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Mycorrhiza

The influence of aqueous extracts of burnt or heated soil on the activity of vesicular-arbuscular mycorrhizal fungi propagules

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Summary. Aqueous extracts of burnt soil, unburnt soil and oven-heated unburnt soil were tested as to their effects on vesicular-arbuscular mycorrhizal (VAM) fungi (spore germination, mycelial propagule activity and root colonization). The extracts of burnt or heated soil inhibited VAM spore germination and extrarrhizal mycelium activity.

Key words: Burnt soil - Soil extracts - Spore germination - VA mycorrhiza - VA propagules

Introduction

Controlled burning, as opposed to ecologically damaging wild fire, is sometimes used to renew vegetation and recycle nutrients. Research has been carried out on the effects of vegetation burning on soil nutrient content (Raison 1979) and microflora (Ahlgren and Ahlgren 1965; Jalaluddin 1969; Jorgensen and Wells 1970), but little is known of the effect on the population of vesicular-arbuscular mycorrhizal (VAM) fungi. Klopatek et al. (1988), who burnt the litter on soils reconstructed in small lysimeters, found that the VAM colonization of plants subsequently grown in the burnt soil depended on pre-fire soil humidity and the temperature reached during burning, and was in all cases less than that of plants grown in unburnt control soils. Dhillion et al. (1988) found that VAM colonization of *Schizachyrium scoparium* in a burnt prairie plot was depressed during the first post-fire growth season but not during the second, and concluded that the depression was due to the effect of fire on the host rather than to its direct action on the VAM fungi. We have found that fire reduces propagule numbers, and that *Acaulospora laevis* spores from burnt sites have lower germination rates than controls from neighbouring unburnt sites (Vilariño and Arines, submitted). Since the latter finding suggested that besides reducing propagule numbers fire might also affect VAM fungi by producing agents inhibiting propagule activity, we have now compared the effects of aqueous extracts of burnt, unburnt and oven-heated soil on VAM propagule activity. Our results are presented in this article.

Materials and methods

Two sites in Galicia (N. W. Spain) that had suffered fires during the summer of 1988 were studied, Oroso and Pedroso. The Oroso site has a slope of less than 5%, a Distric Cambisol soil over basic schists, and vegetation consisting of sparse *Pinus pinaster* L., *Castanea sativa* L. and *Quercus robur* L. and dense *Pteridium aquilinum* L., *Rubus* sp. and grasses. The Pedroso site has a slope of over 20%, a Distric Ranker soil over granite, and vegetation dominated by *Ulex europaeus* L.

At both sites, the burnt area and an adjacent unburnt area of equal slope and vegetation were sampled 1 month after burning (i.e. in September 1988 at Oroso and October 1988 at Pedroso) and in January, May and September 1989. On each occasion, six samples of the top 15 cm of soil were taken within a 75 × 75 cm square, pooled, air-dried and thoroughly mixed; the sub-2-mm fraction was separated by sifting for use in the following experiments.

Germination

Aqueous extracts of the soil samples were obtained by shaking a 1:10 suspension of soil in water for 1 h. The insoluble material was then filtered off and the extracts were brought to pH 5.4, sonicated for 30 min and sterilized by filtration (0.22 μm). Table 1 lists the nutrient contents of the extracts of burnt soil (BS) and unburnt soil (US) from the two sites at the first sampling date.

BS and US extracts and sterile distilled water controls of the same pH (C) were used as supplements in spore germination medium as follows. For each supplement, five petri dishes (replicates) were prepared by adding 10 ml autoclaved agar and 5 ml supplement to each dish when the agar had cooled to 50° C. The dishes were each seeded with ten *Glomus macrocarpum* Tul. et Tul. var. *macrocarpum* spores and incubated in the dark for 20 days at 26° C.

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Table 1. Nutrient concentrations (μg/ml) in aqueous extracts of burnt soil (BS) and neighbouring unburnt soil (US) from sites at Oroso and Pedroso at the first sampling date

	Al	Mn	Fe	K	Ca	Mg
Oroso						
UB	≤1.00	≤1.00	≤1.00	1.45	1.65	1.20
BS	≤1.00	≤1.00	≤1.00	1.56	≤1.00	≤1.00
Pedroso						
UB	≤1.00	≤1.00	≤1.00	≤1.00	≤1.00	≤1.00
BS	≤1.00	≤1.00	≤1.00	1.56	≤1.00	≤1.00

