# FINAL REPORT

## Epidemic Southern Pine Beetle Attacks: A Problem of Fuel-Loading or an Opportunity for Management?

**JFSP Project: 04-2-1-33**

**Project Location**: Clemson Experimental Forest, Pickens, Oconee, and Anderson Counties, SC.

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Other Cooperators: S. Knight Cox, Jr., Forest Manager, Clemson University

## EXECUTIVE SUMMARY

### Overview

The Piedmont Region of South Carolina experienced one of the heaviest attacks of southern pine beetle (*Dendroctonus frontalis* Zimm.) in history during the early part of this decade. Managers with both commercial objectives and restoration objectives needed information on how prescribed burning or mechanical treatments can be used to reduce the heavy fuels resulting from these attacks, without neglecting their primary management objectives. Prescribed burning is of concern because intensities are expected to be high and fires may damage soils, neighboring trees, or target vegetation. Methods of predicting fire behavior and fuel consumption are unavailable. Mechanical treatments will reduce fuels but are expensive and may not control vegetation that would out-compete planted pines, oak sprouts, or other target vegetation.

The project was designed to use beetle-killed areas on a commercial forest, a national forest, and a national military park as treatment areas to compare winter burning, summer burning, and

mechanical fuel reduction. Response variables included vegetation, soil properties, and fuels. Resulting analyses provide local managers a better understanding of the tradeoffs between prescribed fire and mechanical fuel reduction in areas with unusually heavy fuel loads.

Selection of study sites began immediately upon notification of funding. We experienced serious problems almost immediately (identified in each progress report). A change in leadership had occurred on the National Forest where we had made previous arrangements, so the study was no longer supported. We decided to use the Clemson Experimental Forest (Clemson University) and a National Park as representative of commercial and restoration goals, respectively. Study plots were installed at both locations and all pretreatment data were collected. Twelve experimental units of approximately 5 acres were installed in beetle-killed areas representing 3 replications of 4 treatments (completely random design) at each location. Treatments were designed as site-preparation treatments and included a high intensity prescribed burn, a low intensity prescribed burn, mastication, and an untreated control. Each experimental unit was split in half and 1-0 seedlings of loblolly pine (*Pinus taeda*) on one side of the experimental unit at a spacing of 7 by 10 feet. Details of treatments are given in the following attached papers. Briefly, high-intensity fires had a mean temperature, measured by thermocouples 1 foot above ground, of 800$^{o}$F while low-intensity fires burned at approximately 350$^{o}$F. Mastication was conducted by commercial operators using a Gyrotrack® or a Kodiak Kutter® machine which felled standing dead trees and chopped all woody debris to pieces no larger than 8 inches long and 1 inch thick. Chopped woody materials were scattered over each plot.

Treatment installation was completed on the Clemson Experimental Forest in the spring of 2006. However, serious problems continued (Also described in annual progress reports). At that time, the National Park Service informed us for the first time that their regulations required a detailed burn plan which was our responsibility to prepare. This was the first time that either the park manager or the principle investigator had worked with research burns on a National Park, so mistakes in communication were the cause. I immediately hired a consultant to prepare the plan and that plan was approved. The Park Service maintains a traveling burning crew which covers each small park in the southeastern United States on a rotating schedule. As of this date, that burning crew has not visited our plots and burning treatments have not been installed. Control plots and mastication plots were measured before treatment and one year later but were dropped from the study due to unsuccessful installation of burning treatments.

The only remaining portion of the original study was on the Clemson Experimental Forest where all treatments were installed correctly and on schedule. The revised study was not capable of providing all original objectives but many new and beneficial objectives were added. We were able to provide managers with useful information about how burning and mastication impact important variables such as tree survival, fuel loading, log moisture, threats of invasive plants, soil mycorrhizal inoculum potential, greenhouse methods to grow sterile pine seedlings, and energy content of select hardwood leaf species. Each resulting component of this study is the subject of one or more conference or journal papers which are attached and listed in the table of deliverables.

**Lessons Learned**

I. Fuel loading and fire hazard

- Prescribed burning in beetle-killed areas may result in high intensity fires but these fires are easily controlled because heavy fuels are limited to a small area.
- Fires in beetle-killed areas can be difficult to ignite if overstory hardwoods shade the burn unit and moisture conditions are moderate.
- Low intensity fires reduced loading of all fuels but were not significantly different from high intensity fires in loading of 1-hour fuels and 1000-hour fuels and depth of residual litter and duff.
- High-intensity fires consumed significantly higher quantities of 10- and 100-hour fuels than did low-intensity fires.
- High intensity fires provided longer protection from wildfire.  By the second year after burning, woody fuels, litter, and duff were not significantly different between low-intensity plots and unburned controls but all fuels were significantly lower in plots burned at high intensity.
- Mastication resulted in woody fuel loads that were 2.5 to 6 times greater than observed in control plots or those that had been treated with a prescribed burn.  However, these fuels compacted and decomposed quickly resulting in an open stand with little or no fire risk (fig 1).

Figure 1. Experimental units treated with mastication contained few residual logs and were covered in wood chips up to 1.5 feet in depth.

- Late-succession species that replace oaks, in the absence of fire, are thought to have several adaptations that prevent fire.
- An analysis of leaf litter chemistry showed that oak leaves contain higher calorific value and less mineral ash content than do leaves of later-successional species such as red maple and American beech. This suggests that fires in oak forests will burn hotter than fires dominated by red maple and American beech and may be difficult to ignite without oak leaf litter.

II. Logs as fuels – does rainfall wet logs adequately to prevent ignition?

- Using the Keetch-Byram index for fire planning can lead to overestimating log moisture after a heavy short-term rainfall, resulting in high severity soil impacts and long term smoldering.

- A Colorado State rainfall simulator was used to test wetting of air-dried logs under for several scenarios of rainfall amount, duration, and frequency (fig 2).

Figure 2. Colorado State Rainfall Simulator.

- Pine and hardwood logs in the 1000-hr size classes did not increase in moisture content after single rainfall events of up to 4 inches per day, indicating that most rainfall runs off logs and these fuels remain combustible.
- Scenarios where small amounts of rain fell over a period of 1 to 2 weeks showed gradual increases in log moisture content, particularly among the pines. These fuels eventually reach a moisture level where combustion is unlikely.
- Light rainfall (1 inch) that occurred once a week did not increase log moisture content. Rainfall only once a week provided the same results as a single rainfall even though KBDI would be lower during that period.
- The largest increase in log moisture content was associated with a scenario of 1 inch of rain falling 4 times a week for 2 weeks.
- Logs tended to dry to pretreatment levels if rainfall was less frequent than weekly.

- The next step of this study is to apply logs of varying sizes, species, and moisture contents to field burning conditions to determine ignition probability and amount of combustion.

III. Vegetative responses

- Site preparation burning and mastication treatments affected the density of hardwood and pine stems that regenerated after treatment.
- Oak regeneration (<3 ft tall) was significantly higher in areas burned at high intensity than control plots and areas subjected to mastication.  Therefore, these fires may be beneficial for restoration objectives.
- Advanced regeneration (3 to 6 ft tall) of oak was not affected by burning but was significantly reduced by mastication.
- Regeneration of hardwoods, other that oaks, was greatly reduced by the mastication treatment, which may provide the best results for converting to a pure pine forest.
- Advanced regeneration of hardwoods, other than oaks, was not affected by either burning treatment but was significantly reduced by mastication for 2 years.
- Survival of planted pines, one growing season after planting, was unaffected by treatment.  The mastication treatment had the highest survival (85%) which was probably due to a mulching effect.
- Increasing numbers of non-native invasive plant species and the expansion of existing non-native plant populations provide challenges for land managers trying to achieve commercial and restoration goals.
- Each site-preparation treatment initially reduced cover of 7 non-native plants.

- Cover of all invasive species remained low in burned plots for 2 years, except Japanese honeysuckle (*Lonicera japonica*) which increased to pretreatment levels in plots treated with mastication.
- The mastication treatment opens the site to high levels of sunlight which is beneficial to some invasive species, suggesting the need for additional treatments (fire or herbicide) to control these species for commercial and restoration objectives.

IV. Soil Quality and Inoculum Potential

- Successful forest regeneration depends on ecto- (ECM) or vesicular arbuscular mycorrhizal (VAM) fungi which form important symbiotic relationships with most forest plants but the impacts of site-preparation treatments were unknown.
- VAM nor ECM inoculum potential were not significantly different among treatments indicating that there were no negative impacts for either commercial or restoration objectives
- At the sub-experimental unit level, ECM inoculum potential was positively associated with inorganic N concentrations, fire intensity, and residence time, indicating that high-intensity fire could be beneficial for regeneration of ECM-favoring tree genera such as hickory, beech, pine, and oak.
- VAM inoculum potential was negatively associated with inorganic N concentrations, fire intensity, and residence time, suggesting that cooler fires would benefit regeneration of VAM-favoring trees such as maples, ash, yellow-poplar, cherry, and sweetgum.
- Inoculum potential was most highly correlated with pretreatment vegetation species composition.

- Soil fertility was largely unaffected by treatment. Burning caused short-term reductions in concentrations of P, Ca, and Al. Mastication caused short-term reductions in P and Al and an increase in K. No differences were found 2 years after treatment and there was no impact on soil inoculum potential.
- Soil structure was impacted by treatment. Burning reduced litter and duff depth over the 2 years of the study. Mastication covered the existing forest floor with a thick layer (up to 1.5 ft) of wood chips. These changes were not correlated with soil inoculum potential.
- Study of inoculum potential requires that corn and pine seedlings be grown in a mycorrhizal-free environment.
- A high-efficiency particulate (HEPA)-filtered chamber was designed for this study that almost eliminated contamination from the air. Also, seedlings grown in a peat/vermiculite mixture had fewer mycorrhizal short roots than those grown in sand- or soil-based media. These results can improve future efficiency of mycorrhizal research.

**Bottom Line**

Treatments had little impact on soil inoculum potential, soil structure, or soil fertility. The best choice for site preparation to restore beetle-killed areas of the southeastern Piedmont appears to be prescribed burning, particularly high-intensity fires which promoted oak regeneration in this study. These fires reduced heavy fuel loads but were not difficult to control because fuels were heavy in only a small area. However, these fires should only be conducted by trained professionals with proper equipment. High-intensity fires also promote the soil inoculum potential for ectomycorrhizae which are favored by target species such as hickory, beech pine, and oak. Mastication may be the best choice for commercial forests because it nearly eliminated hardwoods. Advanced regeneration was not affected by burning but it was nearly eliminated by mastication for the 2-year sample period. However, mastication increased the cover of non-native invasive plants, particularly Japanese honeysuckle, indicating a need for continued treatment with fire or herbicides. Survival of planted pines was high and did not vary significantly among treatments. The best pine survival was after the mastication treatment which would help meet commercial goals.

**Appendix 1.** Crosswalk between proposed and delivered FFS outreach activities.

| Proposed | Delivered | Status |
|---|---|---|
| "We will learn how to use prescribed fire and mechanical treatments in beetle-killed areas to make recommendations on which treatment best supports regeneration of pines, oaks, and other target vegetation." | The three treatments, high intensity burning, low intensity burning, and mastication, had varying results relative to commercial and restoration objectives. Mastication appears to be the best choice for regenerating a commercial pine forest. This treatment reduces competition from hardwood sprouts, greatly reduces fuel loading and wildfire danger, and has no impact on pine survival, soils, and mycorrhizae. However, invasive plants can become a concern. High-intensity fires were the better choice for restoration objectives. They reduced fuels and wildfire damage, improved soil inoculum potential for mycorrhizae that favor target tree species, controlled invasive plants, and had no lasting damage to soils. | Preliminary results were presented by Phillips and others at the 15th Biennial Southern Silvicultural Research Conference. A paper is in press (see below). A final overview will be presented in a paper to be submitted to the *Canadian Journal of Forest Research.* |
| "An analysis of damage to soils within beetle-killed areas will allow managers to make better-informed decisions relative to the costs of achieving their objectives." | Prescribed burning, even high intensity burning, presented short-term reductions in soil fertility and damage to soil structure (removal of litter and duff). These reductions lasted less than 2 years. Mastication had no short-term impacts other than to cover disturbed soils. Wood chips compacted quickly and began decomposing, which may act as a long-term C sink, but this project was too short to observe C dynamics. Mycorrhizal soil inoculum potential was not impacted across treatment areas but high-intensity fires favored seem to favor species favorable to regeneration of desired tree species. | This portion of the study will be contained within a dissertation from Clemson University and will be used as supporting data for a paper on mycorrhizae for *Forest Science* (draft included in Appendix 2). A portion was already published in *Mycorrhizae* (attached) |
| "The project will provide basic information about the direct relationship of fire intensity to fuel reduction." | Mastication essentially removed all combustible fuels. High intensity fires were easy to control because of the lack of fuels in surrounding areas. High intensity fires provided longer protection from wildfire. By the second year after burning, woody fuels, litter, and duff were not significantly different between low-intensity plots and unburned controls but all fuels were significantly lower in plots burned at high intensity. | This portion of the study will be the contained within a dissertation from Clemson University and will be used as supporting data for a paper on mycorrhizae for *Forest Science* (draft included in Appendix 2). |

**Appendix 1.** Continued.

| Proposed | Delivered | Status |
|---|---|---|
| "Better information will become available to predict the combustion patterns of large woody fuels." | A simulated rainfall study showed that dry logs do not absorb appreciable amounts of moisture from single rainfall events of up to 4 inches or multiple rainfall events if they are spaced more than 1 week apart. Multiple rainfall events within a week are needed to prevent consumption and smoldering of logs after a prolonged drought. | The work was presented by Mohr and others at the 15th Biennial Southern Silvicultural Research Conference (in press). We plan to continue this study to determine ignition probability and consumption in a field setting. |
| Journal Article | Aaron D. Stottlemyer, G. Geoff Wang, Thomas A. Waldrop, Christina E. Wells, Mac A. Callaham. 2009. Examining variability in soil mycorrhizal inoculum potential after fuel reduction treatments in beetle-killed stands. | A draft has been completed and will be submitted to *Forest Science* after internal review. |
| Dissertation | Stottlemyer, Aaron D. 2009. Epidemic Southern Pine Beetle Attacks: A Problem of Fuel-Loading or an Opportunity for Management? Ph.D. Dissertation. Clemson University, Clemson, SC. | Scheduled for completion in May 2009. |
| Journal Article | Stottlemyer, A. ,G. Wang, C.E. Wells, D.W. Stottlemyer, T.A. Waldrop. 2008. Growing non-mycorrhizal loblolly pine seedlings in a greenhouse setting. *Mycorrhiza* (2008) 18:269-275. | Published |
| Journal Article | Waldrop, Thomas A.; Phillips, Ross J.; Stottlemyer, Aaron D. Fuel reduction treatments for restoring beetle-killed areas for commercial or historical objectives. *Canadian Journal of Forest Research.* | Analysis complete. Draft to be submitted for internal review by June 2009. |
| Proceedings Article | Stottlemyer, Aaron D.; Wang, G. Geoff; Waldrop, Thomas A. 2009. Energy content in leaf litter of oaks and species that replace oaks. In: Proc.14th biennial southern silvicultural res. conf. 2007 Feb 26-28; Athens, GA. Gen. Tech. Rep. Asheville, NC: U.S.D.A., For. Serv. | In press. |

**Appendix 1.** Continued.

| Proposed | Delivered | Status |
|---|---|---|
| Proceedings Article | Phillips, Ross J.; Waldrop, Thomas A.; Stottlemyer, Aaron D. 2009. Occurrence and spread of non-native invasive plants in stands treated with fire and/or thinning in the Piedmont of South Carolina. 15th Biennial Southern Silvicultural Research Conference. Hot Springs, AR. 2008. | In press. |
| Proceedings Article | Mohr, Helen H.; Waldrop, Thomas A. 2009. Loading of heavy fuels in beetle-killed areas: a problem of predicting fire behavior. 15th Biennial Southern Silvicultural Research Conference. Hot Springs, AR. 2008. | In press. |
| Abstract | Stottlemyer, A.D., G.G. Wang, T.A. Waldrop, C.E. Wells. 2008. Comparing soil ectomycorrhizal and vesicular-arbuscular mycorrhizal inoculum potential after restoration treatments in beetle-killed stands. Society for Ecological Restoration Coastal Plain Chapter Annual Meeting, Clemson, SC. | Done |
| Abstract | Stottlemyer, A.D., P.H. Brose, G.G. Wang, T.A. Waldrop. 2007. Leaf litter chemistry affects fire behavior in eastern deciduous forests. 92nd ESA Annual Meeting, San Jose, CA. | Done |
| Abstract | Stottlemyer, A.D., G.G. Wang, T.A. Waldrop, C.E. Wells, M.A. Callaham. 2007. Burning and mastication as fuel reduction treatments in beetle-killed stands: effects on ecto-mycorrhizal inoculum potential. 2007 EastFIRE Conference, Fairfax, VA. | Done |
| Abstract | Stottlemyer, A.D., G.G. Wang, C.E. Wells, T.A. Waldrop, and M.A. Callaham. 2007. Burning and mulching to reduce high fuel loading in beetle-killed stands: effects of fuel reduction treatments on ectomycorrhizal inoculum potential. 2nd Annual Fire Behavior and Fuels Conference, Destin, Florida. March 2007. | Done |

**Appendix 1.** Continued.

| Proposed | Delivered | Status |
|---|---|---|
| Poster | Stottlemyer, A.D., R.T. Layton, G.G. Wang, C.E. Wells, T.A. Waldrop, M.A. Callaham. Growing non-mycorrhizal loblolly pine (Pinus taeda L.) seedlings in a standard greenhouse setting. ESA Annual Conference, Memphis, Tennessee, August 2005. | Done |
| Poster | Mohr, Helen H.; Waldrop, Thomas A. 2009. Loading of heavy fuels in beetle-killed areas: a problem of predicting fire behavior. 15th Biennial Southern Silvicultural Research Conference. Hot Springs, AR. 2008. | Done |
| Oral presentation | Stottlemyer, Aaron D.; Wang, G. Geoff; Waldrop, Thomas A. Energy content in leaf litter of oaks and species that replace oaks. 14th biennial southern silvicultural res. conf. 2007 Feb 26-28; Athens, GA. | Done |
| Oral presentation | Phillips, Ross J.; Waldrop, Thomas A.; Stottlemyer, Aaron D. 2009. Occurrence and spread of non-native invasive plants in stands treated with fire and/or thinning in the Piedmont of South Carolina. 15th Biennial Southern Silvicultural Research Conference. Hot Springs, AR. 2008. | Done |
| Oral presentation | Stottlemyer, A.D., G.G. Wang, T.A. Waldrop, C.E. Wells, M.A. Callaham. 2008. Examining variability in soil mycorrhizal inoculum potential after fuel reduction treatment in beetle-killed stands. 15th Biennial Southern Silvicultural Research Conference, Hot Springs, AR. | Done |
| Oral presentation | Stottlemyer, A.D., G.G. Wang, T.A. Waldrop, C.E. Wells. 2008. Comparing soil ectomycorrhizal and vesicular-arbuscular mycorrhizal inoculum potential after restoration treatments in beetle-killed stands. Society for Ecological Restoration Coastal Plain Chapter Annual Meeting, Clemson, SC. Oral presentation. | Done |
| Oral presentation | Stottlemyer, A.D. Studying the role of fire in restoration of eastern deciduous forests. Department of Forestry, Michigan State University, September 2007. | Done |

**Appendix 1.** Continued.

| Proposed | Delivered | Status |
|---|---|---|
| Oral presentation | Stottlemyer, A.D., P.H. Brose, G.G. Wang, T.A. Waldrop. 2007. Leaf litter chemistry affects fire behavior in eastern deciduous forests. 92nd ESA Annual Meeting, San Jose, CA. Oral presentation. | Done |
| Oral presentation | Stottlemyer, A.D., G.G. Wang, T.A. Waldrop, C.E. Wells, M.A. Callaham. 2007. Burning and mastication as fuel reduction treatments in beetle-killed stands: effects on ectomycorrhizal inoculum potential. 2007 EastFIRE Conference, Fairfax, VA. | Done |
| Oral presentation | Stottlemyer, A.D., G.G. Wang, C.E. Wells, T.A. Waldrop, and M.A. Callaham. 2007. Burning and mulching to reduce high fuel loading in beetle-killed stands: effects of fuel reduction treatments on ectomycorrhizal inoculum potential. 2nd Annual Fire Behavior and Fuels Conference, Destin, Florida. March 2007. | Done |
| Oral presentation | Stottlemyer, A.D., P.H. Brose, G.G. Wang, T.A. Waldrop. Energy content in dried leaf litter of some oaks (Quercus spp.) and mixed-mesophytic species that replace oaks. 14th Biennial Southern Silvicultural Research Conference, Athens, Georgia. February 2007. | Done |
| Seminar | Stottlemyer, A.D., P.H. Brose, G.G. Wang, T.A. Waldrop. 2008. Leaf litter chemistry affects fire behavior in eastern deciduous forests. Penn State DuBois Natural Resources Colloquium, DuBois, PA. | Done |
| Teaching – 2 lectures and 2 laboratory exercises | Stottlemyer, A.D. The role of research in silviculture using a study of options for beetle-killed stands. Clemson Univ, Dept of Forestry and Natural Resources, FOR 465 (Silviculture), Spring 2008, Spring 2007. | Done |
| Teaching lecture | Stottlemyer, A.D. Comparing soil ectomycorrhizal and vesicular-arbuscular mycorrhizal inoculum potential after restoration treatments in beetle-killed stands. Clemson University, Department of Forestry and Natural Resources, FOR 812 (Fire Ecology), Spring 2008. | Done |

**Appendix 1.** Continued.

| Proposed | Delivered | Status |
|---|---|---|
| Seminar | Stottlemyer, A.D. Comparing soil ectomycorrhizal and vesicular-arbuscular mycorrhizal inoculum potential after restoration treatments in beetle-killed stands. Clemson University, Department of Forestry and Natural Resources seminar series, Clemson, South Carolina, April 2008. | Done |
| Seminar | Stottlemyer, A.D. and R.J. Phillips. Silvicultural options for fuel reduction in beetle-killed stands. Clemson University, Department of Forestry and Natural Resources FOR 662 (Silviculture II), Spring 2005. | Done |
| Teaching – 4 lectures, 3 laboratory exercises | Stottlemyer, A.D. Fuel sampling methods. Clemson University, Department of Forestry and Natural Resources, FOR 812 (Fire Ecology), Spring 2008, Spring 2007, Spring 2005, Spring 2004. | Done |
| Seminar | Waldrop, T.A. Restoration of beetle kills with fire and mastication. University of Georgia, January 2009. | Done |
| Internet | Results posted on research unit web pages as temporary special features. http://www.srs.fs.usda.gov/disturbance/ | Done |
| Conference paper | Stottlemyer, Aaron D., G. Geoff Wang, Thomas A. Waldrop, Christina E. Wells, Mac A. Callaham. 2009. Comparing first-year pine seedling survival after burning and mulching for site preparation in beetle-killed pine stands. | Draft completed and to be submitted for the $16^{th}$ Biennial Southern Silvicultural Research Conference or other appropriate outlet |

# APPENDIX 2 - PAPERS

## Examining variability in soil mycorrhizal inoculum potential after fuel reduction treatments in beetle-killed stands

[This will be a chapter in the dissertation by Stottlemyer and will be submitted to the *Forest Science*].

