AM-COLONIZED PLANT MATERIALS FOR MINED-LAND RECLAMATION[1]

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Abstract: The production of quality native plants materials is essential to large-scale reclamation efforts being undertaken at mine sites throughout the United States. Some studies have shown that nursery inoculation with arbuscular mycorrhizal (AM) fungi improved survival, growth, and drought tolerance of installed tree and shrub seedlings. The production of AM-colonized plant material, however, has proven problematic due to a lack of integration between the biotechnical firms that produce inoculum and cultural procedures at commercial nurseries. The objective of our research has been to integrate mycorrhizal inoculum successfully into a nursery program, thereby, producing plant materials with improved outplanting performance. Multiple studies were conducted to investigate the efficacy of various inoculum products, the influence of growth media types, and the influence of standard fertilization techniques on the ability of AM fungi to colonize plant materials under nursery conditions. Our research has resulted in a growth media which promotes colonization of plant materials by AM inoculum in a nursery environment. Plants produced by this tecnhique have significantly higher colonization rates than controls. This study reviews the results of greenhouse trials to increase AM-colonization rates in plant materials and outplanting field trials established in Oregon, Montana, and Utah to compare traditionally grown seedlings with those grown in the AM-promoting growth media formulation.

Additional Key Words: reclamation, seedling, VAM, mycorrhiza, plant material.

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Introduction

Mycorrhizal inoculum containing fungal species which form arbuscular mycorrhiza (AM) has become more common as a specification for container grown tree and shrub seedlings utilized in restoration projects throughout the United States (personal observation). Federal legislation such as the Surface Mining Control and Reclamation Act (SMCRA), the Conservation Reserve Program (CRP), and other habitat planting efforts require millions of native seedlings and the expenditure of millions of dollars on sites that are highly disturbed or contaminated from activities such as mining, agriculture, industrial activities, or forest fires (OSMRE 1977; USDA 1997; Robichaud and others 2000; personal communication). Project managers, consequently, are seeking ways to increase plant survival and growth in order to achieve program goals while reducing the cost per surviving seedling.

Ecologists have identified beneficial AM effects such as increased outplanting survival, enhanced seedling growth, increased growth response under nutrient stress, and increased tolerance to drought stress (Allen 1991; Bagyaraj 1992; Killham 1994; Shetty and others 1994, Steinfield and Amaranthus 2003). Increased delivery of ammonium nitrate to plants via mycorrhizal fungi has been documented by others (Marschner and Dell 1994; Jin and others 2005; Tanaka and Yano 2005) and may have a substantial influence on the fitness of outplanted seedlings.

Applied researchers, in turn, have attempted to develop techniques for isolating, bulking, and inoculating nursery-grown seedlings with AM fungi (Biermann and Linderman 1983a; St. John 1992; Brundrett and others 1996; Habte and Osorio 2001). The result has been a nascent biotechnical industry that brings to the restoration marketplace a variety of inoculums with varying species mixes, propagule types, and recommended techniques for introducing the fungi to root surfaces.

Colonization under a nursery environment is influenced by a broad range of nursery cultural practices, such as inocula source, growth media type and chemistry, fertilization practices, and disease control techniques (Biermann and Linderman 1983b; Gianinazzi and others 1990; Corkidi and others 2004). Thus, inoculation may not result in adequately-colonized plant materials if cultural practices are not adjusted to promote the formation of AM. Therefore, inoculation may not result in discernable benefits in the field upon outplanting.

The purpose of this study was to produce AM plant material under contrasting nursery fertilization regimes and to evaluate the field performance of resulting seedling stock. Tree, shrub, and legume seedlings were inoculated and grown under a traditional fertigation regime as well as an alternative slow-release fertilization regime. We subsequently conducted field trials that compared plant survival and growth of outplanted seedlings. We hypothesized that a slow-release fertilization regime would increase colonization rates of seedlings and result in greater survival and growth than seedlings grown under traditional fertigation following outplanting. The results provide restoration practitioners with the ability to evaluate AM inoculum as a specification in planning and they provide horticulturists an improved technique for producing colonized plant material.

