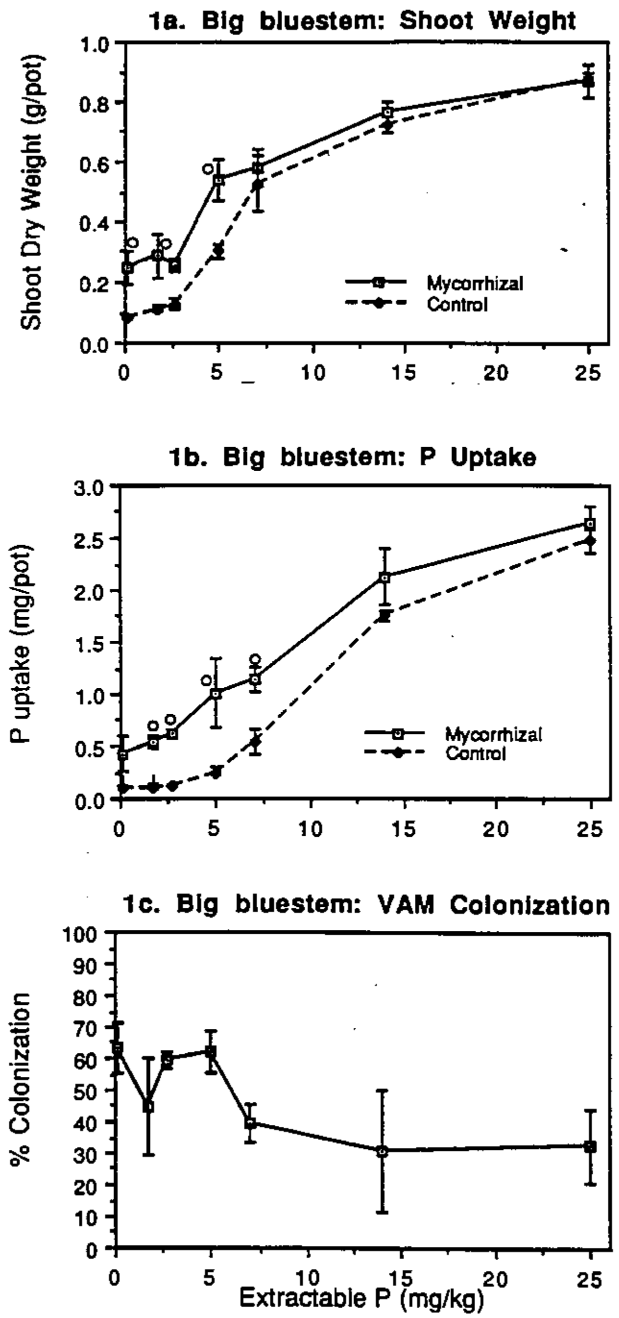
At low and moderate P (0.6 to 5.0 mg kg^{-1}) levels and in the absence of *G. claroideum*, big bluestem plants were stunted, had anthocyanin accumulation in leaves, and older leaves were withered, as compared to mycorrhizal plants. These classic P-deficiency symptoms were not evident at high levels of P. Visual observations correlated with shoot biomass and P uptake measurements (Figure 1). At low and moderate P, mycorrhizal plants had greater shoot biomass (Figure 1a) and greater P uptake (Figure 1b) than control plants. Percent root colonization by *G. claroideum* was greatest (60%) at low to moderate P (Figure 1c) and then leveled at approximately 34%. Under P-limiting conditions(0-4 mg kg^{-1}), mycorrhizal plants showed a significant enhancement of growth as compared to control plants.

Little Bluestem

At low P, control plants expressed visual P-deficiency symptoms similar to those described for big bluestem. Plants inoculated with *G. claroideum* had significantly greater shoot biomass when P availability was moderate to high P (7.0 to 14.0 mg kg^{-1}) (Figure 2a). Shoot biomass at high P was similar to big bluestem. Inoculated plants had greater P uptake at low to moderate P (0.6 to 7.0 mg kg^{-1}) (Figure 2b). Root colonization by VAM was highest at moderate P (and then reached an apparent plateau of approximately 12% (Figure 2c). This level of VAM colonization was the lowest of the three grass species in this experiment.

