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# Evaluation of soil solar heating for control of damping-off fungi in two forest nurseries in France

**Abstract** Field experiments were carried out at two different forest nurseries during the summer of 1994 to examine the efficacy of soil solarization for the control of damping-off. Both soils hosted *Pythium* spp., *Fusarium* spp. and *Rhizoctonia solani* as damping-off agents. Soil samples from solarized, steamed, fumigated and untreated plots were periodically collected and assayed for soil infectivity. Solarization with a double layer of polyethylene film was as effective as steaming or fumigation in reducing soil infectivity in the uppermost layer. During July the temperature of covered beds rose as high as 50 °C at a soil depth of 5 cm. The method achieved good control of *Pythium* spp., the main cause of damping-off at both nurseries, whereas *Fusarium* spp. were more tolerant. The association of *Trichoderma* spp. with a reduction of soil infectivity at the last sampling date strongly suggested that biocontrol processes were induced after solarization. Soil solarization provides a suitable method for control of damping-off.

**Key words** Soil solarization · Damping-off · Soil infectivity · Forest nursery · Biological control · *Pythium* · *Fusarium* · *Rhizoctonia solani*

## Introduction

Damping-off and root rot are among the most widespread and destructive diseases that affect young seedlings of many plant species in forest nurseries around the world. Several fungi can cause the disease but unspecialized fungi such as *Pythium* spp. *Fusarium* spp. (especially *Fusarium oxysporum*), *Rhizoctonia solani* and binucleate *Rhizoctonia* are the main pathogens responsible for severe damage in European nurseries (Perrin and Sampangi 1986). Soil-borne pathogens coexist in most nursery soils especially in old established seedbeds. Disease severity and occurrence of causal agents vary greatly depending on soil characteristics and local conditions (Camporota and Perrin 1994).

Most forest nurseries in the world rely on soil fumigation with methyl bromide or other chemicals such as dazomet (Basamid) to control soil-borne diseases. These techniques are expensive to use, hazardous for users, toxic to the environment and may not be particularly effective (Porter and Merriman 1985). In addition, chemicals are non-selective in their action and they also kill beneficial microorganisms such as mycorrhizal fungi and antagonists. They are known to produce a biological vacuum and, if pathogens are reintroduced such as on seeds, they can reach higher levels than before fumigation, possibly in resulting extensive disease losses (James 1989).

Solar heating is an alternative method of pathogen control widely used in some parts of the world (Katan 1987). Solarization is accomplished by covering moist soil with transparent polyethylene film during the summer months (De Vay 1990) and the success of this practice depends on the soil temperatures reached during the process. In addition to the physical effects of heat induced by solarization including release of volatile degradation products (Zakaria et al. 1980), involvement of a microbial process contributes to disease control (Katan et al. 1984; Greenberger et al. 1987). This is attributed to changes in the populations of soil-borne micro-organisms that occur during and after the process that affect the propagule density and their 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 1990). Among the microorganisms which survive the solar heating process, *Trichoderma* spp. are the main representative of fungal antagonists which interfere with the development of pathogenic fungi (De Vay 1990).

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Soil solarization was found to be effective against a large range of soil-borne pathogens that affect many agricultural crops in various regions of the world (Pullmann et al. 1979; Katan et al. 1976; Melero et al. 1989; Camporota et al. 1986), but very limited data are available on the effect of solarization in forest tree nurseries (Annesi and Motta 1994). This experiment aimed to study the effectiveness of solarization against soil-borne diseases in two forest nurseries in southern France.

## Materials and methods

During 1994 field experiments were conducted in two bareroot forest nurseries located in the southern part of France. These places differ greatly in their geographic location [Saint Jean du Gard (30), 44°10′N latitude, 189 m elevation and Saint Laurent du Cros (05), 44°60′N latitude, 1100 m elevation] and in their physicochemical and biological properties of soil. The soil types were neutral sandy loam at the St. Jean nursery and alkaline sandy clay at St. Laurent.

Soils were 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 done along the seedbeds in a completely randomized block design.

At St. Jean nursery treatments were:
- Noncovered soil (control).
- Steamed soil (15 min at a temperature of more than 80°C as applied by the nursery manager).
- Fumigated soil (dazomet applied as 70 g m$^{-2}$ of the commercial product Basamid and then covered with plastic film for 1 month).
- Solar-heated plots (watered to field capacity, then covered with transparent polyethylene film (40 μm thick), either with a single layer placed over the nursery bed laid 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 began on 4 July 1994.