Materials & Methods

Field Trial Sites

Field trials were established with cooperators whose sites represented a broad range of precipitation, soil conditions, and plant species. Field trials were established at: 1) Bureau of Land Management's Cascade-Siskiyou National Monument (BLM) in Oregon on an abandoned logging landing; 2) Montana State University-Western Agricultural Research Center (WARC) on agricultural land near Corvallis, Montana; and 3) Rio Tinto's Kennecott Utah Copper Concentrator (KUCC) on waste rock material near Magna, Utah. Treatment plant materials were provided to each cooperator who, in turn, installed and monitored the sites. Thus, the general layout and monitoring conducted at each field trial site varied.

Plant Material Preparation

Plant materials for all sites were prepared in a common manner, but were not necessarily grown during the same production cycle. The species evaluated for BLM were: *Amorpha canescens, Dasiphora fruticosa, Juniperus scopulorum, Prunus pumila L. var. besseyi (*hereafter, *P. pumila),* and *Ribes aureum*. Species evaluated for WARC were: *Artemisia canescens, Dasiphora fruticosa, Prunus virginiana, Rosa woodsii,* and *Rhus trilobata.* Species evaluated at KUCC were: *Amelanchier alnifolia*, *Cercocarpus ledifolius, Populus tremuloides,* and *P. virginiana*.

The treatments consisted of inoculated plant material grown under: 1) Traditional Fertilization Regime (TFR) consisting of fertilizers (100 ppm NO_3/20ppm P/90 ppm K, pH 5.9) applied on a minimum weekly basis; and 2) Slow-Release Fertilization Regime + Inoculum

(SRR+ Inoculum) which augmented growth media with the slow-release fertilizer; and 3) Slow-Release Fertilization Regime (SRR) treatment which excluded inoculum (this treatment was implemented on KUCC and WARC sites only). All growth media (Sun Gro Horticulture Canada, Bellevue, Washington: 60% sphagnum peat, 20% perlite, 20% vermiculite) was prepared by hand-mixing MycoApply® Endo (Mycorrhizal Applications, Inc., Grants Pass, Oregon) containing *Glomus intraradices* either at 4 g L^{-1} or 8 g L^{-1} prior to the appropriate treatments. Growth media was placed into 10-cubic inch Ray Leach containers, seeded, and trays placed on a common table, but separated by fertilizer type to accommodate fertilization. Trays were rotated on a weekly basis to minimize local greenhouse effects. All plant material was grown under controlled greenhouse conditions for a period of 12-weeks prior to being transferred to an outside growing facility for a minimum period of two weeks before outplanting.

Prior to outplanting, a subsample of plants from the 2006 growth cycle was destructively sampled to determine mycorrhizal colonization rates per procedures described by Brundrett and others (1996). In addition, caliper (diameter at root collar) was measured on 10 plants of each species treatment to the nearest 0.1 mm. Chlorophyll nitrogen (N) index was measured to investigate an observed difference in leaf color between TFR and SRR plants. A Minolta SPAD 502 Meter (Minolta 2005) was used to determine an indexed chlorophyll N content. A total of five mature leaves were randomly selected from each species-treatment and the SPAD meter placed to avoid leaf margins, midribs, or veins. High SPAD values are indicative of improved plant health due to greater N accumulation. SPAD values were not collected from *J. scopulorum* due to their small leaf size.

Field Trial Design

The general layout of field trial plots varied between sites. All plots were designed in coordination with the site cooperators, thus, they varied in dimension, number of plants per treatment, and other factors (Table 1). All experimental designs, however, consisted of a randomized complete blocks with mycorrhizal and non-mycorrhizal treatments. Plots were all hand-planted with hoedad or similar planting tool.

Data Collection and Analysis

Data collection and analysis also varied by field trial site due to budgetary and time limitations of the cooperator. For the BLM field trial, treatments were tested by Analysis of

Variance and means separated by Tukey honestly significance difference (HSD) ($p<0.05$). For the WARC and KUCC field trials, simple means and standard deviations were calculated.

Table 1. Overview of field trial sites and methods.

