

# Influence of Fire on Mycorrhizal Colonization of Planted and Natural Whitebark Pine Seedlings: Ecology and Management Implications

Paul E. Trusty and Cathy L. Cripps, Department of Plant Sciences and Plant Pathology, Montana State University, Bozeman, MT

## Introduction

Whitebark pine (*Pinus albicaulis*) is a threatened keystone species in subalpine zones of Western North America that plays a role in watershed dynamics and maintenance of high elevation biodiversity (Schwandt, 2006). Whitebark pine has experienced significant mortality due to white pine blister rust, mountain pine beetle outbreaks and successional replacement possibly due to fire suppression (Schwandt 2006; Smith and others 2008). Current management strategies include letting lightning fires burn or applying prescribed fire to provide habitat for natural seedling establishment or the planting of rust resistant seedlings (Keane and Parsons 2010a, 2010b). However survival rates after fire are variable and can be low (Izlar 2007; Keane and Parsons 2010a; Perkins 2004; Tomback and others 2001).

All pines in nature require ectomycorrhizal (ECM) fungi for establishment, growth, health and sustainability (Read, 1998). ECM fungi enhance nutrient uptake and offer protection against drought, pathogens, soil grazers and heavy metals (Smith and Read 1997). Fire can affect ECM communities in soil but impacts are unpredictable and depend on the intensity of the fire, forest type and other factors (Cairney and Bastias 2007). Intense fire has the potential to detrimentally impact ECM communities because of the deep penetration of lethal soil temperatures, the complete loss of the original tree host, and changes in abiotic conditions, including an increase soil surface temperature (Neary and others 1999; Wiensczyk and others 2002). When tree hosts are lost or removed, studies show that ECM fungal viability in the soil declines rapidly after two or three years (Haggerman and others, 1999) and recovery of ECM communities from burning and cutting can take decades (Visser 1995). It is unknown how long ECM fungi, and particularly those specific to whitebark pine, can remain in the soil of ghost forests without presence of a living host.

Fire is historically linked to whitebark pine ecology (Keane and Arno 1993). Fire has the potential to reduce shade-tolerant understory species such as fir; remove canopy for shade-intolerant whitebark pine seedlings; provide openings for nutcrackers to plant seed; reduce rust and beetle infested older trees, and facilitate plantings of rust resistant seedlings (Keane and Parsons 2010a). However, little is known of how fire affects the beneficial fungi on roots of this tree species. This study evaluated the impact of fire on the mycorrhizal communities on planted and naturally

occurring whitebark pine seedlings from an ecological perspective and to address management concerns.

