ARBUSCULAR MYCORRHIZAE IN THERMAL-INFLUENCED SOILS IN YELLOWSTONE NATIONAL PARK

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ABSTRACT.—Mycorrhizae are common plant-fungal symbioses occurring in most land plants. Despite their ubiquity, little is known about the distribution of arbuscular mycorrhizae (AM) in extreme environments. We surveyed for the presence of AM in thermal sites in Yellowstone National Park (YNP) where soils are characterized by extreme pHs, elevated temperatures, and toxic element concentrations. Plants at 5 sites, growing in soils with rooting-zone temperatures up to 48°C and soil pH values as low as 3.4, were mycorrhizal (colonization levels from 4% to 34%). Soils from a sparsely vegetated thermal area and an adjacent, continuously vegetated transition area differed significantly in rooting-zone temperature (35°C vs. 26°C), acidity (pH 3.8 vs. 5.4), electrical conductivity (2.22 vs. 0.49 mmhos cm^{-1}), Fe (181.3 vs. 48.5 mg kg^{-1}), Mn (7.2 vs. 98.2 mg kg^{-1}), and Zn (2.3 vs. 4.5 mg kg^{-1}). Mycorrhizal infectivity potential (MIP) was 77% greater in the transition soils, with colonization levels of 26% and 46% in thermal and transition soils, respectively. Furthermore, colonization of *Agrostis scabra*, *Dichanthelium lanuginosum*, and *Mimulus guttatus* was found to be consistently high throughout the growing season (from 48% to 72%). It is possible that AM are essential for plant life on the edge of thermal areas, and that either or both symbionts are specifically adapted to their environment. Further research is required to elucidate AM function in and specific adaptations to YNP's thermal areas.

Key words: arbuscular mycorrhizae, thermal soils, Yellowstone National Park, extreme environments.

Mycorrhizae, intimate symbioses between plants and fungi, are nearly universal among land plants (Smith and Read 1997). Arbuscular mycorrhizae (AM), also known as endomycorrhizae, include fungal structures both internal and external to roots. Intraradical hyphae, vesicles, and arbuscules occupy space within cortical tissue (Smith and Smith 1990), while the extraradical hyphal network inhabits small soil pores (Smith and Read 1997). The host plant provides carbon for the fungus, while the fungus increases uptake of phosphorus and nitrogen (Johansen et al. 1993, Subramanian and Charest 1999), increases water acquisition (Stahl et al. 1998), and provides protection from pathogens (Newsham et al. 1995).

Fossil evidence shows the presence of AM fungal structures associated with the first land plants (Remy et al. 1994, Redecker et al. 2000), suggesting that AM were important in terrestrial plant evolution (Blackwell 2000). Despite their widespread nature and their evolutionary significance, our understanding of AM is limited to studies of a small fraction of host plants and environments that are amenable to plant growth.

Previous research on AM fungi in harsh environments has been conducted in arid environs as well as acidic soils and metal-contaminated sites. In arid regions we observed increased nutrient status in mycorrhizal plants (Cui and Caldwell 1996, Subramanian and Charest 1999) and increased transpiration rates (Herman 2000). Plant growth in acidic soils is enhanced by AM (Clark et al. 1999, Cuenca et al. 2001), and AM fungi can ameliorate Al toxicity, a common problem associated with acidic soils (Clark 1997). Arbuscular mycorrhizal plants growing in metal-contaminated soils show enhanced plant growth and metal-resistance in some, but not all, studies (Gildon and Tinker 1983, Danielson 1985, Heggo et al. 1990, Hetrick et al. 1994, Shetty et al. 1994). Arbuscular mycorrhizae have increased the biomass of native plants growing on metal-contaminated soils, and AM fungi presence at metal-contaminated sites has resulted in higher colonization levels than AM fungi presence at non-contaminated sites (Moynahan 2002).