Canada Wild Rye

At low P, neither control nor mycorrhizal plants showed the visual P-deficiency symptoms evident in big bluestem and little bluestem. Mycorrhizal plants displayed significantly greater shoot biomass and P uptake at only one P level (1.7 mg kg^{-1}, Figures 3a,b). At very low and very high P there was no benefit conferred by *G. claroideum*. A significant suppression of shoot growth occurred in the moderate P range (Figure 3a) for mycorrhizal plants. The 75% colonization of root length occurred with no added P (Figure 3c) was the highest



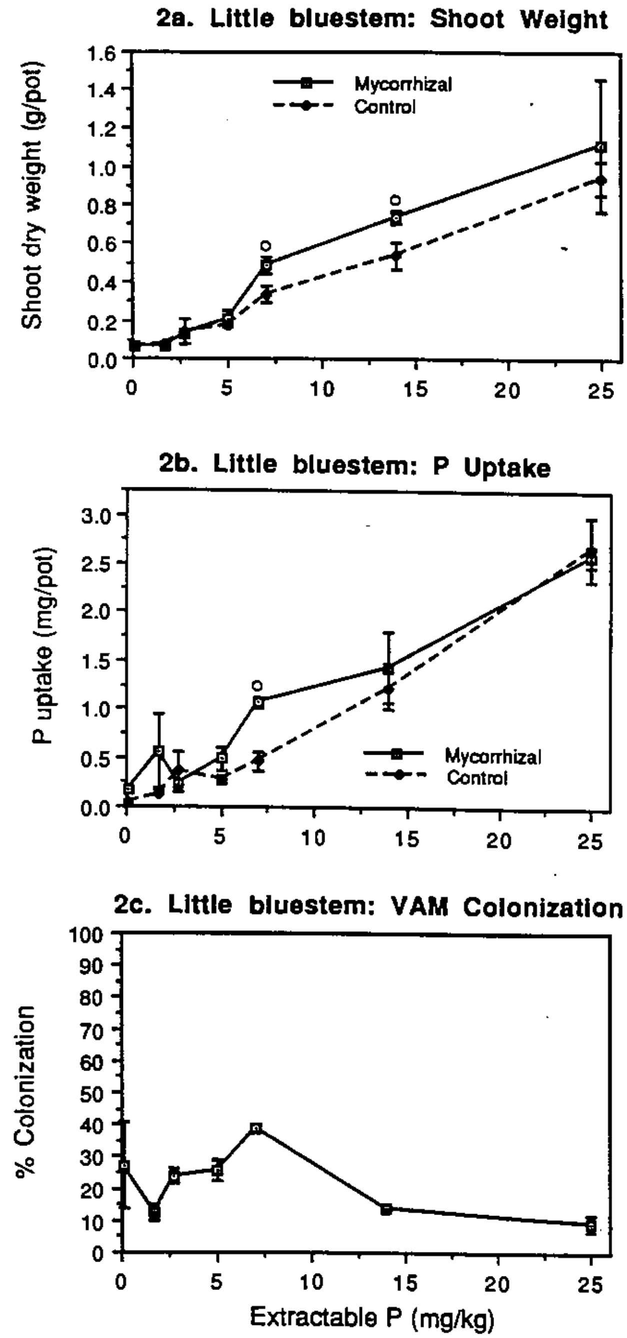


Figure 1. The effect of *Glomus claroideum* on big bluestem in coarse taconite iron ore tailing containing a gradient of P availability. a) Mean shoot dry weight. b) Mean P uptake in shoots. c) Percent root length colonized. Error bars represent S.E. of the mean. Significant differences between treatments are shown by a circle.