Aaron D. Stottlemyer, G. Geoff Wang, Thomas A. Waldrop, Christina E. Wells, Mac A. Callaham

Instructor, Penn State University DuBois, DuBois, PA; Associate Professor, Clemson University, Clemson, SC; Research Forester, USDA Forest Service, Southern Research Station, Clemson, SC; and Associate Professor, Clemson University, Clemson, SC; Research Ecologist, USDA Forest Service, Southern Research Station, Athens, GA, respectively.

Abstract-- Heavy fuel loads were created by southern pine beetle (Dendroctonus frontalis Zimm.) outbreak throughout the southeastern Piedmont during the early 2000's. Prescribed burning and mechanical mulching (mastication) are used to reduce fuel loading, but many ecological impacts are unknown. Successful forest regeneration depends on ecto- (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, soil chemistry, and/or effects on host vegetation. The objectives of this study were to (1) compare soil VAM and ECM inoculum potential after prescribed burning and mulching treatments to no-treatment (control), and (2) examine potential for soil chemistry, fire behavior, and pre-treatment (YR0) vegetation to influence post-treatment soil inoculum potential. Neither VAM nor ECM inoculum potential observed from soil bioassays were significantly different among treatments, but were highly variable within treatments. ECM inoculum potential was positively associated with inorganic N concentrations and fire intensity and residence time. Conversely, VAM inoculum potential was negatively associated with inorganic N concentrations and fire intensity and residence time. Best-subset regression revealed that YR0 vegetation was associated with post-treatment soil inoculum potential. Results of this study suggest that high-intensity, slow-moving prescribed fires may favor VAM host plants and that YR0 vegetation composition and structure may be valuable treatment selection criteria for beetle-killed stands.

### INTRODUCTION
#### Background

There was epidemic southern pine beetle (Dendroctonus frontalis Zimm.) activity in the southeastern United States during the early 2000's. Beetle-killed pine trees fall in one to two 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. Common restoration objectives for beetle-killed stands in this region include: hardwoods, pine plantations, and pine-hardwood mixtures.

It was recently determined in another component to this study that prescribed burning and mechanical fuel reduction differentially affect fuel loads (Appendix 1) and some soil chemical properties (Appendix 2). However, other ecological impacts are unknown including effects on important biological properties of soil.

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 VA mycorrhizal. 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. Furthermore, if old roots and mycelia associated with existing vegetation are the major propagules by which mycorrhizal colonization is initiated with forest regeneration. Therefore, it is possible that the pre-disturbance vegetation community largely determines the type and amount of mycorrhizal fungi available to regenerating plants.

The primary objectives of this study were to (1) compare soil VAM and ECM inoculum potential among treatments, and (2) examine relationships between soil inoculum potential and a) soil nutrients, b) fire behavior and c) pre-treatment (YR0) vegetation composition and structure.

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 18-33 years in age when killed. Mean diameter of *Pinus* spp. stems (live or dead) in the YR0 vegetation community was 21.9 cm. Metal stakes were placed on a 25 meter x 25 meter spacing to create a grid system throughout each stand and permanent references for conducting fuel, vegetation, and soil sampling (Figure 1).

Pre-treatment Vegetation Sampling

Two 10 meter x 50 meter vegetation sampling plots containing of five 10 meter$^2$ subplots were randomly located in each beetle-killed stand (Figure 1). The long axis of the sampling plots were randomly assigned one the four cardinal directions (N, S, E, W). Woody stems rooted inside the 10 meter$^2$ subplots were measured and categorized as saplings (0-10 centimeters DBH), trees (>10 centimeters DBH), or shrubs. Saplings were assigned one of three diameter class ratings (1=<1 percent; 2=1-10; 3=11-25; 4=26-50; 5=51-75; 6=>75). Subsequent calculations were based on the mid-points of the diameter classes.

Diameters were measured for all live and dead trees and that were >1.4 meters in height. Visual estimations of percentage shrub cover were made at the 10 meter$^2$ subplot level.

Four 1 meter2 quadrats were established at the centers of the 10 meter$^2$ subplots (Figure 1). Live non-woody herbaceous plants and tree seedlings and sprouts that were rooted inside the 1 meter$^2$ quadrat and <1.4 meters in height were identified to species and assigned a percentage cover class rating (1=<1 percent; 2=1-10; 3=11-25; 4=26-50; 5=51-75; and 6=76-100). Subsequent calculations were based on the mid-points of the cover classes.

Vegetation survey data were summarized by genus, functional group (herbaceous; seedling/sprout; sapling; shrub; or tree), and mycorrhizal status (VAM; ECM; Ericoid; or non-mycorrhizal). Importance (abundance) values (IV) were calculated for herbaceous plants, shrubs, and seedlings/sprout genera using the formula: IV=(relative cover + relative frequency)/2. New categories were created by summarizing abundance of each genus by functional group and referred to as ‘plant categories’ (e.g., *Quercus* L. seedling and sprout abundance).

Importance values were calculated for live and dead sapling and tree categories using the formula: IV=(relative basal area + relative frequency + relative density)/3 (e.g., live *Liquidambar* L. sapling abundance).

Rank-abundance curves were created for each functional group and used to identify genera that were most abundant across beetle-killed stands in the YR0 vegetation assemblages. For each functional group, a genus was selected if (1) its abundance was greater than 0.05, or (2) its rank placed it above the primary inflection point on the rank-abundance curve (e.g., Figure 2). The selected genera (Table 1) were the most abundant genera in each functional group and were used as independent variables to model post-treatment mycorrhizal dynamics of soil in beetle-killed stands (discussed below).

Fuel Reduction Treatments

The twelve stands were randomly assigned to one of three fuel reduction treatments in an unbalanced design to create three replications of control and mulching and six 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 three-day period between 30-March and 03-May, 2006 using manual strip-head firing.

Fire Behavior Measurement

Type K thermocouples (Onset Computer Corp.) 30 centimeters in length were inserted between the surface of mineral soil and duff at four to six grid point markers that were closest in proximity to the 10 meter x 50 meter vegetation sampling plots (Figure 1) prior to prescribed buring. HOBO® data loggers were set to record temperature every one-and-a-half seconds. The data loggers started collecting temperature readings approximately 2-3 hours prior to prescribed burning and continued collecting data until after the fire was completely extinguished. The raw data were used to determine the maximum heat pulse temperature recorded at the soil-duff interface and create profiles of fire temperature over time. In addition, 50ºC is a temperature threshold above which most soil organisms are compromised (Neary and others 2005). In the current study, 'residence time' was calculated as the amount of time (seconds) that temperature was sustained above 50ºC at each thermocouple.

Mycorrhizal Bioassays

Intact soil cores were obtained for VAM bioassays between 22-May and 8-June 2006. Soil sampling for ECM bioassays was performed between 10-July and 20-July 2006. For each sampling period, a 211 milliliter soil core was obtained from each 1 meter$^2$ quadrat (Figure 1) providing four samples per 10 meter$^2$ subplot and 20 observational units total per 10 meter x 50 meter 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 Greenhouses 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 (*Pinus 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 centimeter corn root segments (<1 millimeter in diameter) were mounted on glass slides after clearing with 10 percent KOH, staining with trypan blue, and de-stained in 50 percent glycerol. Slides were assessed with a compound microscope equipped with a cross-hair eyepiece under 110x magnification. The presence/absence of VAM hyphae was noted at each intersection of the cross-hair and a root segment. VAM colonization values were calculated using the equation: VAM colonization = number intersections at which hyphae were present ÷ 50. In addition, root and shoot dry weights and root/shoot ratios were determined for the seedlings. Root systems of more than 450 corn seedlings were assessed for VAM colonization and root and shoot growth after accounting for seedling mortality and non-germinants.

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 ≥ 6cm in length were randomly selected from each seedling. Each short root was tallied and classified as mycorrhizal or non-mycorrhizal using a dissecting microscope. Non-mycorrhizal 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. In addition, root and shoot dry weights and root/shoot ratios were determined for the seedlings. Root systems of more than 380 pine seedlings were assessed for ECM colonization and root and shoot growth after accounting for seedling mortality and non-germinants.

Statistical Analysis

The 12 beetle-killed stands were experimental units for comparing bioassay seedling variables among treatments. The 24-10 meter x 50 meter plots were experimental units for regression analyses. Sampling unit averages that were calculated from vegetation surveys, fire behavior measurements, soil nutrient sampling, and mycorrhizal bioassays included: importance values of the most abundant YR0 VAM and ECM plant genera in different functional groups (Table 1); maximum heat pulse temperature and residence time of prescribed fire; YR1 soil inorganic N (NH4+-N and NO3--N) concentrations and exchangeable P, and YR1 VAM and ECM colonization, root and shoot dry weight, and root/shoot ratio of bioassay seedlings. The averages were used in subsequent statistical procedures.

Percentages of VAM and ECM colonization were compared among the control, prescribed burning, and mulching treatments using analysis of variance (PROC GLM, SAS Institute, 2003). Next, correlation analysis (PROC CORR) was used to explore associations between percentage VAM and ECM colonization, selected soil nutrients, and maximum heat pulse temperature and fire residence time.

The vegetation importance values had different scales and variances. Therefore, the variables were standardized to mean=0 and standard deviation=1 (PROC STANDARD) prior to the following multivariate statistical procedures. Best-subset regression was used to determine if soil inoculum potential was associated with YR0 vegetation status for each individual treatment. Plant categories were input to the regression model in a stepwise manner. The categories that met a significance level of 0.15 were selected to the model. The models were evaluated using their cross-validated $R^2$ values.

## RESULTS AND DISCUSSION

### Mycorrhizal Colonization of Bioassay Seedlings

Impacts of prescribed fire and mechanical treatments on mycorrhizal dynamics are not fully understood. Furthermore, VAM and ECM have not been studied simultaneously and the importance of existing vegetation to mycorrhizal colonization of new germinants has received little attention. 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 after prescribed burning and mulching (Figure 3). 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.

### Bioassay Seedling Growth

Root and shoot dry weight of corn seedlings grown in soil collected from prescribe burned and mulched sites was significantly higher than that of corn seedlings grown in control soil (Table 2). The bioassay methods used in the current study held water and light equal across treatments which strongly suggests that the growth-limiting factor was in soil. Results from bioassays suggest that soil mycorrhizal inoculum potential did not significantly influence the growth of corn and pine seedlings, but that other soil factors were responsible for observed differences in corn seedling development.

First- (YR1) and second- (YR2) year post-treatment datasets were incomplete for soil bulk density (Appendix 2). However, soil bulk density two years after prescribed burning was not significantly different than the control. Moreover, if mulching would have any impact on soil bulk density, it would likely be compaction rather than loosening (e.g., Moghaddas and Stephens 2008) and the growth of bioassay seedling roots were not restricted based on observed dry weights (Table 2).

Effects of the treatments on exchangeable nutrients in the first year post-treatment (Appendix 2) appear to be responsible for observed differences in corn seedling growth. Aluminum ($Al^{3}+$) toxicity is an important growth-limiting factor in acidic soils, particularly at pH <5.0-5.5 (Havlin and others 1999). Soils in the current study fall within this range with the average pH across beetle-killed stands in YR0 being 5.18 (Appendix 2). $Al^{3}+$ negatively affects plant growth through blockage of Ca and Mg carriers in the plasma membrane, out-competing other cations (particularly $Ca^{2}+$) for binding sites, inhibiting root elongation, and contributing to downstream nutrient and drought stress (Havlin and others 1999; Taiz and Zeiger 2006).

Exchangeable Al was decreased in YR1 by prescribed burning and mulching in the current study (Appendix 2). Other studies found similar results. Boerner and others (2004) reported decreases in extractable Al after fire in a mixed-oak forest in Ohio. Similarly, Kretzschmar and others (1991) found that root growth of pearl millet (*Pennisetum glaucum* L.) was considerably enhanced with millet straw application which the authors attributed to increased soil solution pH and decreased Al in the soil solution. In the current study, corn seedlings grown in soil collected in prescribe burned and mulched stands may have responded favorably to decreased Al accounting for higher root and shoot dry weight (Table 2) compared to corn seedlings grown in control soil.

Sources of Variability in Soil Inoculum Potential: Soil Fertility

There was a high degree of variability in soil mycorrhizal inoculum potential within beetle-killed stands treated with different fuel reduction treatments as well as those that were un-treated (Table 3). Changes in soil mycorrhizal inoculum potential may arise via direct or indirect effects on the abundance or activity of fungal propagules. Herr and others (1994) suggested that variability in ECM colonization of P. strobus L. seedlings two months after prescribed burning may have been related to variability in soil N and P in burned plots.

Generally, low concentrations of soil N and P are conducive to greater mycorrhizal colonization (Herr and others 1994). In the event of limited soil N and P, the plant's investment in C to initiate colonization and support the fungal symbiont is less than the investment in root growth to overcome depletion zones (Smith and Read 2000). In the current study, there were no differences in mycorrhizal colonization of bioassay seedlings despite an observed reduction in exchangeable P after prescribed burning and mulching compared to the control in YR1 (Appendix 2). It is possible that the magnitude of the difference in exchangeable P was not large enough to influence mycorrhizal colonization. Soil inoculum potential was not associated with Total N within or among fuel reduction treatments, but responded to different concentrations of soil NH4+-N and NO3--N in prescribe burned plots (Table 4).

It is unclear why in our study VAM colonization of corn seedlings was negatively correlated with soil NH4+-N and NO3--N concentration after prescribed burning when the activity of VAM fungi has generally been found to increase with increasing soil N (Knorr and others 2003; Libidi and others 2007). The abundance of certain types of ectomycorrhizas (e.g., *Pisolithus tinctorius* (Pers.) Coker and Couch) on loblolly pine roots has been shown to increase as NH4+-N and NO3--N concentrations increase (Marx 1990) which is consistent with our results. However, increasing levels of NO3--N has also been shown to cause a decrease in ECM development of pine (Richards and Wilson 1963).

Soil VAM inoculum potential was marginally associated with NH4+-N and NO3--N across treatments. In addition, it is noteworthy that VAM and ECM inoculum potential exhibited opposite relationships to NH4+-N and NO3--N concentrations after prescribed burning which suggests a possible mechanism by which prescribed burning might encourage some plant taxa while discouraging others. A small proportion of variability in soil inoculum potential was explained by soil chemistry in mulched and control stands.

Soil VAM inoculum potential was not associated with exchangeable P in our study (Table 4). Amaranthus and Trappe (1993) found that VAM colonization of incense-cedar (*Libocedrus* decurrens (Torr.) Florin) seedlings grown in top soil that eroded following forest fire in Oregon was more than 4.5 times that of seedlings grown in residual soil despite P levels in eroded soil being nearly 4 times that of residual soil. It is possible that in the current study the range in exchangeable P in beetle-killed stands was not large enough to produce significant changes in VAM inoculum potential. ECM inoculum potential was marginally associated with exchangeable P in un-treated stands and across all treatments (Table 4). These results indicate that soil nutrients accounted for some of the observed variability in total soil inoculum potential. However, relationships between soil nutrients and percent mycorrhizal colonization of seedlings explained only a small proportion of the variability in soil mycorrhizal inoculum potential after prescribed burning in beetle-killed stands.

Sources of Variability in Soil Inoculum Potential: Fire Behavior

Fire intensity and residence time were variable within burned stands (Table 5) which led to the hypothesis that soil inoculum potential may be associated with fire behavior. When correlations between fire behavior indices and soil VAM and ECM inoculum potential were examined, opposite relationships were observed. Percentage VAM colonization of corn seedlings decreased and percentage ECM colonization of pine seedlings increased as maximum heat pulse temperature increased (Figure 4). Similarly, as fire residence time increased, percentage VAM colonization decreased and percentage ECM colonization increased (Figure 5).

These results suggest that damage and/or mortality of VAM propagules increase as fire intensity and residence time increases with little or no change in active ECM propagules. Different ECM fungi are known to tolerate disturbance differently. For example, Miller and others (1994) and Torres and Honrubia (1997) found that Cenococcum geophilum Fr. sclerotia were more abundant in burned soil than in unburned soil. Therefore, it is possible that in the current study there was proliferation of certain ECM fungal taxa with increasing fire intensity and residence time. It is also possible that there were no changes in the abundance of ECM propagules, but an increase in the activity of existing propagules.

Significant positive correlations were found between percentage ECM colonization of pine seedlings and soil NH4+-N and NO3--N concentrations (Table 4) as previously discussed. Additional correlation analyses revealed that soil NH4+-N and NO3--N concentrations were positively associated with fire intensity and residence time (Table 6). Burning may have accelerated the decomposition of the remaining forest floor releasing NH4+-N. NO3--N is usually low immediately following fire, but increases rapidly during the nitrification of NH4+-N (Neary and others 2005). Therefore, increases in soil ECM inoculum potential observed at higher fire intensities and residence times may have been related to increases in ECM propagules and/or increases in soil NH4+-N and NO3--N concentrations.

Sources of Variability in Soil Inoculum Potential: Pre-treatment Vegetation

The extent to which roots become colonized by mycorrhizal fungi and the dependence on mycorrhizal colonization for nutrient and water acquisition varies among different plant species and with the stage of growth (Smith and Read 2000). For this reason, different plant taxa have different effects on the mycorrhizal fungus content of the soil (Janos 1980). Furthermore, forest structure and composition influence the type and density of the mycorrhizal inoculum (Brundrett and Kendrick 1988) available to new plant germinants (Smith and Read 2000). Therefore, it seemed reasonable that the amount of mycorrhizal colonization exhibited by bioassay seedlings growing in field soil collected after a given treatment may be related to YR0 vegetation status.

Certain plant genera and functional groups were more abundant than others in the YR0 vegetation community in beetle-killed stands (Table 1) and were hypothesized to influence on soil inoculum potential. The new lists were further subjected to best subset regression analyses using VAM or ECM colonization as dependent variables. A fewer number of categories were selected to the models that had a greater influence on soil inoculum potential than non-selected categories (Table 7). Key findings and interpretations from the overall modeling effort follow.

(1)Soil inoculum potential was more highly associated with certain plant genera than others:

In our study, positive associations suggest that the respective plant categories positively influenced soil inoculum potential. Soil inoculum potential increased with increases in the importance of some plant hosts (Table 7). Models of data collected in control stands represent the plant categories that were most highly associated with soil inoculum potential after no-treatment. Negative associations suggest that categories negatively influenced soil inoculum potential

Host genera that were associated with soil VAM inoculum potential were *Vitis, Nyssa, Prunus, Viburnum,* and *Parthenocissus*. Soil ECM inoculum potential was influenced by Quercus and Pinus. To our knowledge, no studies have examined the relative contribution of the relevant VAM and ECM genera to soil inoculum potential. However, it is possible that genera that were positively associated with soil inoculum potential were also associated with a high density of mycorrhizal propagules due to high mycorrhizal dependency (Smith and Read 2000).

(2)Soil inoculum potential was more highly associated with certain plant functional groups than others:

In our study 7 of 12 plant categories selected to models of soil inoculum potential in treated stands were in the 'saplings' functional group. Hart and others (Hart and others 2005) found that ponderosa pine (*Pinus ponderosa* C. Lawson) sapling stands tended to have higher fine root and ECM root biomass than pole and tree stands which suggests a possible reason why areas containing greater sapling abundance prior to treatment were associated with higher soil inoculum potential after treatment.

(3)Increases or decreases in soil inoculum potential were associated with the abundance of certain live plant hosts:

Abundant mycorrhizal propagules are expected under stands of mycotrophic host plants (Janos 1980) and living hyphal networks are important in initiating rapid colonization in seedlings (Smith and Read 1997). Therefore, decreasing inoculum potential with increasing abundance of host genera is less intuitive.

Grasses are the least dependent on mycorrhizal colonization of all mycorrhizal plants (Janos 1980). Increasing abundance of *Panicum* L. (a grass genus) was associated with decreasing VAM inoculum potential in the control (Table 7). Therefore, increasing abundance of *Panicum* may have been associated with decreased abundance of other VAM host plants that are more dependent on VAM colonization and larger contributers to the inoculum pool available to new germinants.