The existing literature suggests that AM fungi may either increase or decrease plant fitness in harsh environments, depending on

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the balance between the cost of carbon allocation to the fungus and the benefits of symbiosis (Johnson et al. 1997). While mycorrhizae have been hypothesized to be important in thermal sites because of limiting nutrient levels (Burns 1997), there are no published data documenting the presence of AM fungi in plants growing in thermal soils. We examined plants in thermal soils across a range of temperatures and pH values with the following objectives. First, we sampled plants from thermal sites around Yellowstone National Park (YNP) to determine whether AM fungi were present and, subsequently, if they had colonized roots at multiple thermal sites. Second, at one site we compared soil conditions, field colonization, and mycorrhizal infectivity potential (MIP) in thermal and transition soils to determine if MIP varied with temperature or soil chemistry. Finally, we monitored AM colonization levels through the summer and into the fall to determine if colonization changed seasonally.

METHODS

Our study areas, the thermal sites of YNP, are located on the Yellowstone Plateau, a 6500-km^2 volcanic region that has been intermittently active for at least 2.2 million years (Christiansen 1984). The chemistry of thermal soils can range from acidic to alkaline as determined by the phase and gaseous components of the rising water, oxidation state of sediments, and presence or absence of shallow groundwater (Henley et al. 1986). This research focuses on plants growing in acidic soils, in which acidification and acid leaching are major processes removing plant nutrients and accelerating rates of mineral weathering relative to nonthermal soils. Moreover, acidic soils tend to be weakly developed, with soil-forming activity limited to the upper 25 cm (Rodman et al. 1996).

Our study included 5 thermal sites in YNP: Rabbit Creek, Fire Hole River, Ragged Hills, Solfatara Plateau, and Lemonade Springs (Fig. 1). These sites contain diverse thermal features, including geysers, fumaroles (steaming vents), thermal pools, thermal springs, and boiling mud pools (Smith and Siegel 2000). In June 2000 we collected *Dichanthelium lanuginosum* (Schmoll) Spellenb. from all 5 study sites (Fig. 1), and, in addition, we collected *Agrostis scabra* Willd. at Solfatara Plateau.

Rabbit Creek is located on the east end of Midway Geyser Basin within the Yellowstone Caldera. Plants adjacent to fumaroles and boiling mud pools were collected. The Fire Hole River is in Lower Geyser Basin, in the western portion of the Yellowstone Caldera, and plants were collected from areas adjacent to thermal pools that border the river. Ragged Hills is within the Norris Geyser Basin, just north of the Yellowstone Caldera boundary and west of Solfatara Plateau. Here, we collected plants from a dry hillside where a recent temperature spike had killed most lodgepole pine (*Pinus contorta* Dougl. ex Loud.) that had colonized the site since the 1988 fire. Solfatara Plateau and Lemonade Springs are located just north of the Yellowstone Caldera. Plants we collected from Solfatara Plateau were growing on a dry hillside with sparse vegetation and elevated soil temperatures. Plants at Lemonade Springs were collected adjacent to the thermal springs.

Soil pH was determined by paste extractions of either 1:2 soil to water for samples from Solfatara Plateau, Ragged Hills, and Lemonade Springs or 1:1 for samples from Rabbit Creek and the Firehole River (Sparks et al. 1996). We measured the pH of each slurry with an Orion meter (model 720A, Beverly, MA) and an Accumet accuTupH pH probe (13-620-185, Tustin, CA). Soil temperature in the rooting zone (5–10 cm belowground) of each plant was recorded at the time of plant collection with a Taylor temperature probe (model 9841, Oak Brook, Illinois).

Plant roots were cleaned in the laboratory under running water, cleared in a 2.5% by volume KOH solution for 48 hours, rinsed in distilled water, acidified in 3% by volume HCl for 12 hours, and stained with Trypan blue for 12 hours (modified method of Phillips and Hayman 1970). Mycorrhizal colonization was quantified using a magnified intersections method (McGonigle et al. 1990). From 40 to 134 intersections were examined per plant to determine colonization levels. Fewer intersections were examined if cortical tissue was stripped in the clearing and staining process. The number of root segment intersections with vesicles, arbuscules, or mycorrhizal hyphae was divided by the total number of intersections examined to determine percent colonization.

