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Solarization in a forest nursery: effect on ectomycorrhizal soil infectivity and soil receptiveness to inoculation with *Laccaria bicolor*

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Abstract Field experiments were carried out in a forest nursery during the summer of 1994 to examine the effect of soil solarization on ectomycorrhizal soil infectivity (ESI) and soil receptiveness to inoculation with *Laccaria bicolor*. Soil samples from solarized, steamed, fumigated and untreated plots were periodically collected and assayed for ESI. Untreated soil exhibited high ESI. Solarization was as effective as steaming or fumigation in reducing ESI in the uppermost layer. Solarization with a double layer of polyethylene film and fumigation were the only treatments which reduced ESI deeper in the soil. During July, the temperature of covered beds reached 50 °C at a soil depth of 5 cm. Ectomycorrhizal fungi were among the soil-borne fungi most sensitive to solar heating. Soil solarization provides an effective disinfection method for controlled mycorrhization in forest nurseries.

Key words Soil solarization · Ectomycorrhizal soil infectivity · Forest nursery · Inoculation · Mycorrhizal receptiveness · *Laccaria bicolor*

Introduction

Soil fumigation with chemicals such as methyl bromide or Dazomet (Basamid) is widely used in forest nurseries to control soil-borne diseases and weeds in a single application. However, these techniques are expensive to use, hazardous for users, toxic for the environment and may not be particularly effective (Porter and Merriman 1985). In addition, such chemicals are not selective in their action and may also destroy desirable organisms such as mycorrhizal fungi (James 1989). Menge et al. (1978) reported that *Glomus* species are more sensitive to fumigants that all soil-borne pathogenic fungi. Nevertheless, by producing a biological vacuum, soil fumigation avoids any kind of competition which is a prerequisite for mycorrhizal inoculation with selected, efficient strains.

Solar heating is an alternative method for controlling soil-borne pathogens and weeds frequently used in regions with a suitable climate. This is accomplished by covering moist soil with transparent polyethylene film during summer months (Katan 1987). It is well known that the success of this practice depends both on the soil temperature reached during the process and the exposure time. Disease control is attributed to changes in the populations of soil-borne microorganisms during and after the process that affect propagule density, aggressiveness and survival (Greenberger et al. 1987). In solarized soils, thermotolerant fungi increase to higher levels and contribute to the induced suppressiveness of soil (De Vay 1991).

Soil solarization affects a wide range of soil microflora (Katan 1987) but very little is known about the effect of solar heating on mycorrhizal fungi. A few reports exist of side effects of solarization on arbuscular mycorrhizal (AM) fungi. Contradictory results have been reported but most suggest that heating the soil does not damage native AM fungi and can enhance mycorrhizal colonization and growth of plants in solarized soil (Pullman et al. 1981; Afek et al. 1991). There is no report dealing with ectomycorrhizal fungi. The aim of the present experiment was to study the effects of solarization on ectomycorrhizal soil infectivity (ESI) in forest nurseries in southern France and the receptiveness of solarized soil to inoculation with a selected strain of ectomycorrhizal fungi.

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Material and methods

During 1994, field experiments were conducted in a bareroot forest nursery located in southern France at Saint Jean du Gard (30) (44°10' N latitude, 189 m elevation). The soil, a neutral sandy loam, was first tilled in spring 1994 and then at the end of June (rototilling) to provide a fine structure and a smooth surface for application of the tarp. Three replications of each treatment (3 m × 1.2 m) were set up along the seed beds in a completely randomized block design.

These treatments were:

1. Non-covered soil (control).
2. Steamed soil (15 min at 80 °C as usually applied by the nursery manager).
3. Fumigated soil (Dazomet applied as 70 g/m2 of the commercial product Basamid and then covered with plastic film for 1 month).
4. Solar heated (watered to field capacity, then covered with transparent polyethylene film 40 μm thick either as a single layer placed over the nursery bed flat against the soil, or a double layer raised as a tunnel 60 cm high over metal structures). The individual plots were left covered for 4, 7 or 11 weeks. The solarization treatment extended from the beginning of July to mid-September 1994.

Soil temperatures were continuously monitored along the centre of one plot of each solarized treatment by thermocouples at 5, 15 and 30 cm depths connected to a data logger.

In order to assess the effect of soil treatment on ESI, soil samples were collected on the day the film was removed 4, 7 or 11 weeks after setting up the experiment, consisting of 6–8 cores (30 cm × 5 cm) collected from individual plots. The soil of each core was divided into three layers 0–5, 5–15 and 15–30 cm. Soil samples from the same layer were pooled to provide a single sample for each individual plot. The samples were passed through a 4-mm sieve and then stored in a cool chamber for biological assays.

