SHORT-TERM EFFECTS OF FUEL REDUCTION TREATMENTS ON SOIL MYCORRHIZAL INOCULUM POTENTIAL IN BEETLE-KILLED STANDS

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Abstract—Heavy fuel loads were created by southern pine beetle (*Dendroctonus frontalis* Ehrh.) outbreak throughout the southeastern Piedmont during the early 2000s. Prescribed burning and mechanical mulching (mastication) were used to reduce fuel loading, but many ecological impacts are unknown. Successful forest regeneration depends on ectomycorrhizal (ECM) or vesicular-arbuscular mycorrhizal (VAM) fungi which form important symbiotic relationships with most forest plants. Fuel reduction treatments may impact mycorrhizal propagule abundance and/or vigor through propagule consumption, changes in soil chemistry, and/or effects on host vegetation. The objective of this study was to compare soil VAM and ECM inoculum potential after prescribed burning and mulching treatments to no treatment (control) using greenhouse bioassays. Neither VAM nor ECM inoculum potential were significantly different among treatments, but were highly variable within treated stands.

INTRODUCTION

Background

There was epidemic southern pine beetle (*Dendroctonus frontalis* Ehrh.) activity in the Southeastern United States during the early 2000s. Beetle-killed pine trees fall in 1 to 2 years, and stands are quickly colonized by herbaceous and early successional woody vegetation. Resulting conditions create a fuel hazard and greatly impede forest management activities.

Natural resource managers in the southeastern Piedmont region requested information about consequences to various ecosystem properties associated with using prescribed fire and mechanical mulching as site preparation treatments.

Mycorrhizas

Mycorrhizas are symbiotic relationships between soil fungi and plant roots and confer drought and disease tolerance to the plant by increasing their absorptive root surface area (Sylvia and others 2005). Most forest plants are dependent on mycorrhizal colonization for their establishment and productivity (Janos 1980). Soil fungi that form associations with the majority of plants in the southeastern Piedmont are glomalean and basidiomycetous fungi and form vesicular-arbuscular (VAM) and ectomycorrhizas (ECM), respectively. Major VAM tree genera in this region are *Acer* L., *Fraxinus* L., *Liriodendron* L., *Prunus* L., and *Liquidambar* L. In addition, many shrub and most herbaceous plants in the region are VAM. Major ECM tree genera are *Carya* Nutt., *Fagus* L., *Pinus* L., and *Quercus* L.

Sources of mycorrhizal propagules in forests are old roots, mycelia, sclerotia, and spores (Brundrett and Kendrick 1988). Therefore, existing vegetation likely plays an important role as refugia for mycorrhizal fungi that colonize forest regeneration. Spores are thought to play a minor role in initiating mycorrhizal colonization in forested ecosystems (Janos 1980) but interestingly were the focus of several studies that concluded that forest disturbance changed the mycorrhizal dynamics in soil.

Changes in mycorrhizal dynamics may be caused by disturbance-related changes in the abundance and/or activity of propagules (Klopatek and others 1988) which has been termed "soil inoculum potential" (Smith and Read 2000). Such changes may arise from direct damage to propagules (Klopatek and others 1988), damage to host vegetation, i.e., indirect damage to mycorrhizal propagules (Buchholz and Gallagher 1982), or changes in soil chemistry (Herr and others 1994).

Total soil inoculum potential is the cumulative potential for all sources of mycorrhizal propagules to initiate colonization with the roots of host plants. It is unclear if prescribed fire and mechanical fuel reduction result in changes in total soil VAM and ECM inoculum potential. Therefore, the objective of this study was to compare short-term soil VAM and ECM inoculum potential among treatments.

METHODS

Study Area

The study was conducted in 12 beetle-killed pine stands each approximately 1 ha in size in the Clemson University Experimental Forest. The stands were artificially planted or naturally regenerated and approximately 18 to 33 years in age when killed. Mean diameter of *Pinus* spp. stems (live or dead) in the year 0 vegetation community was 21.9 cm. Metal stakes were placed on a 25- by 25-m spacing to create a grid

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system throughout each stand and permanent references for conducting fuel, vegetation, and soil sampling.

Fuel Reduction Treatments

The 12 stands were randomly assigned to 1 of 3 fuel reduction treatments in an unbalanced design to create 3 replications each of control and mulching and 6 replications of prescribed burning. The mulching treatment was accomplished using a tracked machine equipped with a hydraulic-driven masticating head. The mulching treatment commenced in late May 2005 and was completed in late June 2005.