At the Rabbit Creek thermal site, we compared MIP and chemistry in soils adjacent to thermal features (thermal soils) and soils 10 m
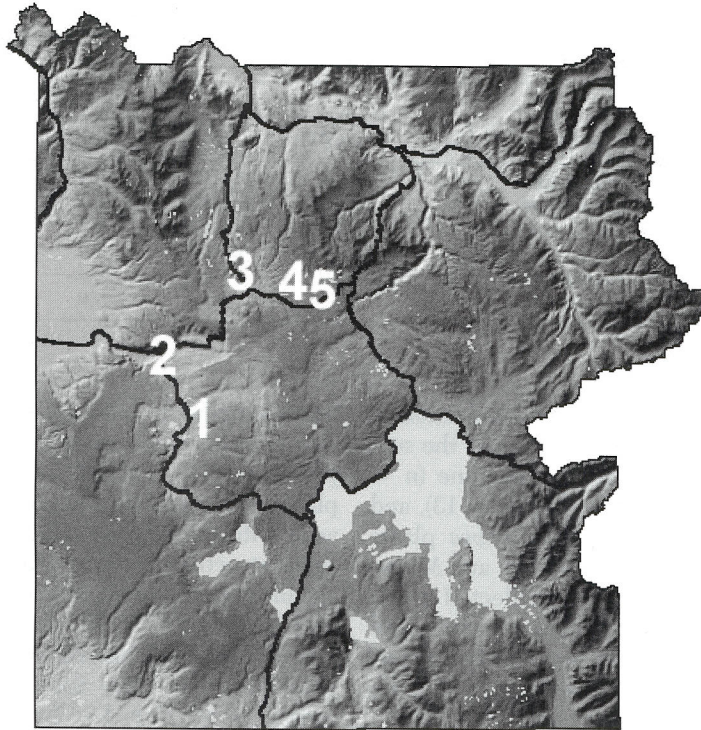
Fig. 1. Relief map of Yellowstone National Park with dark lines depicting major roads and numbers 1 (Rabbit Creek), 2 (Firehole River), 3 (Ragged Hills), 4 (Solfatara Plateau), 5 (Lemonade Spring) indicating locations of sampled thermal areas.

away from thermal features (transition soils). We further distinguished these zones by vegetation cover and rooting-zone temperatures. Thermal soil had less than 50% vegetation cover and temperatures from 35°C to 40°C, and transition soil had near-continuous plant cover and temperatures from 20°C to 30°C. In September 2000 we collected 500 mL of soil from 8 thermal soil and 4 transition soil areas from the rooting-zone of established vegetation. Soils were oven-dried at 95°C for 48 hours and then sieved to 2 mm prior to submission to the Soil, Plant and Water Analytical Laboratory, Montana State University, Bozeman, for analysis. A 1:2 soil to water paste extraction was used for pH and electrical conductivity analysis (Sparks et al. 1996). Available Cu, Fe, Mn, and Zn were extracted by the DTPA-TEA (diethylenetriaminepentaacetic acid triethanolamine) method (Lindsay and Norrell 1969) and analyzed with inductively coupled plasma-atomic emission spectroscopy (ICP-AES, Sparks et al. 1996).

Mycorrhizal infectivity potential, a measure of the relative number of AM propagules found in soils, is determined by growing bait plants in the greenhouse in intact soils collected from the field (Brundrett et al. 1996). Soils from 6 thermal and 6 transition areas were placed in pots (10 cm square and 9 cm deep) and planted with tufted hairgrass, *Deschampsia cespitosa* L. (Beauv.), a plant that is heavily mycorrhizal and able to grow in low pH soils (Moynahan 2002). In addition, 5 pots with sterilized sand (autoclaved twice at 120°C and 25 psi for 90 minutes with a 24-hour resting period) were placed in the greenhouse to verify that AM propagules were not from the local growing environment. After 10 weeks of growth, plants were harvested, cleared, and stained as described above, and examined microscopically to determine AM colonization levels.

Arbuscular mycorrhizal field colonization was monitored across a range of pH and temperature at Rabbit Creek during summer and fall 2001. We collected plants and prepared

TABLE 1. AM colonization levels of plants from thermal areas within Yellowstone; n = number of plants examined for colonization levels, Temp. = temperature in the rooting-zone of the plants (5–10 cm below ground), % AM fungi = average percent of intersections containing AM fungal structures.

