Effect of Restoration Thinning on Mycorrhizal Fungal Propagules in a Northern Arizona Ponderosa Pine Forest: Preliminary Results

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Abstract—The inoculum potential for arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) fungi were investigated in thinned and uncut control stands in a northern Arizona ponderosa pine forest. A corn bioassay was used to determine the relative amount of infective propagules of AM fungi, and a ponderosa pine (*Pinus ponderosa*) bioassay was used to determine the relative amount of infective propagules of EM fungi. Three stands of each treatment were sampled by collecting soil cores along 10 randomly chosen transects within each stand. The relative amount of infective propagules of AM fungi was significantly higher in samples collected from the thinned stands than controls. Conversely, there was a slight decrease in the relative amount of infective propagules of EM fungi in samples collected from thinned stands in comparison to the controls; however, this difference was not significant. These preliminary results indicate that population densities of AM fungi can rapidly increase following restoration thinning in northern Arizona ponderosa pine forests. This may have important implications for restoring the herbaceous understory of these forests because most understory plants depend upon AM associations for normal growth.

Introduction

Low intensity fires carried by grassy understories were prevalent every 2–20 years in southwestern ponderosa pine (*Pinus ponderosa*) ecosystems prior to Euro-American settlement and played a major role in determining the structure, composition, and stability of these ecosystems (Cooper 1960). Current alterations to the structure and function of southwestern ponderosa pine forests are the result of heavy grazing, intensive logging of old-growth trees, and fire suppression by Euro-American settlement around the turn of the 20th century (Covington and others 1997). This has resulted in a large number of small trees with closed canopies and little herbaceous understory production. The current reduction in herbaceous understory diversity and production in ponderosa pine forests has contributed to the alteration of the natural fire regime, loss of habitat for numerous animal species, and overall reduction of species diversity. As a result, a major objective of restoring Southwestern ponderosa pine forests is to increase herbaceous understory diversity and production by reestablishing community structure and function within a range of natural variability.

Mycorrhizae are a major component of soil ecosystems, playing an important role in plant nutrition, nutrient cycling, food webs, and the development of soil structure (Johnson and others 1999). A mycorrhiza is generally a "mutualistic relation between plant and fungus localized in a root or root-like structure in which energy moves primarily from plant to fungus and inorganic resources move from fungus to plant" (Allen 1991). Forest tree species, particularly those within Pinaceae, Fagaceae, Betulaceae, and Dipterocarpaceae form ectomycorrhizal (EM) relationships with basidiomycetes and ascomycetes (Harley and Smith 1983). Over 80 percent of all plants form arbuscular mycorrhizal (AM) relationships with Glomalean fungi, a single order of Zygomycetes. A few exceptional plant families (Cyperaceae, Juncaceae, Caryophyllaceae, Chenopodiaceae, and Brassicaceae) typically do not form any mycorrhizal relationship (nonmycotrophic) (Pendleton and Smith 1983). Numerous researchers have suggested a relationship between the recovery time of disturbed ecosystems and the abundance of infective propagules of fungi (Allen and Allen 1980; Bentwenga and Hetrick 1991; Noyd and others 1995; Reeves and others 1979). Therefore, quantifying the effect of restoration thinning on densities of mycorrhizal fungal propagules may provide insight to the recovery rate of herbaceous understory communities to restoration treatments in ponderosa pine forests.

This is the first known study looking at AM fungal propagule densities in Southwestern ponderosa pine forests. We hypothesized that AM fungal propagule densities would increase and EM fungal propagule densities would decrease in response to restoration thinning in ponderosa pine forests, and that these changes would be correlated to host plant abundance. The specific objectives of this study were to: (1) quantify the effect of restoration thinning on AM and EM fungal propagule densities; and (2) assess the

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relationships between mycorrhizal fungal propagule densities and soil and plant community properties.