## Methods

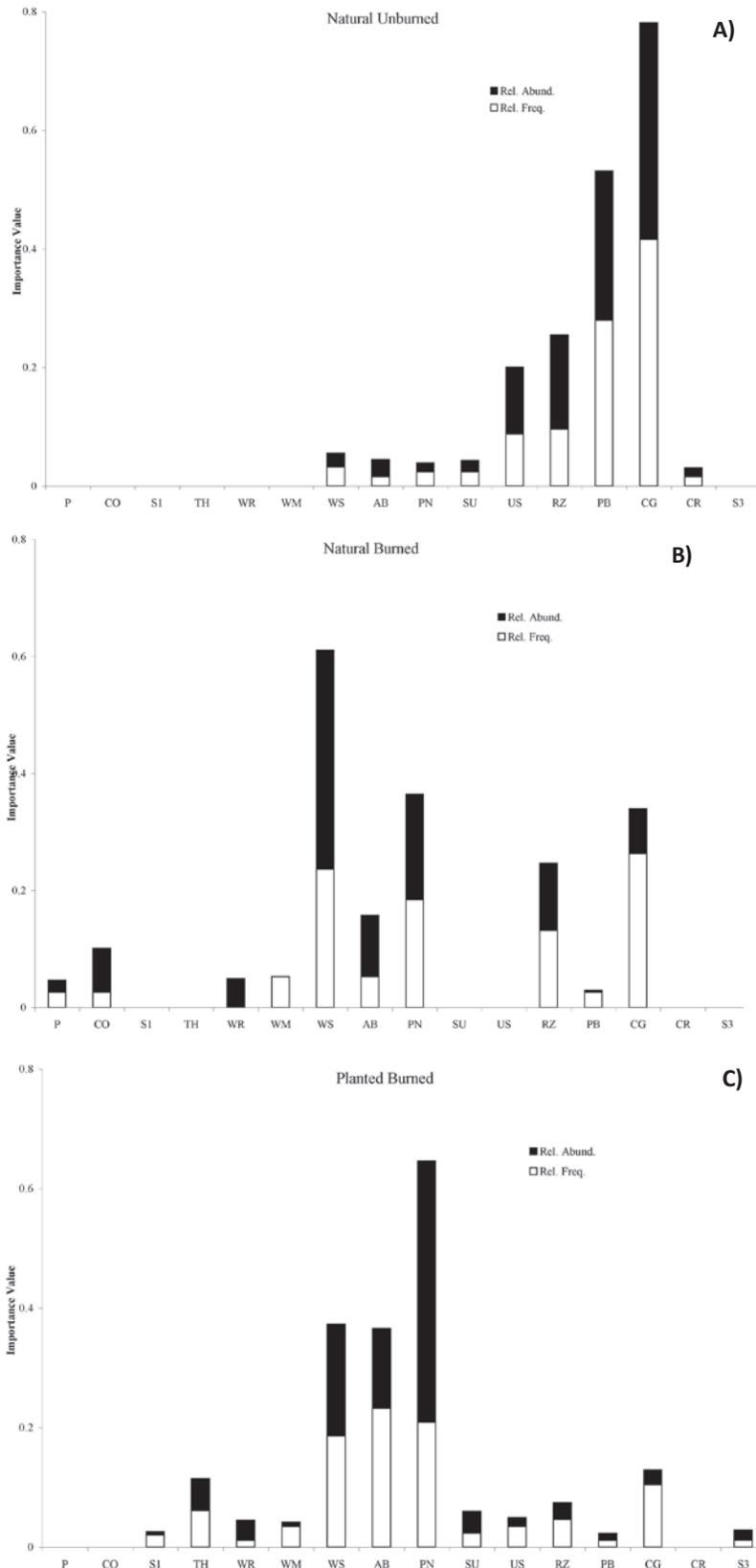
The 2001 Fridley fire (SW Montana) burned a portion of a mature whitebark pine forest; the burn was considered severe and killed many trees (Fridley Fire BAER Team 2001). A year later 20,000 (non-inoculated) rust-resistant seedlings were planted in the burned areas (Trusty 2009). After four years, natural and planted seedlings on the burn and natural seedlings in the adjacent unburned forest were assessed for mycorrhizal colonization levels and diversity of ECM fungi. Root samples were taken along transects in a non-destructive manner and ectomycorrhizae were sorted and counted by species/morphotype for samples in each treatment. Morphotypes were defined by mantle color, presence or absence of rhizomorphs, and other unique characteristics (Trusty 2009). DNA was extracted from samples and identified by matching ITS sequences to reference species in Genbank or UNITE (Trusty 2009). A total of 21,971 root tips from 144 seedlings were assessed and 21 fungal taxa were identified. The relative abundance and frequency for the most encountered ectomycorrhizal fungal taxa (morphotypes) were also determined and summed for importance values (Horton and Bruns 2001). Data from the two different sampling times were pooled after it was determined that there were no statistical differences between them. Shannon's diversity index compared diversity (number and evenness of morphotypes) among groups (done in Program R). Principal Component analysis (PCA) was used to examine the structure of ectomycorrhizal fungal communities based on whitebark pine seedling groupings. PCA of the log-transformed abundances was plotted for the ectomycorrhizal fungal communities and includes loading vectors for taxa that have an absolute value loading score of at least 0.1. This allows for easy interpretation of which taxa are driving sample distances.