At St. Laurent nursery treatments were:
- Noncovered soil (control).
- Solar-heated (covered with a double layer of plastic film as previously described, for either 7 or 9 weeks). The solarization treatment began on 5 July 1994).

Soil temperatures were continuously recorded along the centre of one plot of each solarized treatment by thermocouples connected to a data logger at 5-, 15- and 30-cm depths at St. Jean, or by thermographs buried at 5- and 15-cm depths at St. Laurent.

In order to assess the effect of soil treatment on pathogenic soil infectivity, soil samples were collected the day the film was removed (4, 7 or 11 weeks at St. Jean and 7 and 9 weeks at St. Laurent after setting up the experiment). Soil samples consisted of six to eight cores (30×5 cm) collected from individual plots. The soil of each core was divided into three layer: 0–5, 5–15 and 15–30 cm depths. The parts corresponding to the same layer were pooled together to provide a single sample for each individual plots. Soil samples were passed through a 4-mm sieve and then stored in a cool chamber for biological assays.

### Bioassays for estimation of soil infectivity

Each sample was assayed for pathogenic soil infectivity. Standard bioassay procedures were performed in a greenhouse as previously described (Sampangi 1985). The bioassay involved the cultivation of *Pinus nigra* seedlings on disinfected substrate under controlled conditions for 10 days, corresponding to the most sensitive period to damping-off. The sampled soil was then inoculated to the collar of test plants, by covering the substrate with a layer 1 cm deep, replicated 10 times for each sample. Damping-off occurrence was monitored daily during the following month and disease severity was expressed as the percentage of damped-off seedlings. Damped-off seedlings were collected and the diseased part of the plant was surface sterilized by rinsing in 30% H$_2$O$_2$ 5 seconds before being washed 5 times in distilled sterile water. Disinfected fragments were then plated on acidified malt agar to isolate and determine the causal agents. Some molecular techniques (PCR(RFLP) were also performed for the identification of *Pythium* species.

### Statistical analyses

Data recorded as percentages were arcsin-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, OK 74104, USA).

## Results

The results are presented in graphical form, with the severity of damping-off in relation to the depth of sampling displayed on the left side, and the percentage of main soil-borne pathogens isolated from diseased seedlings shown on the right side, according to the bioassays performed in the greenhouse.

### St. Jean Nursery

The soil infectivity corresponding to the control plots was very high. The percentage of damped-off seedlings was 70% and 80% at 0–5 cm and 5–15 cm depths respectively at the first sampling date, 4 weeks after setting up the field experiment (Fig. 1). There were no significant changes over the following weeks except a decrease in seedling mortality in the upper layer at the third sampling date, associated with the occurrence of *Trichoderma* spp. (Fig. 3). The disease severity corresponding to the deeper layer varied between 55% and 73% according to the sampling date.

*Pythium* species were the main cause of damping-off irrespective of depth. *Pythium ultimum* was the most prominent (95%). Others species were *P. sylvaticum* and *P. oligandrum*. *Fusarium* spp. (mainly *F. oxysporum*) and *Rhizoctonia* sp. were also involved.

Heat steaming resulted in a dramatic decrease in seedling mortality in the two uppermost layers. At first sampling only *F. oxysporum* could be recovered from the diseased seedlings (Fig. 1). The treatment was not effective at 30 cm depth. Damping-off was mainly induced by *Pythium ultimum*. After 7 weeks disease incidence increased significantly in the steamed plots in relation to the occurrence of *Pythium* sp. and *Botrytis cinerea* (Fig. 2). At the latest date of sampling damping-off severity was again very low but only in the upper layer where the only fungus isolated was *Trichoderma* sp. (Fig. 3).

