Mycorrhizae, Survival and Growth of Selected Woody Plant Species in Lignite Overburden in Texas

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ABSTRACT


Seedlings of live oak (Quercus virginiana (Mill.)), Chinese tallow tree (Sapium sebiferum (L.) Roxb.), and Texas mountain laurel (Sophora secundiflora (Ort.) Lag.) were inoculated with either ectomycorrhizal fungi (Pisolithus tinctorius (Mich. ex Pers..) Crk. and Couch) or vesicular-arbuscular mycorrhizal (VAM/endoendomyrrhizal) fungi (Glomus fasciculatum (Thaxter) Gerd. & Trappe, Gigaspora margarita (Becker & Hall), and Glomus mossae (Nicol. and Gerd.) Gerd. and Trappe) in a containerized system and transplanted into lignite overburden at two separate mine sites in the Post Oak Savannah region of Texas. Ectomycorrhizal Q. virginiana and endomycorrhizal S. sebiferum exhibited greater growth, and endomycorrhizal S. secundiflora showed greater survival and growth than noninoculated controls. Overburden at one site was low in P, while the second site was moderately high in P; however, root colonization levels of inoculated plants were high at both sites, while non inoculated plants had low levels of colonization. Both ecto- and endomycorrhizal fungi enhanced growth of the three woody species in these nitrogen-deficient overburden sites, independent of overburden P.

INTRODUCTION

In the Pineywoods, Post Oak Savannah and South Texas Plains vegetation regions of Texas, more than one million hectares has been leased for surface mining of lignite coal. Mixed overburden, composed of soil and overburden mixed together during the mining process, is normally deficient in nitrogen, phosphorus and organic matter (OM) (Dixon et al., 1980). These deficiencies of OM and soil nutrition can be corrected with revegetation systems that utilize introduced herbaceous species, such as coastal bermudagrass, that require high fertility inputs for establishment (Hons et al., 1980). There is considerable
interest in incorporating woody plant species into mineland revegetation programs for multiuse programs, such as wildlife support, recreation, aesthetics and greater plant diversity.

Under natural conditions, symbiotic associations between higher plants and mycorrhizal fungi are the rule rather than the exception (Malloch et al., 1980). Mycorrhizae can improve plant survival and growth through enhanced nutrient uptake (Hayman, 1983; Sweatt and Davies, 1984), improved water and temperature relations (Newman and Davies, 1988), increased disease resistance (Ruehle and Marx, 1979), and phytohormone production (Allen et al., 1980). When ecosystems are disturbed by surface mining, there can be drastic changes in the pH, structure, nutrient availability, and mycorrhizal fungus populations (Allen and Allen, 1980; Dixon et al., 1986; Jasper et al., 1987; Reeves et al., 1979). The success of many revegetation programs may depend, in part, on developing methods to introduce beneficial microorganisms, such as mycorrhizal fungi, into disturbed areas where they are absent or in low numbers. The benefits of mycorrhizae in the revegetation of mine spoils have been documented (Daft and Hacskaylo, 1976; Aldon, 1978; Call and McKell, 1982; Call and Davies, 1988). It is desirable to integrate low maintenance woody and herbaceous species and appropriate soil microorganisms to establish and maintain plant communities under low energy inputs of minimal soil fertility and cultivation.

The three woody plant species utilized in this study are low maintenance (minimum energy inputs of fertility, irrigation, cultivation requirements) landscape plants, which are indigenous to the southern United States. *Quercus virginiana* (Mill.) is an ornamental tree in the white oak group, which is also of value as a timber species. *Sophora secundiflora* (Ort.) Lag. is a woody ornamental shrub, which is quite hardy and drought tolerant. We have observed endomycorrhizal, ectomycorrhizal and rhizobium associations with this species (Strong and Davies, 1982). *Sapindus saponaria* (L.) Roxb. is used as a landscape tree, and is also of value as a biomass species; it establishes well on disturbed sites (Davies and McCully, 1986).

The objectives of this study were to determine if woody species inoculated with either endo- or ectomycorrhizal fungi were conditioned for better survival and growth in a minimal input revegetation program at mixed overburden sites in the Post Oak Savannah region of Texas.