Similarly, increasing abundance of *Quercus* seedlings and sprouts was associated with decreasing ECM inoculum potential after burning and mulching treatments (Table 7). Much of the live vegetation present in the YR0 vegetation assemblages was top-killed by prescribed burning or mulching including oak seedlings and sprouts (data not shown). Re-sprouting depended on C reserves in the roots because little or no photosynthetic tissue remained after treatment (personal observation). It is therefore possible that ECM inoculum potential decreased when C in the roots that is normally available to mycorrhizal fungi was re-directed to build new above-ground tissues (Buchholz and Gallagher 1982).

(4)Increases in soil inoculum potential were associated with the abundance of certain dead plant hosts:

Less is known about the contribution of dead and dying vegetation to the mycorrhizal inoculum pool or how the forest structural attributes of dead vegetation impact soil inoculum potential. In our study, dead saplings in particular were positively associated with soil VAM and ECM inoculum potential (Tables 7). The saplings apparently died as a result of competition with other plants in the years following beetle kill of overstory trees.

Dead saplings of *Prunus, Quercus,* and *Pinus* may have been a symptom of high plant cover and competitive interaction between neighboring plants which commonly occurs in these systems during primary succession (Elliott and Hewitt 1997). Abundance of VAM and ECM inoculum has been shown to increase with vegetation cover after disturbance (Brundrett and others 1996). Therefore, in our study positive associations between abundance of dead stems and soil inoculum potential may have been related to high vegetation cover of host plants which was not directly measured.

It is also possible that mycorrhiza formation was related to favorable environmental conditions created by increasing dead stem abundance. However, this seems unlikely because the factors known to influence mycorrhizal colonization (light; temperature; and moisture) (Brundrett and others 1996b) were controlled during greenhouse bioassays.

Hyphal networks can survive and remain temporarily viable and able to colonize roots when the vegetation with which it developed is dead (Smith and Read 2000). However, it is unclear whether there are certain conditions under which propagules associated with the roots of dead plants are more available to germinating seedlings than ones associated with the roots of healthy plants.

The two hardwood species, *Prunus* and *Quercus*, are vigorous sprouters (Brose and Van Lear 1998). It is possible that although saplings were observed to be dead, many still had a living root system. With the C demand on the plant to support above-ground biomass temporarily reduced, it is possible that more C was available to support a higher abundance of mycorrhizal fungi; thereby temporarily increasing soil inoculum potential in the vicinity of the dead saplings' root systems.

## CONCLUSIONS

Prescribed fire and mechanical mulching have been proposed to reduce high fuel loading in beetle-killed pine stands in the southeastern U.S. 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.

In another component to this study, the treatments were shown to affect downed woody and forest floor fuels differently. In addition, there were significant differences in exchangeable P, Ca, and Al and Ca/Al molar ratio in the first year after treatments were implemented. However, the differences were short-lived and did not appear in the YR2 data.

In the same context, soil biological properties have gone unstudied. Mycorrhizal fungi have the potential to influence the trajectory of vegetation succession after a disturbance. We compared VAM and ECM inoculum potential among treatments and examined possible sources of variation in inoculum potential within treated stands using bioassays of intact soil cores in the greenhouse.

VAM and ECM inoculum potential were variable within post-treatment beetle-killed stands, but neither was significantly different among treatments in the present study. Differences in the root and shoot development of corn seedlings seemed to suggest that plants may benefit from temporary reductions in exchangeable Al caused by prescribed burning and mulching.

A wide range of fire intensities and residence times are likely in beetle-killed stands due to spatial variability in fuel loading. ECM plant hosts may be favored over VAM hosts given opposite relationships of VAM and ECM inoculum potential with fire intensity and residence time observed in our study.

It was unclear why VAM inoculum potential of corn seedlings was negatively associated with inorganic soil N concentrations because the opposite trend has generally been observed in other studies. Our findings that ECM inoculum potential increased with increasing fire intensity and residence time may have been linked to increasing concentrations in inorganic soil N.

YR0 vegetation composition and structure were important sources of variability in YR1 VAM and ECM inoculum potential. However, some of the mechanisms underlying relationships between soil inoculum potential and vegetation status were not intuitive. Rather, interpretations were made drawing on a small literature concerning mycorrhizal dependencies and C dynamics of the relevant plants and treatment-soil inoculum potential interactions. Nonetheless, we feel that our results emphasize the importance of considering vegetation composition and structure when choosing a fuel reduction treatment in support of a given forest management objective.

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Table 1—Pre-treatment vegetation survey data from beetle-killed stands were summarized by genus, functional group (herbaceous cover; seedling/sprout; sapling; shrub; or tree), and mycorrhizal status (vesicular-arbuscular (VAM) or ectomycorrhizal (ECM)). Importance values were calculated for the genera in each functional group. The mycorrhizal genera listed had (1) a relative abundance greater than 0.05, or (2) a rank placing it above the primary inflection point on a rank-abundance curve created for a given functional group

| | **Vegetation Functional Group** | | | | |
|---|---|---|---|---|---|
| **Mycorrhiza type** | **Herbs** | **Seedlings / Sprouts** | **Saplings** | **Shrubs** | **Trees** |
| VAM | *Erechtites* Raf. | *Acer* L. | *Acer* | *Aralia* L. | *Liquidambar* |
| | *Lonicera* L. | *Liquidambar* L. | *Cornus* L. | *Ligustrum* L. | *Liriodendron* |
| | *Panicum* L. | *Liriodendron* L. | *Ilex* L. | *Viburnum* L. | |
| | *Parthenocissus* Planch. | *Nyssa* L. | *Liquidambar* | | |
| | *Rubus* L. | *Prunus* L. | *Prunus* | | |
| | *Smilax* L. | | | | |
| | *Toxicodendron* Mill. | | | | |
| | *Vitis* L. | | | | |
| ECM | | *Carya* Nutt. | *Quercus* | | *Pinus* L. |
| | | *Quercus* L. | | | *Quercus* |

Table 2--Mean root and shoot dry weight and root/shoot ratio 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 in beetle-killed stands that were subjected to different fuel reduction treatments. Standard errors of the means are in parentheses. Means within a row followed by the same letter are not significantly different at the 0.05 level

| | Corn seedlings | | | Loblolly pine seedlings | | |
|---|---|---|---|---|---|---|
| **Treatment** | Root dry wt (g) | Shoot dry wt (g) | Root / shoot ratio | Root dry wt (g) | Shoot dry wt (g) | Root / shoot ratio |
| Control (no-treatment) (n=3) | 0.15 (0.02)b | 0.21 (0.03)b | 0.77 (0.03)a | 9.2E-3 (7.0E-4)a | 6.6E-2 (2.5E-3)a | 0.15 (0.01)a |
| Range | 0.11 - 0.17 | 0.14 - 0.25 | 0.46 - 1.08 | 7.9E-3 - 1.0E-2 | 5.8E-2 - 8.7E-2 | 0.12 - 0.17 |
| Prescribed burn (n=6) | 0.20 (0.01)a | 0.34 (0.02)a | 0.63 (0.02)b | 1.1E-2 (5.0E-4)a | 6.9E-2 (1.8E-3)a | 0.17 (0.01)a |
| Range | 0.13 - 0.25 | 0.21 - 0.43 | 0.49 - 0.92 | 8.1E-3 - 1.5E-2 | 5.9E-2 - 7.5E-2 | 0.13 - 0.38 |
| Mulch (n=3) | 0.19 (0.02)a | 0.29 (0.03)a | 0.66 (0.03)b | 9.9E-3 (8.0E-4)a | 7.0E-2 (2.5E-3)a | 0.14 (0.01)a |
| Range | 0.16 - 0.22 | 0.22 - 0.35 | 0.61 - 0.75 | 6.6E-3 - 1.2E-2 | 6.6E-2 - 7.3E-2 | 0.10 - 0.17 |

Table 3--Ranges in percentage vesicular-arbuscular and ectomycorrhizal colonization of corn and loblolly pine seedlings, respectively. Seedlings were grown in intact soil cores collected in beetle-killed stands that were subjected to different fuel reduction treatments

| | Ranges in percentage mycorrhizal colonization | | |
|---|---|---|---|
| **Mycorrhiza** | Control (no-treatment) (n=6) | Prescribed burn (n=12) | Mulch (n=6) |
| VAM | 16.67 - 24.10 | 8.47 - 20.50 | 11.50 - 37.85 |
| ECM | 14.09 - 45.55 | 10.27 - 37.88 | 10.99 - 41.42 |

Table 4--Correlations between percentage vesicular-arbuscular and ectomycorrhizal colonization of corn and loblolly pine seedlings, respectively, and soil chemical properties. Seedlings were grown in intact soil cores collected in beetle-killed stands that were subjected to different fuel reduction treatments. Soil chemical analyses were performed on soil samples collected in close proximity to the locations where soil cores were collected for mycorrhizal bioassays.

| | **Correlation with:** | | | |
|---|---|---|---|---|
| **Treatment / Mycorrhiza** | **Total N (mg kg-1)** | **NH4-N (mg kg-1)** | **NO3-N (mg kg-1)** | **P (mg kg-1)** |
| **Control (n = 6)** | | | | |
| VAM | 0.1961 | 0.1606 | 0.1154 | -0.2191 |
| ECM | -0.4644 | -0.0909 | -0.2885 | 0.7634* |
| **Burn (n = 12)** | | | | |
| VAM | 0.0109 | -0.7452*** | -0.5486* | -0.0124 |
| ECM | -0.4638 | 0.5822** | 0.5902** | -0.2489 |
| **Mulch (n = 6)** | | | | |
| VAM | -0.2929 | -0.3588 | -0.2980 | -0.1711 |
| ECM | -0.1168 | 0.2903 | -0.0067 | 0.5305 |
| **Across Treatments (n = 24)** | | | | |
| VAM | 0.1686 | -0.3770* | 0.4015* | 0.0886 |
| ECM | -0.0358 | 0.1469 | 0.1061 | 0.3864* |

*P ≤ 0.10; **P ≤ 0.05; ***P ≤ 0.01

Table 5--Means and ranges (in parentheses) of fire behavior indices measured in six treatment units using Type K thermocouples placed at the soil-duff interface and 30 centimeters above the surface of mineral soil. Maximum heat pulse temperature was the highest temperature recorded by remote data loggers at a given measurement location. Residence time was calculated as the amount of time (in seconds) that temperature was sustained above 50°C

| | **Maximum heat pulse temperature (°C)** | | **Residence time > 50°C (s)** | |
|---|---|---|---|---|
| **Prescribe burn unit** | **At soil-duff interface** | **30cm above mineral soil** | **At soil-duff interface** | **30cm above mineral soil** |
| Bombing Range East | 216 (27-751) | 487 (91-766) | 5686 (0-16427) | 5597 (211-21087) |
| Bombing Range West | 224 (61-633) | 386 (101-643) | 4173(254-21308) | 3279 (1260-22349) |
| Dove Field | 54 (17-352) | 181 (37-510) | 319 (0- 2748) | 571 (0- 951) |
| Issaqueena | 248 (27-830) | 384 (91-850) | 2302 (0-11309) | 1304 (546- 3168) |
| Rocky Ford | 37 (17- 86) | 510 (145-815) | 292 (0- 1530) | 1130 (342- 2498) |
| Transfer Station | 42 (12-150) | 152 (32-603) | 276 (0- 1530) | 595 (0- 1452) |
| Across Treatment | 137 (12-830) | 350 (32-850) | 2175 (0-21308) | 2079 (0-22349) |

Table 6--Correlations between NH4+-N and NO3--N concentrations in soil after burning and fire behavior indices measured at the soil-duff interface. Maximum heat pulse temperature was the highest temperature recorded by remote data loggers at a given measurement location. Residence time was calculated as the amount of time (in seconds) that temperature was sustained above 50°C

| | Correlation with: | |
|---|---|---|
| **Soil Variable** | **Maximum heat pulse temperature (°C)** | **Residence time > 50°C (s)** |
| $NH_4^+$-N concentration (mg $kg^{-1}$) | 0.6236<br>(0.0302) | 0.6736<br>(0.0163) |
| $NO_3^-$-N concentration (mg $kg^{-1}$) | 0.5049<br>(0.0941) | 0.6396<br>(0.0251) |

Table 7--The most abundant plant categories in pre-treatment beetle-killed stands (given in Table 1) were independent variables in best-subset regression used to model post-treatment vesicular-arbuscular (VAM) and ectomycorrhizal (ECM) inoculum potential. Plant categories that met a significance level of 0.15 were selected to the model and are listed in the table

| **Mycorrhiza type / Treatment** | **Vegetation Functional Group** | | | | | **Cross-validation $R^2$** |
|---|---|---|---|---|---|---|
| | **Herbs** | **Seedlings / Sprouts** | **Saplings** | **Shrubs** | **Trees** | |
| a. VAM | | | | | | |
| Control | *Vitis* L. +<br>*Panicum* L. - | *Nyssa* L. + | | | | 0.9656 |
| Burn | | | *Ilex* L. +<br>*Prunus* L. +<br>*Prunus* (d) + | *Viburnum* L. + | | 0.7496 |
| Mulch | *Parthenocissus* Planch. +<br>*Vitis* - | | *Prunus* + | | | 0.9958 |
| b. ECM | | | | | | |
| Control | | | *Quercus* (d) + | | *Pinus* (d) + | 0.9332 |
| Burn | | *Quercus* L. - | *Pinus* L. (d) +<br>*Quercus* + | | | 0.5378 |
| Mulch | | *Quercus* - | *Quercus* (d) + | | | 0.4436 |

+ = Positive association
- = Negative association
d = Dead

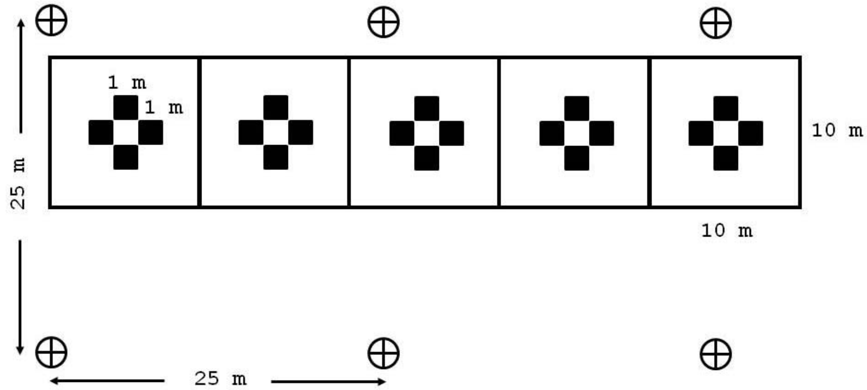


Figure 1--Schematic representation of 10 meter x 50 meter vegetation and soil sampling plot consisting of five 10 meter$^2$ subplots. Crosshairs represent approximate locations of fire behavior measurements relative to vegetation sampling plots. Small, solid squares represent 1 meter$^2$ quadrats. Intact soil cores extracted from 1 meter$^2$ quadrats and used for greenhouse bioassays of post-treatment soil mycorrhizal inoculum potential.

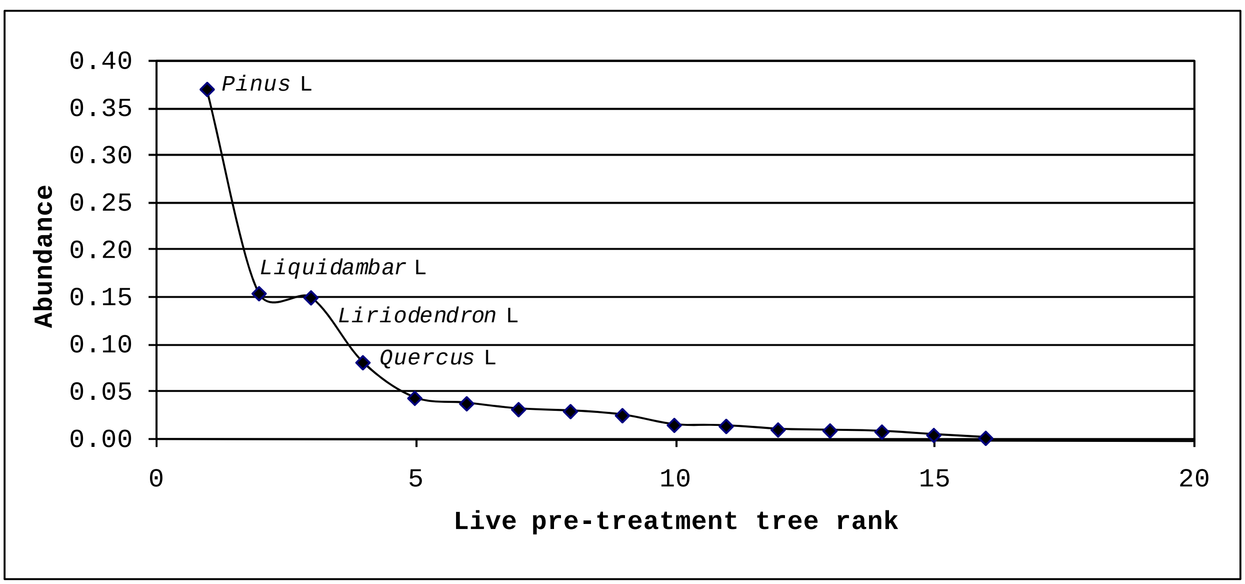


Figure 2--Example of a rank abundance curve used in the current study to identify genera and functional groups that were most abundant across beetle-killed stands in the pre-treatment vegetation assemblages. *Pinus* L., *Liquidambar* L., *Liriodendron* L., and *Quercus* L. were the most abundant tree genera in beetle-killed stands and were used as independent variables to model post-treatment soil mycorrhizal inoculum potential.

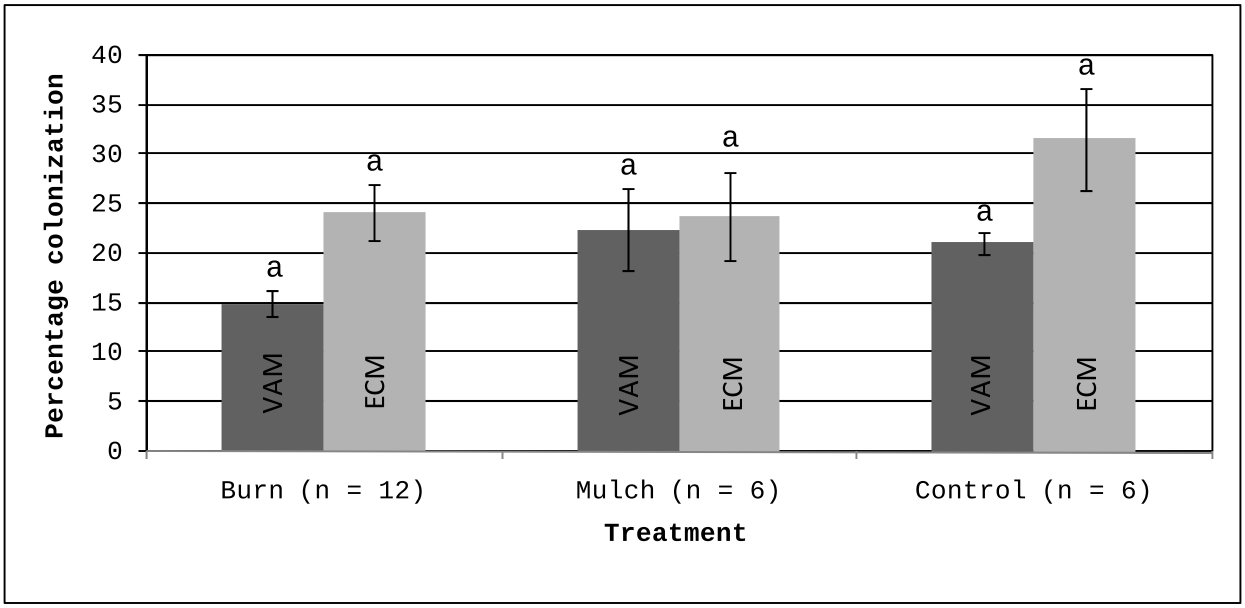


Figure 3—Comparisons of mean percentage vesicular-arbuscular (VAM) and ectomycorrhizal (ECM) colonization of corn (*Zea mays* L.) and loblolly pine (*Pinus taeda* L.) seedlings. Seedlings were grown in intact soil cores collected after beetle-killed stands were subjected to different fuel reduction treatments. Bars represent standard errors of the means. Similar letters indicate that VAM or ECM inoculum potential was not significantly different among treatments.