Bioassays for estimation of ESI

Each soil sample was assayed for infectivity using a standard bioassay performed in a greenhouse as previously described (Duvert 1987; Perrin et al. 1988). The bioassay involved the cultivation of *Pinus nigra* seedlings on disinfected perlite in controlled conditions for 1 month. One-month-old pine seedlings are very receptive to mycorrhizal colonization (Duvert 1987). The seedlings were then transplanted to 175-ml Spencer-Lemaire Rootrainers (Spencer-Lemaire Industries Ltd., Edmonton, Canada) previously filled with sampled soil and replicated 10 times for each sample. Mycorrhizal development was assessed after 1 month growth of transplanted seedlings in controlled conditions. For assessing mycorrhizal colonization, roots were subsampled in three parts corresponding to the upper, middle and lower root systems. A total of 150 root apices was examined and the number of mycorrhizal root apices were counted for each subsample under a dissection microscope and separated into morphological types. ESI is expressed as the mean percent mycorrhizal roots in each treatment.

Receptiveness of solarized soil to mycorrhizal inoculation

Soil samples were collected in the upper soil layer (0–5 cm) in fumigated and solarized plots in spring 1995 (at a time of potential inoculation), i.e. 8 months after the end of the solar-heating treatment. The samples consisted of 8–10 subsamples collected randomly from individual plots. These subsamples were pooled to provide a single sample for each individual plot. Each soil sample was assayed for receptiveness to ectomycorrhizal inoculation with *Laccaria bicolor* strain S238 obtained from R. Molina. Standard bioassay procedures were performed in the greenhouse as previously described (Perrin et al. 1994). The soil was inoculated with mycelium entrapped in alginate beads as described by Mauperin et al. (1987). Each *Pinus nigra* seedling was supplied with 3.5 ml of alginate beads corresponding to a mycelium rate of 14 mg fresh wt. mixed with the soil prior to seedling transplantation. After 2 months growth under controlled conditions, seedlings were harvested and the root system examined under the stereoscopic microscope for mycorrhizae according to the procedure described above. A distinction was made between the typical morphological *Laccaria* type and others. A receptive soil is defined as a soil which allows the development of mycorrhizae from the inoculated strain.

Statistical analyses

Data taken as percentage were arcsin square root-transformed prior to analysis. The transformed data were subjected to analysis of variance (ANOVA) and the treatment means were compared by LSD ($P < 0.05$). All analyses were performed with the STATISTICA program (Statsoft Inc., Tulsa, Okla., USA).

Results

Soil infectivity

Pinus nigra seedlings grown on soil from control plots showed a very high mycorrhizal rate of more than 80% mycorrhizal short roots, regardless of either sampling depth or sampling date (Fig. 1). After 4 weeks, all treatments resulted in a dramatic decrease in ESI in the uppermost layer. Near the soil surface, the percentage of mycorrhizal short roots was 1–12% at the first sampling date. The lowest mycorrhizal percentage was found for the seedlings grown on the steamed soil. Only two treatments, fumigation and solar heating with a double layer of plastic film, significantly reduced the mycorrhizal infection of pine seedlings by indigenous mycobionts grown on soil from deeper layers. However, the decrease in ESI is much less pronounced than near the soil surface, particularly after solarization treatment. The ESI was not significantly affected by any treatment in the 15 to 30-cm layer.

The main indigenous mycorrhiza observed was yellowish-white and *Hebeloma*–like with a cottony mantle surface. Also frequently observed was a brownish, smooth type with the rhizomorph slightly differentiated and a prominent, awl-shaped cystidia with a basal clamp connection, resembling mycorrhizae formed by *Thelephora terrestris* as described by Agerer (1987–1996). Further, less frequently observed, native mycorrhizas were a corraloid white *Rhizopogon* type with typical highly differentiated rhizomorphs, and a *Boletus* type with abundant, highly differentiated rhizomorphs and a smooth, more-or-less silvery mantle surface. The frequencies of these ectomycorrhizal types on bioassay seedlings did not differ between the treatments.