Methods

Experimental Design

Three blocks of four restoration treatments were established during the summer of 1998 within approximately 1,700 acres of the Fort Valley Experimental Forest and adjacent areas near Flagstaff, AZ (fig. 1). Treatment units within each block were randomly assigned one of four treatments: (1) no thinning or burning (control); (2) thinning to a low level of replacement trees and burning; (3) thinning to an intermediate level of replacement trees and burning; (4) thinning to a high level of replacement trees and burning. Specific details of thinning treatment guidelines are outlined in Covington and others 1998. Soils for this study were collected before any of the treatment units had been burned, and therefore these results only reflect the effect of restoration thinning on mycorrhizal fungal propagules. Continuation of this study will investigate the effects of full restoration treatments (thinning and prescribed burning) after all treatment units have been burned.

Field Sampling

Soil samples for "bait-plant bioassays" and soil and vegetation analyses were taken in mid-May, 6 months after thinning, along 10 randomly placed transects within each of the three blocks in the controls (1-1, 2-1, 3-1) and low level of replacement trees thinning treatment units (1-2, 2-2, 3-2) (fig. 1). Soil samples were taken to a depth of 15 cm using a hand trowel. Samples were taken to this depth because AM fungal propagule densities are generally highest in the surface 15 cm (Johnson and others 1991). Two samples from each transect were immediately placed into 4 cm diameter x 20 cm diameter deep Conetainers (Stuewe and Sons, Inc., Corvallis, OR) for "bait-plant bioassays." The other sample for soil analysis was placed in a ziploc bag and stored in a freezer until analysis. A 0.5 x 2 m (1 m^2) plot was located adjacent to each soil sample, and the percent cover of each herbaceous and woody species present along with substrates (litter, soil, and rock) were estimated using cardboard cutouts as visual guides. The soil seed bank, soil disturbance, bulk density, fuel loads, fire behavior, herbaceous biomass production and abundance, overstory tree structure, and understory tree regeneration were also assessed along these 50 m transects.