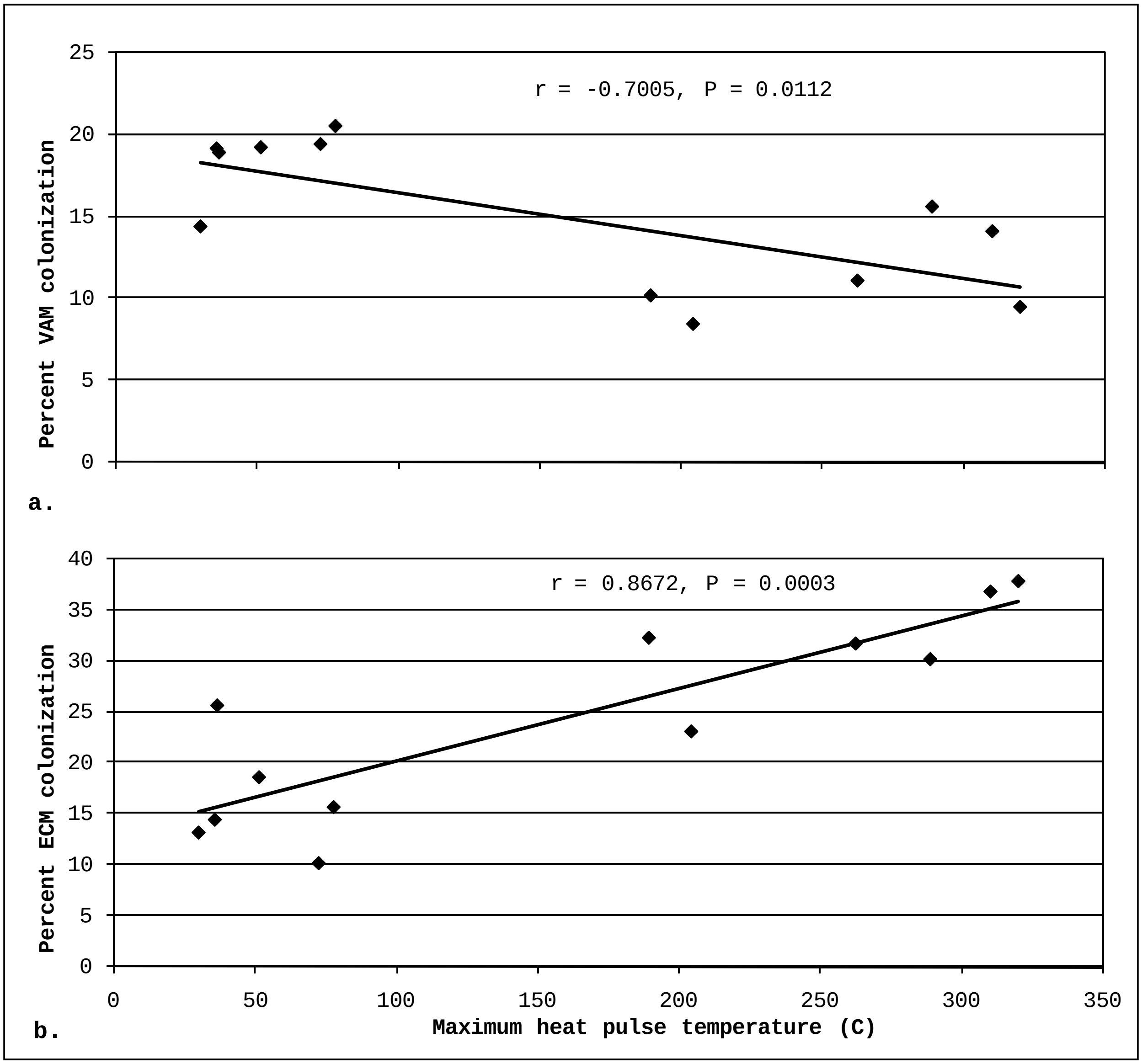


Figure 4--Relationships between percentage vesicular-arbuscular (a.) and ectomycorrhizal (b.) colonization of corn and pine seedlings, respectively, and maximum heat pulse temperature. Measurements were obtained using Type K thermocouples inserted at the interface between the top of mineral soil and the bottom of the duff layer and recorded using remote data loggers.

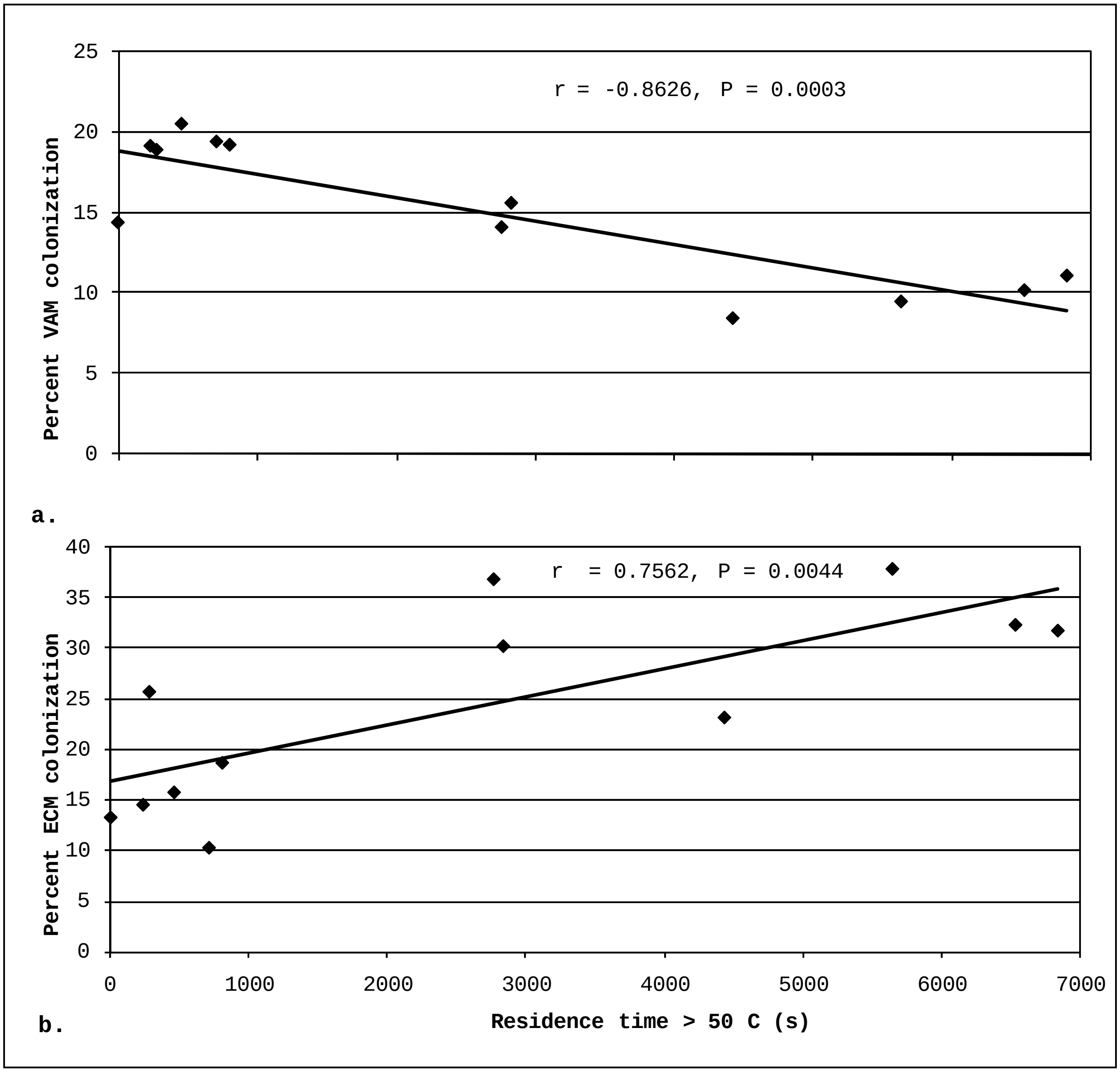


Figure 5--Relationships between percentage vesicular-arbuscular (a.) and ectomycorrhizal (b.) colonization of corn and pine seedlings, respectively, and fire residence time. Residence time was calculated as the amount of time (in seconds) that temperature was sustained above 50°C. Measurements were obtained using Type K thermocouples inserted at the interface between the top of mineral soil and the bottom of the duff layer and recorded using remote data loggers.

Appendix 1 --Fuel loading before and after site preparation treatments in beetle-killed areas of the Clemson Experimental Forest in the Piedmont of South Carolina.

| | 1-hr Weight (t/ac) | 10-hr Weight (t/ac) | 100-hr Weight (t/ac) | 1000-hr Weight (t/ac) | Total Woody (t/ac) | Litter Depth (in) | Duff Depth (in) | Total Floor (in) | Fuel Depth (ft) |
|---|---|---|---|---|---|---|---|---|---|
| Pretreatment | | | | | | | | | |
| Control | 0.41 | 2.1 | 2.2 | 13.6 | 18.3 | 1.5 | 1.0 | 2.5 | 9.4 |
| High intensity | 0.44 | 1.8 | 2.0 | 20.1 | 24.5 | 1.6 | 1.0 | 2.6 | 9.2 |
| Low intensity | 0.43 | 2.2 | 2.4 | 6.2 | 11.2 | 1.4 | 0.8 | 2.3 | 7.5 |
| Mastication | 0.39 | 3.0 | 3.7 | 6.7 | 13.8 | 1.6 | 1.1 | 2.6 | 11.0 |
| Year 1 | | | | | | | | | |
| Control | 0.48a[1] | 1.8a | 3.9a | 25.5 | 31.7 | 1.5a | 1.0a | 2.4a | 15.4 |
| High intensity | 0.08b | 0.4b | 1.5b | 30.8 | 32.8 | 0.2b | 0.1b | 0.2b | 5.6 |
| Low intensity | 0.22b | 1.0ab | 3.2a | 32.8 | 37.2 | 0.4b | 0.2b | 0.6b | 7.8 |
| Mastication | - | - | - | - 205.4* | - | - | - | - | |
| Year 2 | | | | | | | | | |
| Control | 0.49a | 2.0a | 4.4a | 33.9 | 40.8 | 2.1a | 0.7a | 2.8a | 11.8 |
| High intensity | 0.21b | 0.9c | 2.5b | 33.2 | 36.8 | 0.4b | 0.1b | 0.6b | 11.0 |
| Low intensity | 0.36a | 1.3b | 4.3a | 35.7 | 41.7 | 0.6b | 0.3a | 0.9b | 11.7 |
| Mastication | - | - | - | - 105.3* | - | - | - | - | |

[1]Means followed by the same letter within a column and year are not significantly different at the 0.05 level.
*Mulch weights include ground pieces of all fuel types and were not used in analysis of variance.

Appendix 2--Means of mineral soil variables sampled prior to and in two consecutive years following prescribed burning, mechanical mulching, and no-treatment (control) in beetle-killed stands; 'nd' indicates that no data were available. Standard errors of the means are in parentheses. Means within a row followed by the same letter are not significantly different at the 0.05 level.

| **Soil variable/** Treatment | **Sampling year** | | |
|---|---|---|---|
| | Pre-treatment | Year 1 | Year 2 |
| **Soil bulk density (g $cm^{-3}$)** | | | |
| Control (n=3) | 0.79 (0.06)a | nd | 0.75 (0.02)a |
| Prescribed burn (n=6) | 0.84 (0.05)a | 0.93 (0.03)a | 0.80 (0.03)a |
| Mechanical mulch (n=3) | 0.89 (0.04)a | nd | nd |
| **Total C (mg $kg^{-1}$)** | | | |
| Control | 1.68 (0.22)a | 2.16 (0.38)a | 2.18 (0.29)a |
| Prescribed burn | 1.94 (0.17)a | 1.64 (0.11)a | 2.22 (0.23)a |
| Mechanical mulch | 1.91 (0.35)a | 2.07 (0.24)a | 1.80 (0.23)a |
| **Total N (mg $kg^{-1}$)** | | | |
| Control | 0.08 (0.01)a | 0.10 (0.02)a | 0.11 (0.02)a |
| Prescribed burn | 0.08 (0.00)a | 0.07 (0.00)a | 0.10 (0.01)a |
| Mechanical mulch | 0.10 (0.02)a | 0.10 (0.02)a | 0.09 (0.01)a |
| **C/N ratio** | | | |
| Control | 20.69 (1.05)a | 22.64 (1.50)a | 20.79 (0.81)a |
| Prescribed burn | 22.84 (1.55)a | 22.60 (1.42)a | 23.51 (1.94)a |
| Mechanical mulch | 20.12 (1.26)a | 20.54 (1.40)a | 20.51 (1.09)a |
| **pH** | | | |
| Control | 5.36 (0.31)a | 4.91 (0.18)a | 4.99 (0.18)a |
| Prescribed burn | 4.99 (0.06)a | 4.96 (0.08)a | 5.01 (0.09)a |
| Mechanical mulch | 5.18 (0.10)a | 5.13 (0.13)a | 5.14 (0.17)a |

Appendix 2--

| **Soil variable /** Treatment | **Sampling year** | | |
|---|---|---|---|
| | Pre-treatment | Year 1 | Year 2 |
| **P (mg kg$^{-1}$)** | | | |
| Control (n=3) | 3.54 (0.36)a | 2.87 (0.28)a | 3.43 (0.49)a |
| Prescribed burn (n=6) | 3.24 (0.23)a | 1.56 (0.38)b | 2.94 (0.54)a |
| Mechanical mulch (n=3) | 2.97 (0.13)a | 1.62 (0.18)b | 1.56 (0.14)a |
| **Ca (mg kg$^{-1}$)** | | | |
| Control | 167.28 (33.37)a | 154.20 (41.50)ab | 129.86 (22.41)a |
| Prescribed burn | 140.72 (17.56)a | 80.04 (12.35)b | 124.92 (18.59)a |
| Mechanical mulch | 168.80 (44.58)a | 169.58 (37.28)a | 132.96 (29.32)a |
| **Al (mg kg$^{-1}$)** | | | |
| Control | 574.46 (61.05)a | 680.98 (22.81)a | 543.90 (54.69)a |
| Prescribed burn | 481.18 (59.33)a | 460.67 (51.70)b | 489.85 (74.50)a |
| Mechanical mulch | 375.41(133.13)a | 481.48 (66.32)b | 386.97 (88.03)a |
| **Ca/Al Molar ratio** | | | |
| Control | 0.21 (0.06)a | 0.15 (0.04)ab* | 0.16 (0.04)a |
| Prescribed burn | 0.21 (0.03)a | 0.13 (0.02)b | 0.19 (0.04)a |
| Mechanical mulch | 0.32 (0.02)a | 0.24 (0.03)a | 0.24 (0.01)a |
| **Mg (mg kg$^{-1}$)** | | | |
| Control | 30.32 (9.65)a | 27.58 (12.21)a | 25.04 (5.06)a |
| Prescribed burn | 27.10 (5.89)a | 22.26 (3.81)a | 29.55 (4.31)a |
| Mechanical mulch | 31.04 (7.72)a | 39.77 (9.30)a | 37.13 (9.34)a |

Appendix 2--

| **Soil variable /** Treatment | **Sampling year** | | |
| --- | --- | --- | --- |
| | Pre-treatment | Year 1 | Year 2 |
| **K (mg kg$^{-1}$)** | | | |
| Control (n=3) | 33.01 (5.43)a | 26.52 (3.94)b | 25.05 (0.99)a |
| Prescribed burn (n=6) | 28.13 (3.58)a | 22.85 (2.69)b | 29.52 (3.12)a |
| Mechanical mulch (n=3) | 28.42 (2.84)a | 37.83 (3.91)a | 25.43 (1.81)a |
| **$N_{mineralization}$ (mg N kg$^{-1}$ d$^{-1}$)** | | | |
| Control | 0.10 (0.01)a | 0.09 (0.14)a | nd |
| Prescribed burn | 0.10 (0.08)a | nd | 0.49 (0.25) |
| Mechanical mulch | 0.10 (0.06)a | 0.23 (0.37)a | nd |
| **$N_{nitrification}$ (mg N kg$^{-1}$ d$^{-1}$)** | | | |
| Control | 0.05 (0.02)a | 0.23 (0.19)a | nd |
| Prescribed burn | 0.07 (0.03)a | nd | 0.15 (0.02) |
| Mechanical mulch | 0.11 (0.03)a | 0.20 (0.11)a | nd |
| **$N_{proportional}$ (mg N kg$^{-1}$ d$^{-1}$)** | | | |
| Control | 0.02 (0.01)a | 0.02 (0.01)a | nd |
| Prescribed burn | 0.01 (0.01)a | nd | 0.01 (0.00) |
| Mechanical mulch | 0.03 (0.02)a | 0.02 (0.01)a | nd |

Appendix 3 – Vegetative Responses

Regeneration (<3 ft tall) of pine and hardwood tree species before and after site-preparation treatments in beetle-killed areas of the Clemson Experimental Forest in the Piedmont of South Carolina.

| | Oak-Hickory (#/ac) | Pines (#/ac) | Other Hardwoods (#/ac) | All Species (#/ac) |
|---|---|---|---|---|
| Pretreatment | | | | |
| Control | 2,632 | 0 | 6,984 | 9,345 |
| High intensity | 5,162 | 34 | 3,576 | 8,738 |
| Low intensity | 2,530 | 0 | 8,266 | 10,830 |
| Mastication | 2,564 | 34 | 4,689 | 7,287 |
| Year 1 | | | | |
| Control | 1,619 bc | 0 | 6,174 ab | 7,794 a |
| High intensity | 7,321 a | 135 | 4,892 ab | 12,213 a |
| Low intensity | 2,902 ab | 0 | 15,047 a | 18,084 a |
| Mastication | 810 c | 0 | 2,227 b | 3,036 b |
| Year 2 | | | | |
| Control | 2,227 ab | 101 b | 8,232 | 10,560 |
| High intensity | 5,567 a | 438 a | 3,846 | 9,851 |
| Low intensity | 2,193 ab | 371 ab | 9,649 | 12,213 |
| Mastication | 1,721 b | 101 b | 4,723 | 6,545 |

[1]Means followed by the same letter within a column and year are not significantly different at the 0.05 level.

Survival of planted loblolly pine seedlings one growing season after planting

| Treatment | Survival (%) |
|---|---|
| Control | 77 |
| High intensity fire | 83 |
| Low intensity fire | 80 |
| Mastication | 85 |

Means were not significantly different at the 0.05 level.

Advanced regeneration (3-6 ft tall) of pine and hardwood tree species before and after site-preparation treatments in beetle-killed areas of the Clemson Experimental Forest in the Piedmont of South Carolina.

| | Oak-Hickory (#/ac) | Pines (#/ac) | Other Hardwoods (#/ac) | All Species (#/ac) |
|---|---|---|---|---|
| Pretreatment | | | | |
| Control | 578 | 124 | 838 | 1,540 |
| High intensity | 722 | 84 | 470 | 1,275 |
| Low intensity | 321 | 92 | 1,143 | 1,556 |
| Mastication | 231 | 15 | 556 | 802 |
| Year 1 | | | | |
| Control | 497 a | 58 | 931 a | 1,486 a |
| High intensity | 456 a | 30 | 532 a | 1,017 a |
| Low intensity | 193 a | 46 | 884 a | 1,123 a |
| Mastication | 8 b | 0 | 19 b | 27 b |
| Year 2 | | | | |
| Control | 489 a | 40 | 923 a | 1,452 a |
| High intensity | 592 a | 11 | 556 ab | 1,159 a |
| Low intensity | 227 a | 32 | 1,098 a | 1,357 a |
| Mastication | 20 b | 0 | 289 b | 309 b |

[1]Means followed by the same letter within a column and year are not significantly different at the 0.05 level.

# Reducing airborne ectomycorrhizal fungi and growing non-mycorrhizal loblolly pine (*Pinus taeda* L.) seedlings in a greenhouse

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**Abstract**

Atmospheric spores of ectomycorrhizal (ECM) fungi are a potential source of contamination when mycorrhizal studies are performed in the greenhouse and techniques for minimizing such contamination have rarely been tested. We grew loblolly pine (*Pinus taeda* L.) from seed in a greenhouse and inside a high-efficiency particulate air (HEPA)-filtered chamber (HFC) constructed within the same greenhouse. Seedlings were germinated in seven different sand- or soil-based and artificially-based growth media. Seedlings grown in the HFC had fewer mycorrhizal short roots than those grown in the open greenhouse atmosphere. Furthermore, the proportion of seedlings from the HFC that were completely non-mycorrhizal was higher than that of seedlings from the greenhouse atmosphere. Seedlings grown in sterilized, artificially-based growth media (>50 percent peat moss, vermiculite, and/or perlite by volume) had fewer mycorrhizal short roots than those grown in sand- or soil-based media. The HFC described here can minimize undesirable ECM colonization of host seedlings in greenhouse bioassays. In addition, the number of non-mycorrhizal seedlings can be maximized when the HFC is used in combination with artificially-based growth media.

**Keywords:** ectomycorrhizas; soil inoculum potential; bioassays; greenhouse

## Introduction

Mycorrhizal studies that are performed in greenhouses include bioassays of soil inoculum potential (Herr et al. 1994; Teste et al. 2006), assessments of mycorrhizal community structure (Jones et al. 2003; Pilz and Perry 1984), and experimental inoculation with specific ectomycorrhizal (ECM) fungal symbionts (Beckjord and McIntosh 1983; Branzanti et al. 1999; Brundrett et al. 1996; Marx and Bryan 1969). There are two primary methods of assessing soil inoculum potential or mycorrhizal community structure. The first involves obtaining field soil, diluting it with different concentrations of sterile soil or a soilless medium ('most probable number' assay—see Brundrett et al. (1996)) or obtaining intact soil cores and planting ECM host seed in the soil (Boerner et al. 1996). The second method involves producing non-mycorrhizal seedlings of an ECM host species and planting the seedlings in diluted soil (Brundrett et al. 1996) or intact soil cores (Smith et al. 1995), or outplanting the seedlings in undisturbed field soil (Tainter and Walstad 1977). In both cases, the 'trap plants' are left to grow for a period of time, then excavated and assessed for mycorrhizal colonization. However, aerial ECM fungal spores are a potential source of contamination when these tasks are performed in greenhouses (Marx and Bryan 1969). Contamination by *Thelephora terrestris* Ehrh. (an ECM fungus) in greenhouses is particularly vexing due to its worldwide distribution and broad host range (Smith and Read 2002). Measures are often taken to reduce and test for fungal contamination, and it remains unclear whether an air-filtered growth environment is necessary to maintain soil bioassays free of contamination or for the growth of non-mycorrhizal seedlings.

