

ECTOMYCORRHIZAE OF TABLE MOUNTAIN PINE AND THE INFLUENCE OF PRESCRIBED BURNING ON THEIR SURVIVAL

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Abstract—High-intensity prescribed fires have been recommended to regenerate Table Mountain pine (*Pinus pungens*). However, tests of these burns produced few seedlings, possibly due to soil sterilization. This study examined abundance of mycorrhizal root tips in the field after a high-intensity fire and in the laboratory after exposing rooting media to various temperatures. One- and two-year old seedlings in the field had abundant mycorrhizal root tips formed by symbiotic relationships with at least three fungal species. Laboratory tests showed reduced mycorrhizal root tip formation only after prolonged exposure to very high temperatures. This study suggests that poor regeneration after high-intensity prescribed fires was not caused by a lack of mycorrhizal fungi.

INTRODUCTION

There is increasing evidence that fire is an important component of the Table Mountain pine (*Pinus pungens*) community. Although not currently threatened or endangered, Table Mountain pine is being replaced by more shade-tolerant hardwoods in the Appalachian Mountains because of fire exclusion (Van Lear and Waldrop 1989). Early work suggested that Table Mountain pine requires a very high intensity fire in order to promote adequate regeneration (Zobel 1969). However, research to date does not define in sufficient detail the necessary fire regime to provide regeneration.

Attempts at determining the optimum fire regime have had limited success. Waldrop and Brose (1999) found that a high intensity fire was not necessary for successful regeneration of Table Mountain pine. While a lower-intensity fire is easier and safer to conduct, a medium-intensity fire was deemed to be best because it killed most of the overstory, which would allow sunlight to reach the soil surface. High-intensity fires led to the poorest regeneration success in that study. This may have been caused by combustion of cones or the high temperatures may have sterilized the upper soil which would have reduced minimally effective levels of mycorrhizal fungi. This study was conducted to determine the possible deleterious effects of high-intensity fire on ectomycorrhizae in Table Mountain pine seedlings.

METHODS

Study Area

The study area was the same as that used by Waldrop and Brose (1999) and was located in the Chattahoochee National Forest near Clayton, Georgia. Sites 1 and 2 were both 12 ha in size and at elevations of 914 and 884 m, respectively. Site 3 was 18 ha in size and was at 1100 m elevation. All three stands were similar in composition and stocking with an overstory of Table Mountain pine and understory of

various hardwoods. Because of the previous lack of fire, the understory consisted of thickets of mountain laurel (*Kalmia latifolia*) and young hardwoods.

All three stands were burned as one unit on April 4, 1997, a total of 345 ha. Fire intensities within sample plots were classified by Waldrop and Brose (1999). Nine plots were classified as having been burned by low intensity fires, 28 as medium-low, 9 as medium-high, and 14 were high intensity. Site 3 burned at high and medium-high intensities and sites 1 and 2 burned at low and medium-high intensities.

Field Quantification of Mycorrhizae

Three months after the fire, 60 sample plots, 10x20 m² in size, were established throughout the three stands (Waldrop and Brose 1999). Four first- or second-year seedlings of Table Mountain pine were collected in October 1998 on the down-slope side of each of seven arbitrarily selected plots in site 1, eight plots in site 2, and seven plots in site 3. Seedlings were disinterred by carefully removing the attached soil ball and as much of the root system as possible. In the laboratory, each seedling was exposed to running tap water for two minutes to soften and remove soil particles. Seedling size was estimated by measuring stem length, tap root length, and length of lateral and short roots. Each root tip was visually inspected to determine presence of ectomycorrhizae. ANOVA and t-tests were conducted to compare seedling size and presence of mycorrhizae with plot location and seedling age.