SITE DESCRIPTION

The field study sites in the Post Oak Savannah region were located on the Sandow Mine near Rockdale, Texas and the Gibbons Creek Mine at Carlos, Texas which are 152 m and 98 m above sea level, respectively. The two sites were 70 km apart. The climate for the area is subhumid, with a mean annual precipitation of 860 mm for Rockdale and 965 mm for Carlos, TX. The sur-
TABLE 1

Soil nutrient analysis (ppm) of lignite overburden sites at Rockdale and Carlos, TX

<table>
<thead>
<tr>
<th>Site</th>
<th>pH</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>Fe</th>
<th>Zn</th>
<th>Mn</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rockdale</td>
<td>7.2</td>
<td>1</td>
<td>7</td>
<td>150</td>
<td>4000</td>
<td>500</td>
<td>20</td>
<td>2</td>
<td>7</td>
<td>(trace)</td>
</tr>
<tr>
<td>(Sandow Mine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carlos</td>
<td>7.7</td>
<td>1</td>
<td>31</td>
<td>756</td>
<td>4000</td>
<td>500</td>
<td>16</td>
<td>2</td>
<td>10</td>
<td>(trace)</td>
</tr>
<tr>
<td>Gibbons Creek</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rounding Post Oak Savannah vegetation was greatly influenced by cultivation, overgrazing and the exclusion of fire (Call and Davies, 1988). Brush control and good management have shown this area capable of producing abundance of vegetation. Native grass vegetation in the area include bluestem (Andropogon spp.), Indian grass, switchgrass (Panicum spp.) and species of Uniola. The overstory is primarily post oak (Quercus stellata (Wangenh.)), blackjack oak (Quercus marilandica (Muenchh.)) and other brush species (Whiteley and Hossner, 1980). Soils are mostly Alfisols that have loamy to sandy surfaces underlain with cracking clay subsoils.

Following surface mining for lignite, mixed overburden piles were leveled to approximate original contour, fertilized at 25 kg ha⁻¹ N, 9 kg ha⁻¹ P and 19 kg ha⁻¹ K, and seeded with annual rye grass (Lolium perenne). Prior to planting, samples of mixed overburden (25 cm deep) were analyzed for pH (saturated paste), NO₃-N (salicylic-sulfuric acid extraction), and P, K, Mg, and Ca (EDTA-ammonium acetate-hydrochloric acid extraction) (Black, 1965). The two revegetation sites differed in P, which was low (7 ppm) at Rockdale and moderately high (31 ppm) at Carlos (Table 1). At both sites nitrate-N (1 ppm) and Cu were low, while K, Ca, Mg, Fe, Zn, and Mn were high (Table 1).

MATERIALS AND METHODS

Mycorrhizal bioassays and colonization determination

Endomycorrhizal bioassays of mixed overburden taken from the Sandow Mine near Rockdale were conducted with two herbaceous revegetation species: green sprangletop (Leptochloa dubia (H.B.K.) Ness) and side oats 'El Reno' (Bouteloua curtipendula (Michx.)). The two bioassay plants were individually seeded onto mixed overburden in 1-l clay pots. After germination, plants were grown for three months under glasshouse conditions (16 h photoperiod of ambient light supplemented with 5 h incandescent light (20 μmol s⁻¹ m⁻²) for long-day conditions, and 18°C minimum night temperature). Bioassay plants had low colonization levels (0-5%), indicating that soil disturbance contributed to the loss of infectivity and/or number of VA mycorrhizal fungi.
propagules. Percent VAM colonization for bioassays and revegetation test species was determined using the techniques of Phillips and Hayman (1970) and Bevege (1968).

Ectomycorrhizal determination of *Pisolithus tinctorius* (Mich. ex Pers) Crk. and Couch (Pt) was done on *Quercus shumardii* (Buckl.) by subsampling roots and estimating the number of roots infected with Pt. The %Pt development was then calculated (Ruehle and Wells, 1984).

**Culture of mycorrhizal fungi and test plant inoculation**

Starter cultures of endomycorrhizal *Gigaspora margarita*, Becker & Hall, *Glomus fasciculatum* (Thaxt. sensu Gerd.) Gerd. & Trappe, and *Glomus mosseae* (Nicol. & Gerd.) were obtained from R.W. Roncadori (University of Georgia, Athens). We incorporated 50 g of inocula in each pot culture of Sundan (*Sorgum bicolor* (L.) Moench), grown on steam-sterilized 1 sand:1 vermiculite (v/v) medium in a greenhouse under ambient conditions for 69 days. Controls contained medium and roots of uninfected Sundan grass. Pot culture roots and media were chopped into small segments and stored in polyethylene bags under refrigeration at 5°C for 3 weeks prior to use.