The original study plan involved burning in two different seasons to achieve two different levels of fire intensity. However, prescribed burning was delayed in 2005 due to weather. Therefore, all burning was conducted in a 3-day period between March 30 and May 3, 2006, using manual strip-head firing.

Mycorrhizal Bioassays

Plots used to sample vegetation in another component of the current study were used to collect soil samples for mycorrhizal bioassays. Two 10- by 50-m plots were established in each beetle-killed stand and contained five 10- by 10-m subplots. Soil sampling was performed between May 22 and June 8, 2006, for VAM bioassays and between July 10 and July 20, 2006, for ECM bioassays. For each sampling period, 4 intact 211-mL soil cores were obtained from the centers of each subplot providing a total of 20 observational units per vegetation sampling plot. A total of 40 soil cores were obtained from each vegetation sampling plot after collection for VAM and ECM bioassays.

Soil samples were returned to the Clemson University Greenhouse Complex at the end of each sampling day. Soil cores for ECM bioassays were immediately placed in a HEPA-filtered chamber constructed in a greenhouse and previously shown to reduce contamination by airborne ECM fungi (Stottlemyer and others 2008). Soil cores collected for VAM bioassays were planted with corn (*Zea mays* L. 'Viking') seed and were allowed to grow for 4 weeks. Soil cores collected for ECM bioassays were planted with loblolly pine (*P. taeda* L.) seed and were allowed to grow for 6 weeks. All seedlings grew under natural light for the duration of the growing periods and no fertilizers were applied.

At the end of their respective growing periods, corn and pine seedlings were destructively harvested and rinsed free of soil. A subsample of 50 1-cm corn root segments (<1 mm in diameter) were mounted on glass slides after clearing with 10 percent potassium hydroxide (KOH), staining with trypan blue, and destained in 50-percent glycerol. Slides were assessed with a compound microscope equipped with a crosshair eyepiece under 110× magnification. The presence/absence of VAM hyphae was noted at each intersection of the crosshair and a root segment. VAM colonization values were calculated using the equation: VAM colonization = number intersections at which hyphae were present ÷ 50. Root systems of more than 450 corn seedlings were assessed for VAM colonization and root and shoot growth after accounting for seedling mortality and nongerminants.

Pine root systems are heterorhizic with distinct short roots and long (lateral) roots from which short roots subtend (Brundrett and others 1996b). Three lateral roots ≥6 cm in length were randomly selected from each seedling. Each short root was tallied and classified as mycorrhizal or nonmycorrhizal using a dissecting microscope. Nonmycorrhizal short roots were slender and elongated, possessed root hairs and root caps, and lacked fungal mantles. Mycorrhizal short roots were bifurcate or monopodial, possessed fungal mantles, and lacked root hairs and root caps. Colonization values were calculated using the equation: ECM colonization = number of mycorrhizal short roots ÷ total number of short roots. Root systems of more than 380 pine seedlings were assessed for ECM colonization and root and shoot growth after accounting for seedling mortality and nongerminants.

Statistical Analysis

Average percentages of mycorrhizal colonization of corn and pine seedlings were calculated for each beetle-killed stand and compared among fuel reduction treatments using analysis of variance (PROC GLM; SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Mycorrhizal Colonization of Bioassay Seedlings

Impacts of prescribed fire and mechanical treatments on mycorrhizal dynamics are not fully understood. Past studies that used "most probable number" bioassay methods showed that prescribed burning decreased soil VAM (Rashid and others 1997) and ECM (Torres and Honrubia 1997) inoculum potential in different forest ecosystems. However, this methodology involves soil collection, dilution with sand, and mixing prior to growing bait plants. Mixing soil likely disrupts old root systems and mycelia networks (Horton and others 1998) which are the primary mode of colonization of forest regeneration (Brundrett and others 1996a).

In the current study, there were no significant differences in percentage VAM and ECM colonization of bioassay seedlings among fuel reduction treatments in beetle-killed stands that were subjected to different fuel reduction treatments (fig. 1). Fungal mycelia may have escaped injury or resistant propagules including spores and sclerotia may have initiated mycorrhizal colonization with corn or pine seedlings. Therefore, the possibility for multiple propagules to initiate mycorrhizal colonization after forest disturbance highlights the importance of bioassay methods that assess total soil inoculum potential.