Sample locations	Species	n	Temp. (°C)	pH	% AM fungi		
					Vesicles	Arbuscules	Total
Rabbit Creek	D. lanuginosum	4	31–42	3.4–4.8	2.5	0.5	6
Fire Hole River	D. lanuginosum	2	21–38	5.2–6.5	9.0	2.5	34
Ragged Hills	D. lanuginosum	4	48 .	3.6–4.1	0.2	1.2	4
Solfatara Plateau	A. scabra	2	38–41	3.9–4.7	1.0	0.5	23
Solfatara Plateau	D. lanuginosum	2	33–41	3.7–3.9	0.0	0.0	6
Lemonade Springs	D. lanuginosum	2	34	3.6–3.7	4.0	1.0	34

roots as previously described. Colonization of *D. lanuginosum* was monitored across the season; plants were collected on 20 June (n = 13), 26 July (n = 12), 21 August (n = 13), and 23 October (n = 10). *Agrostis scabra* plants were collected on 26 July (n = 12), and *Mimulus guttatus* DC. plants were collected on 20 June (n = 11). *Agrostis scabra* was collected only in July because it had senesced by August. *Mimulus guttatus* was collected only in June because it had senesced by July.

Colonization data were ln-arcsin transformed prior to 1-way ANOVA to test the null hypothesis that AM colonization did not differ between sites. Site was used as a random factor, and LSD post hoc tests were used to determine which sites were significantly different. Soil chemistry data from thermal and transition area soils were analyzed to test the null hypothesis that soil chemistry did not differ between zones, using independent samples and 2-tailed t tests after testing for equality of variance by Levene's test. Mycorrhizal infectivity potential data were analyzed to test the hypothesis that the MIP of the thermal and transition area soils was not different, using independent samples t tests. In all cases, SPSS (10,0.1) was used for analysis and α was set to 0.05.

RESULTS

Soils from all thermal sites sampled were acidic, with the pH ranging from 3.4 to 6.5 (Table 1). The only samples with pH values greater than 4.8 were collected at the Fire Hole River. Rooting-zone temperatures of the plants collected ranged from 21°C to 48°C (Table 1), with the warmest soils found at Ragged Hills. Arbuscular mycorrhizae were present in all plants, with average colonization levels ranging from 4% to 34% (Table 1). Vesicles or arbuscules were present in 11 of 16 plants sampled, and hyphae in the remaining 5 plants were aseptate and consistent in appearance with the hyphae associated with mycorrhizal structures in other plants. Sites differed significantly in overall colonization levels ($F_{4,11}$ = 4.15, P = 0.027). Plants from Ragged Hills and Rabbit Creek had significantly lower colonization levels than those from Lemonade Springs or the Fire Hole River.

Thermal and transition area soils at the Rabbit Creek site differed significantly in chemical profile (Table 2). Electrical conductivity was 4.5 times higher in thermal soils (Table 2). Copper did not differ between thermal and transition area soils (t_{10} = 0.59, P = 0.57), Fe was almost 4 times as high in thermal soils (t_{10} = 2.09, P = 0.063), while Zn (t, unequal variances $_{3.1}$ = 4.53, P = 0.019) and Mn (t_{10} = 2.90, P = 0.016) were significantly lower in thermal soils. Mycorrhizal infectivity potential was 77% higher in transition soils than thermal soils (t_{10} = 2.07, P = 0.065), with 26% of *D. cespitosa* root length colonized by AM fungi when grown in thermal soils, in contrast to 46% colonization levels when grown in transition soils (Table 2). Vegetation of thermal areas was depauperate compared to transition areas. Thermal areas included *A. scabra, D. lanuginosum, M. guttatus,* and *Panicum capillare* L. Transition areas included all species present in the thermal area in addition to *Achillea millefolium* L., *Antennaria* sp., *Aster* sp., *Fragaria virginiana* Miller, *Gentianella detonsa* (Rottb.), *Rumex acetosella* L., *Agrostis stolonifera* L., G. Don, *Elymus spicatus* (Pursh) Gould, and *Poa compressa* L.