Fumigation with dazomet was the most effective treatment even in the deeper layer, where few seedlings died because of infection with *Botrytis cinera* and *Fusarium oxysporum*. After 11 weeks a slight increase in seedling
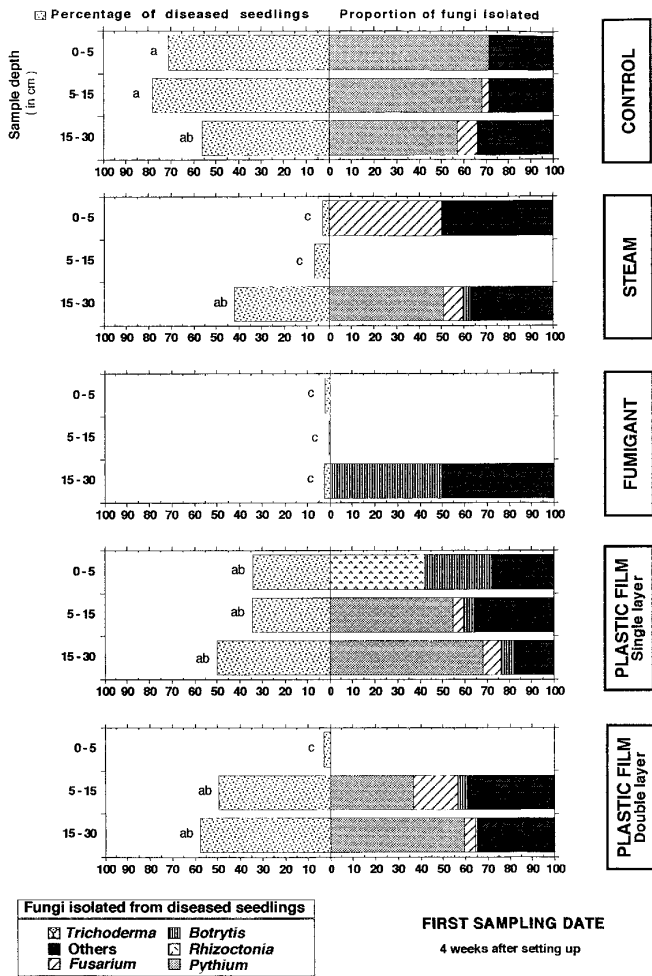
**Fig. 1** Soil infectivity, expressed as seedling mortality and proportion of fungi isolated from damped-off seedlings according to the bioassay performed on soil samples collected after 4 weeks of treatment at the St. Jean nursery. *Values followed by the same letter* are not significantly different (LSD test at *P*=0,05)
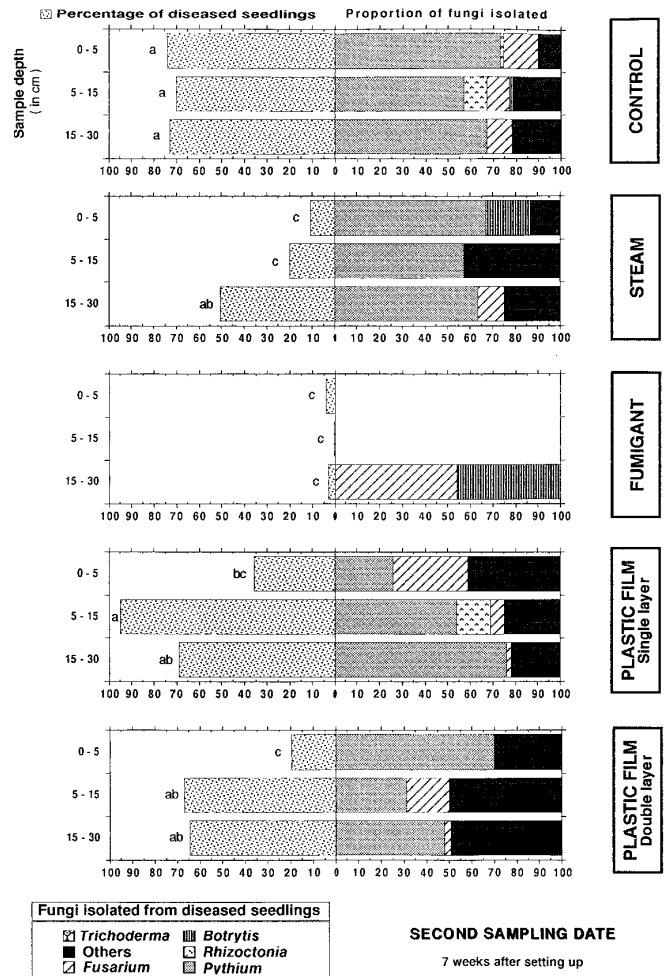
**Fig. 2** Soil infectivity, expressed as seedling mortality and proportion of fungi isolated from damped-off seedlings according to the bioassay performed on soil samples collected after 7 weeks of treatment at the St. Jean nursery. *Values followed by the same letter* are not significantly different (LSD test at *P*=0,05)

mortality occurred, particularly in the deeper layer in relation to the involvement of some *Pythium* sp. in spite of *Trichoderma* occurrence (Fig. 3).