Seeds of *Sapium sebiferum* and *Sophora secundiflora* were surface-sterilized with 10% NaClO for 10 min before sowing. The growth medium was steam-sterilized 1 part builders sand:1 part sandy loam soil (Dothan fine sandy loam) amended with 74 g m⁻³ Peters (W.R. Grace Co.) frittered trace elements and either with 0 or 1.2 kg m⁻³ 18N-6P₂O₅-12K₂O Osmocote, a slow release fertilizer (Sierra Chemical Co.). Before the addition of fertilizer, the medium pH was 7.2 with 4 ppm phosphorus. Spencer Lemiare® plastic seedling trays (5.0 cm x 3.8 cm x 17.5 cm) (Edmonton, Canada) were used as containers. *Sapium sebiferum* containers were filled with medium containing either 50 g each (100 g total) of combined *G. fasciculatum* and *G. margarita* inoculum or were left mycorrhizal free as a control medium; mycorrhizal *Sophora secundiflora* was inoculated with *G. fasciculatum* and *G. mosseae* at the rates of 50 g each.

Ectomycorrhizal *P. tinctorius* (IMRD Pt isolate No. 298) was grown under standard procedures with modified Melin–Norkrans medium (Ruehle and Wells, 1984). The Pt isolate used is reported to be effective over a wide range of conditions, including dry sites (D.H. Marx, personal communication, 1989).

Half of the *Q. virginiana* seedlings were inoculated with the ectomycorrhizal fungus, *P. tinctorius* (1 inoculum: 8 medium (v/v)) (Marx and Artmen, 1979; Ruehle and Wells, 1984); controls were left uninoculated. The medium for inoculated and noninoculated *Q. virginiana* treatments contained 1 kg m⁻³ 18N-6P₂O₅-12K₂O Osmocote.

All plants were grown for 4 months under glasshouse conditions (16 h photoperiod of ambient light supplemented with 5 h incandescent light (20 µmol s⁻¹ m⁻²) for long-day conditions, and 18°C minimum night temperature).
Test species were then hardened off outdoors under 50% saran shade cloth for two weeks before transplanting at mine revegetation sites. In the transplanting of the woody species, a completely randomized design was used consisting of 3 woody species × 2 mycorrhizal levels with a minimum of 20 replications (transplants) per treatment. Plants were spaced on 1 m centers and given 1 l of water at the time of planting. Data were recorded for 3 and 2 growing seasons at the Sandow and Gibbons Creek Mines, respectively. Measurements included percent survival, height, root-collar diameter and root-collar diameter squared × total height (D^2H), which is an estimator for above-ground biomass of young tree seedlings (Hatchell et al., 1985). Analysis of variance and Duncan's multiple range test (0.05 significance level) were used in statistical analyses.

RESULTS

Mycorrhizal colonization in mixed overburden from the Sandow mine revegetation site was very low as indicated by endomycorrhizal bioassays of *L. dubia* (5%) and *B. curtipendula* (0%). Noninoculated ectomycorrhizal *Q. virginiana* and noninoculated endomycorrhizal *Sapium sebiferum* had 15% and

<table>
<thead>
<tr>
<th>Mine site</th>
<th>Species</th>
<th>Mycorrhizae¹</th>
<th>Infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>At trans-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>planting</td>
</tr>
<tr>
<td>Sandow Mine (Rockdale, TX)</td>
<td>Chinese tallow</td>
<td>Noninoculated</td>
<td>0*</td>
</tr>
<tr>
<td></td>
<td><em>(Sapium sebiferum)</em></td>
<td>G. f. and G. mar.</td>
<td>35*</td>
</tr>
<tr>
<td></td>
<td>Live oak</td>
<td>Noninoculated</td>
<td>0*</td>
</tr>
<tr>
<td></td>
<td><em>(Quercus virginiana)</em></td>
<td>P. t.</td>
<td>30*</td>
</tr>
<tr>
<td>Gibbons Creek Mine (Carlos, TX)</td>
<td>Texas mountain laurel</td>
<td>Noninoculated</td>
<td>0*</td>
</tr>
<tr>
<td></td>
<td><em>(Sophora secundiflora)</em></td>
<td>G. f. and G. mos.</td>
<td>25*</td>
</tr>
<tr>
<td></td>
<td>Live oak</td>
<td>Noninoculated</td>
<td>0*</td>
</tr>
<tr>
<td></td>
<td><em>(Quercus virginiana)</em></td>
<td>P. t.</td>
<td>25*</td>
</tr>
</tbody>
</table>

¹Mycorrhizae treatments: *P. t.*, *Pisolithus tinctorius*; *G. f.*, *Glomus fasciculatum*; *G. mos.*, *Glomus mosseae*.