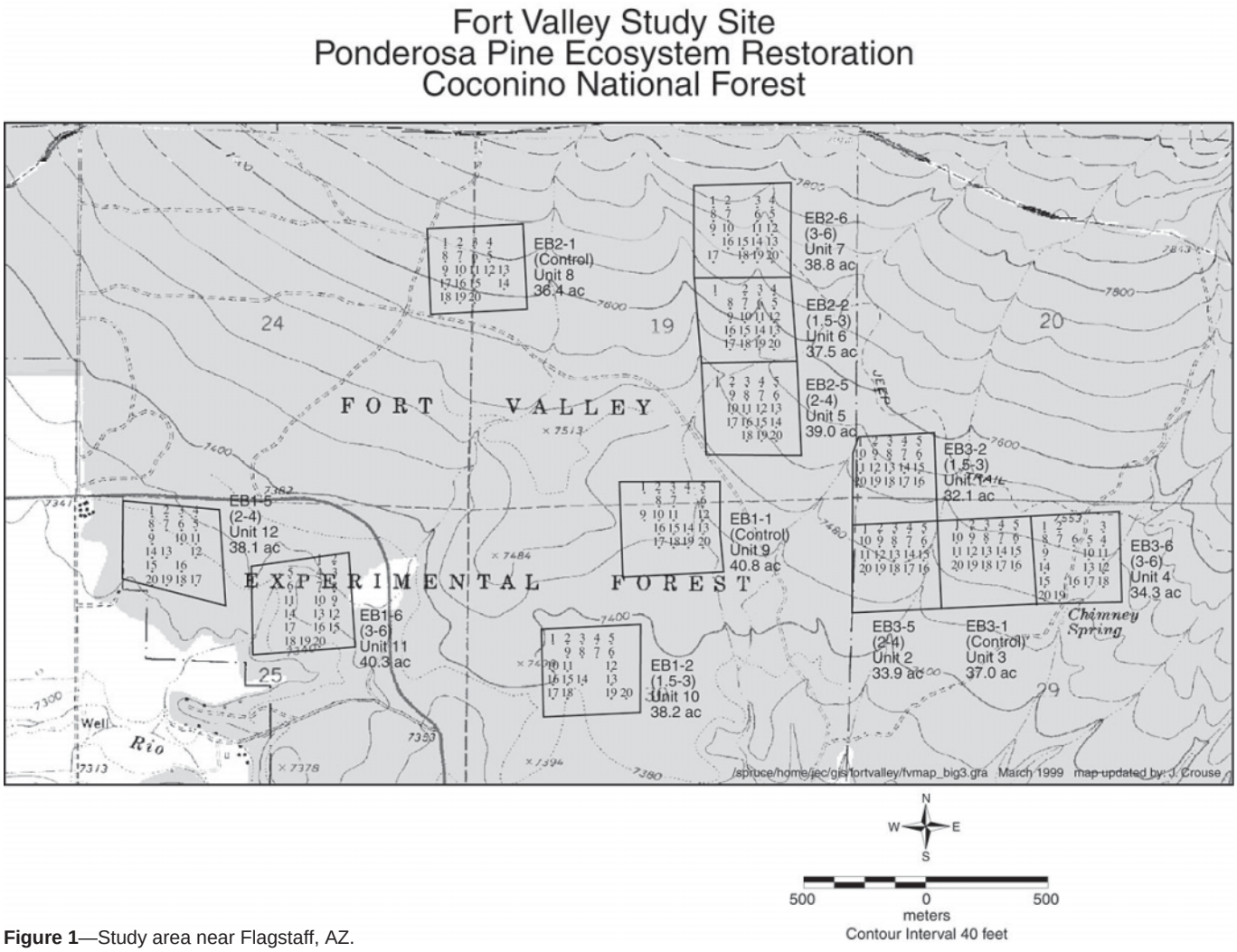

Figure 1—Study area near Flagstaff, AZ.

Lab Analysis

A corn (*Zea mays*) bioassay was used to determine the relative amount of infective propagules of AM fungi, and a ponderosa pine bioassay was used to determine the relative amount of infective propagules of EM fungi. Corn is a strongly mycotrophic plant and grows rapidly and uniformly, and its advantages outweigh the disadvantage of not using native host plants (Johnson and others 1999). "Bait-plant bioassays" are designed to detect all types of viable mycorrhizal fungal propagules including spores, fragments of mycorrhizal roots and extraradical hyphae and therefore may more accurately quantify total mycorrhizal fungi than direct counts of sporocarps, spores, or colonized root lengths (Brundrett and others 1994; Johnson and others 1999). Plants were placed in a greenhouse and watered every 3 days until corn plants were harvested at 6 weeks and pine plants were harvested at 12 weeks. Roots were carefully washed free from the soil and were measured for length and weighed. Following mycorrhizal analysis, roots were dried in an oven at 70 °C for 24 hours and then were weighed again. Shoot lengths and dry weights were also measured. Corn roots were prepared for fungal propagule density measurements by taking a random subsample of roots of a known mass, clearing roots in 5 percent KOH, and then staining roots with trypan blue (Johnson and others 1999). The gridline intersect method using a dissecting microscope was used to measure the proportion of root length containing AM fungal structures: arbuscules, vesicles, coils, internal mycorrhizal hyphae and external mycorrhizal hyphae (Givanetti and Mosse 1980). Pine roots were measured for fungal propagule density through direct examination using a dissecting microscope to quantify the proportion of root tips colonized with EM fungi (Gehring and Whitham 1991). Root tips were also classified as dead or living.

Soil Analysis

Soil samples from each transect were analyzed for pH, total N, total P and organic C at the Bilby Research Soil Analysis Laboratory, Flagstaff, AZ. Soil pH was determined in a 1:1 slurry by pH meter. The general method used for N and P was a Kjeldahl digestion of the soil material followed with the analysis of N and P by automated colorimetry using a Technicon auto-analyzer (Parkinson and others 1975). Organic matter was determined by loss on ignition. Samples were heated in crucibles in a muffle furnace at 450 °C and net weight loss was estimated as organic matter.

