Importance of Indigenious VAM Fungi for the Reclamation of Coal Refuse Piles[1]

by

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Abstract. During the initial phases of vegetation establishment on drastically disturbed soils, vesicular-arbuscular mycorrhiza (VAM) can enhance plant establishment and growth. A similar beneficial response would be very advantageous in efforts to reclaim coal refuse piles. Coal refuse, a by-product of eastern coal cleaning plants, presents a particularly difficult problem for reclamationists. In preliminary field trials conducted in Mercer Co. WV, results indicated that survival of containerized white clover (Trifolium repens) was enhanced after outplanting when VAM colonized plant roots. Roots extending into the coal refuse were found to be devoid of VAM. A subsequent growth chamber study was undertaken to evaluate various white clover-VAM combinations to increased concentrations of coal refuse in plant growth media. Five combinations of coal refuse and soil were used. Treatments included two VAM species and a non-VAM control. After 60 days of growth a significant increase in root and shoot dry weights was observed in VAM colonized seedlings. As the percentage of coal refuse in the growth media increased, those plants colonized by an indigenous VAM species had improved S:R ratios, leaf water relations and leaf areas. Tissue concentration of P, K, Ca and Mg were higher for VAM colonized seedling, with indigenous VAM infections providing increases as the percentage of coal refuse in the growth media increased. These data suggest that VAM do enhance plant growth and establishment in coal refuse; however ecotypic variations in VAM must be studied further.

Additional Key Words: vesicular-arbuscular, mycorrhiza, coal refuse, reclamation.

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Introduction

Coal refuse piles present a unique environment for reclamation efforts. Modern coal cleaning plants produce, as a by-product, thousands of tons of coal refuse annually. This material consists primarily of rock inclusions and other contaminating, often phytotoxic materials that are removed from coal before it is burned. Fragments ranging from 20 cm in diameter to sand-sized and smaller are separated from the coal using various sedimentation processes. After separation, the refuse material is transported to a permanent disposal site. As a result of the inherent mineralogical variability in coal seams and the different compounds used in the cleaning processes, coal refuse

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exhibits a wide range of chemical characteristics. Such materials often represent an environmental hazard because of toxic substances that can develop in and leach from them. Recently enacted laws (Surface Mining Control Act of 1977) require that all permanent refuse disposal sites be vegetated with a permanent self-sustaining plant cover.

The inherent low fertility and recent deposition of coal refuse presents a set of growth-limiting factors similar to those encountered by pioneer plant species. Advancing on this premise, several studies (Schramm 1966; Daft and Hacskaylo 1976; Khan 1978) have been conducted to determine possible adaptations by successful colonizing species to overcome the stresses presented.

Schramm (1966) concluded that plants successfully colonizing bare, nutrient-deficient coal wastes are generally nitrogen-fixing, mycorrhizal, or both. A majority of plants growing on coal refuse piles in Scotland (Daft and Hacskylo 1974) and Australia (Khan 1978) was found to be infected by mycorrhiza-forming fungi.

The benefit to the "host plant" in a mycorrhizal association is widely believed to be due to improved phosphorus nutrition of the host (Daft and Hacskylo 1976; Lambert and Cole 1980). The possible alteration of other physiological processes that would enhance colonization of stressful sites has also been suggested (Schramm, 1966; Khan 1978).

A major purpose of the studies presented in this work was to examine the possible links between successful plant colonization of coal-refuse piles and the presence of vesicular-arbuscular mycorrhizae (VAM). In many situations, the presence of VAM does not confer an advantage to the host plant (Hetrick et al. 1984); i.e., VAM infection cannot always be viewed as beneficial. While the VAM-host-plant interaction may be highly integrated both structurally and physiologically, better growth of the host is not automatically a consequence of the relationship. Host-plant responses vary widely when conditions of nutrition, soil, and other environmental factors are changed. Isolates (ecotypes) of the same VAM species collected at locations with differing rainfall patterns have been shown to have substantially different effects on host-plant water relations (Stahl and Smith 1986). Especially at the species level, the presumption that differing host-plant responses would occur seems valid.

The ability of a VAM association to enhance host plant growth has been called symbiotic efficiency (Smith and Gianinazzi-Pearson 1988). Several growth parameters have been used to quantify the symbiotic efficiency of a VAM isolate with a particular host species. In situations of environmental stress good indicators of symbiotic efficiency are relative growth rate and root:shoot ratio (Smith and Gianinazzi-Pearson 1988). Under nutritional stress, possible determinants of symbiotic efficiency of a VAM isolate or species are host nutrient uptake and deployment as measured by tissue analysis (Smith and Gianinazzi-Pearson 1988).

A central (implicit) hypothesis in these studies is that VAM species isolated from environments most closely matching the experimental conditions of a study, will exhibit a higher degree of symbiotic efficiency. Commonly, VAM isolates are selected for their ability to promote growth under non-stressful conditions. By selecting ecotypes that have evolved on or near refuse piles the possibility of finding a VAM-producing fungus that will enhance its hosts tolerance of these stresses is much improved.