Figure 2. The effect of *Glomus claroideum* on little bluestem in coarse taconite iron ore tailing containing a gradient of P availability. a) Mean dry shoot weights. b) Mean P uptake of shoots. c) Percent root length colonized. Error bars represent S.E. of the mean. Significant differences between treatments are shown by a circle.

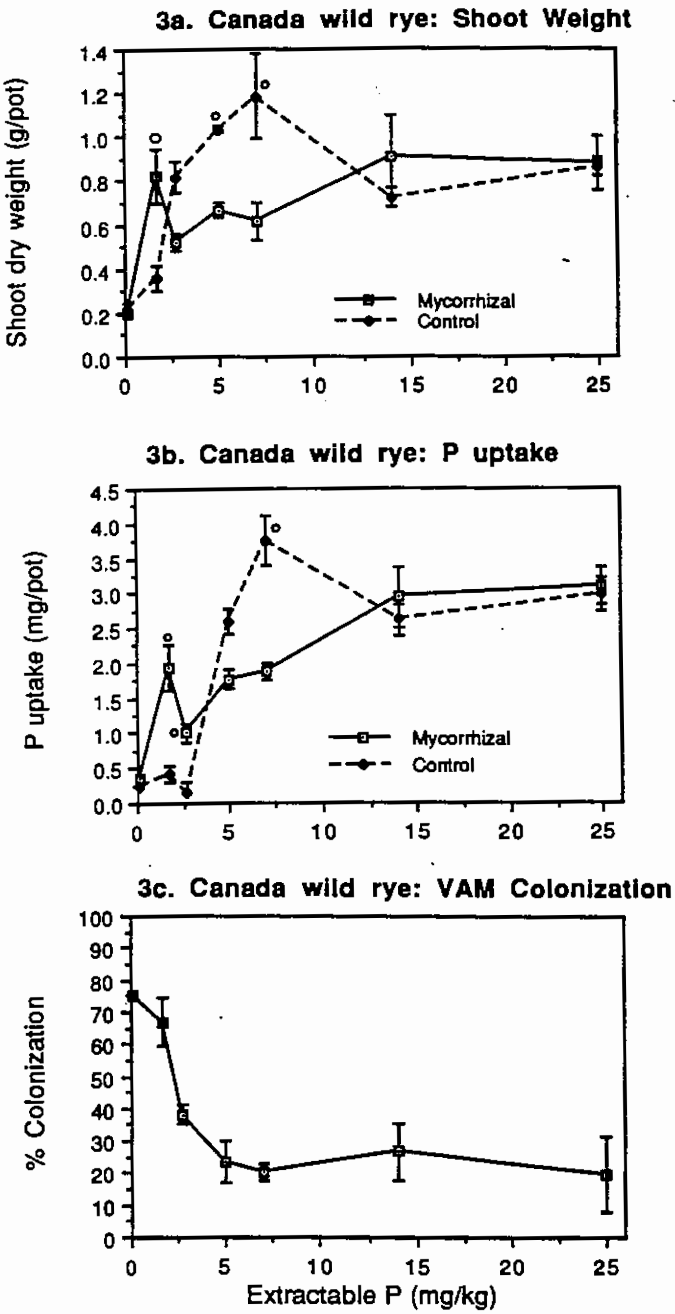


Figure 3. The effect of *Glomus claroideum* on Canada wild rye in coarse taconite iron ore tailing containing a gradient of P availability. a) Mean dry shoot weights. b) Mean P uptake of shoots. c) Percent root length colonized. Error bars represent S.E. of the Mean. Significant differences between treatments are shown by a circle.

colonization level observed in this experiment.

Discussion

All plants increased shoot biomass with additions of P. This demonstrates that P is a growth-limiting nutrient in the coarse taconite tailing substrate. Many experiments measuring plant responsiveness to P use applied P as their independent variable (Pairunan *et al.* 1980, Jasper *et al.* 1979, Sainz and Arines, 1988, Abbott and Robson 1977). This value may not represent the actual P concentrations in the soil solution that influence the growth of plant roots and mycorrhizal hyphae. To overcome this problem, we used extractable P concentrations as the independent variable upon which our plant and mycorrhizal fungi measurements were made.