Statistical Analysis

Analysis of variance for a randomized complete block design was conducted using SAS JMPIN version 3.2.1 (SAS Institute 1997) to determine the effect of thinning on mycorrhizal propagule densities. Root infection data were log (x + 1) transformed prior to analysis, and bait plant length and weight, herbaceous plant abundance, and soil properties were ln transformed. Simple correlation analyses were conducted to assess the relationship between infectivity, soil and plant community properties, and bait plant characteristics (length and weight) using SAS JMPIN. Significance of correlations and analysis of variances were accepted at alpha ≤ 0.05.

Results

Mycorrhizal fungi colonized all "bait-plant" roots grown in soil from the thinned and control units. Bioassay results indicated that infective propagule densities of AM fungi were significantly higher in the samples collected from the thinned units than controls ($F = 5.4437$, $P < 0.005$) (fig. 2). Also, corn grown in thinned soils had relatively more vesicles and less hyphae than corn grown in control soils (fig. 3). Conversely, there was a slight decrease in the relative amount of infective propagules of EM fungi in samples collected from thinned stands in comparison to the controls ($F = 2.1601$ $P > 0.05$); however, this difference was not significant (fig. 2). Sparsely branched bifurcated tips were the dominant EM morphotype with tuberoid mycorrhiza also dominant.

AM fungal infectivity was not correlated with corn shoot weight, corn root weight or percent herbaceous cover ($r = -0.037$, $r = 0.031$, and $r = 0.035$ respectively, $N = 60$, $P \geq 0.05$). EM fungal infectivity was not correlated with pine shoot weight ($r = 0.11$) but was positively correlated with pine root weight ($r = 0.28$, $N = 60$, $P \geq 0.05$). The only soil property that was correlated with AM fungal infectivity was pH ($r = 0.27$,

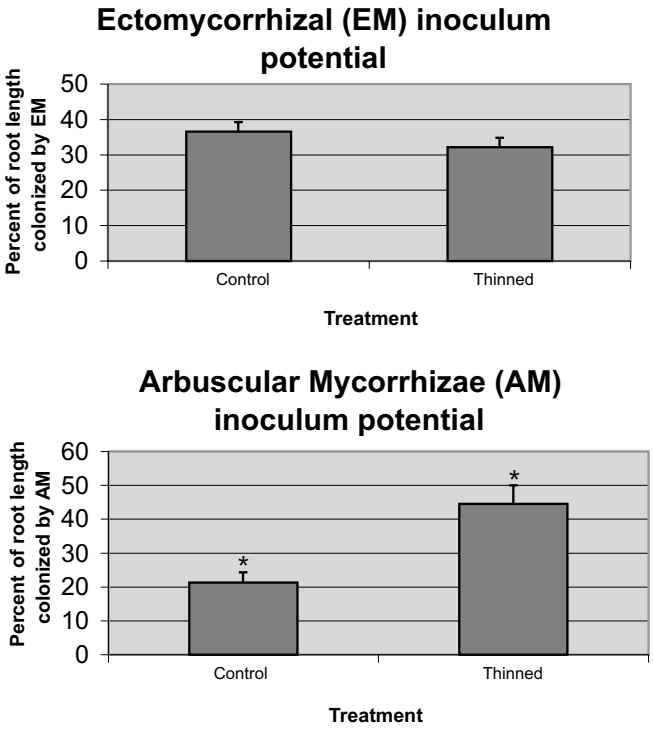

Figure 2—The inoculum potential for EM fungi (top graph) and AM fungi (bottom graph) from "bait-plant bioassays" in the thinned and control units. Differences between the thinned and control units were determined by analysis of variance for a random block design on log (x + 1) transformed data ($F = 2.1601$, $P > 0.05$, $F = 5.4437$, $P < 0.005$, respectively).

