Native Plants and Vesicular-Arbuscular Mycorrhizal Fungi in Reclamation of Coarse Iron Mine Tailings in Minnesota[1]

by

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Abstract. Studies were initiated to determine the occurrence and rate of immigration of VAM spores into recently deposited coarse and fine tailings. Plant roots, soil, and coarse and fine tailings were collected every two weeks from May through September 1990 and analyzed for VAM species present, spore numbers, and percent root colonization. Average spore numbers ranged from 0.7 to 56/g soil and percent root colonization ranged from 4.4 to 18.4%. VAM species present were: *Acaulospora bireticulata*, *Glomus aggregatum*, *G. claroides*, *G. intraradix*, and *G. mosseae*. *Glomus intraradix* was the most frequently recovered species from all sites. Field plots of seventeen plant species native to Minnesota were established to evaluate seedling establishment, growth, and survival. Half of the plots received 3 g root/soil inoculum of *G. intraradix*. One thousand seeds of each species were planted into one square meter plots. Ammonium nitrate fertilizer was applied to all plots at a rate equal to 220 kg/ha. Stand counts were taken 6 weeks after planting with the following results expressed as a percentage: *Amorpha canescens* 54, *Andropogon gerardi* 78, *Elymus canadensis* 83, *Lespedeza capitata* 70, and *Schizachyrium scoparium* 57. A greenhouse study was conducted with these five plant species in combination with *G. intraradix* for the evaluation of its effect on plant biomass in coarse tailings. Plant biomass and colonization by *G. intraradix* were significantly greater than noninoculated plants.

Additional Key Words: reclamation, vesicular-arbuscular fungi, native plants, coarse iron mine tailings.

Introduction

In 1991, USX Corporation shipped over 13.3 million gross tons of iron ore pellets from its Minntac plant at Mountain Iron, Minnesota (Skillings' 1992). For every ton of iron ore pellets produced, approximately two tons of crushed bedrock, or tailings, are produced as a waste product (Norland, pers. comm.). Crushing and magnetic separation processes segregate the tailings by size into coarse and fine particles. Deposits of coarse and

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fine tailings are subject to reclamation rules instituted in 1980 by the Minnesota Department of Natural Resources (6130.3600). According to these rules, 90% cover of vegetation must be established on tailing deposits within three years. Stable, self-sustaining plant communities must be established in these areas within ten years. Current revegetation practices use agronomic crops such as alfalfa, buckwheat, red fescue, and perennial ryegrass that are adapted to fertile soils and require high inputs of labor and fertilizer. This practice is successful on the fine tailing deposits primarily due to the small particle size and moisture retention. However, successful revegetation of coarse tailing deposits has proved to be more difficult because the tailings are a very poor plant growth medium (Stewart and Pfleger 1985). Unlike most soils, coarse tailings lack adequate levels of organic matter, nitrogen and phosphorus, and have a very low water-holding capacity (2% at approximate field capacity).

An alternate approach is the selection of long-lived native perennial grasses and forbs that demonstrate stress-tolerant survival strategies. These plants are more suitable for revegetating and establishing self-sustaining plant communities in coarse tailings. Call and McKell (1982) advocated the use of stress-tolerant plants in the revegetation of disposed oil shale. According to Grime (1979), stress-tolerant plant species adapted to infertile habitats are long-lived, spread extensively by vegetative propagation, and have low maximum growth rates. Grime (1979) termed plants with this survival strategy stress tolerators. Adapted for long-term survival, stress-tolerators conservatively utilize water and mineral nutrients in favor of maximizing the capture of these resources (Grime 1979).

The roots of stress-tolerators form vesicular-arbuscular mycorrrhizae (VAM), an association with a group of soil-borne fungi that enhance the plant's ability to acquire water and minerals. Many reports show that this association confers considerable advantages to a host plant such as improving tolerance to water stress (Bildusas et al 1986, Stahl and Smith 1984, Allen and Boosalis 1983; Auge, Schekel, and Wample 1986; Johnson and Hummel 1985) and enhancing its ability to uptake minerals, especially phosphorus (Jeffries 1987).