Maximal soil temperatures achieved under a single layer plastic were ca. 46 °C near the soil surface, and ca. 43 °C and 33 °C at 15 cm and 30 cm depths, respectively. Daily soil temperatures remained above 46 °C only at

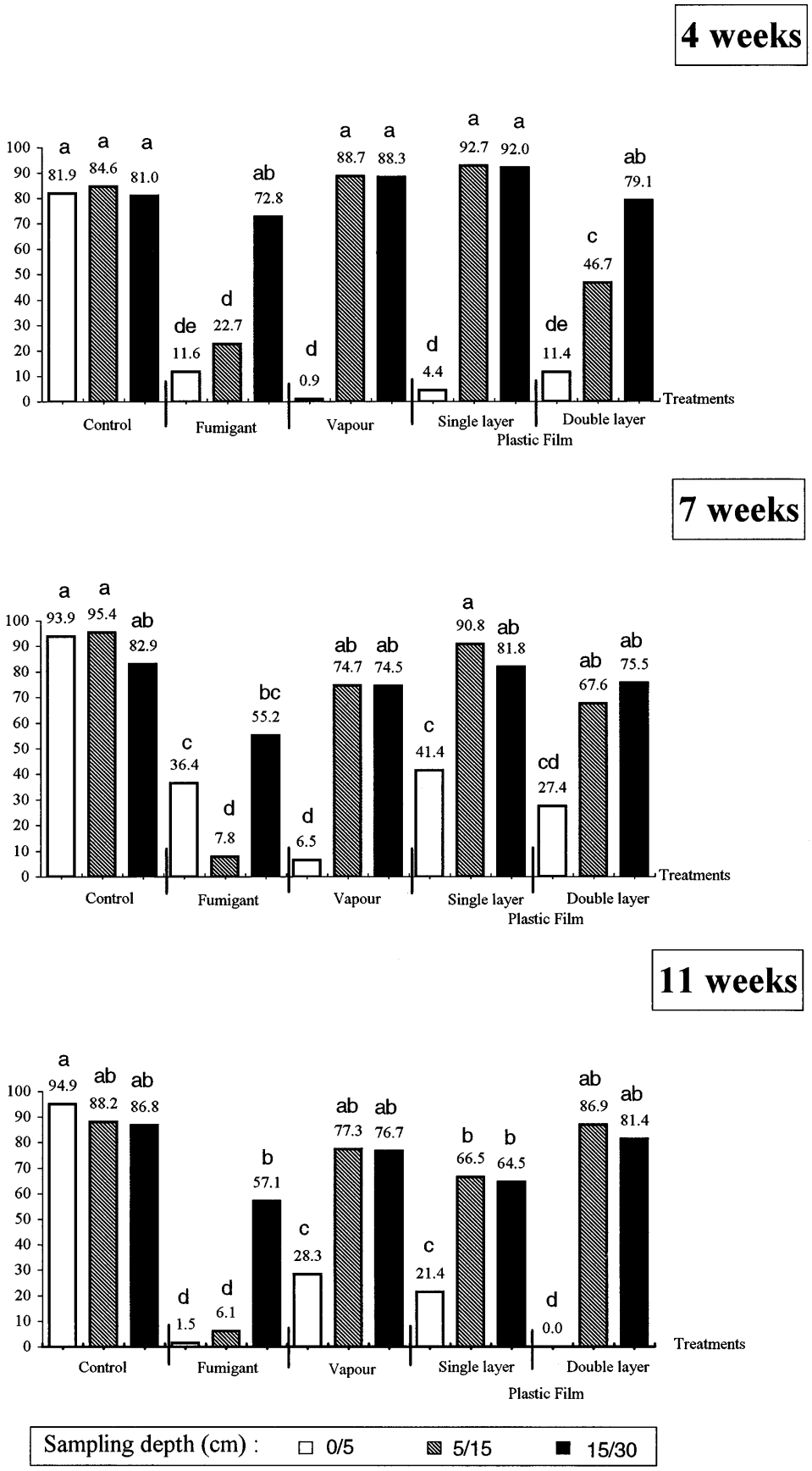

Fig. 1 Changes in ectomycorrhizal soil infectivity (ESI) expressed as percentage mycorrhizal roots according to a bioassay performed on soil samples collected after 4, 7 and 11 weeks of disinfection treatments in the Saint Jean nursery at different soil depths. Values followed by the same letter are not significantly different ($P = 0.05$)

5 cm depth and for a total of 13 h. The highest temperatures recorded under the double layer were 49.9 °C, 44 °C and 40 °C at 5 cm, 15 cm and 30 cm, respectively. Daily soil temperatures exceeded 46 °C only near the soil surface but extended for 75 h, distributed among five different periods. Temperatures above 48 °C, and 78% of total exposure time to temperatures exceeding 46 °C occurred during the first 4 weeks of solar heating.