Some groups have produced large numbers of non-mycorrhizal seedlings using only standard greenhouse space with different types of sterilized growth media (Boerner et al. 1996; Smith et al. 1995; Tainter and Walstad 1977). Others have used growth rooms with air filtration systems to remove airborne spores of mycorrhizal fungi from the atmosphere (Marx 1973; Ruehle 1982). However, Marx and Bryan (1969) indicated that non-mycorrhizal pine seedlings of at least 11 different species (including the focus of the current study, loblolly pine (*Pinus taeda* L.)) cannot be grown in field or greenhouse culture due to the pervasiveness of ectomycorrhizal propagules. Consequently, an electronically air-filtered, air-conditioned, growth room was used to produce non-mycorrhizal seedlings (Marx and Bryan 1969) and to keep greenhouse contaminants from colonizing seedlings inoculated with specific ECM fungal symbionts (Marx 1973). Limited information suggests that non-conifers (e.g., *Quercus* spp.) may be less susceptible to airborne contamination than conifers during greenhouse bioassays (Dickie et al. 2004) for unknown reasons.

Conventional growth chambers and laminar flow cabinets are costly, space-limited, and/or may not provide the level of air filtration necessary to reduce contamination of ECM bioassays. High-efficiency particulate air (HEPA)- filtering technology may maintain lower levels of aerial contamination by ECM spores. Although HEPA-filtering has not previously been used in a greenhouse setting, it has been successfully used in mushroom cultivation to prevent contamination from undesirable fungal propagules (Tisdale et al. 2006).

Various sterile growth media have been used in combination with filtered and unfiltered growth environments to grow non-mycorrhizal seedlings with variable success; these include sand, soil, vermiculite, and perlite (Boerner et al. 1996; Brundrett et al. 1996; Pilz and Perry 1984; Smith et al. 1995; Tainter and Walstad 1977). Whether specific growth media are structurally and/or nutritionally less conducive to mycorrhiza formation remains unclear.

The objectives of our study were to (1) test whether a HEPA-filtered chamber (HFC) was successful in reducing ECM colonization through reduction in aerial contamination and (2) determine whether sand- or soil-based and artificially-based growth media promote different amounts of ECM colonization in pine seedlings.

## Material and Methods

### HEPA-filtered chamber

The study was conducted at the Clemson University Greenhouse Complex, Clemson, South Carolina, USA (lat. 34° 40' 8"; long. 82° 50' 40") which was built in 2002. The HFC was built inside the greenhouse to reduce the level of ECM spores in the growth atmosphere. The frame of the HFC was constructed of 5.1 cm x 10.2 cm wood studs that enclosed a 152 cm x 244 cm greenhouse bench (Figure 1). The top and four sides of the HFC were lined with clear 2.65 mil, 7UVM Mylar film and all seams were sealed with clear polyethylene tape. HEPA-filtering of the HFC atmosphere was accomplished using a MAC 10® Fan Filter Unit (Envirco Corp.). The spores of ECM fungi range in diameter from 4 to 30 µm (Brundrett et al. 1996). The fan-filter unit was rated to be 99.99 percent efficient at removing particles with a mean diameter of 0.3 µm and for circulating air at 1,105 $m^3 h^{-1}$. All air entered the HFC at one end through the fan-filter unit and exited through the open bottom of the HFC. The HFC was thoroughly cleaned using a 10 percent household bleach solution (Izzo et al. 2006) and was purged with the fan filter unit running for 2 wk prior to its use. A positive-pressure, HEPA-filtered environment was maintained inside the HFC for the duration of the study.

### Atmospheric measurements

Particle counts of total atmospheric particulate were performed using an electronic particle counter (Royco) at five positions distributed evenly in the HFC and in the greenhouse atmosphere 22 d prior to the start of the study. Additionally, five Petri-dishes containing potato-dextrose agar (PDA) (Difco) (Cerrato et al. 1975) were placed at five different positions on the bench inside the HFC and on the bench immediately adjacent the HFC in the greenhouse to capture atmospheric particulate (Marx and Bryan 1969) 20 d prior to commencing the study. The Petri-dish traps were intended to confirm that the greenhouse water did not contain fungal spores (Cerrato et al. 1975). Petri-dishes were covered and cultured in the dark for 41 d at 22°C after 24 h of exposure. Fungal colonies were then counted on each Petri-dish.

Measurements of photosynthetic photon-flux density (PPFD), ambient temperature, and relative humidity were obtained during the growth period both inside and outside of the HFC. PPFD was measured between 12:00-16:00 on four different days at six positions distributed evenly across the benches in the HFC and greenhouse environments using an AccuPAR LP-80 ceptometer (Decagon Devices, Inc.). PPFD was measured under different amounts of cloud cover, and with and without 50 percent shade cloth draped across the growth area on four separate days. Ambient temperature and relative humidity were measured simultaneously both inside and outside of the HFC continuously for seven consecutive days using hygrothermographs (WEATHERtronics Corp.).

### Sand- or soil-based and artificially-based growth media

Ten 90 ml cavities of IPL® Rigi-Pots™ (Stuewe and Sons, Inc.) tube trays were filled with seven types of growth media, five of which have been used in previous greenhouse mycorrhizal studies (Boerner et al. (1996) Brundrett et al. (1996); Smith et al. (1995); Pilz and Perry (1984); Tainter and Walstad (1977)). Growth media were classified as either sand- or soil-based or artificially-based depending on the amount of peat moss, vermiculite, and/or perlite in the mixture. The four artificially-based media contained >50 percent peat moss (Lambert), vermiculite (Palmetto), and/or perlite (Milolite) by volume and included a commercial mix (Fafard), 1:1 (v:v) vermiculite/perlite (Smith et al. 1995), 1:1:1 (v:v:v) peat moss/river sand/vermiculite (Tainter and Walstad 1977), and pure vermiculite. The river sand was collected in Oconee County, South Carolina, USA. The commercial mix contained 5:3:1:1 (v:v:v:v) peat moss/processed pine bark/perlite/vermiculite and a packaged starter fertilizer (15:11:32).

The three soil-based media were river sand (Brundrett et al. 1996), 1:1 (v:v) river sand/peat moss (Boerner et al. 1996), and a forest soil (Pilz and Perry 1984). The forest soil was an ultisol (suborder Udult) collected from a loblolly pine stand in Clemson, South Carolina, USA and included mineral A and B horizons to 10 cm in depth. Two tube trays filled with each type of growth media were autoclaved for 15 min at 122°C under 0.14 MPa (Fergus 1969) while inside a 64 cm x 76 cm polypropylene autoclave bag which was closed with a wire tie. Trays remained inside the autoclave bags until the time of planting.

The study was a randomized complete block design with the HFC and greenhouse representing blocks and the two media categories representing treatments. One tube tray containing each media type was randomly distributed and positioned on the bench inside the HFC and on a bench immediately adjacent the HFC in the greenhouse. The positions of the tube trays were not re-randomized during the experiment.

### Ectomycorrhizal bioassays

Thirty-day stratified loblolly pine seeds (mixed lot, Telfair County, Georgia, USA) were surface sterilized in 10 percent household bleach/water solution for five minutes (Dickie et al. 2001). Two sterilized seeds were planted in each tray cavity to a consistent depth using a plastic dowel marked at 0.25 in (0.64 cm). Germinates were thinned to one per cell after 14 d and allowed to grow under natural light for 14 additional wk (16 wk total) during which no fertilizer was applied. Seedlings were watered to field capacity 2 to 3 times per wk with tap water. For each watering event, an irrigation hose and wand entered the HFC through an access opening (Figure 1) during which positive-pressure was maintained. Tap water samples were collected 20 d prior to commencing the study. The water was cultured on PDA media in Petri-dishes for 41 d at 22°C to confirm that the water source did not contain fungal spores. The bioassay seedlings were harvested at 16 wk, and excess growth medium was carefully removed from the root systems under running tap water.

Pine root systems are heterorhizic with distinct short roots and long (lateral) roots from which short roots subtend (Brundrett et al. 1996). Three lateral roots ≥ 6 cm in length were randomly selected from each root system. Each short root was tallied and classified as mycorrhizal or non-mycorrhizal using a dissecting microscope (Brundrett et al. 1996). Non-mycorrhizal 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. No attempts were made to identify the mycorrhizas in any way. Percent ECM colonization was calculated by dividing the number of mycorrhizal short roots by the total number of short roots. In addition, the bioassay seedlings were dried (40°C for 24 h) and weighed to obtain measurements of root and shoot dry weight and root/shoot ratio.

A sub-sample of four seedlings in each of the seven growth media (28 seedlings total) were randomly selected and used to evaluate the methods used for recognizing mycorrhizas. For each of these seedlings, short roots were tallied and classified as mycorrhizal or non-mycorrhizal. The same roots were subsequently cleared with 10% KOH (6 to 12 h at 75°C), stained in trypan blue (6 h at 22°C), and de-stained in 50% glycerol (Brundrett et al. 1996). Each short root was classified as mycorrhizal or non-mycorrhizal based on the presence of Hartig net hyphae as observed under 110x magnification using a compound microscope. Percentage colonization values were calculated as described previously.

Although we used only one HFC and one greenhouse environment, the 70 seedlings in each environment (HFC and greenhouse) were grown in individual 90 ml cavities of tube trays containing growth media. These 70 seedlings were treated as experimental units. However, we acknowledge that the seedlings grown in the same environment were not completely independent.

## Statistical analysis

ECM colonization percentages were arcsin transformed prior to statistical analyses. Analysis of variance was performed using PROC GLM (SAS Institute, 2003) to determine if there was a significant effect on ECM colonization of seedlings due to the category of growth media (sand- or soil-based or artificially-based) that the seedlings were grown in.
Mean ECM colonization, shoot and root dry weight, and root/shoot ratio of seedlings were calculated for the two media categories in each environment. Additionally, the proportions of non-mycorrhizal seedlings produced in the two environments and in the two media categories were calculated. Chi-square tests were performed using PROC FREQ to compare the proportion of non-mycorrhizal seedlings produced in the two media categories. Due to the lack of replication of the environment factor, means were calculated, but no statistical comparisons were made between the HFC and greenhouse environments with respect to percentage ECM colonization, seedling growth, or proportion of non-mycorrhizal seedlings.

The sub-sample of 28 seedlings was used to evaluate how closely associated the percentage ECM colonization values determined using morphological characteristics and dissecting microscopy were to those determined using staining and compound microscopy. Percentage colonization values determined using different methodologies were compared to one another using correlation analysis (PROC CORR) with each seedling representing one observation. All data were expressed as the mean ± standard error of the mean.

**Results**

Electronic particle counts revealed that the mean number of particles ≥ 0.5 μm in diameter per $m^3$ air differed significantly between the air of the greenhouse environment (1.34 x $10^6$ ± 66,800 particles) and that of the HFC (30 ± 23 particles) ($P < 0.0001$). The average number of fungal colonies on Petri-dish traps exposed to the greenhouse atmosphere (27 ± 2.6) was also significantly higher than that on traps exposed to the HFC atmosphere (0.2 ± 2.9) ($P < 0.0001$).

Measurements of PPFD and relative humidity revealed that the climatic conditions of the HFC and greenhouse environments were similar. Mean PPFD in the HFC (249.4 ± 29.4 mmol $m^{-2} s^{-1}$) was not significantly different than that in the greenhouse (293.8 ± 48.1 mmol $m^{-2} s^{-1}$) ($P = 0.4610$). The difference in mean daily temperature between the HFC (25.7 ± 0.24°C) and the greenhouse environment (24.7 ± 0.27°C) was statistically significant ($P = 0.0104$) probably because the motor of the fan filter unit warmed the HFC environment slightly. Mean daily relative humidity in the HFC (60.4 ± 2.9 percent) was not significantly different than that of the greenhouse (60.4 ± 2.6 percent) ($P = 0.9954$). There were not differences in PPFD, ambient temperature, or relative humidity, due to bench position, neither in the HFC nor in the greenhouse (data not shown). Culturing of tap water revealed that no fungal spores were present in the water source 20 d prior to commencing the study.

Analysis of variance revealed a significant blocking effect due to environment ($P = 0.0026$). When all seven media were considered together, the mean percent ECM colonization of seedlings grown in the HFC (10.8 ± 2.3 percent) was lower than that of greenhouse seedlings (23.1 ± 3.0 percent). In addition, the proportion of seedlings that were non-mycorrhizal from the HFC was higher (by approximately 30%) than that of seedlings that were non-mycorrhizal from the greenhouse.

The mean percent ECM colonization of seedlings grown in artificially-based media (10.6 ± 2.1 percent) was significantly lower than that of seedlings grown in sand- or soil-based media (25.3 ± 3.2 percent) ($P = 0.0407$). Mean percent colonization of seedlings, root and shoot dry weight, and root/shoot ratio are also given for individual growth media for the reader's information (Table 1). The category of media did not influence the proportion of non-mycorrhizal to mycorrhizal seedlings ($P = 0.5532$).

Percentage colonization values determined for 28 seedlings using morphological characteristics and dissecting microscopy were strongly correlated with those determined for the same seedlings using the staining procedure and compound microscopy ($r = 0.7910$, $P < 0.0001$). This result provided verification that a dissecting microscope provided a sufficient level of magnification for the lab worker to effectively distinguish between mycorrhizal and non-mycorrhizal short roots based on morphological characteristics.

Mean seedling root dry weight was not significantly different between the two media categories ($P = 0.2069$). Shoot dry weight of seedlings grown in artificially-based media (0.30 ± 0.02 g) was significantly higher than that of seedlings grown in sand- or soil-based media (0.22 ± 0.01 g) ($P < 0.0001$). The root/shoot ratio of seedlings grown in artificially-based media (0.26 ±

0.02 percent) was significantly lower than that of seedlings grown in sand- or soil-based media ($0.31 \pm 0.02$ percent) ($P = 0.0053$).

**Discussion**

The results of this study strongly suggest that maintaining a reduced level of atmospheric fungal contaminants substantially reduces the amount of undesirable ECM colonization and increases the production of non-mycorrhizal seedlings. Furthermore, artificially-based growth media discourage the formation of mycorrhizas compared to sand- or soil-based media.

Electronic particle counts and Petri-dish traps exposed for 24 h indicated that fungal spores were abundant in the greenhouse atmosphere and essentially absent from the HFC atmosphere 22 d prior to the start of the study. Marx and Bryan (1969) found similar amounts of atmospheric fungal contamination in their filtered growth room after exposing malt extract agar, which is also non-selective and grows a variety of fungi (Atlas 2005), for only 30 min.

Some contamination may have been carried into the HFC on the surface of irrigation equipment, instruments for measuring the atmospheric environment, or clothing; thus accounting for some of the colonization observed on the seedlings in the HFC. A sub-irrigating watering system would eliminate physical entry to the HFC through the access openings during weekly watering events and may further reduce contamination.

Many of the climatic conditions known to influence mycorrhiza formation (e.g., water, light, temperature, and relative humidity) were either controlled or measured with differences between the HFC and greenhouse atmospheres found to be not statistically significant. Although the environment factor lacked true replication, these results strongly suggest that the reduced level of ECM colonization exhibited by seedlings and the increased production of non-mycorrhizal seedlings was due to reduced airborne ectomycorrhizal contamination provided by the HFC.

Little difference in seedling root and shoot development suggests that mycorrhizal colonization was influenced by structural and/or nutritional differences between the media categories. Soil characteristics including nutrient content, pH, water-holding capacity, and porosity are known to influence the activity of mycorrhizal fungi (Marks and Kozlowski 1973; Schüepp et al. 1987). Schüepp et al. (1987) found that growth of vesicular-arbuscular mycorrhizal hyphae occurred at lower rates in calcined clay, peat moss, and chopped hay than in various sand and soil-based media. They suggested that the effects of nutrition and/or pH on fungal growth, ease of hyphal penetration, texture, or moisture content may have caused differences in the rate of hyphal growth through the different media. Therefore, we suspect that peat moss, vermiculite, perlite, and pine bark may have had similar effects on fungal activity and mycorrhizal colonization in the present study.

Trap plants were used in some greenhouse studies to test for atmospheric contamination. Boerner et al. (1996) found that control seedlings growing in a sterilized sand/peat moss mixture were free of ECM colonization after 9 wks in a greenhouse study of soil inoculum potential. In another study, non-mycorrhizal loblolly pine seedlings were propagated in an un-filtered greenhouse using a sterilized peat moss moss/vermiculite/sand mixture (Tainter and Walstad 1977). These results suggest that contaminants were not present in their greenhouse or were present but did not colonize control seedlings because the media mixture discouraged the formation of mycorrhizae with the seedlings. Results of the present study demonstrate that trap plants grown in control media that are dissimilar from the experimental soil or media mixture may misrepresent the amount of atmospheric contamination. However, such problems can be avoided by growing trap plants in the same type of media that is under investigation.

The combination of sterilized artificially-based growth media and a reduced level of atmospheric propagules should minimize the amount of undesirable colonization of seedlings during greenhouse studies involving ectomycorrhizas. Furthermore, the HFC is relatively inexpensive to build (< $1,000 USD) and appears to provide an atmosphere of reduced contamination that is needed for bioassays of soil ECM inoculum potential, studies of mycorrhizal community structure, or those that involve the isolation of specific fungal symbionts.

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**Table 1 -** Mean percent of ectomycorrhizal (ECM) colonization, root and shoot dry weight, and root/shoot ratio of loblolly pine (*Pinus taeda* L.) seedlings that were germinated in sand- or soil-based and artificially-based growth media. Sand- or soil-based media included: river sand; 1:1 (v:v) sand peat; and forest soil. Artificially-based media included: a commercial potting mix; 1:1 vermiculite/perlite; 1:1:1 peat/river sand/vermiculite; and 100% vermiculite. Two trays of each media type were sterilized and placed into a greenhouse and a HEPA-filtered chamber that was intended to reduce ECM fungal spore contamination. Standard errors of the means are in parentheses**.**

| | Greenhouse (n = 70) | | | | HEPA-filtered chamber (n = 70) | | | |
|---|---|---|---|---|---|---|---|---|
| **Growth media** | % ECM colonization | Root dry wt (g) | Shoot dry wt (g) | Root / shoot ratio | % ECM colonization | Root dry wt (g) | Shoot dry wt (g) | Root / shoot ratio |
| River sand | 31.0 (6.8) | 0.07 (0.01) | 0.18 (0.01) | 0.40 (0.05) | 12.3 (8.2) | 0.07 (0.01) | 0.17 (0.01) | 0.34 (0.03) |
| 1:1 (v:v) sand/peat | 20.5 (8.6) | 0.08 (0.01) | 0.26 (0.02) | 0.26 (0.01) | 11.8 (7.9) | 0.08 (0.01) | 0.27 (0.01) | 0.30 (0.05) |
| Forest soil [a] | 41.4 (9.7) | 0.06 (0.00) | 0.26 (0.01) | 0.24 (0.03) | 13.5 (7.6) | 0.07 (0.01) | 0.20 (0.02) | 0.34 (0.04) |
| Commercial mix [b] | 19.1 (7.8) | 0.14 (0.01) | 0.48 (0.03) | 0.26 (0.01) | 11.4 (6.5) | 0.15 (0.01) | 0.67 (0.04) | 0.21 (0.03) |
| 1:1 vermiculite/perlite | 9.3 (5.5) | 0.05 (0.00) | 0.18 (0.01) | 0.22 (0.01) | 7.4 (5.0) | 0.06 (0.00) | 0.20 (0.01) | 0.32 (0.02) |
| 1:1:1 peat/river sand/vermiculite | 21.4 (5.6) | 0.08 (0.01) | 0.24 (0.01) | 0.35 (0.03) | 14.6 (5.2) | 0.05 (0.00) | 0.24 (0.01) | 0.26 (0.01) |
| Vermiculite | 18.6 (6.3) | 0.06 (0.00) | 0.18 (0.01) | 0.28 (0.03) | 4.8 (3.2) | 0.04 (0.00) | 0.20 (0.00) | 0.16 (0.01) |

[a] The forest soil was an ultisol (suborder Udult) collected from a loblolly pine stand in Clemson, South Carolina, USA and included mineral A and B horizons to 10 cm in depth

[b] The commercial mix contained 5:3:1:1 (v:v:v:v) peat moss/processed pine bark/perlite/vermiculite and a packaged starter fertilizer (15:11:32)

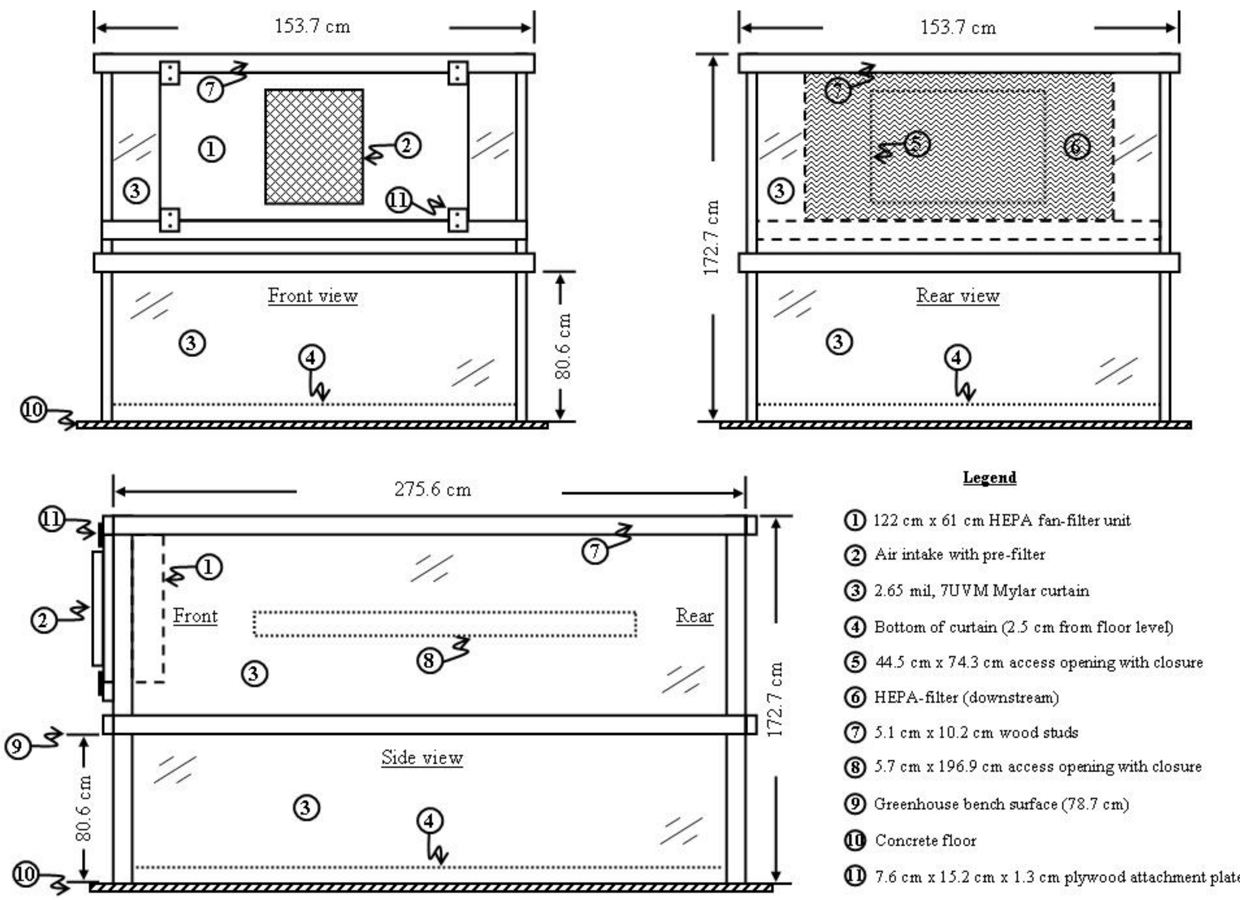


Figure 1 - Schematic diagram of a HEPA-filtered chamber that was built inside a greenhouse to reduce contamination by ectomycorrhizal fungal spores in the growth atmosphere.