At P concentrations above 14 mg kg^{-1}, a level considered adequate for most crops in agricultural soils, *G. claroideum* did not confer a significant increase in P uptake or shoot biomass for any of the three plant species. These results are consistent with the general hypothesis that the beneficial effects of VAM are reduced above nonlimiting levels of available P (Mosse 1973, Abbott and Robson 1977).

Mycorrhizal plants in all three prairie species showed a significant increase in shoot biomass and P uptake to mycorrhizal inoculation at one or more levels of extractable P. Big bluestem, little bluestem, and Canada wild rye were very different in the degree to which they responded to *G. claroideum*. The warm-season grasses, big and little bluestem, benefitted significantly more from mycorrhizal formation over a wider range of available P and showed a greater positive responsiveness to *G. claroideum* than the cool-season grass, Canada wild rye. These results support the hypothesis that cool-season grasses with highly branched fibrous root systems can grow moderately well in the absence of mycorrhizal symbiosis, where warm-season grasses with coarser, less frequently branched root systems are more responsive to formation (Baylis 1970, Hetrick *et al.* 1988a). In contrast to Hetrick *et al.* (1988a), who concluded that cool-season grasses were weakly dependent on mycorrrhizae, we found that *G. claroideum* caused a significant suppression of shoot biomass and P uptake of Canada wild rye at moderate P. This negative effect may be due to competition between the host and the fungus for photosynthetically-derived carbon, with Canada wild rye being incapable of regulating the interaction. In contrast, the warm-season grasses may have greater control on the extent of colonization by *G. claroideum*. However, the warm-season grass species responded positively to *G. claroideum* formation at different P availabilities which supports the view that VAM responsiveness is determined by the P status of the soil (Abbott and Robson 1984).

The mycorrhizal responsiveness of potentially useful plant species is important information in formulating a revegetation strategy. Big and little bluestem showed positive growth response to *G. claroideum*. These grass species are highly competitive and dominate infertile sites (Tilman and Wedin 1991). Thus if these grasses are expected to establish in coarse tailing, an adequate level of VAM fungal inoculum must be present in the substrate. A previous study showed that coarse tailing deposits have very low levels of VAM fungal inoculum and immigration rates are low (Noyd *et al.* 1992). Therefore, constructing suitable soil conditions for the growth of competitive plant species may require inputs of VAM fungal inoculum or other methods to increase populations of VAM propagules. One method of increasing VAM fungal inoculum was precropping using a highly mycotrophic species such as *Sorghum* spp. (Dodd *et al.* 1990). Even though Canada wild rye responded negatively to *G. claroideum* at moderate P, at low P it was well colonized. Therefore, Canada wild rye in combination with an initial source of VAM fungal inoculum may be useful as an early colonizer of taconite tailing and increase the mycorrhizal infectivity potential of the tailing. The subsequent increase in VAM inoculum fungal would then favor the establishment of grasses such as big bluestem and little bluestem, which are adapted for a long term, self-sustaining plant community.

The use of plants adapted to harsh sites in combination with their co-adapted VAM symbionts may represent the most efficient consumers of available P in taconite tailing and would reduce the amount of P fertilization necessary to establish plant cover. The shoot biomass of mycorrhizal little bluestem plants at 7 mg kg^{-1} were equal to the nonmycorrhizal plants at 14 mg kg^{-1} (Figure 2a). Mycorrhizal big bluestem plants at 1.7 mg kg^{-1} had equal shoot biomass to nonmycorrhizal plants at 5 mg kg^{-1} (Figure 1a). Choosing the lowest level of fertilization necessary to optimize plant growth and stability of the soil ecosystem is both cost-effective and environmentally rational. Field trials will be necessary to establish whether the combination of moderate P fertilization and VAM inoculation can provide adequate vegetative cover to meet current regulations.

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