Solar heating using a single-layer film reduced the percentage of diseased seedlings to 35% in the upper soil layer. At the first sampling date, *Pythium* sp. was controlled but damping-off was induced by *Rhizoctonia solani* and *Botrytis cinerea*. Later on there were no significant changes in disease incidence or in the main causal agent irrespective of the date or the depth of sampling. Maximal soil temperatures achieved under single-layer plastic were around 46 °C near the soil surface, and around 43 °C and 33 °C at 15-cm and 30-cm depths, respectively. Daily soil temperatures remained above 46 °C only at 5 cm depth, for a total of 13 h, for a period of 5 consecutive days, for an average of 2.6 h day$^{-1}$ (Table 1). At the second sampling date (7 weeks) the plastic films used to cover the single-layer plots had been completely torn off. So solarization was interrupted 2 weeks previously (according to the temperature records). After 11 weeks a slight decrease in seed-

ling mortality and *Trichoderma* sp. occurrence were observed.

Four weeks of double-layer soil solarization was sufficient to provide good control of the disease only at 0–5 cm (Fig. 1). The disease incidence was reduced to a low level (3%) similar to that achieved by fumigation or steaming. At 15 cm depth a lower percentage of *Pythium* was recovered and there was only a slight decrease in disease severity (50%, vs. 80% control). Solar heating had no effect deeper in the soil. The highest temperature recorded under double-layer solarization was 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 during 75 h, distributed over five different periods of a maximum of 5 consecutive days, lasting 3.75 h day$^{-1}$ on average (Table 1). Temperatures above 48 °C and 78% of the total time exposure to temperatures exceeding 46 °C were achieved during the first 4 weeks of solar heating. After 7 weeks the uppermost layer of plastic film started to break off. As a consequence, increases in temperature
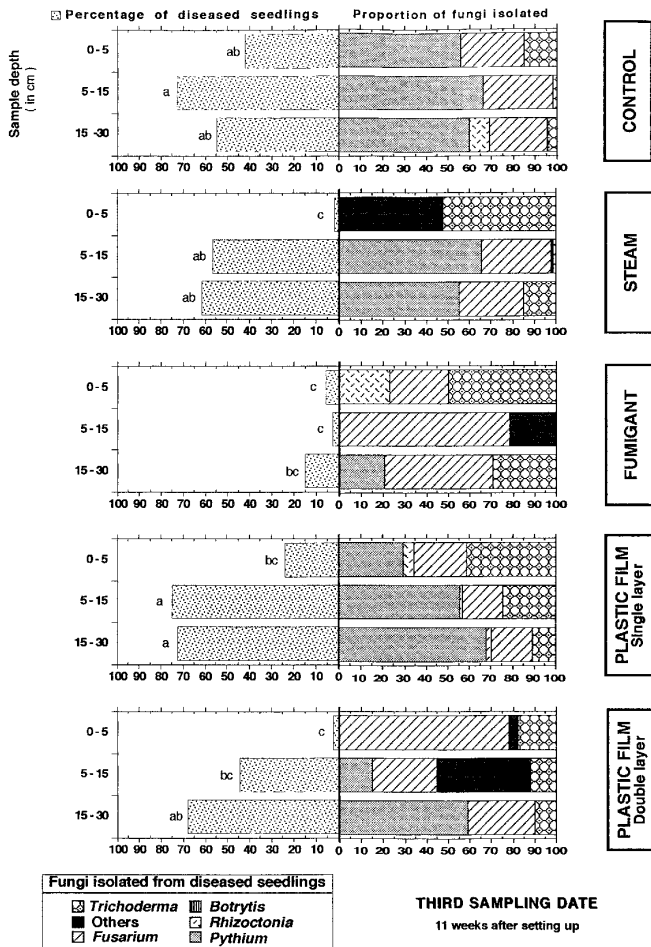
Fig. 3 Soil infectivity, expressed as seedling mortality and proportion of fungi isolated from damped-off seedlings according to the bioassay performed on soil samples collected after 11 weeks of treatment at the St. Jean nursery. *Values followed by the same letter* are not significantly different (LSD test at $P=0.05$)