Site Name	BLM	WARC	KUCC
Year of Establishment	Fall 2005	Fall 2006	Spring 2007
Treatments	TFR, SRR	TFR, SRR, SRR + Inoculum	TFR, SRR, SRR + Inoculum
Plot Type	Randomized Complete Block	Randomized Complete Block	Randomized Complete Block
Repetitions (plants/repetition)	4 (5)	6 (10)	8 (10)
Data Collected	Root Length Colonized Chlorophyll Index, Survival , Height, Caliper	Survival, Height, Caliper	Survival, Height
Analysis	Oneway Analysis of Variance (ANOVA)	Means/Standard Deviations	Means/Standard Deviations

Results & Discussion

Greenhouse Mycorrhizal Verification

Proportion of Plants Colonized. Proportion of plant colonized represents the percentage of plants evaluated on which mycorrhizal structures occurred. In all occurrences, the SRR + Inoculum produced a greater proportion of colonized plants than the TFR (Table 2). The SRR + Inoculum ranged from 16 to 92% of plants exhibiting mycorrhizal structures compared to 0 to 20% for the TFR by species. *Amorpha canescens, D. fruticosa,* and *P. pumila* treated with SRR + Inoculum produced a significantly higher proportion of colonized plant than their TFR counterparts. *Ribes aureum* demonstrated a trend toward a higher proportion of plant colonized in SRR + Inoculum, but was not signicantly different.

Root Length Colonized. Root Length Colonized (RLC) is a measure of the intensity of colonization and represents the percent of root segments in which mycorrhizal structures were

located. The SRR + Inoculum produced statistically higher levels of colonization than the TFR in all occurrences with exception to *A. canescens* (Table 2). The SRR + Inoculum ranged from 7 to 51% RLC in contrast to 0 to 3% for the TFR. *Dasiphora fruticosa, P. pumila,* and *R. aureum* treated with SRR + Inoculum were significantly higher than their TFR counterparts. *Amorpha canescens* demonstrated a trend toward a higher RLC in SRR + Inoculum , but was not significantly different.

Table 2. Colonization characteristics of plants grown under two contrasting fertilization regimes at BLM field trial site (N=25).[zyx]

Species	Treatment	Proportion Colonized (%)	Root Length Colonized (%)
Amorpha canescens			
	Traditional Fertigation	0a (0.0)	0a (0.5)
	Slow-Release Fertilizer + Inoculum	16b (7.5)	7.0a (3.3)
Dasiphora fruticosa			
	Traditional Fertigation	20a (8.9)	3.8a (3.1)
	Slow-Release Fertilizer + Inoculum	92b (4.9)	51.4b (5.1)
Juniperus scopulorum			
	Traditional Fertigation	ND	ND
	Slow-Release Fertilizer + Inoculum	ND	ND
Prunus pumila			
	Traditional Fertigation	4a (4.0)	0.2a (1.3)
	Slow-Release Fertilizer + Inoculum	56b (14.7)	30.5b (6.1)
Ribes aureum			
	Traditional Fertigation	20a (0.0)	1.3a (0.7)
	Slow-Release Fertilizer + Inoculum	36a (9.8)	17.0b (5.3)

[z]Means with different letters within a column are significantly different ($p<=0.05$) as determined by oneway ANOVA and Tukey HSD. [y]Standard error for means in parentheses. [x]Data for Proportion Colonized (%) transformed by arc-sine square root prior to analysis. Actual values as percentage are displayed. ND= No data.

Pre-Installation Chlorophyll N Index, Height, and Caliper. A significantly higher chlorophyll N index was observed in the SRR + Inoculum treatment for all species ($p<0.05$) (Table 3).

Chlorophyll N index values ranged from 25 to 64% higher in the SRR. The only legume in the study, *Amorpha canescens*, demonstrated the greatest differential between its TFR counterpart followed by *P. pumila*, *D. fruticosa*, and *R. aureum* in decreasing order. Mean height and caliper were not significant between treatments with exception to *R. aureum*. Regarding *R. aureum*, TFR plants were significantly taller with greater caliper than their SRR + Inoculum counterparts.