Histology

After the preceding measurements were completed, representative root tips were severed and fixed in 3.5 percent glutaraldehyde, dehydrated, and embedded in plastic resin (JB-4 embedding kit, Fisher). Transverse sections 6-8 Fm thick were prepared using glass knives and a JB-4.3, JB-4A Porter Blum microtome. Sections were affixed to clean microscope slides, dried, stained with toluidine blue and

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Table 1—Statistics of Table Mountain pine seedlings from the field collections

Site	n	Mean Length (mm)		Mycorrhizal	
		Stem	Root	Total	Root tips
First-year seedlings					
1	2	8.5a ¹	5.3a	13.8a	7.0a
2	10	9.3a	6.5a	15.8a	25.0a
3	28	8.3a	9.4a	17.7a	20.0a
Second-year seedlings					
1	26	13.7a	8.7a	22.4a	42.5a
2	22	15.4a	10.1a	25.5a	36.7a

¹Means with the same lowercase letter within a column are not significantly different at the 0.05 level.

safranin-fast green, and viewed under 100X magnification. Four sections from each root tip were examined and the following measurements were recorded: 1) mantle thickness and morphology, 2) diameter of cortical cells, and 3) morphology and diameter of intercellular hyphae. Comparison of these measurements with published data (Trappe 1962, Jackson and Mason 1984, Marx 1977, Hacskaylo 1961) indicated that *Cenococcum* spp., *Pisolithus tinctorius*, and *Suillus granulatus* were the most common symbionts of Table Mountain pine seedlings.

Axenic culture

In order to confirm that these species were, indeed, capable of forming ectomycorrhizae with Table Mountain pine, cultures of *Cenococcum graniforme*, *C. geophilum*, *P. tinctorius*, and *S. granulatus* were obtained from the American Type Culture Collection (Beltsville, MD). The cultures were maintained on Melin-Norkrans media.

Seeds of Table Mountain pine were surface disinfected by swirling in a 1 percent solution of sodium hypochlorite for three minutes, rinsed in three changes of sterile distilled water, and then plated on acidified potato-dextrose agar to test for surface sterility and to induce germination.

Axenic growth chambers consisted of 1-quart canning jars with a 1.27 cm dia piece of PVC pipe glued in a hole in the lid and plugged with cotton. The open end was covered loosely with a piece of aluminum foil to allow for aeration but to minimize dust contamination. Each jar contained a mixture of 400 ml of vermiculite and 256 ml of Melin's (1921) nutrient solution as modified by Norkrans (1949). The jars were autoclaved for 30 minutes at 121°C two separate times with a 24-hour period in between to allow for germination of heat-resistant spores. A layer of aluminum foil was wrapped around the bottom half of each jar to exclude light from the root zone. The jars were placed in a growth chamber programmed for an 18-hour photoperiod and a constant temperature of 22°C. Average light intensity was 21.83 microeinsteins (FE /sm²).

An aseptic seed with a radicle 1-3 mm in length was transplanted into each jar at a depth of 6 mm. At the same time, 2-10 mm discs of inoculum from 30-day-old cultures of the respective fungus were placed on the vermiculite surface. After four months incubation, the seedlings were removed, measured as with the field quantifications, and inspected for mycorrhizal development.

The experiment included a total of 60 jars, with 5 treatments which included each of the four fungi and a control with only an aseptic seedling and 12 replications within each treatment. The entire experiment was performed twice. Results were analyzed by ANOVA and t-tests.

Heat treatments

A heated water bath was used to determine the resistance of mycorrhizal fungi to heat. Pure cultures of each of the respective mycorrhizal fungi were grown in 1-quart canning jars as described above, except that pine seedlings were not placed in the jars for the first part of the experiment. After 30 days incubation, the jars containing their respective fungal cultures were placed in a water bath at 25°C (control), 50°C, 60°C, or 80°C, respectively, for 60 minutes. After cooling for 24 hours, two aseptic seeds of Table Mountain pine each with a radicle 1-3 mm in length were planted in each jar. The jars were incubated for 90 days, after which time the seedlings were removed, measured as with the field quantifications, and inspected for mycorrhizal development. Sixty jars were used, separated into five sets of twelve each. Each set included the four fungi and a control. The experiment was repeated twice. ANOVA and t-tests were conducted.