## Results

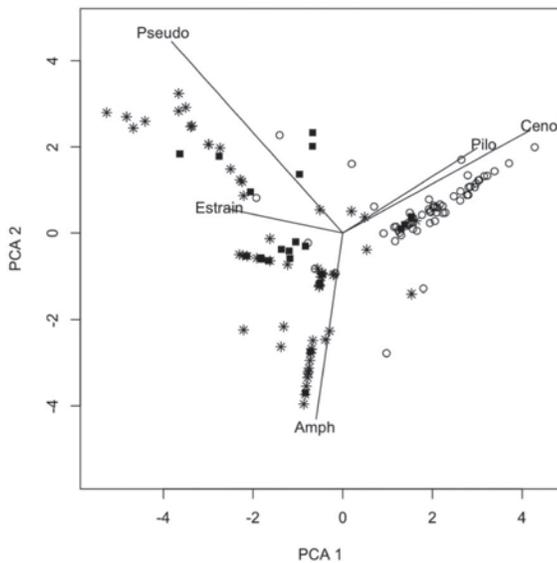
All whitebark pine seedlings sampled were well-colonized by ECM fungi (over 90 percent for all treatments) although a portion may be residual E-strain fungi for those from the

nursery. Seedlings on the burn (both planted and natural) supported a lower diversity of ECM fungi (0.21 and 0.32, respectively) compared to those in the unburned area (0.56) according to the Shannon diversity index. This reduction in 40-60 percent of the ECM fungal diversity was assessed 5 years after the fire. There was a dramatic shift (change) in

the dominant fungal species between those establishing in the adjacent unburned forest and those in the burn (Fig. 1). There were smaller differences in the ECM community between planted and natural seedlings within the burn (Fig. 1, Band C).



**Figure 1.** Importance values (frequency + abundance) for 10 most common taxa of ectomycorrhizal fungi on whitebark pine seedlings. A. regenerating naturally in the unburned forest, B. regenerating naturally in burned area, C. planted in burned area. Abbreviations: P-*Phialocephala fortinii*, S1-Species 1, CO- *Coltricia* sp., TH-Theleporoid spp., WM- *Wilcoxina mikolae*, WR- *Wilcoxina rhemii*, WS- *Wilcoxina* spp., AB- *Amphinema byssoides*, PN- *Pseudotomentella nigra*, SU- *Suillus* spp., US- Unknown suilloids, RZ- *Rhizopogon* spp., PB- *Piloderma byssinum*, CG- *Cenococcum geophilum*, CR- *Cortinarius* spp., S3- Species 3.



**Figure 2.** Principal components analysis plot of abundances of ectomycorrhizal fungal species according to group status: circles (○) = natural unburned, squares (■) = natural burned, stars (\*) = planted burned. Pseudo= *Pseudotomentella*, E-strain= *Wilcoxina*, Amph= *Amphinema*, Pilo= *Piloderma*, Ceno= *Cenococcum*. Loading vectors are for taxa with loading values greater than 0.1.

Natural seedlings in the unburned forest hosted mainly *Cenococcum geophilum*, *Piloderma byssinum* and suilloid fungi. *Cenococcum* does not produce spores and *Piloderma* species are often associated with woody organic matter in mature forests, thus their dispersal potential into the burn is limited. The suilloids are specific for 5-needle pines and important associates of whitebark pine (Cripps and others 2008; Cripps and Antibus 2011; Mohatt and others 2008). Seedlings in the burn (both natural and planted) were dominated by *Wilcoxina* species (E-strain), *Amphinema byssoides* and *Pseudotomentella nigra*. The latter was more dominant on planted seedlings and there is some historical question as to its pathogenicity. E-strain is common on nursery seedlings but was also found on naturally establishing seedlings. All are considered non-host specific fungi known to occur on burned soil. Suilloid fungi (*Rhizopogon*, *Suillus*), known to be important in pine establishment, were found on seedlings in all treatments, although colonization rates were lower in the burn. Suilloids were less frequent (10–13 percent) on seedlings in the burn compared to those establishing in the mature unburned forest (25 percent). Spores of suilloids are imported into burns by wind and animal vectors via fecal pellets from nearby inoculum sources (Ashkannejhad and Horton 2005). An important note is that roots of planted seedlings sampled still retained the shape of containers after four years and roots had not yet spread out into the soil.

