

Effects of simulated fire on vesicular-arbuscular mycorrhizae in pinyon-juniper woodland soil

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Abstract

Effects of fire on vesicular-arbuscular mycorrhizal fungi were tested using microcosms constructed from soil, litter, and duff collected beneath canopies of pinyon pine, Utah juniper, and in the open space (interspace). Burning was conducted over wet and dry soils. Soil temperatures were monitored continuously throughout the microcosms during burning. Plants grown in soils burned when dry had a lower VAM colonization than soils burned when wet. Juniper soils demonstrated the greatest reduction, over 95%, compared to their respective controls. Plants grown in interspace soils burned when wet were least affected. There was a positive correlation ($r^2 = 0.90$) between the decrease in VAM colonization and the soil temperature as a result of the fire. Temperature effects, and associated reductions in VAM, were related to amount of litter burned in each microcosm and the moisture content of the soils.

Introduction

It is well known that the symbiotic relationship formed between mycorrhizal fungi and their host plants result in an efficient nutrient and water uptake system (Mosse, 1973; Safir *et al.*, 1972). Numerous papers have demonstrated the impact of disturbance on this symbiotic relationship (Daft and Nicholson, 1974; Janos, 1980; Klopatek and Klopatek, 1984; Reeves *et al.*, 1979). These reports have shown that there is a corresponding loss of vesicular-arbuscular mycorrhizae (VAM) propagules and a decrease in VAM colonization as a function of increased disturbance. As severity of disturbance increases, *i.e.* from the grazing of animals (Reece and Bonham, 1978) to surface mining (Allen and Allen, 1980; Zac and Parkinson, 1982), there is a corresponding decrease in the frequency of VAM propagules and/or colonization.

The pinyon-juniper ecosystem is the third most expansive vegetation type across the United States (Klopatek *et al.*, 1979). It is undergoing a number of different perturbations including overgrazing, extensive fuel wood harvesting, and coal mining. Both wild and prescribed fires are recurring phenomena in this woodland (USDA Forest Service, 1981). Tress and Klopatek (1987) have reported the effects of fire on the successional processes of the vegetation. We wanted to determine how fire affected mycorrhizae in this ecosystem. Additional information on fire effects on other below-ground processes are reported elsewhere (DeBano and Klopatek, 1988).

This woodland has both endo- and ectomycorrhizal components. We chose to investigate only the vesicular-arbuscular endomycorrhizal component because the majority of plants present in mature pinyon-juniper communities are colonized

Table 1. Chemical and physical properties of unburned mineral soils

Cover	Soil properties					
	Organic C	Total N	Total P	pH	Texture	
	%	g/kg		Sand	Silt	Clay
Pinyon	24.6	1.28	0.47	7.8	50.9	22.4
Juniper	28.7	2.16	0.50	8.1	49.2	26.2
Interspace	6.8	0.55	0.44	8.2	45.8	30.4

with VAM and therefore are probably important for recovery after disturbance (C C Klopatek, unpublished). The objective of this study was to determine whether VAM propagules are reduced following fire and if fire affects the propagules' ability to colonize host plants.

Material and methods

Site description

Soils for this study were collected from a pinyon-juniper community, referred to as the Dillman site, located 30 km south-southeast of Grand Canyon, Arizona, in the Kaibab National Forest. *Pinus edulis* Engelm. is the pinyon pine species in this area and the codominant juniper is *Juniperus osteosperma* (Torr.) Little. The dominant interspace and understory species is blue grama grass [*Bouteloua gracilis* (H.B.K.) Lag.]. Site elevation is approximately 2030 m, soils are classified as Lithic Ustochrepts, which were derived from Kaibab Limestone. Climatic data were obtained from a weather monitoring station located 20 km from the study site. Precipitation averages 350 mm/y, occurring during summer as convectional thunderstorms and during the winter from frontal systems. Annual mean monthly temperatures range from -1.4 to 21.1°C , with a mean temperature of 9.2°C . Further site descriptions can be found in Klopatek (1987).

Experimental design

To account for spatial variability, materials were collected from beneath pinyon and juniper trees and interspaces on three sites in the study area. On each site, five subsamples of soil (to a depth of

10 cm), duff, and litter were collected by shovel and composited separately from beneath pinyon and juniper trees. Composite samples of only litter and soil were collected from interspaces because no duff was present. In total, 24 separate composite samples consisting of nine soil, nine litter, and six duff samples were collected for study. Soil, duff, and litter were used to reconstruct laboratory microcosms for pinyon trees, juniper trees, and interspaces in 35-cm-high clay irrigation pipes having an inside diameter of 20 cm. The bottom of the pipes were sealed and a 10-cm layer of sterilized, acid washed sand (containing $< 1 \text{ mg kg}^{-1}$ inorganic N and $1 \text{ mg kg}^{-1} \text{ PO}_4$) was placed in the bottom of each pipe. The sand was covered with filter paper to separate it from the above soil. Nine microcosms were constructed for each of the three cover types (9 pinyon, 9 juniper, 9 interspaces) for a total of 27 individual microcosms. In one series (nine microcosms), three replicates from each cover type, were wetted with sterile distilled water to reach fifty percent field capacity, prior to burning. In the second series, nine microcosms were burned having soil moisture content similar to that of field dry soils (approximately 10% on a weight basis). The third series of microcosms, also at field dried moisture conditions, were not burned, and represented the controls.