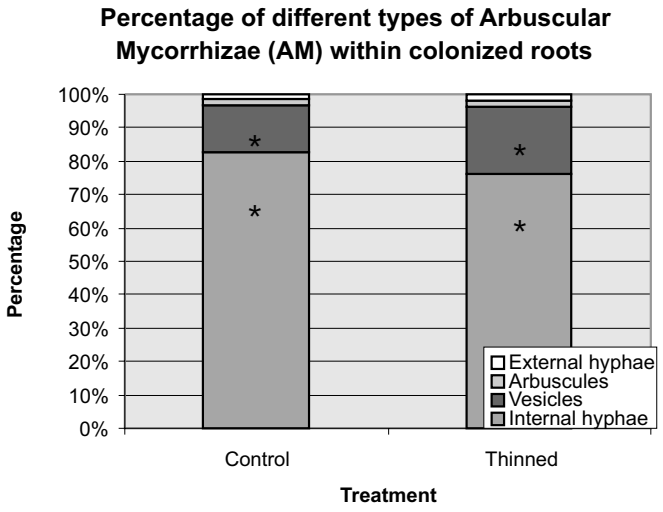

Figure 3—The percentage of external hyphae, arbuscules, vesicles, and internal hyphae within colonized corn roots for each treatment. An asterisk indicates that one type of AM is significantly different ($P < 0.05$) between treatments as determined by Analysis of Variance for a random block design on log $(x + 1)$ transformed data ($F = 8.3001$, $P > 0.005$, $F = 15.4818$, $P > 0.0002$, respectively).

$N = 60$, $P \geq 0.05$). There were no significant differences between soil properties in the thinned and control stands (table 1). However, pH levels were higher in the thinned units than the controls, which is congruent with the findings that thinned units had greater propagule densities of AM fungi than the controls.

Both the thinned and control units were dominated by graminoids followed by nonlegume forbs, legumes, and woody shrubs. Unidentified sedges, *Carex* sp., dominated the herbaceous cover for both units followed by C3 grasses including *Elymus elymoides* (squirreltail), *Poa fendleriana* (muttongrass) and *Festuca arizonica* (Arizona fescue). *Muhlenbergia montana* (mountain muhley) was the only dominant C4 grass in both units; however, its presence was less than the dominant C3 grasses. The dominant forbs in both the thinned and control units were *Cirsium wheeleri* (thistle) and *Solidago* sp. (goldenrod). There were slightly higher species richness, Simpson's diversity, and herbaceous cover in the thinned units in comparison with the control units; however, these differences were not significant (table 2).

Table 1—Soil parameter comparisons between thinned and control ponderosa pine treatments. Average values are presented, N = 30. Standard error of the mean (SEM) for each value is in parentheses.

	Thinned		Control	
pH	6.06	(0.756)	5.85	(0.352)
Total P	0.86	(0.148)	0.95	(0.199)
Total N	1.57	(0.209)	1.65	(0.174)
Organic C	10.29	(1.158)	10.59	(0.994)

Table 2—Plant species richness and Simpson's diversity index (SI) for 1999 herbaceous transects. Minimum, maximum and average richness is by individual transects, not units.

Treatment	Average richness	Min.	Max.	Average SI	Min.	Max.
Control	5.1	1	13	4.34	0.58	12.78
Thin	5.8	1	12	4.71	0	13.75

Discussion

Restoration thinning increased the cover of herbaceous, AM hosts and decreased the cover of ponderosa pine, EM hosts. These aboveground changes were accompanied by the expected belowground changes: propagule densities of AM fungi increased by 23 percent while those of EM fungi decreased by 6 percent. These preliminary results indicate that mycorrhizal fungal population densities respond rapidly to restoration thinning in northern Arizona ponderosa pine forests. Two main processes control population densities of mycorrhizal fungi following disturbance: immigration of new propagules from nearby areas and survival and spread of residual propagules (Warner and others 1987). Rapid colonization of AM fungi has been illustrated in other studies. For example, Johnson and McGraw (1988) found that unreclaimed taconite tailings devoid of AM fungi were colonized within weeks of reclamation. It was hypothesized that spores were transferred to the reclaimed tailings by biotic (animals) and abiotic (wind and water) vectors (Johnson and McGraw 1988). In the present study it is likely that AM fungi spread from preexisting mycorrhizal hyphae in living and dead roots.