Experimental Methods

Seedling Establishment

White clover (*Trifolium repens*) seedlings were established in plastic cone-tainers that had been filled with various mixtures of soil and coal refuse. The soil, a Groseclose silt loam, was mixed with coal refuse to achieve 0, 25, 50, 75 or 100 % coal refuse content. Nutrient status and pH for the five soil:refuse combinations is shown in Tables 1 and 2. Prior to mixing, soil was fumigated with methyl bromide and thoroughly aerated. Coal refuse samples taken from several seams in the Pocahontas formation of West Virginia were used after being autoclaved at 180°C for 30 minutes, allowed to cool for 18 hour and re-autoclaved. In preparation for this experiment, the coal refuse was treated with lime at 2 Mg/ha 18 months prior to experimentation.

At planting, soil preinoculated with either *Glomus etunicatium* (Ge) or *Glomus sp.* (Gs) or filtrate from sievings of the two spore cultures (none) was added to each cone-tainer. After seedling emergence, but prior to the appearance of the first trifoliolate leaf, plants were thinned to one per pot.

Table 1. Chemical characteristics of soil: coal refuse mixtures.

Growth Media	pH	E.C.
% Refuse		dS/cm
0	5.6	0.22
25	6.8	0.23
50	7.3	0.34
75	7.4	0.42
100	7.4	0.69

Table 2. Plant available nutrient content of soil:coal refuse mixtures.

Growth Media	NH4OAC Extractable P	K	Ca
% Refuse	mg/kg		
0	3.8	47.4	216.0
25	4.8	46.8	412.0
50	7.6	57.4	648.0
75	7.4	64.4	777.6
100	9.4	68.2	871.2

Plants were grown in a growth chamber under a 14 h daylength, temperatures of 25° C day /18oC night and relative humidities of 70 to 80 %. All plants were irrigated with 5 ml of 50% Hoaglands nutrient solution minus P - containing salts (Hoagland and Arnon, 1950) daily. At biweekly intervals, all mycorrhizal treatments were fertilized with 10 ml of 50% Hoaglands nutrient solution with P salts added, non-mycorhizal treatments received 10 ml weekly.

Water Relations Studies

When the plants were 55 days old, all pots were brought to field capacity and wrapped to prevent evaporative water loss, and water was withheld for five days. Relative water content (RWC)(Matin et al. 1989) was determined for an excised leaf at day five. Determination of RWC was performed using the formula:

$$RWC = [(FW - DW)/(TW - DW)]*100$$

where FW = fresh weight
DW = dry weight
TW = turgid weight (after rehydration in distilled water).

Post-Harvest Measurements

Plants were removed from all pots 60 days after planting, and the soil:refuse materials were washed from roots. Root length was estimated using the line intersect method (Tennant 1975). Roots and shoots were dried separately at 70 C for 18 hours and weighed. Rehydrated roots were stained (Kormanik et al. 1980) and the VAM colonization assessed by a modified line-intersect method (Biermann and Lindemann 1981). Concentrations of P in dried leaves was determined using inductive coupled plasma spectroscopy (Donahue 1986). Statistical analysis was conducted using a split-plot design. Data compilation was performed using GLM (General Linear Models) procedure in SAS (SAS Institute, Inc. 1985).

Results

When compared to the non-VAM control, total biomass production of white clover (Tables 3 and 4) was enhanced by the presence of VAM at all soil:refuse combinations. Top growth was particularly enhanced by VAM. The isolate Ge, a ubiquitous mycorrhizal species, produced higher shoot dry weights at 0 and 25% mixtures of coal refuse. Shoot biomass was greater with Gs when coal refuse contents were greater than 50%. Root biomass production was enhanced with VAM infection at higher coal refuse contents. In general, root biomass was greater in Gs infected plants where coal refuse was added to the growth medium.

Table 3. Root production as influenced by various soil refuse mixtures and two mycorrhiza (Glomus etunicatum (Ge) and Glomus sp. (Gs)).

Growth Media	VAM Symbiont Ge	Gs	None	LSD (0.05)
% Refuse	dry wt.(g)			
0	0.92	0.59	0.45	0.91
25	1.09	1.50	0.61	1.34
50	0.75	2.38	0.47	0.48
75	0.60	0.74	0.55	0.48
100	0.46	0.62	0.44	0.39
LSD (0.05)	0.96	0.96	0.36	

With increasing content of coal refuse in the growth medium, VAM-infected plants responded differently in the partitioning of dry matter (Table 5). A decrease in root production by Gs-infected plants at 75 and 100% refuse shifted the shoot:root

Table 4. Shoot production as influenced by various soil refuse mixtures and two mycorrhiza (Glomus etunicatum (Ge) and Glomus sp. (Gs)).

Growth Media	VAM Symbiont Ge	Gs	None	LSD (0.05)
% Refuse	dry wt.(g)			
0	1.49	3.38	0.85	2.92
25	1.93	4.96	1.06	2.89
50	3.52	3.39	0.85	1.91
75	3.97	2.68	0.84	2.64
100	3.01	1.87	0.50	1.84
LSD (0.05)	2.52	3.19	0.64	

ratio sharply to favor shoot production. While the partitioning ratio for Ge-infected plants was unchanged with refuse additions, total biomass declined.