There was a high degree of variability in soil mycorrhizal inoculum potential within beetle-killed stands treated with different fuel reduction treatments (table 1). Variability in soil inoculum potential may have been caused by variation in the intensity of the treatments. For example, a wide range of fire intensities and residence times are likely in beetle-killed stands due to spatial variability in fuel loading. In addition, existing hyphal networks and old roots in soil are the primary

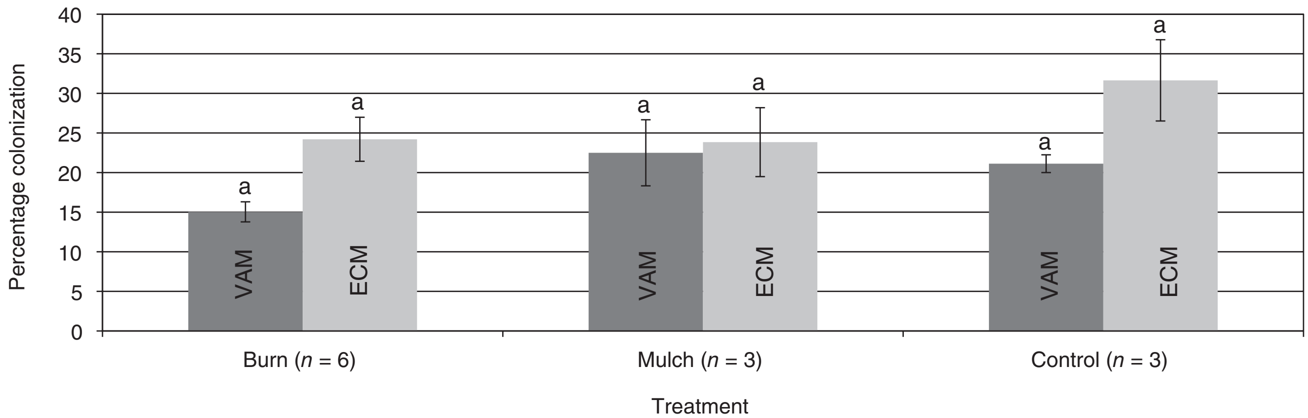

Figure 1—Comparisons of mean percentage vesicular-arbuscular mycorrhizal (VAM) and ectomycorrhizal (ECM) colonization of corn (*Zea mays* L.) and loblolly pine (*Pinus taeda* L.) seedlings. Seedlings were grown in intact soil cores collected after beetle-killed stands were subjected to different fuel reduction treatments. Bars represent standard errors of the means. Similar letters indicate that VAM or ECM inoculum potential was not significantly different among treatments.

Table 1—Ranges in percentage mycorrhizal colonization of corn (*Zea mays* L.) and loblolly pine (*Pinus taeda* L.) seedlings used for greenhouse bioassays of total soil mycorrhizal inoculum potential. Seedlings were grown in intact soil cores collected following different fuel reduction treatments in beetle-killed pine stands.

	Ranges in percentage mycorrhizal colonization		
Mycorrhiza	Control (no treatment) (*n* = 3)	Prescribed burn (*n* = 6)	Mulch (*n* = 3)
Vesicular-arbuscular mycorrhizal	16.67–24.10	8.47–20.50	11.50–37.85
Ectomycorrhizal	14.09–45.55	10.27–37.88	10.99–41.42

source of mycorrhizal inoculum for new plant germinants. Therefore, the composition and structure of pretreatment vegetation has the potential to influence posttreatment soil inoculum potential.

CONCLUSIONS

Prescribed fire and mechanical mulching have been proposed to reduce high fuel loading in beetle-killed pine stands in the Southeastern United States. Forest managers in the region are interested in whether these treatments offer viable options for reducing fuels without jeopardizing site productivity. Until recently, the effectiveness of the treatments at reducing fuels in extremely high fuel loading and their impacts on important ecosystem processes were largely unknown.

Mycorrhizal fungi have the potential to influence the trajectory of vegetation succession after a disturbance. We compared VAM and ECM inoculum potential among treatments using bioassays of intact soil cores in the greenhouse. Neither VAM nor ECM inoculum potential were significantly different among fuel reduction treatments but were highly variable within posttreatment beetle-killed stands. Understanding how pretreatment vegetation, fire behavior, and soil fertility influenced soil inoculum potential is the focus of current research.

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