There is evidence that the particular VAM species or intraspecific variants can elicit different physiological responses in host plants in the field (Bethlenfalvay et al 1989). Stahl and Smith (1984) report that different geographic isolates of *Glomus macrocarpum* Tul. & Tul. and *Glomus microcarpum* Tul. & Tul. had different effects on the water relations of the rangeland grass *Agropyron smithii* Rydb. This supports the view of Mosse (1975) that VAM fungi adapted to different habitats may have different influences on the physiology of the host plant.

Studies have shown that VAM fungi are able to disperse into new areas by many different vectors such as small mammals (Warner, Allen, and MacMahon 1987), birds, insects, earthworms (McIlven and Cole 1976), and on the hooves of ungulates (M. Allen 1987). Spores were also found in the pericarp of weed seeds (Taber 1982). Wind has been shown to be an important vector and can carry VAM spores (M. Allen 1987).

One of the most vulnerable stages in the life history of a plant to stressful growth conditions is from seed germination to the seedling stage of development (Harper 1977). Thus, native plant species that are candidates for revegetation must show high levels of recruitment of seeds to the seedling stage in coarse tailings under field conditions. Recruitment refers to a plant's ability to germinate, emerge, and establish itself as an independent plant (Harper 1977).

Objectives of this research were to 1) determine the occurrence and rate of VAM immigration into coarse tailings, 2) evaluate native plant species for use in revegetation, and 3) select a VAM fungi/host plant combination that will grow and survive in coarse tailings.

Methods and Materials

Study Site

Studies were conducted in the northwest sector of tailing deposits at the USX Corporation Minnesota Ore Operations, Minntac, Mountain Iron, Minnesota. The study site is located approximately 220 miles north of Minneapolis in the Mesabi Iron Range of northeastern Minnesota and has a mean annual precipitation of approximately 73 cm (28 in). Seasonal air temperatures vary considerably from -38°C (-36°F) to 33°C (91°F). Summer surface temperatures on the coarse tailings have been recorded at 39°C (102°F) (Stenlund unpublished). Open areas of fine tailing basins occupy the central portion of the 3000 hectare deposit site. Surrounding the basins are level coarse tailing benches and roadways. The study site on coarse tailing benches is bordered by an upland site consisting of aspen (*Populus* spp.) and mixed conifers (*Pinus* spp., *Picea* spp.) and a low wetland area dominated by *Glyceria canadensis* (Michx) Trin. and *Typha* spp.

VAM Occurrence and Immigration

A survey was conducted to determine the occurrence of VAM fungal species in three areas: 1) undisturbed vegetated areas adjacent to coarse tailing benches, 2) coarse tailings, and 3) fine tailings. Samples were collected every two weeks from May 26 through September 28, 1990. A 2-cm wide soil probe was used to collect three soil or tailing cores adjacent to living plants if present. Soil cores from each location were bulked and placed in plastic bags, and transported on ice to the laboratory.

Bulked tailings and soil samples were air dried and thoroughly mixed prior to removal of a 10, 15, or 25 g subsample of soil, fine or coarse tailings respectively. VAM fungal spores were extracted using a wet-sieving method adapted from McKenney and Lindsey (1987). Sieve sizes were 250 μm, 90 μm, and 25 μm. VAM fungal spores were counted and identified to species according to Schenk and Perez (1990). Spores were placed on roots of sudan grass plants (Piper Sudangrass, Northrup King Co.) in sterilized sand to determine viability and percent colonization by the fungus.

Two types of spore traps were constructed to collect, quantify, and monitor the immigration rate of VAM spores into coarse and fine tailings. One type of trap was a rectangular-shape with a total area of 0.25 m^2 and constructed with plastic window screen attached to a frame of hemlock wood. Traps were 5.2 cm deep and contained a 2 cm layer of sand overlaid with coarse taconite tailings. Sand and tailings were steam pasteurized. Every two weeks the entire contents of each trap were collected and refilled with pasteurized tailings and sand. A 500 mL sample was assayed for species of VAM present and spore numbers. Nine spore traps of this type were randomly located on undisturbed vegetated areas, coarse and fine tailings areas. The second type of trap collected VAM spores from rainfall. Each rain trap consisted of a funnel (8.5 cm wide) that flowed into a PVC tube. At the base of the tube was a cellulose filter (Gelman, 47 mm) paper (0.22 μm pore size). It was supported by a plastic mesh to allow water to flow through the trap. The filter paper was replaced every two weeks and examined for the presence of VAM spores. Rain traps were installed at two random locations on coarse tailings and at one location on the upland site within vegetation.