Table 3. Similarity of chlorophyll index, height, and caliper in fertilization regime treatments prior to outplanting at BLM field trial site (N=4).[xzy]

Species	Treatment	Chlorophyll Index	Height (cm)	Caliper (mm)
Amorpha canescens				
	Traditional Fertigation	17.2a (0.82)	9.9a (0.53)	1.9a (0.00)
	Slow-Release Fertilizer + Inoculum	28.2b (0.90)	11.7a (0.80)	2.0a (0.10)
Dasiphora fruticosa				
	Traditional Fertigation	30.6a (1.96)	21.1a (1.56)	3.3a (0.21)
	Slow-Release Fertilizer + Inoculum	43.0b (1.10)	20.3a (0.90)	3.4a (0.16)
Juniperus scopulorum				
	Traditional Fertigation	ND	11.1a (0.64)	3.6a (0.37)
	Slow-Release Fertilizer + Inoculum	ND	9.9a (0.80)	2.7a (0.26)
Prunus pumila				
	Traditional Fertigation	31.3a (0.85)	25.6a (0.61)	3.8a (0.13)
	Slow-Release Fertilizer + Inoculum	47.7b (0.73)	23.2a (1.05)	3.4a (0.16)
Ribes aureum				
	Traditional Fertigation	28.2a (0.65)	6.1a (0.59)	5.6a (0.16)
	Slow-Release Fertilizer + Inoculum	35.2b (1.92)	4.4b (0.50)	4.6b (0.16)

[z]Means with different letters within a column are significantly different ($p<=0.05$) as determined by oneway ANOVA and Tukey HSD. [y]Standard error are in parentheses. [x]Chlorophyll index N=5, Height N=10, Caliper N=10. ND= No data.

BLM Field Trial Results

Survival. Survival was monitored after one growing season following outplanting. Overall, SRR + Inoculum plants survived at significantly ($p<0.0001$) higher levels than the TFR plants (Table 4). The SRR + Inoculum plants averaged 79% survival while the TFR plants averaged

38% survival. The most substantial increases in survival were seen in *A. canescens*, *J. scopulorum*, and *R. aureum* with SRR + Inoculum plants surviving at more than double the rate of Traditional Fertigation. *Amorpha canescens* survival in the SRR + Inoculum was 85% compared to 20% for TFR. SRR + Inoculum *J. scopulorum* (95%) and *R. aureum* (70%) similarly survived at much higher rates than their fertigated counterparts at 40% and 30% respectively. Although they attained the highest RLC rates, SRR + Inoculum *D. fruticosa* (65%) and *P. pumila* (85%), although they attained the highest RLC rates, increased to a lesser but significant survival rate above TFR rates of 40% and 60% respectively.

Growth. All living plants were remeasured following one year of growth (Table 4). Caliper ($p=0.096$) and height ($p=0.097$) were not significantly different between TFR and SRR + Inoculum plants.

WARC Field Trial Results

Survival. Survival was monitored after one growing season following outplanting. Survival between treatments was similar with all species and treatments achieving 100% survival with exception to *R. trilobata* TFR and SRR which achieved 93% survival and *A. cana* SRR + inoculum which achieved 93% survival.

Growth. Growth parameters of caliper, height, and total wet weight were measured through destructive sampling after one growing season (Table 5). Substantial differences in caliper, height, and total wet weight were observed in all species with exception to *A. cana*. The greatest differential occurred in *R. trilobata* in which the SRR + Inoculum treatment produced 202 grams of mass compared to 30 grams of mass in the TFR treatment. *Prunus virginana* in the SRR + Inoculum treatment was more than double (271 g versus 96 g) than the TFR treatment Fig. 1 and 2). *Artemisia cana* contradicted this trend with a slight reduction in caliper, height, and total weight when SRR + Inoculum were compared with TFR treatments. This could be the result of *A. cana* allocating resources acquired from colonization disproportionately to roots or that this species does not readily form mycorrhiza with the fungal species utilized. Mycorrhizal structures have not been observed on the *Artemisia* genus during greenhouse production (personal observation), but are commonly reported on native collected samples.