RESULTS AND DISCUSSION

Field Quantification of Ectomycorrhizae

Sites 1 and 2 had burned with similar low- to medium-fire intensities whereas site 3 burned at a much higher intensity. Because of this, there was no difficulty in finding an adequate number of either first- or second-year

Table 2—Statistics of Table Mountain pine seedlings in axenic culture, in first/second experiments

Fungal Species	# of Seedlings	Length (mm)	Total # root tips (avg.)	# mycorrhizal root tips (avg.)
<i>C. gran.</i>	11/	15.6a ¹	56.6a/	0.6a/
	11	14.4a	29.4a,b	5.3b
<i>C. geop.</i>	12/	21.4a/	45.8a,b/	3.8a/
	12	13.3a	21.2a,b	7.5b
<i>S. gran.</i>	10/	28.2b/	52.2a,b/	4.5a/
	10	16.4a	31.3a	21.1a
<i>P. tinc.</i>	10/	18.0a/	20.6c/	4.4a/
	11	13.2a	13.6b	7.6b
Control	11/	16.0a/	31.2b,c/	0.0a/

seedlings at the first two sites but it was very difficult to find either first- or second-year seedlings at site 3. Hence, only a limited number of first-year seedlings could be found and there are no second-year seedling data for site 3.

There was no significant difference in average stem length, root length, total length, and average number of root tips with mycorrhizae among first-year and second-year seedlings of Table Mountain pine between the study areas (table 1). However, there was a significant difference in mycorrhizal root tips between first- and second-year seedlings. Approximately 70 percent of the root tips from all three sites

Table 3—Average number of mycorrhizal root tips formed at various temperatures and by different fungal species

Temperature (°C)	Experiment 1	Experiment 2
25	6.1 a,b ¹	5.4 a
50	7.7 a	2.1 a,b
60	2.3 b,c	3.8 a,b
80	0.1 c	0.3 b

Fungus	Experiment 1	Experiment 2
Control	0.0 b	0.0 b
<i>C. geo.</i>	0.0 b	0.9 b
<i>C. gran.</i>	1.7 b	1.1 b
<i>S. gran.</i>	8.9 a	5.8 a
<i>P. tinct.</i>	9.7 a	5.8 a

¹In each experiment, means followed by the same lowercase letter are not significantly different at the 0.05 level.

were mycorrhizal, suggesting that mycorrhizal development began in the first growing season after the fire and continued into the second season. The data also suggest that soil temperatures did not reach lethal levels, even with the high-intensity fire.

Histology

There were three distinct morphological types of ectomycorrhizae observed on the roots of Table Mountain pine. These matched published descriptions of *Suillus granulatus*, *Pisolithus tinctorius*, and *Cenococcum* sp. (Chambers and Cairney 1999, Riffle 1973, Marx and others 1969). The most important diagnostic attributes were color, type of branching, root tip length and diameter, mantle diameter, presence of the Hartig net, and size of cortical cell. Visual examination of mycorrhizal root tips and the histological sections indicated that *P. tinctorius* was the slightly more abundant of the three fungal symbionts in all three sites in both first- and second-year seedlings. The occurrence of mycorrhizal root tips on first-year seedlings in site 3 suggests that soil sterilization did not occur.

Axenic Culture

The two axenic culture experiments produced relatively low levels of mycorrhizal root tips and the results were somewhat variable (table 2). Total seedling length with any of the fungi did not differ significantly from the control except in the first experiment where total seedling length of 28.2 mm for *S. granulatus* was significantly greater than that for any of the other fungi or the control. In the first experiment, only seedlings with *C. graniforme* were associated with a larger number of root tips (56.6) than the control (31.2), yet this difference was not observed in the second experiment, nor was there any difference in the average number of mycorrhizal root tips between any of the fungi and the control. In the second experiment, only *S. granulatus* produced a significantly greater number of mycorrhizal root tips (21.1) than the control (0.0). Mycorrhizal fungi are notoriously difficult to work with in axenic culture and it is suspected that the low infection rates seen here are due to the lack of a clear understanding of all the subtle growth variables necessary for successful infection.