From soil core samples collected, 0.25 g of dry secondary roots were placed into tissue cassetes and cleared in 10% KOH for 48 hours. After rinsing and neutralizing the solution with 5 mL 5M HCl for 5 minutes, roots were stained in 0.01% (w/v) acid fuchsin for 24-48 hours at room temperature. Colonization of plant roots by VAM was determined by a gridline intersect method following the procedure outlined in Kormanic and McGraw (1982). Several infected roots were mounted in polyvinyl alcohol (PVA) for voucher slides and further microscopic examination.

Field Plot Studies - Native Plants

Seventeen native plant species were evaluated to determine suitability of these species for revegetation of coarse tailings. Selection of these plant species was based on drought and frost tolerance, seed availability, and ease of propagation. Seeds of native plants were sown June 11, 1991 to evaluate their ability to germinate and become established in coarse tailings under field conditions. Native plant species included cool- and warm-season perennial grasses, as well as leguminous and nonleguminous forbs (Table 1). Legume seeds (Prairie Restorations, Inc.) were predusted with appropriate species of nitrogen-fixing bacteria (*Rhizobium*). One thousand seeds of each of the native grasses and forbs (Table 1) were sown into one square meter plots using a completely randomized design. Two replications were inoculated with *Glomus intraradix* Schenck & Smith. The *G. intraradix* inoculum was produced in the greenhouse by inoculating roots of sudan grass with soil and roots collected from adjacent undisturbed vegetation. After a three month growth period, roots of sudan grass were cut into small segments. The inoculum consisted of 3 g of root tissue and 500 mL sand/soil mixture that contained extraradical spores. It was spread evenly and raked into plots. Two other replications were not inoculated with VAM fungi. Ammonium nitrate (34-0-0) was added to all plots at a rate equal to 220 kg/ha. Living plants were observed, counted, and measured at 6 and 10 weeks. At 10 weeks, selected plant roots were carefully extracted to assess root growth and the presence of VAM infection.

Greenhouse Studies - Native Plants

Greenhouse studies were conducted to determine the potential benefit of VAM fungi on the growth of native plants in coarse tailings. Two-week-old seedlings of the those native plant species that emerged on the coarse tailings in field plots were transplanted into conetainers (Ray Leach, Inc.) containing coarse taconite tailings. Each conetainer contained two plants. Eight conetainers were planted with each plant species. Four conetainers were inoculated with *G. intraradix*. Inoculum was produced by the same method as described for field plots. Inoculum consisted of 5 g root/soil mixed with 10 mL fine sand. Plants were fertilized three times per week with 20 mL of a low phosphorus (4 ppm/application) 10% Long-Ashton solution (Hewitt 1966). Survival and overall plant vigor was continuously monitored and biomass determinations were made after 150 days.

Results and Discussion

Occurrence of VAM Fungi

Five different species of VAM fungi were sieved from soil samples. These included *Acaulospora bireticulata* Rothwell & Trappe, *Glomus aggregatum* Schenck & Smith, *Glomus claroides* Schenck & Smith, *Glomus intraradix* Schenck & Smith, and *Glomus mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe.

Glomus intraradix occurred on wetland, forest soil, coarse and fine tailings sites, and was the most frequently encountered VAM species. *Glomus intraradix* has been reported to reduce water stress and increase drought recovery in plants growing in soils of low water potentials (Auge, Schekel, and Wample 1987). This VAM species was found colonizing roots of goldenrod (*Solidago* spp.) growing in isolated pockets on the coarse tailings. These pockets contained dead leaves blown in from adjacent undisturbed vegetation. Presently, sites like this on the coarse tailings, that contain large numbers of *G. intraradix* spores, may represent the best sources of environmentally-adapted VAM inoculum for use in revegetation.

Table 1. Seed weights and recruitment rates for plant species native to northern Minnesota used to revegetate coarse iron tailings.