Soil disturbance has been reported to generally reduce AM propagule densities due to the destruction of the hyphal network during the break-up of the soil macrostructure (Reeves and others 1979; Fairchild and Miller 1988). Consequently, one may predict that propagule densities should decrease following thinning due to soil disturbance from mechanized logging equipment. This effect was not observed in our study, which may indicate that soil disturbance was not severe enough to have destroyed AM fungal propagules, or that the sites were rapidly colonized by AM fungal spores and residual hyphae. Another study, conducted by Rives and others (1980), showed that there was no reduction in population densities of AM fungi following soil disturbance. It has been suggested that environments with a high proportion of grasses are more tolerant of disturbance because their fiberous root systems generate high densities of infective propagules (Jasper and others 1991). A successional study of AM propagule densities across a grassland to forest chronosequence showed AM inoculum potential increased with increasing grass cover and decreased in later successional sites with EM trees (Johnson and others 1991). Similarly, a study by Benjamin and others (1989) illustrated that dominant herbaceous plants had lower AM colonization as tree density increased, possibly because these plants had insufficient photosynthetic capability to support AM infection. Finally, some research indicates that while the fungal propagule densities do not decrease following disturbance,

AM species composition may change (Johnson and Pfleger 1991). Specifically, AM species from the Glomaceae family depend more on hyphae for reproduction than spores. In contrast, AM species from the Gycosperaceae family depend more on spores for reproduction than hyphae (Biermann And Linderman 1983).

Implications for Restoration

Pre Euro-American ponderosa pine understory communities were dominated by warm-season (C4) grasses, which are often obligately mycotrophic (Cooper 1960; Wilson and Hartnett 1998). However, current ponderosa pine forests are dominated by species that form no mycorrhizal associations (*Carex* sp.) or C3 grasses that are often facultatively mycotrophic. This pattern is contrary to other studies that suggest succession proceeds from dominance of nonmycotrophic and facultative mycotrophic species to obligate mycotrophic species in later successional stages (Janos 1980; Allen and Allen 1984). This reversed pattern has been established because the natural fires that historically would minimize competition between ponderosa pine and herbaceous plants have been suppressed, and now shade-tolerant C3 grasses are better adapted to these environments. Therefore, without the reduction of EM tree competition and reintroduction of fire, the understory of Southwestern ponderosa pine forests will likely continue to be dominated by nonmycotrophic *Carex* and facultatively AM species. Noyd and others (1995) demonstrated that obligate C4 grasses, *Andropogon gerardii* (big bluestem) and *Schizachyrium scoparium* (little bluestem), were unable to grow or survive as seedlings in soil where AM fungi were eliminated; however, *Elymus canadensis* (C3 grass) establishment was unaffected by AM fungi availability. Similarly, Hetrick and others (1994) found that C4 grasses were competitively superior to C3 grasses when grown in the presence of AM fungi; but C3 grasses were competitively superior in soils without AM fungi. As a result, one can predict that increased populations of AM fungi in the thinned restoration units may promote increased C4 grass cover. Finally, recent mycorrhizal fungi research in a variety of environments has shown that mycorrhizal interactions may be important determinants of plant diversity, ecosystem variability, and productivity (Hartnett and Wilson 1999; van der Heijden and others 1998a,b). Microcosm experiments simulating European and American grasslands have shown that increasing AM fungal diversity can increase diversity and overall community structure (Klironomos and others 2000; van der Heijden and others 1998b).

Conclusions

Restoration thinning impacts propagule densities of EM and AM fungi. Reducing pine cover and increasing herbaceous cover shifts the system toward a more AM dominated community. Increasing propagule densities of AM fungi may favor the establishment of a C4 grassland community. As a result, knowledge of AM fungal propagule densities and AM fungal species composition will be crucial to understanding the successional response of herbaceous understory communities to Southwestern ponderosa pine forest restoration thinning and prescribed burning treatments.

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