Values with the same superscript within a species are not significantly different, Duncan's multiple range test (*P*<0.05).
### TABLE 3

Effect of mycorrhizal fungi on survival and growth of *Quercus virginiana* and *Sapindus sebiferum* on a lignite site in Rockdale (Sandow Mine) measured over three growing seasons (1983-1985)

<table>
<thead>
<tr>
<th>Species</th>
<th>Mycorrhizae(^1)</th>
<th>Survival (%)</th>
<th>Height (cm)</th>
<th>Root-collar diam. (mm)</th>
<th>(D^2H) (cm(^2))</th>
<th>(\Delta) Height (cm)</th>
<th>Root-collar diam. (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinese tallow</td>
<td>Noninoculated</td>
<td>95(^a)</td>
<td>51.2(^a)</td>
<td>61.3(^b)</td>
<td>25.1(^b)</td>
<td>30.9(^b)</td>
<td>322.6(^b)</td>
</tr>
<tr>
<td>(S. sebiferum)</td>
<td>G. f. and G. mar.</td>
<td>90(^a)</td>
<td>55.8(^a)</td>
<td>75.1(^a)</td>
<td>29.2(^a)</td>
<td>41.1(^a)</td>
<td>475.8(^a)</td>
</tr>
<tr>
<td>Live oak</td>
<td>Noninoculated</td>
<td>96(^b)</td>
<td>26.9(^b)</td>
<td>34.6(^b)</td>
<td>10.3(^b)</td>
<td>13.3(^b)</td>
<td>28.5(^b)</td>
</tr>
<tr>
<td>(Q. virginiana)</td>
<td>P. t.</td>
<td>93(^a)</td>
<td>37.6(^a)</td>
<td>55.9(^a)</td>
<td>14.7(^a)</td>
<td>22.5(^a)</td>
<td>81.2(^a)</td>
</tr>
</tbody>
</table>

\(^1\)Mycorrhizae treatments: see Table 2.

Values with the same superscript within a species are not significantly different, Duncan's multiple range test \((P<0.05)\).
TABLE 4

Effect of mycorrhizal fungi on survival and growth of *Sophora secundiflora* and *Quercus virginiana* in a lignite overburden site at Carlos, TX (Gibbons Creek Mine) after two growing seasons

<table>
<thead>
<tr>
<th>Species</th>
<th>Mycorrhizae</th>
<th>Survival (%)</th>
<th>Height (cm)</th>
<th>Root-collar diam. (mm)</th>
<th>D³H (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Texas mountain laurel (S. secundiflora)</td>
<td>Noninoculated</td>
<td>80b</td>
<td>6.4b</td>
<td>9.6b</td>
<td>5.9b</td>
</tr>
<tr>
<td>G. f. and G. m.</td>
<td>100*</td>
<td>11.2*</td>
<td>14.9*</td>
<td>24.9*</td>
<td></td>
</tr>
<tr>
<td>Live oak (Q. virginiana)</td>
<td>Noninoculated</td>
<td>100*</td>
<td>21.6b</td>
<td>9.8b</td>
<td>20.7b</td>
</tr>
<tr>
<td>P. t.</td>
<td>100*</td>
<td>25.7*</td>
<td>12.7*</td>
<td>41.5*</td>
<td></td>
</tr>
</tbody>
</table>

*Mycorrhizae treatments: see Table 2.
Values with the same superscript within a species are not significantly different, Duncan's multiple range test (P < 0.05).

10% colonization levels, respectively, by the third growth season, indicating low mycorrhizal re-establishment (Table 2). Colonization levels of inoculated ecto- and endomycorrhizal plants increased from 30 to 70% and 35 to 71%, respectively.

Neither ecto- nor endomycorrhizal colonization enhanced the high survival rates of *Q. virginiana* or *Sapindum sebiferum*; however, mycorrhizae enhanced height, root-collar diameter and D³H in *Sapindum sebiferum* by the third growing season, and in *Q. virginiana* by the second growing season (Table 3).

At the Gibbons Creek Mine, mycorrhizal colonizations were low as indicated by non-inoculated *Q. virginiana* and *Sophora secundiflora*, which had 10 and 5% colonization levels, respectively, by the second growing season (Table 2). Colonization levels of inoculated ecto- and endomycorrhizal plants increased to 45 and 35%, respectively. Endomycorrhizae enhanced survival of *Sophora secundiflora*, as well as all other growth parameters (Table 4).