Mycelium infectivity

For each supplement (extract or control) prepared as above on the first, second and fourth sampling occasions, five pots were made up with 100 ml of a 5:1 mixture of sterile sand and unburnt Oroso soil that had been passed through a 125-μm sieve to remove spores and mycorrhizal roots and ensure that the only VAM propagules present were free mycelium fragments. In each pot, three *Trifolium pratense* L. plantlets were grown for 27 days, during which time they were treated every 2 days with 10 ml of one of the supplements. The infectivity (I) of the propagules was determined as the percentage of root that had been infected at the end of the experiment, measured by Giovannetti and Mosse's (1980) gridline intersection method; the relative abundance of arbuscules (Arb) was determined by microscopic examination of fragments of colonized root at X 40 magnification (from each pot, ten 1-cm fragments were obtained and mounted on a single slide, and 100 fields of each slide were examined; see Arines et al. 1988).

Oven-heated soils

Samples of US from the Oroso site were spread to form a layer less than 5-mm thick, heated in an oven at 200° C for 5, 15, 30, 60, 120 or 180 min, and extracted as above (HS extract). US extract, controls and HS extracts from the samples heated for 5, 15, 30, 60 and 180 min were then subjected to the *Glomus macrocarpum* germination test as above. US extracts, controls and HS extract from the sample heated for 120 min were used to determine non-sporal VAM propagule activity by Porter's (1979) most probable number method as follows. Unburnt Oroso soil was passed through a 125-μm sieve and dilutions were made with sterile sand at 10-fold intervals from 10 to 10000. For each combination of dilution and sample type (HS, US or control), three *Trifolium pratense* L. plantlets were grown in 100 ml diluted soil in each of five pots and treated every 2 days with 10 ml of the corresponding supplement. After 6 weeks, the most probable number of propagules was determined, and for the 10-fold dilution soils I (%) and Arb (%) were determined as above after roots had been stained (Phillips and Hayman 1970).

One-way analysis of variance was performed after the Kolmogorov-Smirnov test had confirmed the normality of the data distribution at the $P=0.01$ level. Means were compared using the Tukey's W-test.

Results

VAM spore germination in the presence of extracts of BS

The germination rate of *G. macrocarpum* spores was always less in the presence of BS extracts from either site than in the presence of the corresponding US extracts or controls (Fig. 1). The difference between the results for BS and US extracts was always statistically significant ($P=0.01$) at both sites, and for the Oroso site the BS results also differed significantly from those of the controls.

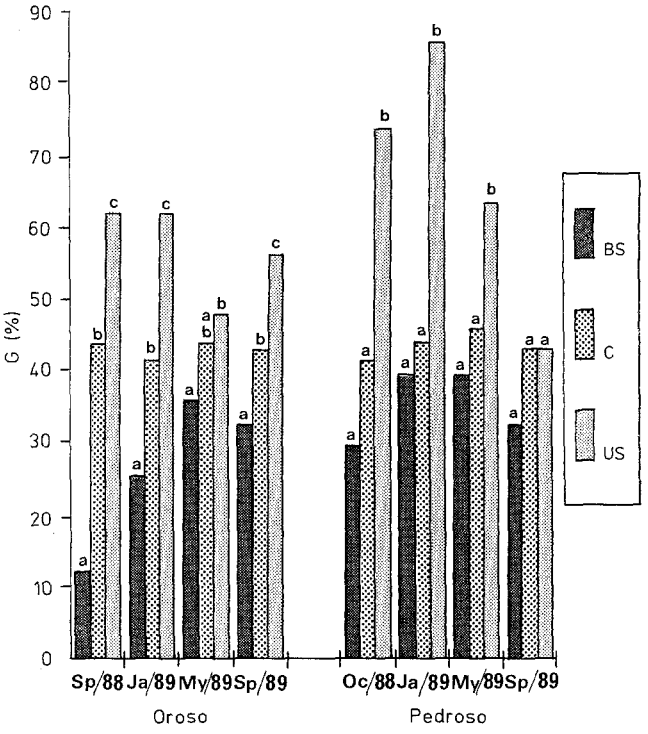

Fig. 1. Percentages of *Glomus macrocarpum* spores germinating in the presence of aqueous extracts of burnt soil (*BS*) or unburnt soil (*US*) from Oroso or Pedroso, and in control media (*C*). For a given site, means differing significantly ($P=0.05$) have different letters attached

VAM colonization in the presence of extracts of BS

Plants treated with Oroso BS extracts were always significantly less colonized ($P=0.01$) than those treated with Oroso US extracts or controls, whereas for Pedroso extracts this was true only for those obtained from samples taken shortly (1 month) after burning (Fig. 2). For extracts from both sites, Arb (%) was always significantly less ($P=0.05$) for BS than for US (Fig. 3).