# ENERGY CONTENT IN DRIED LEAF LITTER OF SOME OAKS AND MIXED-MESOPHYTIC SPECIES THAT REPLACE OAKS

Stottlemyer, Aaron D.; Wang, G. Geoff; Waldrop, Thomas A. 2009. Energy content in dried leaf litter of some oaks (Quercus spp.) and mixed-mesophytic species that replace oaks. In: Stanturf, John A. ed. Proceedings of the 14th biennial southern silvicultural research conference. 2007 February 26-28; Athens, GA. Gen. Tech. Rep. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station. [In press].

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Abstract-- Mixed-mesophytic hardwood tree species are replacing upland oaks in vast areas of the eastern US deciduous forest. Some researchers have suggested that the leaf litter of mixed-mesophytic, oak replacement species renders forests less flammable where forest managers wish to restore a natural fire regime. We performed chemical analyses on dried leaf litter from select oak and oak replacement tree species. The litter of oak replacement species was lower in calorific value and higher in mineral ash content than that of oaks. These results support a feedback theory that the flammability of oak litter favors the perpetuation of oaks over fire-sensitive species. Incorporating this information into fuel and fire behavior models will assist forest managers in planning prescribed burning operations in areas where mixed mesophytic hardwood tree species are replacing oaks.

## INTRODUCTION

Prescribed fire is an important silvicultural tool in the managmement of oak (*Quercus* spp.)-dominated forests in the eastern United States (Brose and Van Lear 1998). However, some researchers have suggested that the replacement of upland oaks by mixed-mesophytic hardwood tree species changes the fire regime in eastern deciduous forests. Specifically, the litter of oak replacement species is suggested to be less flammable than that of oaks (Abrams 2005), although differences in leaf litter quality among eastern deciduous tree species have not been documented. The objective of this study was to determine if there were differences in chemical properties of the litter of select oaks and oak replacement species.

## METHODS

### Leaf Litter Collection and Sample Preparation

We collected freshly fallen leaf litter of three oaks and two species suggested to replace oaks in two or three different stands in the Clemson University Experimental Forest, Clemson, SC. Oak tree species included scarlet oak (*Q. coccinea* Muenchh.), southern red oak (*Q. falcata* Michx.), and post oak (*Q. stellata* Wangenh.). Oak replacement tree species included red maple (*Acer rubrum* L.) and American beech (*Fagus grandifolia* Ehrh.). Dried leaf litter was milled to 60-mesh in a Wiley mill and pressed into 0.5 g pellets for calorimetry and remained in powder form for mineral ash content analysis.

### Calorimetry and Mineral Ash Content Analysis

Fuel chemistry is concerned with the total amount of chemical energy in a fuel and its availability to the combustion process (Mutch 1970). Calorific value is a measure of the thermal energy released when a fuel is burned (Dickinson and Kirkpatrick 1985) and was measured using an IKA® C200 oxygen bomb calorimeter using four subsamples per litter species per stand. Mineral ash content affects combustible fuel mass, gas evolution, and ignitability (Broido and Nelson 1964) and was measured as the percent of dry mass remaining after complete combustion of three subsamples per litter species per stand in a muffle furnace (at 600° C for 2 hr). Calorific values and mineral ash contents of the litter of the oak species and oak replacement tree species were averaged and compared using one-way analysis of variance.

## RESULTS AND DISCUSSION

The calorific value of the leaf litter of oak replacement tree species (17,977 ± 248.49 J $g^{-1}$) was less than that of oak litter (18,676 ± 242.95 J $g^{-1}$) ($P = 0.0322$). Further, the mineral ash content of the leaf litter of oak replacement tree species (7.27 ± 0.74 percent) was greater than that of oak litter (3.93 ± 0.73 percent) ($P = 0.0028$).

The results of this study suggest sites dominated by mixed-mesophytic hardwood tree species may exhibit lower fire intensities than those dominated by oaks because calorific value greatly influences heat output (Dickinson and Kirkpatrick 1985). Additionally, sites dominated by mixed-mesophytic species may be less flammable and/or burn more heterogeneously than those dominated by oaks because increasing mineral ash content decreases ignitability (Broido and Nelson 1964).

## CONCLUSION

In our study, the leaf litter of oak replacement tree species had lower calorific value and higher mineral ash content than that of oak species. These results support a feedback theory that the flammability of oak fuel complexes favors the perpetuation of oaks over mixed-mesophytic, fire-sensitive tree species. Oak replacement might be expected to make it increasingly difficult to accomplish silvicultural objectives with prescribed fire. However, incorporating information regarding fuel quality into fuel and fire behavior models should assist forest managers in modeling and manipulating prescribed fire in areas where mixed-mesophytic hardwood tree species are replacing oaks.

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# OCCURRENCE AND SPREAD OF NON-NATIVE INVASIVE PLANTS IN STANDS TREATED WITH FIRE AND/OR MECHANICAL TREATMENTS IN THE UPPER PIEDMONT OF SOUTH CAROLINA

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Abstract—Increasing numbers of non-native invasive plant species and the expansion of existing non-native plant populations provide challenges for land managers trying to achieve commercial and restoration goals. Some methods used to achieve these goals (e.g., prescribed fire and mechanical treatments) may result in disturbances that promote the establishment and spread of invasive species. Natural disturbances (e.g., insect infestations) can also provide opportunities for non-native plant expansion. We examined the effects of fuel reduction treatments on the occurrence and abundance of non-native invasive plants for mixed *Pinus taeda*/*Pinus echinata* stands that had sustained southern pine beetle infestations and those that had not. Invasive plant abundance appeared to be greatest 3 – 5 years after disturbance. For stands not affected by southern pine beetles, the combination of mechanical treatment plus burning resulted in the largest increases for invasive species. Stands suffering pine beetle damage and subjected to mechanical treatment showed higher invasive abundance as compared to other treatments. Some invasive species responded differently to treatments. This information will help direct land decisions.

## INTRODUCTION

Invasions of non-native plant species have received considerable attention over the last few decades as land managers are faced with increasing issues of exotic plant control, which can affect biodiversity, forest productivity, and disturbance regimes (Gordon 1998, Levine and others 2003, Vitousek 1990). Fuel reduction treatments intended to reduce fuel loading and restore community composition and structure may be contributing to non-native plant invasion and expansion (e.g., Metlen and Fiedler 2006); therefore, managers need information on the changes to community structure, environmental variables, and invasive plant dynamics in response to these treatments.

Natural disturbances can also provide opportunities for non-native plant expansion. Outbreaks of southern pine beetle (*Dendroctonus frontalis* Zimm.) occur periodically in the southern United States with severe outbreaks causing extensive damage to large areas of pine forests. In South Carolina, southern pine beetle infestations from 2000 until the winter of 2002 affected over 13.5 million hectares (USDA Forest Service 2003a) and caused losses over $250 million for 2002 alone (USDA Forest Service 2002). For existing non-native plant populations in the understory of these affected areas, overstory mortality can lead to expansion of these plant populations as limiting resources become more available.

It has been estimated that economic impacts of invasive species exceed more than $4 billion a year (USDA Forest Service 2003b). With invasive species comprising up to 48% of the total flora for some states and the expected increase of non-native species as globalization continues and climate conditions change (Dukes and Mooney 1999), dealing with these species will continue to be a major issue for land managers.

For this paper we examined the effect fuel reduction treatments had on non-native invasive plant abundance and how this was influenced by additional natural disturbances (i.e., southern pine beetle infestation). We also looked at the changes in understory species composition over time as it related to differences in stand structure with particular emphasis on non-native invasive species.

## STUDY SITE

The study site is in the South Carolina Piedmont on the Clemson Experimental Forest (34°40'N, 82°49'W) in Anderson, Oconee, and Pickens Counties. The dominant forest type is *Pinus taeda* L. and *Pinus echinata* Mill. growing over highly degraded soils. Most of the forest is second- or third-growth timber resulting from reforestation programs of abandoned agricultural fields in the early 1900s. The area also had a history of intentional introductions of non-native plants for erosion control, wildlife forage, and/or horticultural purposes (Sorrells 1984). The climate of the region is characterized by mean monthly temperatures between 5°C to 26°C and mean annual precipitation of 1372 mm distributed evenly throughout the year (NOAA 2002).

## METHODS

We used a randomized complete block design with each treatment replicated three times. The treatments for intact pine stands (i.e., no presence of pine beetles at study initiation) included: (1) mechanical treatment by means of a single entry thinning from below (conducted in the winter of 2000 – 2001) with a target basal area of 18 $m^2$/ha; (2) prescribed burning during the spring every three years; (3) the combination of mechanical treatment plus burning; and (4) an untreated control. The first prescribed burns were performed in 2001 for the burn only treatment, whereas the burns for the mechanical + burn treatment occurred the following year. A second round of burns was conducted in 2004 and 2005 for the burn treatment and mechanical + burn treatment, respectively. Pine beetle damage was so extensive in the original burn treatment that a second set of burn treatments was established (designated as “burn1” for the original and “burn2” for the replacements). For the initial burns, we recorded maximum temperatures of 253–399°C in the burn1 treatment; 177–253°C for the burn2 treatment, and 177–253°C in the mechanical + burn treatment. Maximum temperatures for the second burn in the mechanical + burn treatment ranged from 204–816°C. Details on fire behavior and weather are reported by Phillips and Waldrop (2008). For stands that sustained southern pine beetle damage, we selected treatment units from stands where all overstory pines had been killed over an area of at least 0.25 ha and that had active pine beetles within two years prior to plot establishment. To reduce fuel loading for these stands, we used: (1) a mastication treatment, which removed all dead overstory trees and turned large woody fuel into mulch; (2) low-intensity site prep burns conducted in the late spring of 2006; and (3) high-intensity site prep burns, also in the spring of 2006. An untreated control was established for comparison. Maximum temperatures measured at 1 m above the forest floor for the beetle-killed prescribed burns ranged from 181°C to 216°C for the low-intensity burn treatment and from 291°C to 320°C for the high-intensity burn treatment.

Understory vegetation (less than 1.4 m tall) was measured on twenty 1 $m^2$ subplots nested within 0.1-ha plots (50 x 20 m in size). All tree seedlings/sprouts were recorded by origin (germinant, established, sprout), height category (< 10 cm, 10 to 50 cm, and 50 to 139 cm) and cover class. Shrubs and herbaceous species were recorded by origin and cover class. Cover classes were: 1 = <1 percent; 2 = 1 to 10 percent; 3 = 11 to 25 percent; 4 = 26 to 50 percent; 5 = 51 to 75 percent; and 6 = >75 percent. Each cover class was assigned the value of the class midpoint for data analysis. We selected a subset of non-native invasive species to analyze based on maximum abundance, number of occurrences, and threat classification. All species selected are classified as "severe threat" for South Carolina, defined by the SC Exotic Pest Council (2008) as posing significant risk to composition, structure, or function of natural areas. These species included: *Lonicera japonica* Thunb., *Lespedeza bicolor* Turcz., *Microstegium vimineum* (Trin.) A. Camus, *Ligustrum sinense* Lour., *Albizia julibrissin* Durazz., *Ailanthus altissima* (Mill.) Swingle, and *Pueraria montana* (Lour.) Merr.

We applied repeated measures ANOVA to test differences of selected non-native invasive plants and their responses to each fuel reduction treatment. To account for pre-treatment differences, we compared change over time by subtracting pre-treatment values from each post-treatment measurement. We made post-hoc comparisons using linear contrasts for each site separately and interpreted significant treatment and/or treatment*year interactions at $\alpha = 0.05$ as evidence of treatment effects. Data from stands unaffected by pine beetles were analyzed separately from beetle-killed stands.

Non-metric multidimensional scaling (NMS) was used to examine changes in understory vegetation composition over time due to treatment effects with particular emphasis on non-native species. After removing rare species (occurring in less than 2% of sampled plots) from the data set, we conducted ordinations using the Sørensen distance measure with 250 runs of real data and 250 runs of randomized data in six dimensions (McCune Mefford 2006).

## RESULTS AND DISCUSSION

Prior to fuel reduction treatments, total cover of non-native invasive species was considerably less in stands not impacted by pine beetles (0.8 percent) as compared to those that had sustained southern pine beetle damage (3.9 percent). Pine beetle infestations caused extremely high mortality for all overstory pines resulting in basal areas of ≤1 m$^2$/ha, whereas intact stands were characterized by basal areas of 25 m$^2$/ha or greater (Phillips and Waldrop 2008).

Time since disturbance appeared to be the most important factor affecting species abundance with species responding differently to the treatments (Table 1). The greatest increases in cover were observed 3 to five 5 after treatment, primarily in the mechanical + burn treatment, as species were able to take advantage of more available resources quickly recovering from treatment disturbances or becoming established following treatment. In the mechanical + burn treatment, *L. japonica* was significantly greater than all other treatments (p-values = <0.0313) three years after treatment and greater than the mechanical treatment (p-value = <0.0001) and control (p-value = 0.0008) five years after treatment. *L. sinense* also showed significant increases as compared to the burn1 treatment (p-value = 0.0084), mechanical treatment (p-value = 0.0065), and control (p-value = 0.0087) after three years, but not the burn2 treatment (p-value = 0.0823). *M. vimineum* was only recorded in the mechanical + burn treatment and the control with significant differences observed for the sampling period three years after treatment (p-value = 0.0074). Burning appeared to encourage growth and/or establishment for *L. bicolor*, *L. sinense*, and *A. julibrissin*, whereas *L. japonica* demonstrated a decrease in cover in the burn treatments but showed a slight increase in abundance after initial mechanical + burn treatment. However, successive burns for this treatment reduced cover to below pre-treatment values. In contrast, the mechanical treatment resulted in continued growth of *L. japonica* as it was able to take advantage of more available light.

Our results are consistent with other studies showing greater abundance of non-native invasive plants in areas subjected to mechanical treatment combined with prescribed burning (Dodson and Fiedler 2006, Griffis and others 2001). Large reductions in overstory basal area and disturbance to the forest floor provided suitable seedbed habitat for non-native species to germinate and expand. Implications from these findings suggest fuel reduction treatments could potentially have effects opposite from intended purposes (e.g., ecosystem restoration) by negatively impacting natural species regeneration (Oswalt and others 2007) and altering disturbance regimes (Brooks and others 2004).

Results from the fuel reduction treatments in beetle-killed stands (Table 2) cover only the first two years since disturbance; therefore, conclusions are preliminary. Initial reductions in invasive cover did not persist over time as relatively few differences between pre-treatment and 2-year post-treatment values were evident. *L. japonica* was the most abundant species with other species periodically recorded during the study period. Surrette and Brewer (2008) demonstrated that high cover of *L. japonica* was associated with areas of high disturbance, low fire frequency, and high soil compaction, which are characteristics of both the mechanical and mastication treatments. For these stands, *L. japonica* is likely to out-compete other species preventing stand development unless fire, or another means for limiting its growth (e.g., herbicides, manual removal), is incorporated. Burning successfully reduced *L. sinense*, however, *P. montana* and *L. bicolor* became established following treatment. Continued burning will likely increase the abundance of *L. bicolor* (Tesky 1992) as well as *P. montana* (Munger 2002) unless accompanied by herbicide application (Miller 2003).

Distinct differences in community composition between intact pine stands and beetle-killed pine stands were evident prior to treatment, but these differences appeared to diminish over time, except for the control and mastication treatments for the beetle-killed stands (Figure 1). The final NMS ordination converged on three axes with a final stress of 7.4. The amount of variance explained by each axis was 36.3 percent for Axis 1, 29.0 percent for Axis 2, and 28.9 percent for Axis 3 (cumulative $R^2$ = 94.2 percent). Over time plots generally moved from left to right across Axis 1, which may be a gradient for soil moisture. Data on soil moisture were not available, but we speculate there were differences between stands that were not affected by beetles and those which had little, if any, overstory trees following beetle infestation. These changes in stand structure and disturbance to the forest floor may have affected soil moisture thus influencing species composition. Axis 3 appeared to be associated with light availability as demonstrated by the positive correlation to basal area (r = 0.829) and the movement of plots down this axis for intact pine stands, where basal area decreased over time, contrasted by the progression of beetle-killed plots up this axis.

With respect to invasive species, *A. julibrissin* and *L. sinense* were positively associated with Axis 1, whereas *M. vimineum* showed positive correlation with Axis 3 (Table 3). *L. japonica* was correlated with both Axis 1 (r = 0.829) and Axis 3 (r = -0.535). Even though *L. japonica* can persist under low-light conditions, it is more prolific in high-light environments as demonstrated by a strong negative correlation with Axis 3. In contrast, *M. vimineum*, which is well adapted to low-light conditions and prefers disturbed sites that are shaded and more mesic (Barden 1987), had a positive association with this axis.

Abundances of some of these non-native species are relatively small and statistically significant differences may not represent biological differences, but their presence and responses to these treatments indicate that land managers need to consider these species when planning hazard reduction or restoration treatments. The establishment of non-native species following treatment, even at low levels, needs to be addressed in a timely manner as cost for eradication increases over time (Rejmánek and Pitcairn 2002).

## CONCLUSIONS

Based on understory vegetation composition, it appears that pine stands treated with prescribed fire and mechanical fuel reduction techniques and sustaining southern pine beetle damage are converging over time; however, the effects of these treatments on non-native invasive species differed. Burning stimulated growth and/or establishment for *L. bicolor*, *L. sinense*, and *A. julibrissin*, whereas it decreased *L. japonica* cover. The mechanical treatments, both thinning and mastication, resulted in greatest abundance of *L. japonica*. Depending on the presence of invasive species prior to fuel reduction treatment, land mangers can decide which treatment is best suited for preventing invasive expansion while accomplishing the goal of hazard fuel reduction.

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Table 1—Invasive plant cover in pine stands treated with fuel reduction techniques in the upper Piedmont of South Carolina

| Years Since Treatment | Treatment | LONIJAP | LESPBIC | MICRVIM | LIGUSIN | ALBIJUL | AILAALT | PUERMON |
|---|---|---|---|---|---|---|---|---|
| 0 | Control | 0.47 | 0.00 | 0.01 | 0.09 | 0.00 | 0.00 | 0.00 |
| | Mechanical | 0.65 | 0.00 | 0.00 | 0.05 | 0.00 | 0.00 | 0.00 |
| | Burn 1 | 0.16 | 0.00 | 0.00 | 0.01 | 0.00 | 0.01 | 0.00 |
| | Burn 2 | 1.18 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 |
| | Mechanical+Burn | 0.72 | 0.00 | 0.19 | 0.41 | 0.00 | 0.00 | 0.00 |
| 1 | Control | 0.62 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 |
| | Mechanical | 0.37 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Burn 1 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.22 | 0.00 |
| | Burn 2 | 0.36 | 0.10 | 0.00 | 0.16 | 0.15 | 0.00 | 0.00 |
| | Mechanical+Burn | 0.38 | 0.53 | 0.31 | 0.24 | 0.35 | 0.14 | 0.00 |
| 3 | Control | 0.66 | 0.00 | 0.00 | 0.03 | 0.00 | 0.00 | 0.00 |
| | Mechanical | 0.84 | 0.00 | 0.00 | 0.02 | 0.00 | 0.01 | 0.00 |
| | Burn 1 | 0.13 | 0.00 | 0.00 | 0.00 | 0.01 | 0.05 | 0.00 |
| | Burn 2 | 0.44 | 0.07 | 0.00 | 0.26 | 0.00 | 0.00 | 0.00 |
| | Mechanical+Burn | 1.77 | 1.16 | 1.84 | 1.13 | 0.26 | 0.00 | 0.00 |
| 5 | Control | 2.43 | 0.00 | 0.13 | 0.08 | 0.00 | 0.00 | 0.00 |
| | Mechanical | 2.99 | 0.00 | 0.00 | 0.08 | 0.00 | 0.00 | 0.00 |
| | Burn 1 | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| | Burn 2 | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| | Mechanical+Burn | 1.12 | 5.73 | 0.97 | 1.48 | 0.51 | 0.01 | 0.00 |
| 7 | Control | 0.45 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 |
| | Mechanical | 1.13 | 0.00 | 0.00 | 0.05 | 0.00 | 0.03 | 0.00 |
| | Burn 1 | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| | Burn 2 | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| | Mechanical+Burn | 0.59 | 0.02 | 0.10 | 0.50 | 0.25 | 0.00 | 0.00 |

n/a = Data not available because burning was on a 3-year rotation.
“Years since treatment” corresponds to pre-treatment sampling (0); first year immediately after initial treatment (1); three years after initial treatment (3), etc. Species codes: LONIJAP = *Lonicera japonica*; LESPBIC = *Lespedeza bicolor*; MICRVIM = *Microstegium vimineum*, LIGUSIN = *Ligustrum sinense*; ALBIJUL = *Albizia julibrissin*; AILAALT = *Ailanthus altissima*; PUERMON = *Pueraria montana*.