DISCUSSION

This is the first report to conclude that endo- and ectomycorrhizae were beneficial to the growth and development of *Sapindum sebiferum*, *Sophora secundiflora* and *Q. virginiana* on lignite overburden sites. Endomycorrhizae also enhanced growth and survival of perennial grasses in the Sandow Mine overburden site (Call and Davies, 1988). The benefits of mycorrhizae in the revegetation of mine spoils have been documented with other woody and herbaceous species (Alden, 1978; Marx and Arment, 1979; Call and McKell, 1982).

Growth of *Sophora secundiflora*, which can form endomycorrhizal, ectomycorrhizal and *Rhizobium* associations (Strong and Davies, 1982), was enhanced in this study when inoculated with endomycorrhizae. *Sophora* was the
slowest growing of the 3 woody transplant species, and was the only plant whose survival was enhanced by mycorrhizal colonization.

Indigenous mycorrhizal fungi were sparse at both sites before transplanting was initiated. The noninoculated endo- and ectomycorrhizal species did become partially colonized, but after 3 growing seasons, only reached colonization levels of 10 and 15%, respectively. In a recent study of VAM fungi in one- and ten-year-old mixed overburden and unmined soil in the Post Oak Savannah region of Texas, endomycorrhizal (VAM) associations reached pre-mining levels 3 to 7 years after disturbance, but production of spores by VAM fungi was greatly reduced in all spoils (Mott and Zuberer, 1987). Apparently during surface mining, topsoil disturbance and storage without plant growth decreases both spore number and the infectivity of endomycorrhizal fungi propagules (Jasper et al., 1987). The reintroduction of ectomycorrhizal spores in these drastically disturbed environments is primarily an abiotic dispersal mechanism (wind), while the dispersal of endomycorrhizae via spores, infected root and mycelial fragments is dependent on human and animal vectors, flowing water, soil erosion, etc. (Ponder, 1979).

Fungal hyphae enable mycorrhizal roots to compensate for deficiencies of immobile ions by mining a greater volume of soil than nonmycorrhizal roots, and thus enhancing nutrient uptake (Tinker, 1984). At the Sandow Mine site, inoculated perennial grasses had higher levels of N and P in above-ground biomass than non-inoculated plants (Call and Davies, 1988). Using the same VAM species, uptake of K, Ca, S, Zn, and Mn was enhanced with *Rosa multiflora* (Thunbo.) grown under low fertility in a containerized medium (Davies, 1987). In this experiment, both overburden sites were low in soil N, slightly alkaline, and high in K, Ca, Mg, Fe, Zn, and Mn. The Gibbons Creek Mine site had moderately high P (31 ppm), yet colonization levels were not suppressed and growth responses of ecto- and endomycorrhizal transplants were better than controls (independent of overburden P). This suggests that factors other than nutrition, such as mycorrhizal enhanced water relations and stress tolerance, play a role in revegetation systems (Marx and Artmen, 1979; Newman and Davies, 1988).

This research documents the benefits of mycorrhizal infection in the revegetation of mine spoils with woody plant species. Considering potential growth and survival responses, it would be economically feasible to use seedling transplants of woody species using containerized systems of plugs that allow for transplanting preinoculated plants with intact root systems into overburden sites. Colonization levels need not be high, since significant growth enhancement can be obtained even with low root colonization levels (Furlan et al., 1983). The success of many revegetation programs may depend, in part, on developing methods to introduce beneficial microorganisms, such as mycorrhizal fungi, into disturbed areas where they are absent or in low numbers.
ACKNOWLEDGMENT

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REFERENCES


Hons, F.M., Hossner, L.R. and Whitely, E.L., 1989. Reclamation and yield potential of various forages on surface-mined soil. In: L.R. Hossner (Editor), Reclamation of Surface-mined Lignite Spoil in Texas. Res. Monogr. No. 10, Texas Agricultural Experimental Station, Texas A&M University, College Station, TX, pp. 36-47.


Whiteley, E.L. and Hossner, L.R., 1980. Lignite resources and environmental considerations in Texas. In: L.R. Hossner (Editor), Reclamation of Surface-mined Lignite Spoil in Texas. Res. Monogr. No. 10, Texas Agricultural Experiment Station, Texas A&M University, College Station, TX, pp. 5-11.