There were few significant changes in ESI within the following weeks. At the second sampling date, ESI increased significantly near the soil surface in the fumigated plots and under both single- and double-layer polyethylene films. The former increase was restricted to the upper soil layer and was related to a very high level of mycorrhizal infectivity in one of the three individual fumigated plots. Moreover, all the mycorrhizae belonged to a single *Boletus* type. After 7 weeks, the plastic films covering the single-layer plots were completely worn out (from UV radiation, animals and wind), and solarization was interrupted 2 weeks previously according to the temperature records. At the same time, the upper plastic film of the double-layer treatment began to deteriorate. As a consequence, increases in temperature were less pronounced as the damage developed.

At the third sampling date (after 11 weeks), the ESI remained at a low level, similar to that observed at 4 weeks in the upper soil layers for all treatments. The lowest mycorrhizal percentages were recorded on seedlings grown either in the fumigated soil (up to 15 cm depth) or in double-layer solarized soil. In the latter, no mycorrhizae developed during the time of the bioassay. After 11 weeks, the plastic was almost totally destroyed.

Soil receptiveness

Typical ectomycorrhizae of *L. bicolor* developed on all root systems regardless of soil treatment (Fig. 2). Inoculated isolate S238 of *L. bicolor* formed typical ectomycorrhizas in the bioassay with *P. nigra* seedlings grown either in fumigated or solarized soil (Fig. 2). However, significant variation occurred between the treatments in the rate of root colonization. The highest level of total mycorrhizal colonization (83% root tips including native fungi) was found on seedlings transplanted in soil collected 8 months after the end of solar heating with a single layer of polyethylene film. The proportion of native mycorrhizae was very low (2%). Both total mycorrhizal frequency and percentage colonization by *L. bicolor* were significantly lower on seedlings grown on fumigated soil than the other treatments. In contrast, the proportion of mycorrhizae other than those developed by the inoculated strain was significantly higher. There was no significant difference between the two solarized treatments.

Discussion

The technique was successful in generating soil temperatures comparable to those reported under field conditions by Katan et al. (1976), i.e. 46–54 °C at 5 cm depth and known to be lethal or sublethal for many soil-borne fungi. Effects of solarization are clearly related to soil temperatures attained during the process. Soil solarization achieved under climatic conditions prevailing in southern France provides a satisfactory control of damping-off (Le Bihan et al. 1997).

Nothing was previously known about either the viability and survival of native propagules of ectomycorrhizal fungi or the effect of solar heating on ectomycorrhizal soil infectivity. Information available on the use of soil solarization for control of soil-borne diseases concerns only occasionally the side effects and only endomycorrhizal fungi and endomycorrhizal colonization (Pullman et al. 1981; Stapleton and Devay 1984; Nair et al. 1990; Afek et al. 1991). Apparently heating the soil does not damage native endomycorrhizal colonization and growth of inoculated plants in solarized soil.

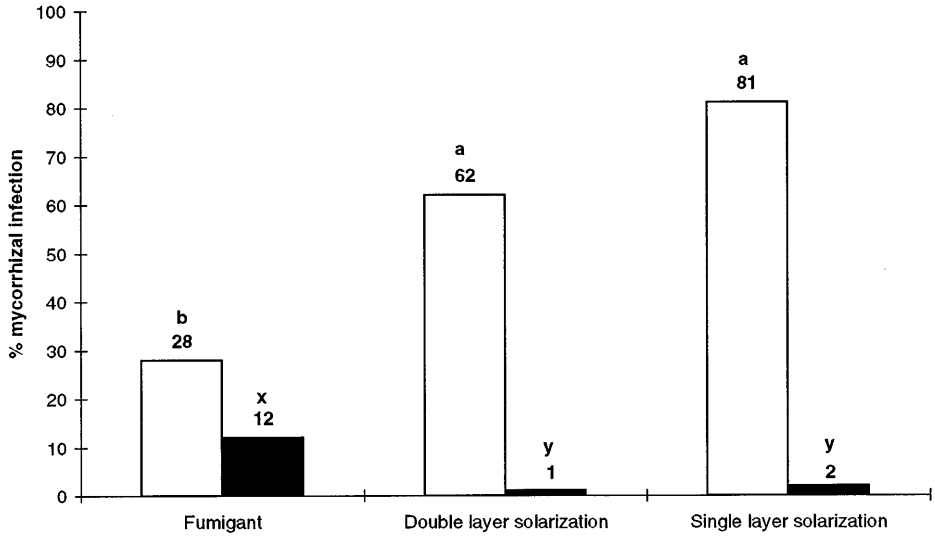

Fig. 2 Influence of fumigation and solar heating on mycorrhizal receptiveness expressed as percentage mycorrhizal roots according to a bioassay performed on soil samples collected 8 months after treatment of a nursery soil inoculated with *Laccaria bicolor* S238. Values followed by the same letter are not significantly different ($P = 0.05$). *Symbols:* □ Laccaria bicolor; ■ native