Plant spp.	Weight per 1000 seeds (g)	Recruitment Rates /1000 seeds 6 weeks	10 weeks
Amorpha canescens Pursh. (Lead plant)	6.8	543	636
Elymus canadensis L. (Canada wild rye)	6.8	836	696
Lespedeza capitata Michx. (Prairie bush clover)	5.0	702	873
Heliopsis helianthoides (L.) Sweet. (Common ox eye)	4.8	310	175
Bromus kalmii Gray. (Kalm's bromegrass)	3.2	25	2
Andropogon gerardi Vitm. (Big bluestem)	3.2	781	834
Petalostemum purpurpeum (Vent.)Rydb. (Purple prairie clover)	3.2	125	132
Petalostemum candidum (Willd.)Michx. (White prairie clover)	2.5	183	223
Panicum virgatum L. (Switch grass)	2.4	58	85
Sorghastrum nutans (L.) Nash. (Indian grass)	2.4	254	326
Schizachyrium scoparium Michx. (Little bluestem)	2.3	570	672
Bouteloua curtipendula (Michx.)Torr. (Side oats grama)	2.0	101	105
Koeleria cristata (L.) Pers. (June grass)	1.0	0	0
Aster azureus Lindl. (Azure aster)	0.8	0	0
Rudbeckia hirta L. (Black-eyed susan)	0.2	0	0
Achilla millefolium L. (Yarrow)	0.2	0	0
Juncus greenei Oakes & Tuckerm. (Rush)	<0.1	0	0

The VAM survey revealed that the adjacent undisturbed vegetation had spore numbers 10 times greater than those found in the fine tailings deposits. The average number of spores/g in adjacent vegetation, fine tailings, and three sites on the coarse tailings were 56, 5.6, 1.4, 1.0, and 0.7 spores/g respectively. Analysis (Scheffe F-test, ANOVA $P<.05$) indicates that the number of spores/g in the fine and coarse and fine tailings were not significantly different.

The greatest numbers of spores in the coarse tailings occurred in early August, whereas, the peak in the fine tailings was early July. In soils of adjacent undisturbed vegetation, the highest average spore counts occurred at the end of September (Figure 1).