Heat Treatments

This experiment was conducted twice, with similar results produced both times. Hence, only the results of experiment one are presented. At the control temperature of 25° C, 74 percent of *S. granulatus* root tips were mycorrhizal, 49 percent of *P. tinctorius* root tips were mycorrhizal, 16 percent of *C. graniforme* root tips were mycorrhizal, and *C. geophilum* and sterile seedlings had no response (figure 1). The mean mycorrhizal count for all the fungal species was 6.1 per seedling.

At 50° C, 79 percent of *P. tinctorius* root tips were mycorrhizal, 75 percent of *S. granulatus* root tips were mycorrhizal, 10 percent of *C. graniforme* root tips were mycorrhizal, and *C. geophilum* and sterile seedlings had no response (figure 1). The mean mycorrhizal count for all the fungal species was 7.7 per seedling.

At 60° C, 31 percent of *P. tinctorius* root tips were mycorrhizal, 13 percent of *S. granulatus* root tips were

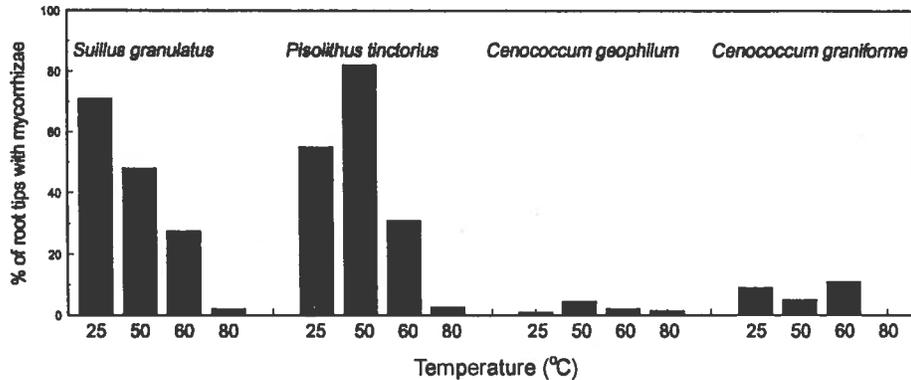


Figure 1—Percentage of Table Mountain root tips with mycorrhizae by fungal species formed after heat treatments at various temperatures.

mycorrhizal, and the two *Cenococcum* species and the control had no response (figure 1). The mean mycorrhizal count for all the fungal species was 2.3 per seedling.

At 80° C, there was almost no mycorrhizal growth (figure 1), with *S. granulatus*, *P. tinctorius*, and *C. geophilum* producing only 3 percent to 6 percent of mycorrhizal root tips. The mean mycorrhizal count for all the fungal species was 0.1 per seedling.

There was a significant difference in mycorrhizal count among the various temperatures (table 3). Mycorrhizal abundance tended to drop at temperatures over 50° C and was almost eliminated at 80° C. There was also a significant difference in mycorrhizal count among fungi, with both *P. tinctorius* and *S. granulatus* different from *C. graniforme* and *C. geophilum* as well as the control. *S. granulatus* and *P. tinctorius* gave the most favorable results in the heat treatment experiments (figure 1). Both species grew well at the lower temperatures and, except for some variation at 50° C in experiment 2, both fungi grew in the same relative temperature range. Neither survived well at temperatures reaching 80° C.

CONCLUSIONS

Table Mountain pine was confirmed to be symbiotic with at least three mycorrhizal fungi, all of which are known for their preference for dry habitats and, hence, are very well adapted to form beneficial relationships with Table Mountain pine. This research also showed experimentally that these fungi cannot survive a prolonged temperature exceeding 80° C. Regardless of fire intensity, it is unlikely that temperatures of 80° C would be achieved to any significant soil depth. In the experimental burned area exposed to a high-intensity fire, the behavior of mycorrhizal formation in first- and second-year seedlings suggests that the mycorrhizal fungi either survived the intense fire intensities or they recolonized the site quickly. While it is probably desirable to perform prescribed burns at something less than a medium-high intensity, it seems clear from the results of the present research that even a medium-high intensity probably does not seriously harm the mycorrhizal symbionts in the soil of the burned areas.

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