The results reported here show that 4 weeks soil solarization is sufficient for a strong decrease in native ESI in the upper soil layer. The mycorrhizal infection of test seedlings was reduced to a low level (< 11.5%), similar to those achieved by fumigation or steaming. At 15 cm depth, there was a slight decrease in ESI only under a double plastic layer. Solar heating was without effect deeper in the soil. Our data do not allow an accurate determination of the lethal temperatures for native ectomycorrhizal fungi. Nevertheless, the relationship between mycorrhizal infection and recorded temperature at various depths under single- or double-layer film show that temperatures higher than 45 °C have a suppressive effect on ectomycorrhizal infectivity. Such lethal conditions were frequently achieved during the first 4 weeks of treatment both under single- and double-layer film near the soil surface. Thus, ectomycorrhizal fungi are among the species of soil borne microorganims less tolerant of soil solar heating. Numerous plant pathogens, such as species of *Pythium and Fusarium* and *Rhizoctonia solani* or endomycorrhizal fungi are reported to survive higher heat treatments. (De Vay 1991).

Fumigation was the most effective treatment for reducing ESI even at a 15-cm depth. The temporary increase in ESI of fumigated soil at the second sampling date is likely due to an accidental contamination following spore deposition a few days before sampling. Reinfestation from spores was prevented or highly reduced by the plastic film in solarized plots, at least for the double-layer treatment during the course of the trial.

In spite of partial rupture of the plastic cover, the maximum temperature fluctuated between 35 and 40 °C under the double layer during the last 4 weeks of the experiment. Such temperatures are often considered to be sublethal for pathogenic fungi less sensitive to heat than ectomycorrhizal fungi. The mycelial thermal death point of mycorrhizal fungi ranges from 30 to 41 °C (Ivory unpublished data in Harley and Smith 1984). Pullman et al. (1981) reported control of *Verticillium dahliae* and *Pythium spp.* in soils heated to 36–38 °C. The decline in the viability of soil-borne microorganisms during solarization depends on both soil temperature and exposure time, which are inversely related. Total exposure to sublethal temperatures was higher than 50 h. The reduction in ESI under double-layer film at the last sampling date suggests the occurrence of sublethal heat effects. Heat damage accumulates gradually to a point beyond which the very sensitive ectomycorrhizal propagule cannot recover. Near the soil surface, temperatures are subject to diurnal fluctuations, known as pulse effects, which increase heat damage to fungal propagules (Stapleton and De Vay 1986). Sub-lethal heating weakens propagules and results in greater sensitivity to volatiles and various other stresses.

Ectomycorrhizal fungi generally have negligible competitive saprophytic ability (Harley and Smith 1984). Consequently, ectomycorrhizal reinfestation from deeper layers or adjacent infested beds did not occur or occurred later than with most soil-borne fungi, particularly the thermotolerant species such as *Penicillium*.

The receptiveness of upper soil layers to the selected ectomycorrhizal strain of *L. bicolor* is greater after solarization than after fumigation. This is likely due to differences in the competitive microbial population developing during the fews months between disinfection and sowing. Fumigation or steaming commonly used for soil disinfection in forest nurseries result in more dramatic decreases in microbial populations than solar heating. Patterns of microbial reinfestation vary greatly with the mode of disinfection.

Our results indicate the potential of soil solarization for forest nursery application as an alternative to fumigation. These results disagree with the generally accepted idea that solarization favours beneficial microorganisms (Katan 1987). Unfortunately, soil solarization also seems to injure ectomycorrhizal fungi included in the pool of beneficial microorganisms but not some of the other biological control agents such as *Trichoderma sp*. or *Penicillium sp*.

Soil solarization provides a non-chemical, non-hazardous method suitable for control of some damping-off pathogens in forest nurseries and allows controlled mycorrhization with selected ectomycorrhizal strains. Consequently, solar heating can contribute significantly to the principles of sustainable agriculture. Application of solarization in summer, during the growing season, may have disadvantages compared with other soil disinfection methods, but this problem is mitigated by seedling rotation, including one fallow season, practised in most bareroot nurseries.

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