Spore germination and propagule activity in the presence of extracts of oven-heated soil

All the HS extracts inhibited spore germination in comparison with the 72% germination rate achieved in the presence of US extracts, and those from soil heated for 30 min or more also exerted inhibition ($P=0.05$) in comparison with the 34% rate of the controls (Fig. 4). The extract of soil heated for 120 min reduced the number of propagules, the intensity of colonization and the relative abundance of arbuscules (Table 2).

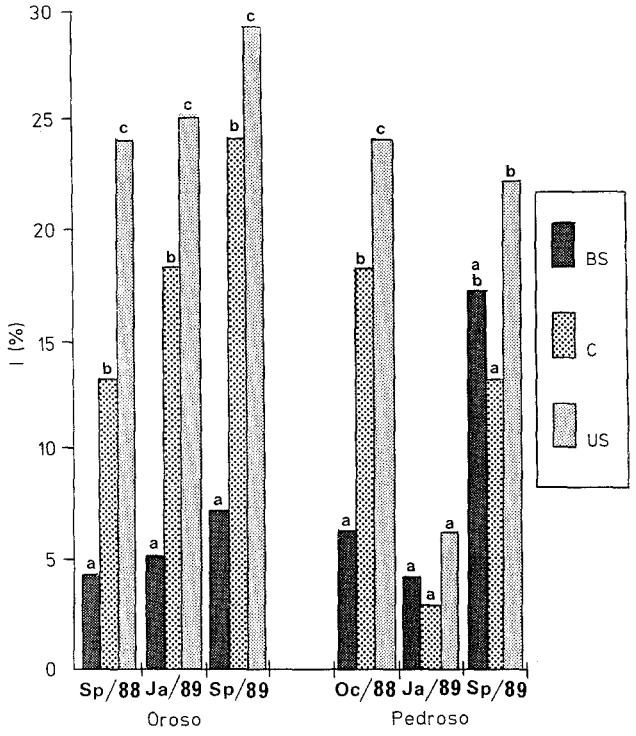

Fig. 2. Percentages of *Trifolium pratense* root colonized by vesicular-arbuscular mycorrhiza (VAM) in the presence of aqueous extracts of burnt soil (*BS*) or unburnt soil (*US*) from Oroso or Pedroso, and in control media (*C*). For a given site and sampling date, means differing significantly ($P=0.01$) have different letters attached

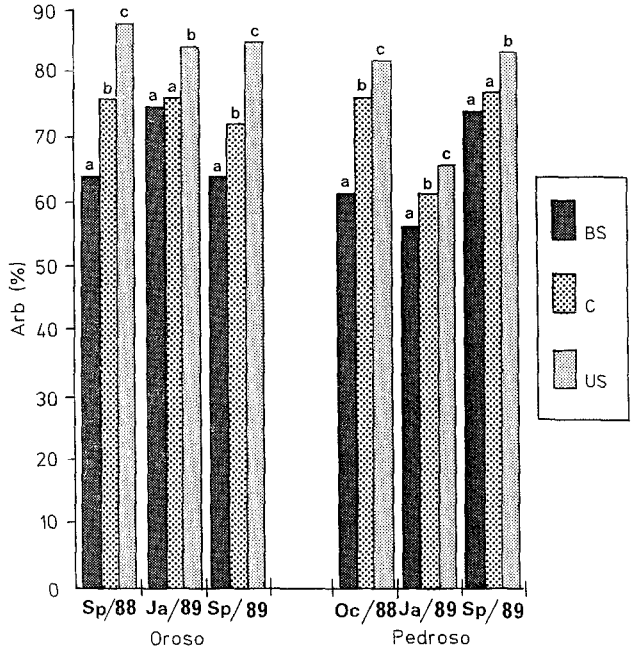

Fig. 3. Percentages of infected root length containing arbuscules in plants of *Trifolium pratense* grown in the presence of aqueous extracts of burnt soil (*BS*) or unburnt soil (*US*) from Oroso or Pedroso, and in control media (*C*). For a given site and sampling date, means,differing significantly ($P=0.01$) have different letters attached