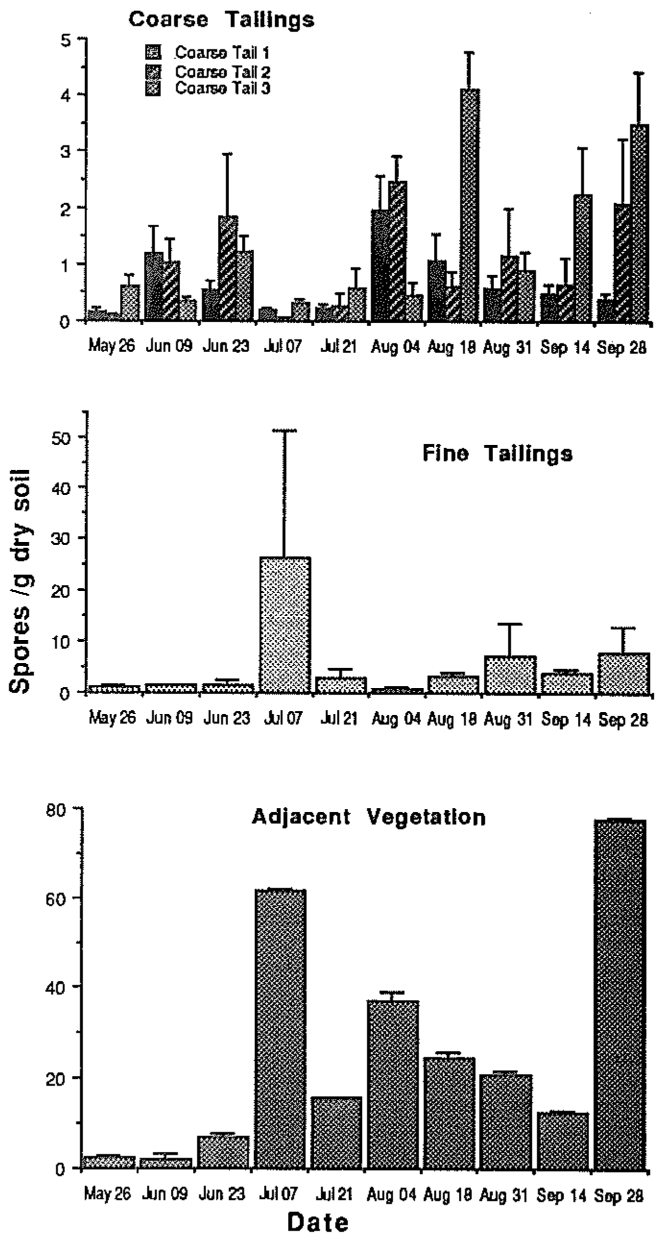

Figure 1. Site survey of VAM fungal spores per gram^{-1} of dry soil collected from undisturbed adjacent vegetation, coarse and fine tailing areas in 1990. Data are pooled average values from 10 samples.Three different coarse tailing benches are reported.

Colonization by VAM fungi was not significantly different in roots of adjacent undisturbed vegetation from roots of plants growing in coarse tailings. Percent infection was measured at 15.2%, 12.9%, and 18.4% for coarse tailings and 18.4% for adjacent undisturbed vegetation. Roots of plants growing on fine tailings were colonized at a much lower 4.4%.

Immigration of VAM Fungi

Freshly deposited coarse tailings are devoid of VAM propagules such as spores, hyphae, or colonized roots. The presence of VAM spores in coarse tailings indicates that these fungi migrated into the study area. Observations at the study site included several examples of animal immigration vectors. Moose and deer tracks were observed on the tailings. A small mammal's seed cache, as well as, wolf scat found on coarse tailings were found to contain large numbers of *G. intraradix* spores. Ground beetles (*Benbidium* spp.) found on the tailings, however, did not have VAM spores on the surface of their bodies.

The coarse and fine tailings sections of the study site are open and expansive areas with strong westerly winds. Winds blowing dust into and around taconite tailings are a likely vector for VAM spore dispersal and immigration. Wind-transported spores are deposited by settling or impact. Vegetation reduces wind speed and provides a point of impact. From spore trap data, greater numbers of spores/g were recovered from soils in adjacent vegetated sites than from the open coarse and fine tailings areas. Vegetation spore traps collected an average of 3.3 spores/g dry soil, whereas average spore numbers recovered from traps on the coarse tailings were 1.0, 1.4, and 0.7 spores/g and fine tailings traps caught an average of 5.6 spores/g. Spore numbers for coarse and fine tailing sites were not significantly different from each other, but were different from adjacent undisturbed vegetation (Scheffe F-test, ANOVA $P < .05$).

Rain traps established to collect

VAM spores washed from the atmosphere by rainfall traps yielded less than one spore per trap/two weeks and many traps yielded no spores. The effectiveness of rain traps was hampered by physical problems and, in part, to collecting and replacing the filter paper once every two weeks. Traps often contained large amounts of debris that prevented the flow of water through the trap creating ideal conditions for saprophytic activity that was evident on the spores.

VAM spore immigration peaked from early June through early July, 1990 on all locations (Figure 2). Spore numbers after that time were significantly lower per gram dry soil through the end of September.

<u>Recruitment Patterns of Native Plants</u>

Seeds of 17 native plant species were evaluated in coarse tailings under field conditions for their possible use in revegetation. Table 1 shows recruitment rates at 6 and 10 weeks for each of the plant species. Plant species showing the greatest recruitment rates were *Elymus canadensis* L, *Andropogon gerardi* Vitm., *Lespedeza capitata* Michx., *Schizachyrium scoparium* Michx., and *Amorpha canescens* Pursh., Lower recruitment rates for *E.canadensis* (cool-season grass) in August over July were due to early senescence. Shoot growth rates for all plant species were low, at six weeks, surviving plants were approximately 2-3 cm tall, at ten weeks plants were 3-5 cm tall.