Table 4. Survival, height, and caliper characteristics of plants grown under two contrasting fertilization regimes following one growing season at BLM site.[zy]

Species	Treatment	Survival (%)	Height (cm)	Caliper (mm)
Amorpha canescens				
	Traditional Fertigation	20a (9.2)	9.4a (0.55)	2.7a (0.42)
	Slow-Release Fertilizer + Inoculum	85b (8.2)	13.1a (0.71)	2.9a (0.26)
Dasiphora fructicosa				
	Traditional Fertigation	40a (11.2)	26.5a (1.24)	4.6a (0.26)
	Slow-Release Fertilizer + Inoculum	65b (10.9)	26.2a (0.86)	5.4a (0.18)
Juniperus scopulorum				
	Traditional Fertigation	40a (11.2)	13.4a (0.60)	5.5a (0.38)
	Slow-Release Fertilizer + Inoculum	95b (5.0)	11.6a (0.63)	4.4a (0.27)
Prunus pumila				
	Traditional Fertigation	60a (11.2)	29.0a (1.54)	5.4a (0.26)
	Slow-Release Fertilizer + Inoculum	80b (9.2)	28.2a (1.86)	5.1a (0.41)
Ribes aureum				
	Traditional Fertigation	30a (10.5)	28.7a (5.80)	7.2a (0.54)
	Slow-Release Fertilizer + Inoculum	70b (10.5)	34.2a (2.31)	5.5a (0.42)

[z]Means with different letters within a column are significantly different ($p<=0.05$) as determined by oneway ANOVA. [y]Standard error are in parentheses.

Table 5. Caliper, height and total wet weight of plants grown under three contrasting fertilizer regimes following one growing season at WARC field trial site (N=30).[z]

Species	Treatment	Caliper (mm)	Height (cm)	Total Weight (g)
Artemisia cana				
	Traditional Fertigation	29.9 (5.0)	68.8 (11.9)	1108 (380)
	Slow-Release Fertilizer	28.9 (7.0)	61.9 (13.7)	979 (432)
	Slow-Release Fertilizer + Inoculum	28.6 (7.8)	64.0 (13.7)	963 (516)
Dasiphora fruticosa				
	Traditional Fertigation	14.1 (4.0)	42.1 (9.2)	203 (111)
	Slow-Release Fertilizer	16.4 (3.7)	45.6 (6.8)	243 (85)
	Slow-Release Fertilizer + Inoculum	20.0 (4.0)	48.1 (6.8)	336 (109)
Prunus virginana				
	Traditional Fertigation	8.9 (2.5)	50.2 (21.7)	96 (61)
	Slow-Release Fertilizer	8.0 (2.6)	38.3 (22.4)	69 (69)
	Slow-Release Fertilizer + Inoculum	13.4 (2.7)	82.9 (25.5)	271 (136)
Rhus trilobata				
	Traditional Fertigation	6.7 (2.4)	30.0 (18.2)	30 (29)
	Slow-Release Fertilizer	10.1 (2.8)	54.5 (14.6)	102 (55)
	Slow-Release Fertilizer + Inoculum	14.4 (2.9)	74.2 (17.0)	202 (90)
Rosa woodsii				
	Traditional Fertigation	11.4 (4.9)	50.4 (28.0)	144 (166)
	Slow-Release Fertilizer	13.8 (3.4)	57.0 (20.5)	191 (102)
	Slow-Release Fertilizer + Inoculum	16.6 (4.2)	68.7 (21.0)	259 (156)

[z]Means with standard deviation in parentheses.

Figure 1. *P. virginiana* treatment at WARC.

Figure 2. *P. virginiana* with inoculum at WARC.

KUCC Field Trial Results

Survival. Survival was monitored two years after outplanting (Table 6). Overall, survival of seedlings was high at this field trial site with exception to *A. alnifolia* (Fig. 1). *Populus tremuloides* achieved the highest survival with no loss of plants (100% survival in TFR and SRR treatments; 99% survival in SRR + Inoculum treatment). *Prunus virginana* survival rates were similar between the TFR (85%) and SRR (84%) treatments with a minor loss of plants in the SRR + Inoculum (77%) treatment (Fig. 3 and 4). Several *C. ledifolius* seedlings noted as dead in 2007 were resprouting resulting in increased survival rates for SRR + Inoculum (65%) and SRR (81%) treatments with the TFR (75%) remaining similar. *Amelanchier alnifolia* survival remains almost identical to 2007 with the TFR (50%) surviving at significantly higher levels than the SRR + Inoculum (30%) and SRR (20%) treatments. Again, the *A. alnifolia* had individual plants that were assumed dead in 2007, but resprouted in 2008.

Growth Caliper and height were not substantially different between treatments. Growth factors noted, but not measured were leaf area and stem caliper. Despite negligible increases in height, leaf area and caliper appeared to have increased in the SRR + Inoculum as evidenced by a comparison of photodocumentation.