Table 2—Invasive plant cover in beetle-killed pine stands treated with fuel reduction techniques in the upper Piedmont of South Carolina

| Years Since Treatment | Treatment | LONIJAP | LESPBIC | MICRVIM | LIGUSIN | ALBIJUL | AILAALT | PUERMON |
|---|---|---|---|---|---|---|---|---|
| 0 | Control | 4.74 | 0.00 | 0.00 | 0.01 | trace | trace | 0.00 |
| | Mastication | 8.07 | 0.00 | 0.00 | 0.15 | 0.00 | 0.00 | 0.00 |
| | Low Burn | 2.32 | 0.00 | 0.00 | 0.16 | trace | trace | 0.00 |
| | High Burn | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1 | Control | 7.36 | 0.00 | 0.00 | 0.06 | 0.13 | 0.00 | 0.00 |
| | Mastication | 1.35 | 0.00 | 0.00 | 0.00 | trace | 0.00 | 0.00 |
| | Low Burn | 1.53 | 0.00 | 0.00 | 0.15 | trace | 0.00 | 0.11 |
| | High Burn | 0.10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | Control | 4.48 | 0.00 | 0.00 | 0.10 | 0.05 | 0.00 | 0.00 |
| | Mastication | 7.57 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Low Burn | 1.63 | 0.00 | 0.00 | 0.06 | 0.05 | 0.00 | trace |
| | High Burn | 0.22 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

"Years since treatment" corresponds to pre-treatment sampling (0); first year immediately after initial treatment (1); and two years after initial treatment (2). Species codes: LONIJAP = *Lonicera japonica*; LESPBIC = *Lespedeza bicolor*; MICRVIM = *Microstegium vimineum*, LIGUSIN = *Ligustrum sinense*; ALBIJUL = *Albizia julibrissin*; AILAALT = *Ailanthus altissima*; PUERMON = *Pueraria montana*.

Table 3—Parametric (Pearson's r) and non-parametric (Kendall's tau) correlations of invasive species with NMS axes for pine stands in the upper Piedmont of South Carolina

| | Axis 1 | | Axis 2 | | Axis 3 | |
|---|---|---|---|---|---|---|
| Species | r | tau | r | tau | r | tau |
| AILAALT | -0.190 | -0.129 | 0.020 | 0.097 | 0.119 | 0.209 |
| ALBIJUL | 0.430 | 0.340 | -0.406 | -0.413 | 0.069 | -0.038 |
| LESPBIC | 0.035 | 0.048 | -0.317 | -0.389 | 0.232 | 0.240 |
| LIGUSIN | 0.423 | 0.304 | -0.210 | -0.178 | 0.276 | 0.175 |
| LONIJAP | 0.829 | 0.613 | -0.080 | -0.060 | -0.535 | -0.263 |
| MICRVIM | 0.030 | -0.096 | -0.181 | 0.029 | 0.353 | 0.435 |
| PUERMON | 0.187 | 0.216 | -0.356 | -0.293 | -0.148 | -0.149 |

Species codes: LONIJAP = *Lonicera japonica*; LESPBIC = *Lespedeza bicolor*; MICRVIM = *Microstegium vimineum*, LIGUSIN = *Ligustrum sinense*; ALBIJUL = *Albizia julibrissin*; AILAALT = *Ailanthus altissima*; PUERMON = *Pueraria montana*.

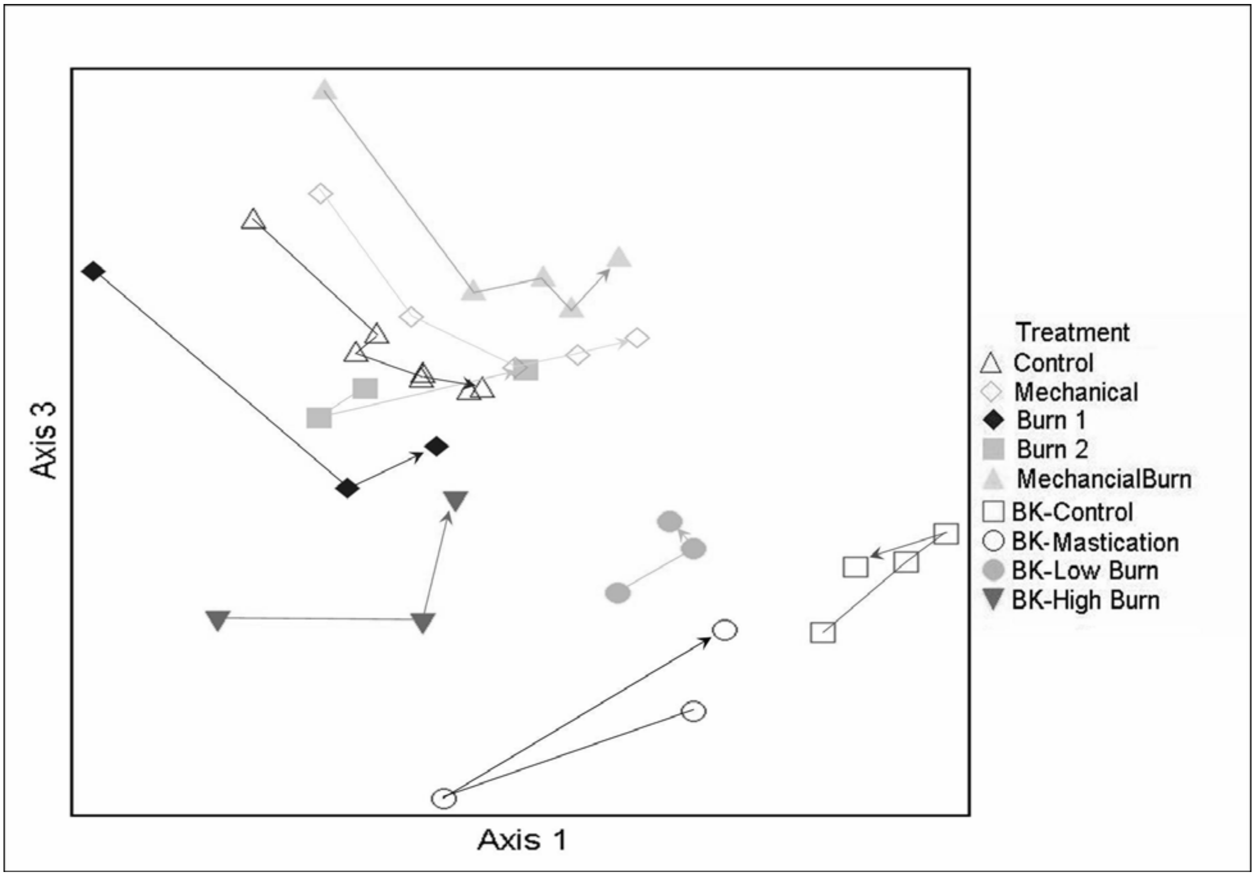

Figure 1—NMS ordination of plots in species space depicting change over time for pine stands treated with fuel reduction treatments in the upper Piedmont of South Carolina.

# IMPACT OF RAINFALL ON THE MOISTURE CONTENT OF LARGE WOODY FUELS.

Mohr, Helen H.; Waldrop, Thomas A. 2009. Loading of heavy fuels in beetle-killed areas: a problem of predicting fire behavior. 15th Biennial Southern Silvicultural Research Conference. Hot Springs, AR. 2008. [In press].

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Abstract—A prolonged drought in the southeastern United States increased the severity of southern pine beetles (Dendroctonus frontalis) throughout the region. Since 2001, South Carolina alone has detected over 92,000 beetle killed spots and the death of approximately 25.5 million trees. After these attacks as dead trees fall, a particular concern is heavy fuel loading and how to predict when large fuels will ignite. Fire behavior models typically do not account for these fuels, but when ignited, logs can increase fire intensity and / or duration and become a problem for smoke management (Haywood and others 2003). Variables such as rainfall amount and duration greatly impact the ignition probability of large woody fuels but these relationships have not been measured. We conducted a study of log moisture content after simulated rainfall events ranging from 4 inches in one day to 1 inch four times in four weeks. Log moisture content was measured before and after each rainfall event to determine how a dry log absorbs moisture over time.

## INTRODUCTION
Land managers are commonly faced with prescribed fires that have higher intensities than expected after prolonged droughts. One reason for this is that land managers are using the Keetch-Byram Drought Index (KBDI) alone to estimate fire behavior. The KBDI Index is part of the National Fire Danger Rating System (NFDRS) and is the most widely used drought index for the fire danger rating. The KBDI Index was developed by John Keetch and George Byram to look at the effects of long term drying on litter and duff and subsequently, on fire activity. The index ranges from 0 to 800 with 0 being saturated and 800 the worst drought condition. The index measure is in hundredths of an inch and is based on a measurement of eight inches of available moisture in the upper soil layers. The available moisture can be used by vegetation for evapotranspiration. The index indicates deficit inches of available water in the soil. A KBDI of 250 means that there is a deficit of 2.5 inches of ground water available to the vegetation (Melton 1990). During a long dry period KBDI could be in the 600+ range and large fuels have experienced deep drying. A single rain event could cause KBDI to drop into the 200 to 300 range. While the 1-hr and 10-hr fuels would be immediately impacted by the rain event the larger 100-hr and 1000-hr fuels would still be extremely dry on the interior. Fire behavior could be much different than expected if the KBDI alone is used as a fire behavior predictor.

In Georgia, the number of fires and acres burned between 1957 and 2000 was highest in the months of February and March. This is when KBDI is typically lowest with August having the highest KBDI. Wildland fire incidents in Georgia corresponded especially to the spring fire season not the high KBDI months of summer (Chan and others 2004). The influence of fuel moisture on fire behavior is not well known for large 100-hr and 1000-hr fuels. This is particularly a problem in the southeastern United States where there is extensive southern pine beetle damage. Many southern forests have heavy 100-hr and 1000-hr fuel loading due to the southern pine beetle. This study applied six different rainfall events and monitored fuel moisture of 100-hr and 1000-hr logs to see how moisture contents changed with different rainfall amounts and durations.

## METHODS

This study was conducted on the Clemson Experimental Forest in Clemson, SC. We collected two species of logs red oak and loblolly pine (Pinus taeda L.). The loblolly pine collection consisted of live tree logs and dead tree logs. The red oak logs were all collected from live trees. The three log size classes were 3-5 inches, 5-7 inches and 7-9 inches in diameter. Each log was 3 feet in length. Trees were cut down and then cut up to meet the size classes required. The dead loblolly logs were collected from the forest floor and cut according to size class as well. The dead loblolly logs were left from a southern pine beetle cut and had been lying on the forest floor for approximately one year.

The cut logs were placed into a barn where they were well protected from rain. This barn was not temperature controlled. The logs stayed in the barn for a total of two years. In the second year fans blew air onto the logs for 24 hours a day to help in the drying process. Log moistures were checked over two years to monitor the drying process. When the logs reached equilibrium they were removed from the barn as needed to apply rainfall treatments.

Rainfall events were simulated with a Colorado State rainfall simulator. The simulator was placed on an open gravel lot in full sun. The gravel was covered with three inches of wood mulch to simulate the forest floor. Twenty-four logs from each log type totaling 72 logs were placed on the mulch for each rainfall event. There were six rainfall events: 4 inches in one day, 2 inches two times in one week, 1 inch four times in one week, 1 inch four times in two weeks, 1 inch four times in three weeks, and 1 inch four times in four weeks.

Log moisture was sampled in two locations on each log pre-treatment and post-treatment after each rainfall event. The post-treatment log moistures were taken 24 hours after each rainfall event to allow time for the exterior of the logs to dry. We used a Delmhorst J-series compact wood moisture meter with a type 26-ES two pin hammer probe electrode. The probes were driven into the log one foot from each end giving two measurements per log. The moisture meter probes reached a depth of 1-1/8 inches.

## RESULTS

Pre-treatment log moisture ranged from 11.18 percent to 23.19 percent with an average of 15.44 percent. Oaks and live pines had the lowest pre-treatment moisture contents with the dead pine slightly higher. The dead pine had the highest moisture contents for all of the post-treatment measurements. The oak, live pine and dead pine log moisture contents stayed close after the first rainfall in all treatments except for the 4 inches one time in one day treatment. This treatment showed the dead pine with higher moisture content than the live pine and oak (Figure 1). The one inch four times in one week treatment rained on logs every other day for one week. All of the log moisture contents increased at a steady rate. The pines had the highest moisture contents ranging from 27.28 to 33.22 percent (Figure 2). The two inches two times in one week treatment followed the same trend with increased log moistures after each rainfall event (Figure 3). The oaks once again show a slower increase in moisture content than the live and dead pine. One inch of rain four times in two weeks produced the wettest logs after the last treatment (Figure 4). The watering for this treatment had more time between rainfall events but not enough time to let the logs dry. All pine logs and the three to five inch size class oak logs all had moisture contents greater than 30. Watering the logs one inch four times in three weeks showed the same steady increase with lower moisture contents than the one inch four times in two weeks treatment (Figure 5). With all of the treatments there was a steady increase in fuel moisture over time except for the 1 inch four times in four weeks treatment (Figure 6). The amount of time between rainfalls may have been too great and allowed for drying. Our treatments show that 1 rainfall event does not impact the log moisture content. To reach log moistures in the high 20's to low 30's we had to apply frequent rain over a two to three week period. We got the wettest logs by applying 1 inch of rainfall four times over two weeks. The logs that received 2 inches of rain two times in one week also had a higher moisture content of 27.07 percent.

## CONCLUSIONS

We know that KBDI alone is not a good indicator of fire behavior and intensity in the southeast. After a single rainfall event KBDI can drop drastically but moisture contents of large woody fuels do not increase very much. In this preliminary study we were able to monitor log moisture content over time with different rainfall treatments. This study gives us a picture that one rainfall event will not increase the moisture content of 100-hr and 1000-hr fuels. It takes several rainfall events over time. The next step in this study is to take the wet logs to wooded areas that are scheduled for prescribed burns. Burning these logs in forested conditions would allow us to measure consumption due to the fire. The combination of log moisture data and log consumption may give us a clearer picture of fire behavior due to large woody fuels.

## ACKNOWLEDGEMENTS

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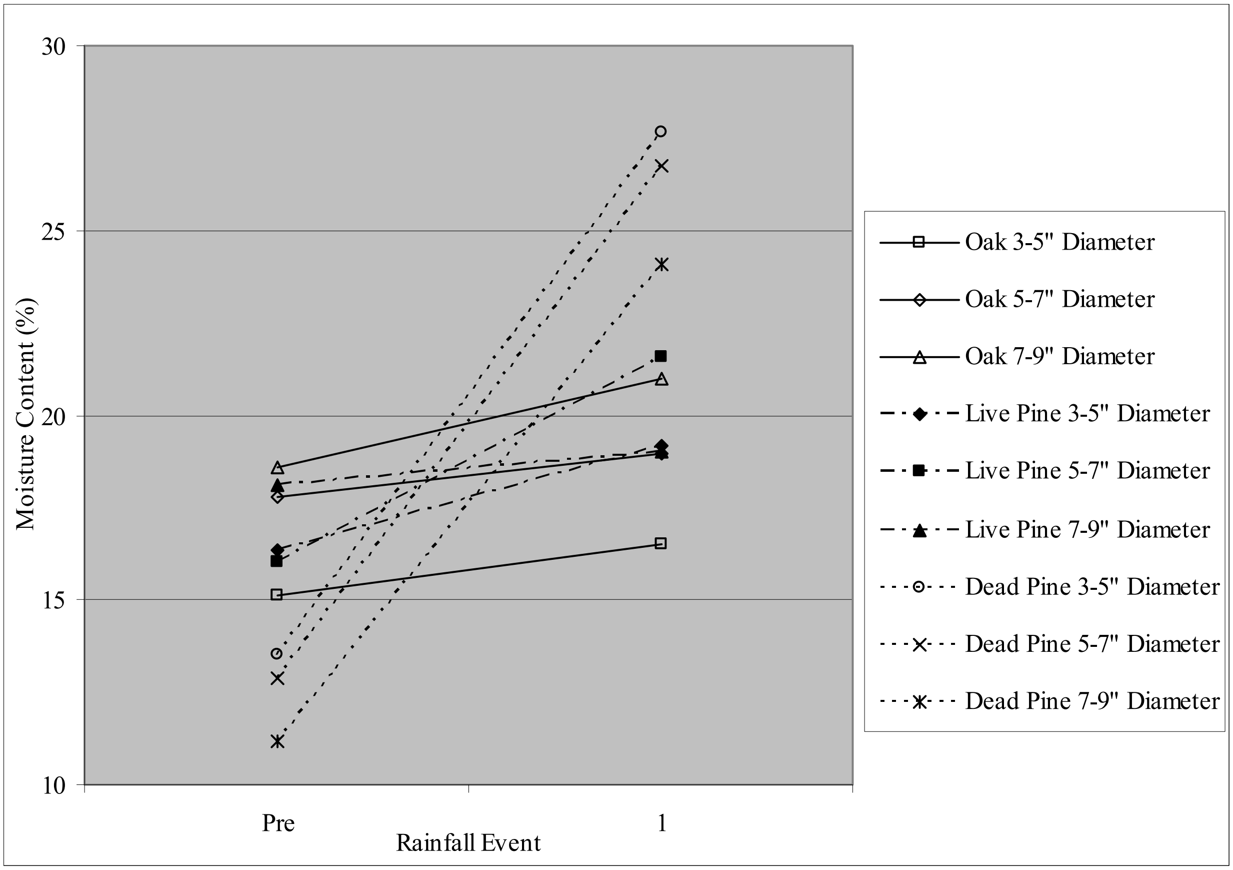


Figure 1—Average log moisture content pre-treatment and 24 hours after each rainfall event. Treatment: Four inches one time in one day.

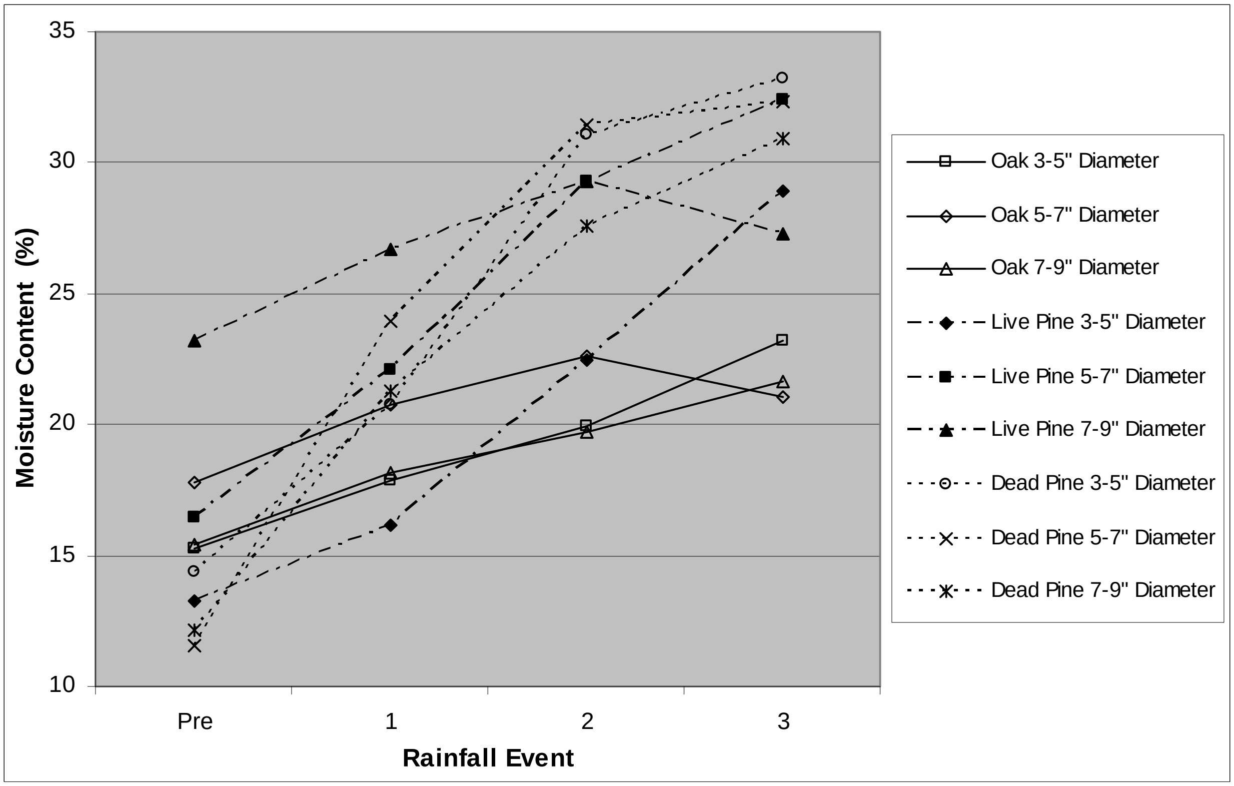


Figure 2—Average log moisture content pre-treatment and 24 hours after each rainfall event. Treatment: One inch four times in one week.