Temperature probes were inserted at 7 different depths (litter, duff, soil surfaces and 1, 2, 5, and 10 cm soil depths) to record fire temperatures (complete temperature profiles are reported in DeBano and Klopatek, 1988). A fire was simulated on top of each microcosm by using six 500-watt infrared heat lamps to produce instantaneous ignition of the litter. Each treated microcosm was heated with the lamps for a period of 15 min. All litter was consumed by fire within this period and those microcosms containing duff continued to smolder for several hours depending on the moisture content of the soils. Samples were collected from the 2–5 cm soil depth of the microcosms 24 h after ignition. All microcosms were brought up to 50% field moisture capacity following the first sampling period (to maintain uniform environmental conditions).

Mineral soil samples taken previous to burning were analyzed for organic carbon (Walkley-Black), total Kjeldahl N and phosphorus (Olsen and Sommers, 1982), and pH (1:1 soil:water) and soil texture by hydrometer (Day, 1965) (Table 1). No

statistical differences were noted in these soil properties at the 5 cm depth due to burning (DeBano and Klopatek, 1988).

VAM bioassay

Soil from each microcosm were bioassayed to determine the effects of fire on VAM propagules. Sudan grass [*Sorghum bicolor* (L.) Moench] was used as the host plant. Test tubes (18 × 150 mm) were filled to a depth of 70 mm with autoclaved sand (121°C for three 2 h intervals) followed by a 50 mm depth of inoculum (soil to be tested), and a 20 mm top layer of autoclaved sand. Germinating grass seeds were placed in the top 20 mm layer and grown in a growth chamber under a temperature regime of 25/15°C (day/night), and a 12 h photoperiod. A set of three test tubes for each of the 27 microcosms were grown with Sudan grass for 25 days. Preliminary testing indicated that at twenty five days post-germination, the pattern of colonization was such that, each point of colonization occurred from an individual propagule, and not from the spread of a preexisting colonization along the roots. It should also be noted that the authors when referring to the word 'propagule' is adhering to its strict definition — *i.e.*, "consisting of chlamydospores or azygospores, soil borne vesicles, and mycelium or infected root pieces," (anything that induces a VAM infection) (Daniels and Skipper, 1982).

Harvested plants were stained using a modified version of the Phillips and Hayman (1970) method. Fine roots were cut into 50–1-cm portions, scored for the presence or absence of infection points and data were converted into percentages. A positive identification was observed as either an appressorium formation or the presence of internal VAM hyphae.

Arcsin transformations were made of all percent colonization data prior to statistical analysis. VAM colonization data were compared among treatments and cover types using analysis of variance computed on the means for each microcosm. Significant differences ($p = < 0.01$) in colonization among experimental treatments and cover types were isolated using Tukey's honest significant difference measure within the SAS statistical software (SAS Institute, Inc., 1985). Simple correlation

analyses of the data were used to test the relationship between VAM colonization and maximum temperatures reached in each treatment. A decrease in VAM colonization was defined as: percent colonization of the control minus percent colonization of treatment divided by the percent control colonization.

Results and discussion

There were no statistical differences among unburned controls, which averaged 43.7%, 41.4%, and 36.4% colonization for juniper, pinyon, and interspaces, respectively (Table 2). Such uniform colonization was not surprising for the following reasons. Juniper is a VAM dependent species. Pinyon pine, although ectomycorrhizal, has been reported to have numerous VAM propagules around its base (Klopatek and Klopatek, 1987). This is likely due to aeolian deposition of spores. Wind deposits sand particles under pinyon pine (Barth, 1980; Klopatek, 1987) and probably deposits spores along with the sand. Thus, pinyon may indeed be an important repository for VAM propagules and consequently we included soil samples obtained beneath these trees. All grasses in the interspace areas served as hosts for VAM fungi, giving rise to numerous VAM propagules (Klopatek and Klopatek, 1987).