Table 6. Survival and height of plants grown under three contrasting fertilizer regimes following one growing season at KUCC field trial site (N=8).[z]

Species	Treatment	Survival (%)	Height (cm)
Amelanchier alnifolia			
	Traditional Fertigation	50 (50)	19.3 (4.9)
	Slow-Release Fertilizer	20 (40)	17.0 (5.5)
	Slow-Release Fertilizer + Inoculum	30 (46)	18.2 (5.6)
Cercocarpus ledifolius			
	Traditional Fertigation	75 (44)	10.7 (4.6)
	Slow-Release Fertilizer	81 (40)	14.3 (6.0)
	Slow-Release Fertilizer + Inoculum	65 (48)	13.5 (6.2)
Populus tremuloides			
	Traditional Fertigation	100 (0)	26.4 (5.0)
	Slow-Release Fertilizer	100 (0)	29.4 (5.7)
	Slow-Release Fertilizer + Inoculum	99 (11)	32.4 (7.5)
Prunus virginana			
	Traditional Fertigation	85 (37)	13.9 (5.4)
	Slow-Release Fertilizer	84 (37)	14.8 (5.0)
	Slow-Release Fertilizer + Inoculum	77 (43)	15.2 (6.2)

[z]Means with standard deviation in parentheses.

Figure 3. *P. Virginiana* control treatment at KUCC.

Figure 4. *P. virginiana* with inoculum at KUCC.

Discussion

Growth increases varied between field trial sites with the WARC site demonstrating substantial increases in biomass for the inoculated plant material while the BLM and KUCC did not. The primary difference between these three sites likely relates to initial soil fertility. Although none of the sites received fertilization prior to planting, the WARC site was former agricultural land with original topsoil and the capacity to support successional stands of vegetation. In contrast, the BLM and KUCC sites consisted primarily of waste rock or parent material in which organic matter and nutrients had been largely removed. The compensation for harboring mycorrhizal fungi under nursery conditions is the improved supply of mineral nutrients of low mobility in the soil, such as phosphorus, as well as greater access to water through the external mycelium. These nutrients, however, must be present at a critical level to confer those benefits. Our interpretation of the data is that the WARC site demonstrates that inoculated plants have much higher potential for growth given sufficient nutrients for growth. With modest levels of nutrients present on the degraded sites, greater growth benefits would be anticipated. In contrast, excessive depletion of nutrients and moisture at the KUCC site resulted in a lack of growth benefits. Another interpretation is that the fungal species used within the inoculum were not adapted to the edaphic conditions of the more disturbed soils. While AM fungi are not necessarily host specific to plant species, research has demonstrated some specificity to the soil environment (Abbot and Robson 1982).

Survival rates also varied between the BLM and the WARC and KUCC sites. The BLM field trial demonstrated a substantial survival increase for the SRR + Inoculum treatment over TFR. In contrast, no substantial differences in survival were observed between mycorrhizal and non-mycorrhizal treatments at the WARC and KUCC sites. Increased survival rates were likely related to the broad range of benefits previously recognized in general studies of mycorrhiza ecology, such as greater access to phosphorus and nitrogen resources, increased drought tolerance, increased disease resistance, among others (Allen 1991; Killham 1994; Shetty and others 1994, Steinfield and Amaranthus 2003). The lack of difference at other sites may be related to factors such as better production practices prior to outplanting or site factors more conducive to survival following outplanting.

Conclusions

The fundamental questions to be answered by this research were whether mycorrhizal inoculum can be successfully introduced into a nursery environment and whether it confers survival and growth benefits to seedlings upon outplanting. Our study demonstrates that contrasting fertilization regimes utilized during greenhouse production can have dramatic effects on mycorrhizal colonization rates, chlorophyll N index, and plant performance. This study emphasizes the need to consider greenhouse fertilization procedures in order to effectively produce AM-colonized plant materials. Our field data indicates there is strong potential for mycorrhizal inoculation to increase growth and survival following outplanting of seedlings. Factors such as initial soil fertility, however, may strongly influence these benefits. Mycorrhizal colonization level as a measure of fitness reflects the potential for survival and growth of outplanted seedlings on harsh planting sites.

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