Colonization of *D. lanuginosum* at Rabbit Creek averaged 53% and did not change

TABLE 2. Soils data from Rabbit Creek Basin, Yellowstone National Park. All metals are given as the available concentration, extracted by DTPEA-TEA and analyzed by ICP-AES.

| | Thermal area soils (High stress, $n = 8$) | | Transition area soils (Low stress, $n = 4$) | |
	Average	Range	Average	Range
Temperature (°C)	35	30–40	26	26–28
EC (mmhos cm^{-1})	2.22	0.93–5.61	0.49	0.13–1.17
pH	3.8	3.1–5.1	5.4	4.9–5.7
Copper (mg kg^{-1})	0.81	0.49–2.30	0.62	0.50–0.71
Iron (mg kg^{-1})	181.3	22–395	48.5	17–65
Manganese (mg kg^{-1})	7.2	1.5–17.5	98.2	50.3–131.6
Zinc (mg kg^{-1})	2.3	1.22–5.33	4.5	3.45–5.79
Mycorrhizal Infectivity Potential	26%		46%	

TABLE 3. Colonization levels for 2001 from Rabbit Creek Basin, Yellowstone National Park, shown as the mean observed colonization level ± the standard error; n = number of plants examined for colonization levels.

| Species | Month | n | % AM fungi | | |
			Vesicles	Arbuscules	Total
D. lanuginosum	June	13	10.5 ± 1.9	4.5 ± 1.6	63 ± 7
	July	12	7.9 ± 1.1	1.8 ± 0.4	49 ± 4
	August	13	8.8 ± 3.1	6.1 ± 0.4	48 ± 8
	October	10	7.1 ± 2.1	3.5 ± 0.9	51 ± 9
	Average		8.7 ± 1.1	2.6 ± 0.5	53 ± 3
M. guttatus	June	11	12.8 ± 4.6	11.4 ± 2.5	72 ± 5
A. scabra	July	12	16.5 ± 5.1	1.7 ± 0.5	66 ± 4

through the summer months and into the fall (October). Additionally, *M. guttatus* and *A. scabra* colonization levels were 72% and 66% in the monitored months of June and July, respectively (Table 3).

CONCLUSIONS

Arbuscular mycorrhizae are present in multiple thermal areas across YNP, despite conditions that are limiting to plant growth, including acidic soils and elevated rooting-zone temperatures. Furthermore, AM fungal propagules are able to survive in extreme soil conditions, albeit at lower levels than in adjacent, less extreme soils. Thus, even where vegetation is sparse and soil conditions unfavorable for plant growth, colonizing plants will have access to AM fungi. Although our data characterizing these sites are limited, thermal soils are known to exhibit extremely heterogeneous soil chemistry (Rodman et al. 1996). This fact underscores the plasticity of both the plants and the AM fungi to a wide range of environmental conditions.

Colonization levels of *A. scabra, D. lanuginosum,* and *M. guttatus* were sampled more intensively at Rabbit Creek during 2001 and were consistently higher than colonization levels of *D. lanuginosum* at Rabbit Creek during 2000. The lower colonization in 2000 might be attributed to different microsite conditions and to variability between each year's weather conditions, but it is also confounded by small sample size. Nonetheless, colonization levels of *D. lanuginosum* were constant through summer and fall. The pervasiveness of AM in thermal areas, coupled with its historic importance for plants colonizing marginal habitats (Blackwell 2000), implies that AM may be critical for plant growth in thermal environments.

We observed significant changes between thermal and transition soils in rooting-zone temperatures, soil chemistry, plant species diversity, and MIP. Decreased plant species diversity in the thermal areas provides evidence that the conditions limit plant growth. In addition, because of the age of the thermal areas (Christiansen 1984), we expect that either the plant or fungal symbionts, or both, may have

developed adaptations to their environment. However, because changes are occurring in the plant community, the rooting-zone temperature, and the soil chemistry simultaneously, it is difficult to predict what those adaptations are.

Arbuscular mycorrhizae may be a key factor in plant life on the edge of thermal areas, and either plants or fungal symbionts, or both, may be specifically adapted to the unique thermal environments. Because plants encounter multiple limiting environmental factors in thermal soils, carbon fixation and biomass accumulation are restricted, and AM could provide plants with increased nutrient acquisition. However, if nutrient needs are low for plants with diminutive stature (Koide 1991), mycorrhizae may be parasitic with higher costs than benefits (Johnson et al. 1997). Further research is required to clarify AM function in thermal soils and specific adaptations by either symbiont that might alter the function of this intimate symbiosis.

ACKNOWLEDGMENTS

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