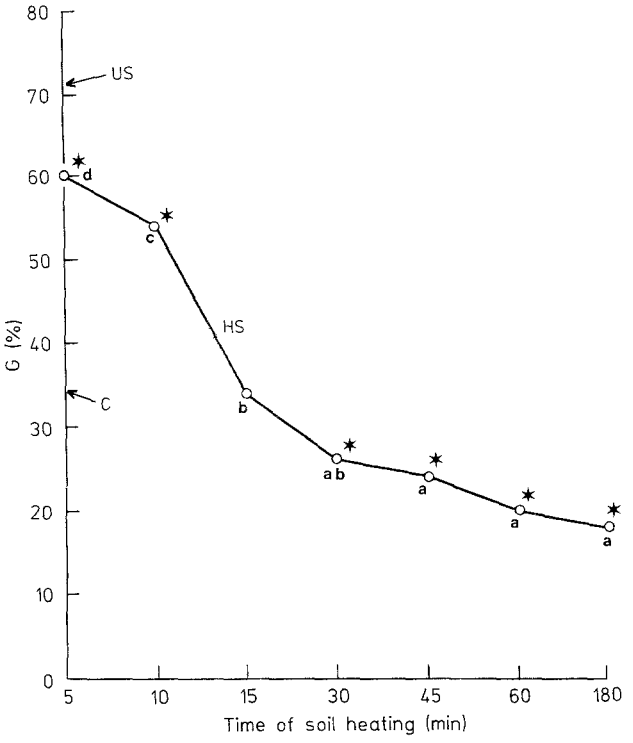

Fig. 4. Percentages of *Glomus macrocarpum* spores germinating in the presence of aqueous extracts of unburnt Oroso soil samples oven-heated at 200° C (*HS*) for different times, and the corresponding germination rates in the presence of extracts of unburnt Oroso soil (*US*) or distilled water (*C*). For a given duration of heating, *asterisks* indicate significant differences ($P=0.05$) between HS and C. Differences between times of heating are denoted by the letters attached ($P=0.05$)

Table 2. Most probable numbers of VAM fungi propagules in the substrate of *Trifolium pratense* L. plants treated with aqueous extracts of oven-heated soil (HS) or unheated soil (US) or with sterile water (C) (MPN, with 95% confidence interval in parentheses), together with the VAM colonization percentage (I), and the relative abundance of arbuscules (Arb) in the plants grown at the first of a 10-fold dilution series for MPN test of unburnt Oroso soil (means ± standard error)

	HS	C	US
MPN	96 (34-299)	162 (57-529)	272 (91-901)
I[a]	28±3a	33±5a	44±2b
Arb[a]	52±6a	64±3b	76±4c

[a] Different letters indicate significant differences ($P\leq 0.01$) among soil treatments

Discussion

Glomus macrocarpum germination rates, root colonization intensity and arbuscule formation were all less in the presence of BS extracts than in controls (at least shortly after burning), and the control figures were always less than those obtained in the presence of extracts from US. Since these results are unlikely to have been affected by the low nutrient content of the extracts (Table 1), they suggest that BS contains water-soluble

agents reducing both the quantity and the quality of VAM colonization, and that US contains water-soluble agents promoting VAM colonization and the germination of the spores of VAM fungi. The reduction in germination rates, VAM colonization intensity, arbuscule formation and propagule density caused by extracts of oven-heated soil support the idea that the presence of the inhibitors in the BS was brought about by the high temperatures suffered during burning.

The possibility that sterilization by heat treatment might produce germination inhibitors was put forward by Daniels and Graham (1976). Raison (1979) reported the appearance of toxic substances in soil as the result of fire. Wilson (1984) observed that autoclaving inhibited the germination and growth of germinal hyphae of *Gigaspora* sp. but pointed out that the inhibition might be due to the formation of inhibitors or to the elimination of the micro-organisms that are involved in both VAM spore germination (Daniels and Trappe 1980) and germinal hyphae growth (Azocon 1989). In our experiments, all the experimental media were sterilized and the inhibition exerted by BS extracts was detected by comparison with the performance of US extracts and controls; microbial effects, therefore, were absent from all media, and the inhibition must have been due to the formation of toxic substances.

Eltantawy (1980) reported that the alteration of soil organic matter by heating to 200° C consists chiefly of dehydroxylation, decarboxylation, volatilization and oxidation. The effects of organic soil components on microbial metabolism has been studied by Gaur and Bhardwaj (1971), Barton and Ruocco (1981) and Pflug and Ziechmann (1982), but there is no published information concerning their effects on VAM fungi.

The fact that the inhibitory effect was less intense and, in particular, of shorter duration at Pedroso than at Oroso may be attributed to the much greater slope of the former site, which would favour the leaching of inhibitors. However, the simultaneous involvement of other factors, such as the nature of the soil organic matter, cannot be ruled out.

To sum up, the above results show that the spores and propagules of VAM fungi are inhibited by substances formed when the soil is overheated. The characterization of these inhibitors, of the effect of fire on native VAM populations and of the role of symbiosis in the restoration of soils affected by fire are medium- to long-term research goals.

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