Over 60 percent of the variation in abundances of ECM fungi can be accounted for by the variable *burning* according to Principal Component Analysis (Fig. 2). This is primarily driven by larger abundance of *Pseudotomentella*, E-strain and *Amphinema* in the burn (for both planted and natural seedlings) and by large abundance of *Cenococcum*

and *Piloderma* in the unburned area. Differences between communities of fungi on planted and natural seedlings within the burn were not well defined as data points from planted seedlings were scattered within those for the natural seedlings (Fig. 2).

## Discussion

The functional significance of the shift (change) in ECM fungal species after fire in terms of seedling survival is not yet known. However, it is assumed to have physiological implications since fungi vary in benefits to seedlings and as a community (Teder 2009). Five years after the fire, planted and natural seedlings in the burn were partially colonized by suilloids likely due to the availability of a nearby inoculum source (the adjacent unburned forest), the presence of vectors (deer, small mammals) that import inoculum and a management plan that included planting one year after the burn (Trusty 2009). These factors should be considered when planting in severe burns (Wiensczyk and others 2002). While some fungal species can survive and rapidly re-colonize after fire, removal of the duff layer can be problematic for other ECM fungi (Smith and others 2005).

Mycorrhizal colonization was high for seedlings planted on the burn, but seedling survival was still low, assessed at 42 percent during the study (Izlar, 2007). This suggests that other factors (biotic and abiotic) might be involved in seedling mortality or that the timing and type of mycorrhizal colonization might be problematic. A lag time before colonization and lack of appropriate fungi could still be factors. Soil temperatures were about 8°C higher at sampling dates during the study, and this in itself can affect ECM fungal communities (Wiensczyk and others 2002).

Fire is historically important in whitebark pine forests (Keane and Arno 1993), and it is often assumed that the ECM system is also adapted to this disturbance. However, the time and space scales needed for recovery without the loss of fungi specific to whitebark pine are not known. One concern is to determine if intense fires on a large scale coupled with losses from white pine blister rust and mountain pine beetles contribute to irretrievable losses of ECM fungi specific to whitebark pine. These fungi have a co-evolutionary history with stone pines spanning thousands of years which suggests their importance in the system.

Ponderosa pine seedlings inoculated with suilloid fungi (for 2–3 needle pines) showed an increase in survival rate of 40 percent on harsh, dry sites compared to controls (Steinfeld and others, 2003). European stone pines (*Pinus cembra*) have been inoculated with suilloids specific to 5-needle pines for over 50 years and coupled with improved planting techniques, survival has increased from 50 to 90 percent (Weisleitner, personal communication). Whitebark pine seedlings have been successfully inoculated with native fungi (Cripps and Grimme 2011), but out-plantings have not yet been assessed.

## Management Implications

In general, the overall benefits of fire in whitebark pine systems need to be weighed against potential microbial impacts. When planting whitebark pine in severe burns, managers should consider planting as soon as possible (within a year) before ECM fungi decline further in the soil, minimizing distances to an inoculum source (living whitebark pine) for prescribed burns, and promoting animal vectors that import inoculum (unless seedling damage is problematic). Monitoring ECM colonization in risky situations and inoculation of planted seedlings with native fungi are management strategies that can be used on severe burns where appropriate fungi do not exist (Brundrett and others 1996; Cripps and Grimme 2011). Inoculation and soil transfer can be considered when plantings are in areas not previously in whitebark pine. There is no information on the persistence of the suilloid fungi in ghost forests but we are working to answer this question. Preservation of the ECM fungi specific to whitebark pine should be considered in management strategies as well.

There is no evidence to date that light burns affect ECM fungi in whitebark pine forests and results reported here are for one fire, thus implications are limited. The impact of the shift of ECM fungal *species* reported in this study on seedling survival is not known particularly since overall mycorrhizal colonization levels were high on seedlings in the burn five years after the fire.

## Acknowledgments

We would like to thank Robert Keane, USDA Forest Service, Rocky Mountain Research Station at the Missoula Fire Sciences Laboratory for partial funding of this project.

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