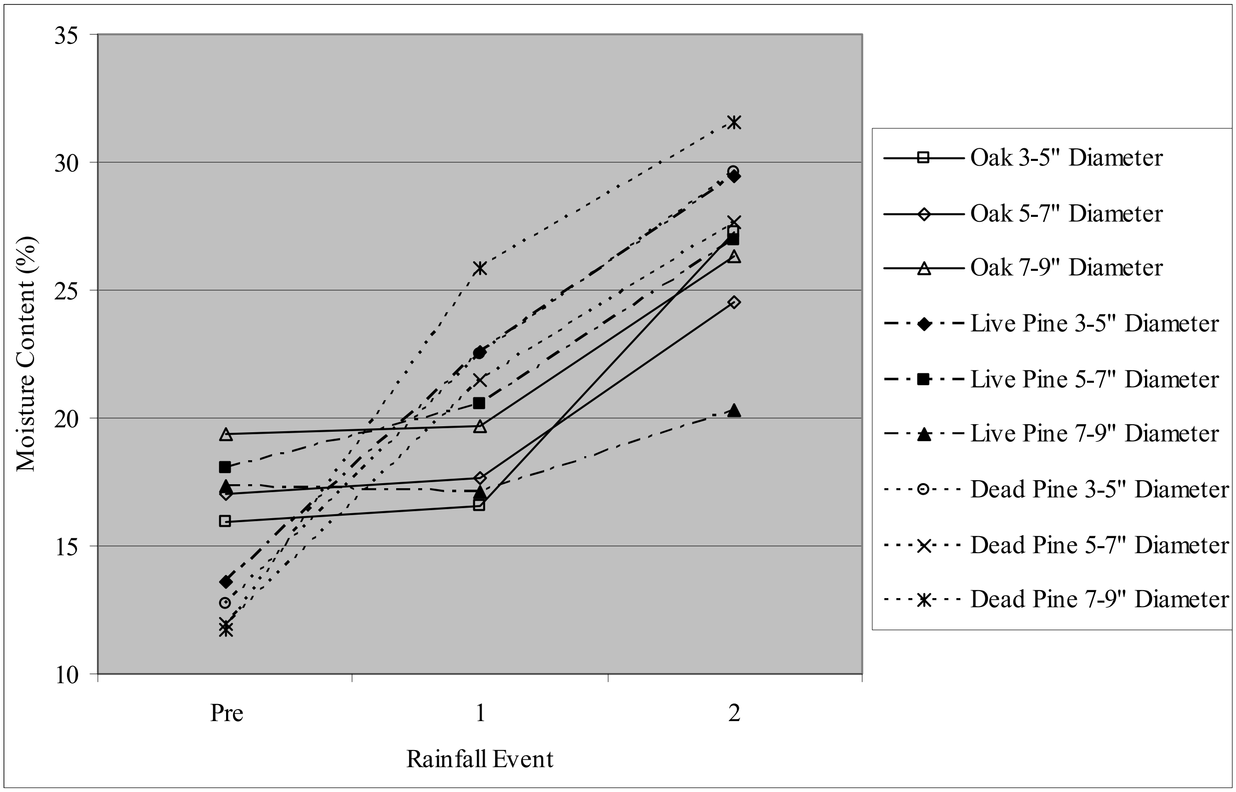


Figure 3—Average log moisture content pre-treatment and 24 hours after each rainfall event. Treatment: Two inches two times in one week.

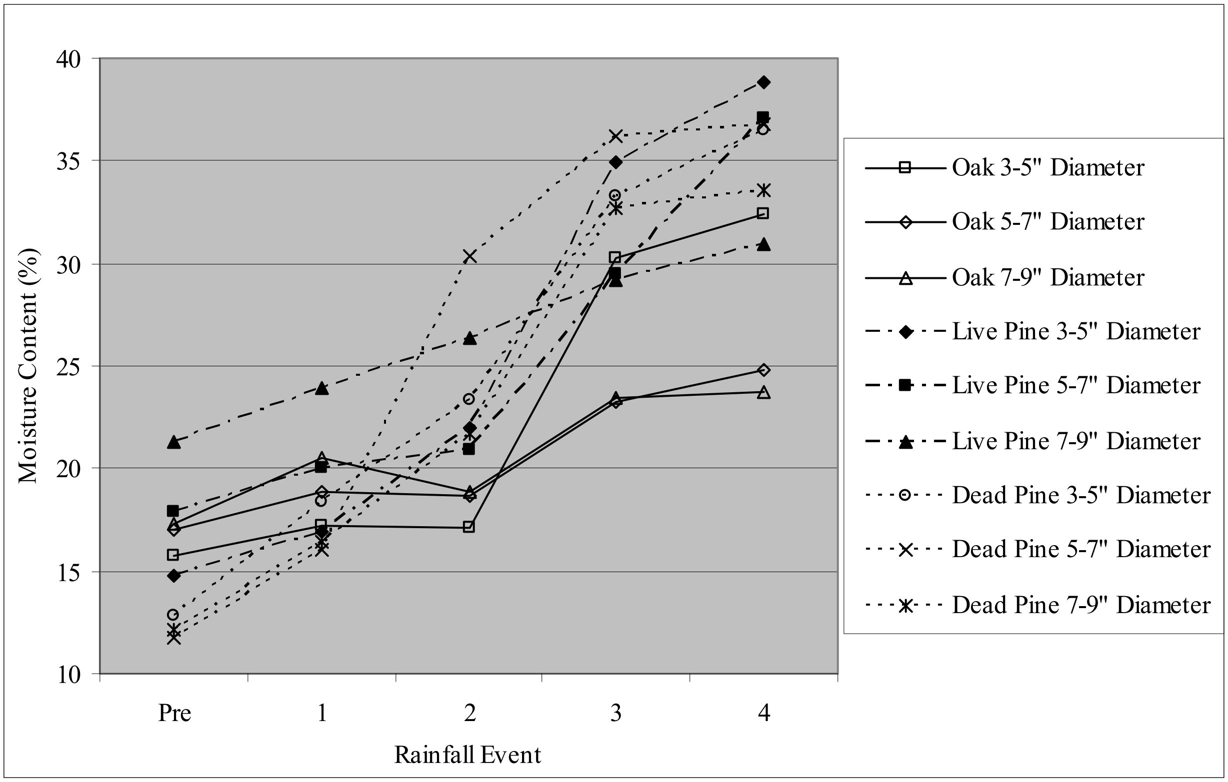


Figure 4—Average log moisture content pre-treatment and 24 hours after each rainfall event. Treatment: One inch four times in two weeks.

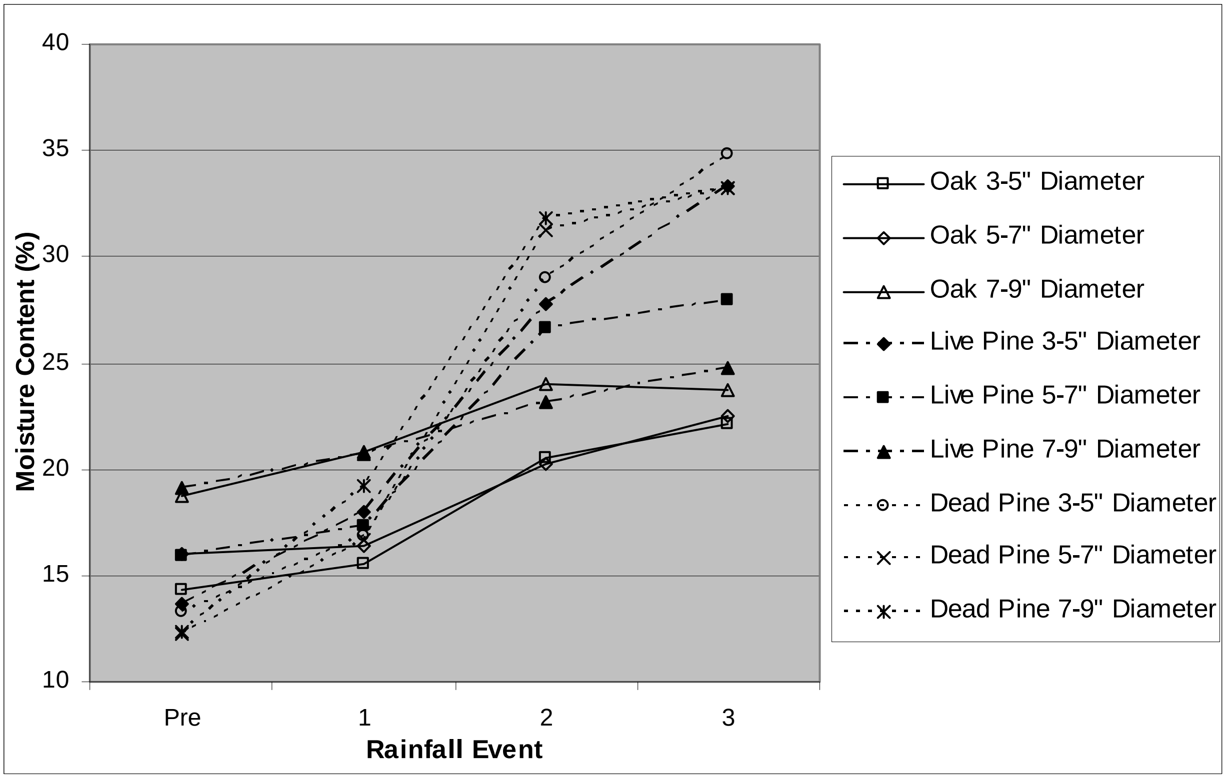


Figure 5—Average log moisture content pre-treatment and 24 hours after each rainfall event. Treatment: One inch four times in three weeks.

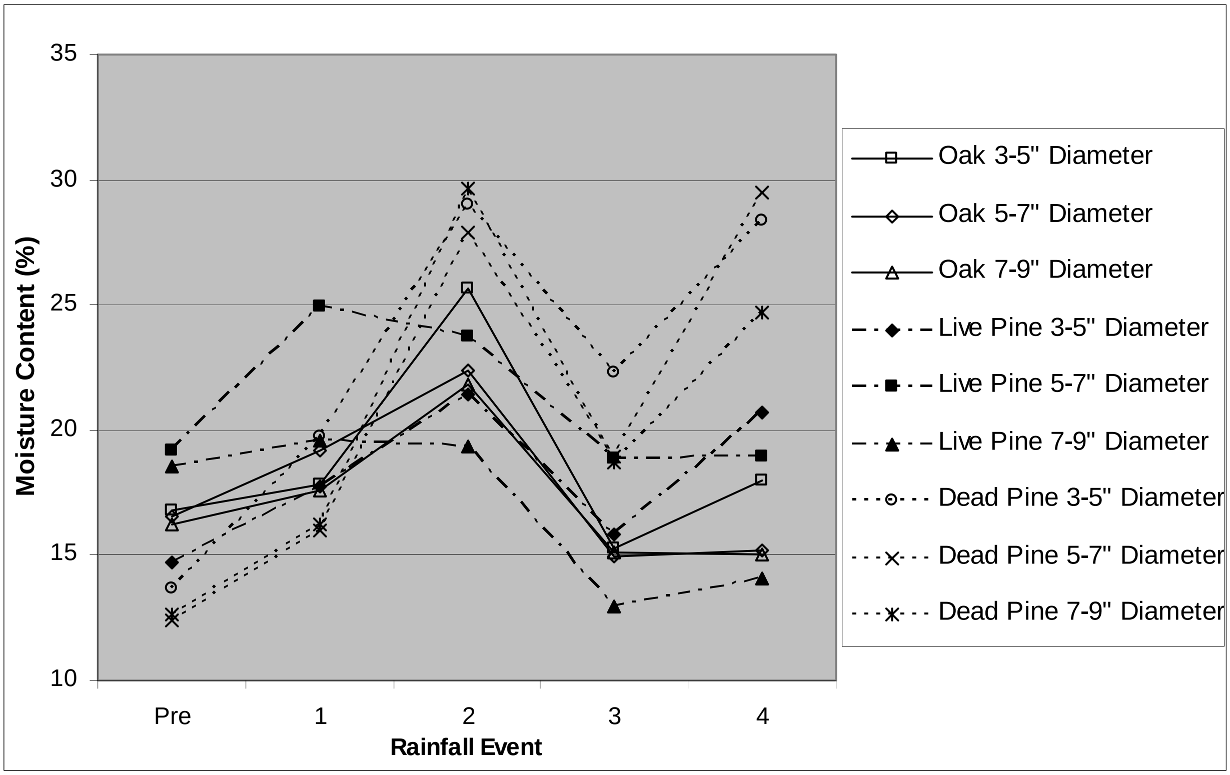


Figure 6—Average log moisture content pre-treatment and 24 hours after each rainfall event. Treatment: One inch four times in four weeks.

# COMPARING FIRST-YEAR PINE SEEDLING SURVIVAL AFTER BURNING AND MULCHING FOR SITE PREPARATION IN BEETLE-KILLED PINE STANDS

To be submitted for the 16th Biennial Southern Silvicultural Research Conference or other appropriate outlet.

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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 2000's. Prescribed burning and mechanical mulching (mastication) have been proposed but have not been tested for reducing downed-woody material and interfering vegetation in beetle-killed conditions to prepare sites for artificial regeneration. The objective of this study was to examine loblolly pine (*Pinus taeda* L.) seedling survival one year following different site preparation treatments in beetle-killed stands in the upper South Carolina Piedmont. Prescribed burning and mulching (both followed by chemical herbicide application) were effective at reducing downed-woody material and interfering vegetation. Pine seedling survival was highest after mulching (85 percent) compared to prescribed burning and the control (herbicide only) (82 and 77 percent, respectively). Higher seedling survival after mulching was possibly due to prolonged weed control and favorable soil conditions provided by the mulch. More definitive conclusions can be made regarding the effectiveness of these treatments as we follow the growth of the seedlings over the next few years.

## BACKGROUND

Southern pine beetle (SPB) (*Dendroctonus frontalis*) is a native insect to southern forests in North America but is the most destructive pest of this regions pine forests (Ward and Mistretta 2002). One of the regions in which outbreaks have been most severe and persistent is the Piedmont of Alabama, Georgia, and South Carolina (Ward and Mistretta 2002). The Piedmont region's long legacy of human influence through crop agriculture, livestock pasturing, and exploitative timbering caused erosion and depleted the soil resource (Callaham and others 2006) which has been linked to increased susceptibility of stands to SPB outbreaks (Karpinski and others 2003). Dense, naturally-regenerated stands and un-thinned plantations of loblolly pine and shortleaf-hardwood mixtures are the most common stand types in which SPB infestations are found in the Piedmont.

The Piedmont region's pine and pine-hardwood forests experienced significant SPB attack in the early 2000's. All pines in large, contiguous stands may be killed during active SPB infestations. Dead trees fall in 1-2 years and herbaceous vegetation quickly takes advantage of the increased availability of light, water, and nutrients and colonizes the beetle-killed sites. Trees pile one on another, some trees stand whole or break half-way up the stem, and under-brush grows at thicket-like densities. The resulting condition is dangerous, unsightly, and vexing for society and resource managers in the region.

Interests in other objectives have been expressed for beetle-killed areas (e.g., Hart Vincent (2003)), but perhaps the most common objective of regional managers is the re-establishment of pine plantation. However, planting activities are greatly hindered by the large amounts of course woody debris and the dense layer of understory vegetation. Prescribed fire and various mechanical site preparation treatments are available (Schultz 1997), but limited information exists on the impact of these treatments on ecosystem integrity when used in extremely high fuel loading.

The treatments were previously shown to differentially impact downed-woody and forest floor fuels and certain soil chemical properties (see Chapter X). However, the response of desired vegetation to changes in ecosystem processes has received only limited attention with the use of greenhouse bioassay procedures (see Chapter X). Therefore, the objective of this study was to examine loblolly pine (*Pinus taeda* L.) seedling survival one year following different site preparation treatments in beetle-killed stands in the upper South Carolina Piedmont. This is part of a larger study that examined several variables including fuels, fire behavior, soil fertility, and vegetation.

## METHODS

### Study Area

The study was conducted in 12 beetle-killed pine stands that were approximately 0.5 ha in size in the Clemson University Experimental Forest. The stands were artificially planted or naturally regenerated and approximately 18-33 years in age when killed. Mean diameter of *Pinus* spp. stems (live or dead) in the pretreatment vegetation community was 21.9 cm.

### Fuel Reduction Treatments

The twelve stands were randomly assigned to one of three fuel reduction treatments in an unbalanced design to create three replications of control and mulching and six 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 three-day period between 30-March and 03-May, 2006 using manual strip-head firing.

### Herbicide Application and Tree Planting

Chemical herbicide was used to kill early successional vegetation that escaped the initial site preparation treatments or that colonized the stands in the interim. The herbicide was prepared by mixing 236.6 milliliters of glyphosate and 29.6 milliliters of surfactant per 11.4 liters of water. The herbicide was applied to the foliage of all live vegetation <2 meters in height using backpack sprayers. Herbicide application in the 12 stands occurred between 02-October and 13-October 2006. Two-year old loblolly pine seedlings (mixed lot) were planted on 12-February 2007. Planting was conducted by a contracted crew throughout each stand at 2.1 meter x 3.0 meter spacing (1587 seedlings per ha).

In summary, the site preparation treatments used in our study are: (1) prescribed burning with herbicide ("burn"); (2) mulching with herbicide ("mulch"); and (3) herbicide only ("control").

### Seedling Survival Estimation

Two 3.7 meter-wide transects were walked parallel to the long axes of the 12 treatment units between 30- and 31-January-2008 to obtain estimates of pine seedling survival. The live and dead seedlings that fell within the 3.7 meter-wide transects were tallied.

### Statistical Analysis

Percentage seedling survival estimates were determined by dividing the total number of live seedlings by the total number seedlings (live + dead) encountered by walking the two transects. Seedling survival percentages were compared among the three fuel reduction treatments using analysis of variance (PROC GLM, SAS Institute, 2003).

## RESULTS AND DISCUSSION

Pine seedling growth is usually most rapid when competing vegetation is removed prior to regeneration activities (Schultz 1997). Extreme coarse woody debris loading and dense vegetative cover occupy growing space, cast heavy shade on the forest floor, and impede planting activities in beetle-killed stands. In the current study, loblolly pine seedling survival was increased with mulching when compared to burning and the control (Table 1).

Martin and Shiver (2002) demonstrated that prescribed burning ranked worst and the combination of herbicide and burning ranked among the best in terms of improving loblolly pine seedling growth. In the current study, pine seedling survival after prescribed burning followed by herbicide was not significantly different than the control (i.e., herbicide only). In their study, more complete and/or prolonged weed control was achieved using the 'brown and burn' (chemical herbicide followed by burning) technique. It is unknown whether the brown and burn method would be more effective at controlling interfering vegetation in beetle-killed stands. In addition, the follow-up herbicide may have produced better woody plant control had the application occurred in the summer rather than in early fall (Schultz 1997).

It is important to note that one of the objectives of the larger study was to determine whether prescribed burning would effectively reduce downed-woody debris and interfering vegetation without jeopardizing site productivity. Using first-year pine seedling survival as an indicator, it appears that it was not.

Loblolly pine seedling survival following the mulching treatment was approximately 8 percent higher than the control. Haywood (1999) found that three year old loblolly pine seedlings mulched with three different cellulose-based materials were taller and had larger ground-line diameters than unmulched seedlings. The mulches were also found to reduce weed cover by 48 percent, on average. The authors suggested that soil conditions (moisture and temperature) were also made more favorable to seedling growth (Haywood 1999). In our study, mulch depths ranged from 2 to 16 centimeters. Mulching greatly reduced interfering vegetation (data not shown) and the mulched material likely improved soil conditions around planted seedlings. In addition, the mulch provides a means of passive weed control which may persist through seedling establishment.

Planting seedlings so that their roots are completely covered by mineral soil is made more difficult in areas where thick mulch layers overlay the soil. Failing to completely cover seedlings' root systems with soil may lead to greater seedling mortality in mulched stands.

## CONCLUSION

Prescribed burning and mulching have been proposed but not attempted in beetle-killed conditions to prepare the sites for artificial regeneration. The objective of this study was to examine loblolly pine seedling survival one year following these treatments in beetle-killed stands in the upper South Carolina Piedmont. Prescribed burning and mulching (both followed by chemical herbicide application) were effective at reducing downed-woody material and interfering vegetation. Pine seedling survival was highest after mulching compared to burning and the control (herbicide only) possibly due to prolonged weed control and favorable soil conditions provided by the mulch. More definitive conclusions can be made regarding the effectiveness of these treatments as we follow the growth of the seedlings over the next few years.

## ACKNOWLEDGMENTS

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**Table 1--**Mean loblolly pine (*Pinus taeda* L.) seedling survival. SEM = standard errors of the means. Seedlings were planted in beetle-killed stands that were subjected to different fuel reduction treatments in the upper South Carolina Piedmont. Means within a column followed by the same letter are not significantly different at the 0.05 level

| | Survival (%) | |
|---|---|---|
| **Treatment** | Average | SEM |
| **Control (n = 3)** | 76.7 b | 3.3 |
| Prescribed burn (n = 6) | 81.7 ab | 1.1 |
| Mulch (n = 3) | 85.0 a | 0.0 |