Plants grown in dry burned soils of each cover type experienced the greatest decrease in VAM colonization (Table 2). Juniper soils burned when dry were the most severely affected, having an av-

Table 2. Percentage VAM colonization on Sudan grass grown in soils from under pinyon and juniper canopies and interspaces subjected to different burning treatments. Mean equals the average of three replicate microcosms having three subsamples each. ± SE are in parentheses and represent between plot variability

Treatment	Microhabitat		
	Pinyon	Juniper	Interspace
	%		
Control	41.4 (2.8)a, x ^a	43.7 (1.9)a, x	36.4 (2.5)a, x
Dry burn	21.6 (2.7)a, z	9.0 (3.9)b, z	22.4 (0.9)a, y
Wet burn	32.9 (1.7)b, y	25.6 (1.0)c, y	40.4 (3.4)a, x

^a Means in a row having the same first letter (a to c) and in columns having the same second letter (x to z) are not significantly different at the 0.01 level.

erage percent colonization on Sudan grass of only 9.0%. The highest fire temperatures were reached in the juniper soils (up to 94°C) (DeBano and Klopatek, 1988). This was probably due to large amounts of litter and duff in addition to near total combustion of the duff under these conditions, which contributed to a more intense burn. The high temperatures were also maintained for a long duration due to smoldering of duff material. Magnitude and duration are the two principal factors causing heat injury to plants (Hare, 1961) and are also likely to be deleterious to VAM fungus propagules. Percent VAM colonization of plants grown in pinyon and interspace burned-over dry soils averaged 21.6% and 22.4%, respectively. Both were also significantly lower than their corresponding controls, although not as low as juniper soils.

Plants grown in pinyon and juniper soils burned when wet demonstrated a significant decrease ($p < 0.01$) in percent colonization compared to their controls but not as large of a decrease as for soils burned dry (Table 2). Pinyon soils burned when wet had a statistically greater average percent colonization (32.9) than juniper (25.6) soils, consistent with the pattern in soils burned when dry. Those plants grown in the interspace soil burned when wet demonstrated a slight increase (though not statistically significant) over the controls, with a colonization of 40.4%. There was little litter and no duff to burn in the interspaces, resulting in a significantly lower ($p < 0.05$) burning temperature than either the pinyon or juniper burned when wet microcosms. This, in combination with the wet soil conditions minimized any temperature effects on VAM.

A significant correlation ($r^2 = 0.90$, $p < 0.01$) was found between soil temperature and percent decrease in VAM infection (Fig. 1). Most soil temperatures ranged from 40 to 60°C which corresponded to a decrease in VAM colonization of 20% to 50% (Fig. 1). VAM colonization was severely reduced when soil temperatures reached 80–90°C and at 94°C there was a near total loss of VAM colonization (Fig. 1).

In summary, fire moderately affected VAM propagules when soil temperatures were less than 50°C. Substantial decreases (> 50%) did not occur until soil temperatures reached 60°C. Temperatures of 50 to 60°C did result in a significant reduction of VAM colonization. It may be that a large portion

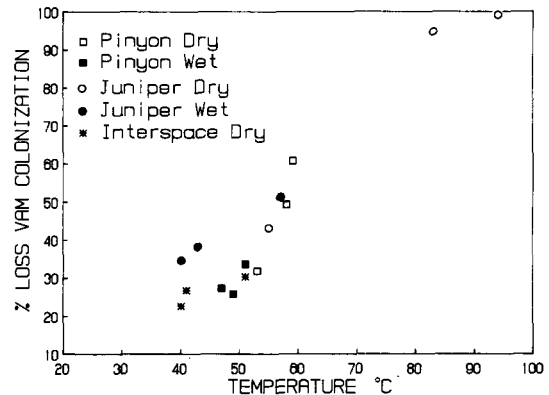


Fig. 1. Percent decrease in VAM colonization as a function of soil temperature. VAM is the average of $N = 3$ subsamples from each microcosm, temperature is the average maximum temperatures recorded in each microcosm. $R^2 = 0.90$, $p < 0.001$ from data which was arcsin transformed (see text).

of the infecting mycorrhizal propagules were in the form of infected root and hyphal pieces and were more vulnerable to these temperatures. Hyphae are known to ramify throughout the upper soil horizon, *i.e.* litter and duff (St. John *et al.*, 1983) once burned or exposed to high temperatures they may be considered to be lost from the VAM propagule population. This loss may also have contributed to a lower VAM colonization.

At an ecosystem level, fire intensity (increased soil temperatures and duration of these temperatures resulting from combustion of organic debris on the soil surface) in combination with soil moisture content at the time of burning, may affect the length of time required for mycorrhizal dependent plant species to recolonize an area. Plant recolonization following a fire is therefore influenced by VAM availability, in addition to other reported losses such as seed availability (Hare, 1961; Martin *et al.*, 1975). The decrease in VAM may be an important factor in the long-term productivity of pinyon-juniper woodlands and therefore should be considered when assessing fire effects in this ecosystem.

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