Table 5. Shoot:root ratio based on the dry weight of plants grown in various soil refuse mixtures and two mycorrhiza (Glomus etunicatum (Ge) and Glomus sp. (Gs)).

Growth Media	VAM Symbiont			LSD
	Ge	Gs	None	(0.05)
% Refuse	----- S:R ratio -----			
0	2.55	5.15	3.91	5.18
25	2.09	4.96	2.03	1.62
50	1.50	4.51	1.64	2.01
75	5.69	4.59	1.51	1.53
100	4.41	2.89	1.89	2.57
LSD (0.05)	1.81	3.55	2.73	

The RWC for all treatment combinations (Table 6) did not follow a consistant trend. VAM-infected plants had moderately better RWC as the refuse content increased to 50, 75 and 100 %. While several of the non-VAM treatments exhibited RWC below 90 % none were oberserved to have lost turgidity.

Vesicular-arbuscular mycorrhiza increased white clover tissue P concentrations at all levels of refuse addition (Table 7). Increased concentrations of coal refuse in the media did not affect the P concentration in the plant tissue.

Table 6. Relative water content of plants grown in various soil refuse mixtures and inoculated with two mycorrhiza (Glomus etunicatum (Ge) and Glomus sp. (Gs)).

Growth Media	VAM Symbiont			LSD
	Ge	Gs	None	(0.05)
% Refuse	------ % Water -------			
0	92.5	87.5	94.2	6.9
25	89.9	91.8	93.1	4.1
50	92.7	95.4	89.9	2.9
75	93.4	95.0	84.8	3.9
100	89.9	92.3	88.4	6.3
LSD (0.05)	4.4	4.0	5.9	

Table 7. Tissue P concentrations of plants grown in various soil refuse mixtures and inoculated with two mycorrhiza (Glomus etunicatum (Ge) and Glomus sp. (Gs)).

Growth Media	VAM Symbiont		
	Ge	Gs	None
% Refuse	--------- % ----------		
0	0.18	0.21	0.12
25	0.25	0.17	0.09
50	0.25	0.17	0.10
75	0.22	0.16	0.08
100	0.19	0.17	0.09
Mean	0.21	0.16	0.09
LSD (0.05)	0.04		

Discussion

Vesicular-arbuscular mycorrhiza enhanced white clover growth in coal refuse. Mycorrhizal plants are often observed to have dramatic increases in dry matter production (Smith and Gianinazzi-Pearson 1988). In this experiment interesting trends in dry-matter production and allocation developed as the content of coal refuse increased in the growth medium. As content increased to 50%, the amount and proportion of dry matter devoted to roots increased sharply in Gs infected plants, while little or no increase was observed for the Ge-infected plants. At the same time, shoot biomass increased in the Ge-infected plants from 0 to 25% coal refuse and declined with additional refuse inputs. Conversely, as refuse concentrations increased Gs-infected plants produced more shoot biomass. Infection with the Gs isolate appeared to enable the host plant to allocate more resources to shoot production. Increased shoot production with reduced root production indicates an increase in root efficiency.

Dry matter allocation is best illustrated as root:shoot ratios. Across all coal refuse combinations Ge infected plants maintained a stable S:R ratio. An overall reduction in root and shoot dry matter production was responsible for this stability. Root:shoot ratios for Gs-infected plants shifted sharply to favor shoot production at high concentrations of coal refuse, but were similar to non-VAM plants at lower concentrations (0, 25 and 50%). At some point beyond 50% refuse content Gs-infected plant roots became more successful in maintaining sufficient metabolic processes to support increased shoot production.

Both VAM producing species were able to support higher P and plant water status at all soil:refuse combinations. Increases in P uptake have been linked to several parameters involved in the maintenance of good plant water relations (Smith and Gianinazzi-Pearson 1988). Plants infected with the Gs isolate had a generally higher RWC the higher refuse concentrations with a reduction in dry matter allocation to roots. While the leaf RWC was adequate to maintain turgor in the non-VAM plants, growth may have been limited by reductions in RWC as the concentration increased.

While the interactions between the Gs-isolate and higher coal refuse concentration are quite striking in these experiments, changes in the physical and chemical characteristics of the growth media may account for some of the observed results. As the concentration of refuse increased, both the pH and the level of soluble salts increased. These changes are a probable result of the addition of $CaCO_3$ (lime) prior to this study. Changes in the texture and bulk density of the growth media also occurred as the more coarse textured coal refuse was added to the finer textured soil.

Summary

VAM infection enhanced white clover growth in coal refuse. Two VAM isolates produced different dry matter allocation patterns. An indigenous VAM isolate maintained a higher P and water status. Improvements in root uptake efficiency are likely responsible for better growth of white clover in coal refuse. Selection of a VAM isolate from a stressful site may have merit for use in improving plant establishment on coal refuse piles.

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