Even though shoot growth was sparse, root growth was extensive. For example, at 10 weeks roots of *Bromus kalmii* Gray. had extensive secondary roots and exceeded 15 cm in length. These observations were not surprising because prairie plants such as perennial grasses and forbs are known to initially allocate most of their energy into the development of extensive root systems (Weaver 1939). Root growth was vertical rather than lateral which may have

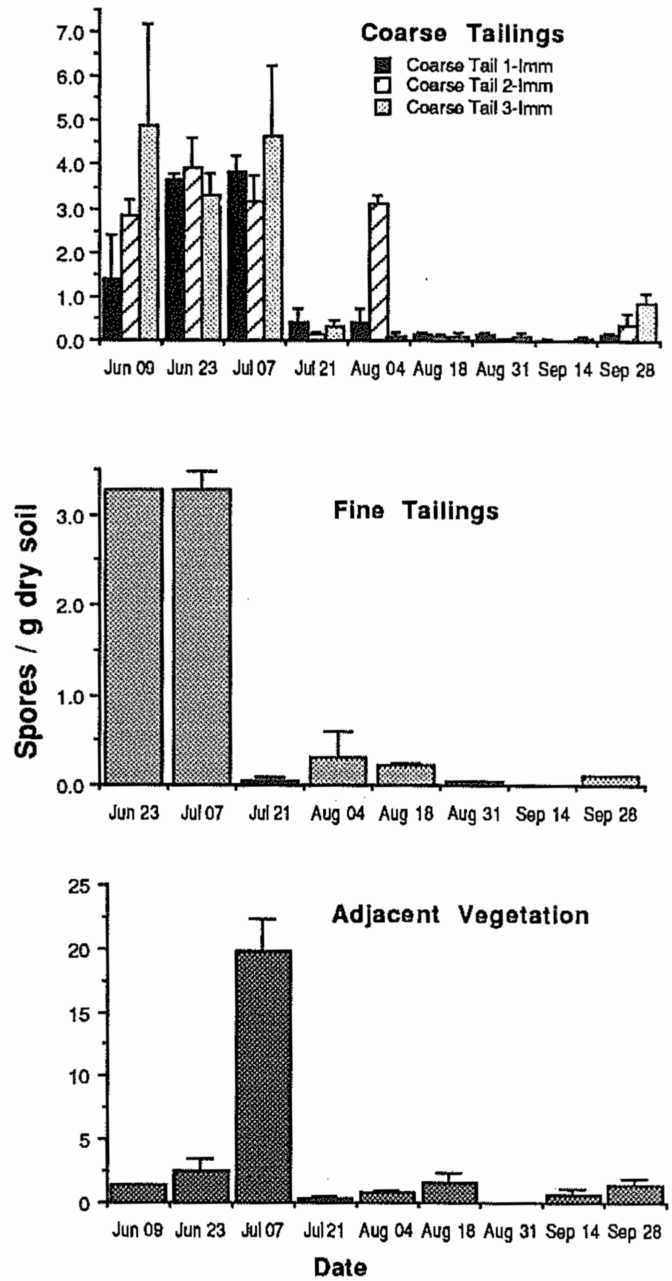

Figure 2. Immigration VAM fungal spore numbers per gram^{-1} of dry soil collected from spore traps set in 1990. Data are pooled average values from 10 samples. Traps set on three different coarse tailing benches are reported.

played a role in limiting contact between roots and the VAM inoculum. Additionally, the level of inoculum may have been too low because there was no evidence of infection of inoculated plants and recruitment rates did not reflect any difference between inoculated and noninoculated plots. Winter survival and further recruitment will be monitored in spring 1992.

Seed weight among plant species varied considerably and offered a possible explanation for the lack of recruitment for some species. Species with seeds weighing under one gram per thousand did not emerge. We speculate that rains washed these small light seeds down through the porous tailings, preventing emergence.

Greenhouse Studies of Mycorrhizal Benefit

Five-month-old plants inoculated with VAM fungi showed significantly greater survival and biomass (ANOVA $P<.05$). All plant species that were inoculated with mycorrhizae showed greater vigor and had a mean biomass of 0.216 g, compared to nonmycorrhizal plants with lower vigor and a mean biomass of 0.068 g. This preliminary study indicates that VAM have the ability to confer significant survival benefits to native plants growing in coarse tailings at a single level of phosphorus. Further studies will be conducted generating growth response curves at several levels of phosphorus similar to those advocated by Abbott and Robson (1984). This work will increase our understanding of the potential benefit of using VAM in revegetation with native plants.

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