**Table 1** Exposure time (h) at various temperature levels recorded at 5-, 15- and 30-cm depths under double-layer (DL) and single-layer (SL) plastic sheets between 5 July and 15 September 1994 at the St. Jean nursery

| Temperatures | DL 5 cm | DL 15 cm | DL 30 cm | SL 5 cm | SL 15 cm | SL 30 cm |
|---|---|---|---|---|---|---|
| >40 °C | 338 | 243 | 0 | 224 | 96 | 0 |
| >42 °C | 244 | 98 | 0 | 149 | 5 | 0 |
| >44 °C | 150/5.17[a] 5p/12d[b] | 3 | 0 | 66/3.67[a] 6p/7d[b] | 0 | 0 |
| >46 °C | 75/3.75[a] 5p/5d[b] | 0 | 0 | 13/2.6[a] 1p/5d[b] | 0 | 0 |
| >48 °C | 16/1.77[a] 4p/4d[b] | 0 | 0 | 0 | 0 | 0 |
| >49 °C | 6/2[a] 3p/2d[b] | 0 | 0 | 0 | 0 | 0 |

[a] Average daily exposure time (h)
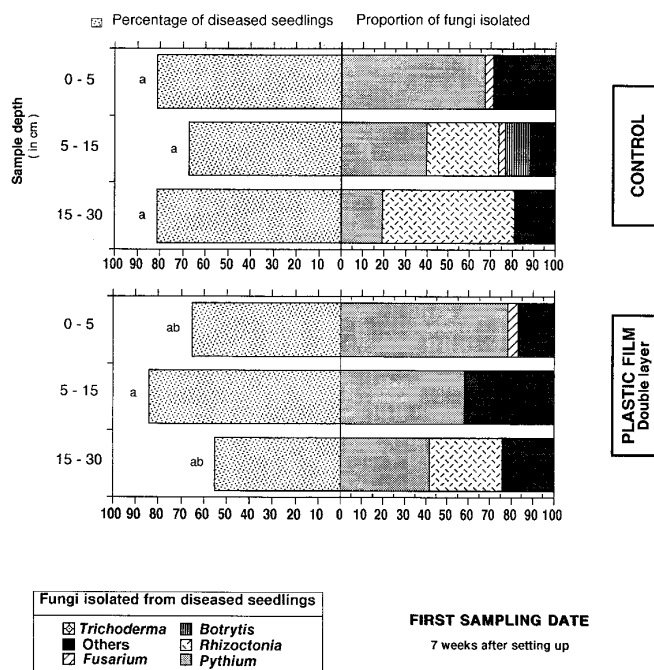[b] Number of periods/maximum number of consecutive days



Fig. 4 Soil infectivity, expressed as seedling mortality and proportion of fungi isolated from damped-off seedlings according to the bioassay performed on soil samples collected after 7 weeks of treatment at the St. Laurent nursery. *Values followed by the same letter* are not significantly different (LSD test at $P=0.05$)

were then less pronounced as the damage developed. There was a slight increase in seedling mortality in relation to the occurrence of *Pythium ultimum*. After 11 weeks the plastic was almost totally torn off. As previously observed with the other treatments, disease incidence was strongly reduced where *Trichoderma* sp. could be isolated from the plants.

St. Laurent nursery

All the soil samples collected from the control plots showed a very high infectivity. Damping-off affected up to 80% of *Pinus nigra* seedlings within the bioassay (Fig. 4). It was mainly induced by *Pythium* sp. at 5 cm depth but the relative importance of *Pythium* sp. decreased whereas that of *Rhizoctonia solani* increased with depth (Fig. 4). The disease incidence was curiously lower (5%) at the last sampling date for the deeper soil layer.

There were no significant changes in disease incidence and pathogen occurrence, since *Pythium ultimum* remained the main cause of damping-off after 7 weeks of soil solarization (Fig. 4). Temperatures reached a maximum of 46 °C and 44.5 °C at depths of 5 cm and 15 cm, respectively. Temperatures remained above 45 °C for 4 h only near the soil surface (Table 2).

Disease incidence decreased greatly after 9 weeks of soil solarization in the upper layer (Fig. 5). *Fusarium oxysporum* was the only pathogen isolated from the few damped-off seedlings. A slight decrease in seedling mortality at 15 cm depth could be related to the control of *Rhizoctonia solani*.

**Table 2** Exposure time (h) at various temperature levels recorded at 5-cm and 15-cm depths under double-layer (DL) plastic sheets between 7 July and 8 September 1994 at the St. Laurent nursery

| Temperatures | DL 5 cm | DL 15 cm |
|---|---|---|
| >41 °C | 36/2.77 [a] 5p/5d [b] | 34/2.81 [a] 5p/5d [b] |
| >43 °C | 10/2 [a] 3p/2d [b] | 8/2 3p/2d [b] |
| >45 °C | 4/2 [a] 2p/1d [b] | 0 |

[a] Average daily exposure time (h)
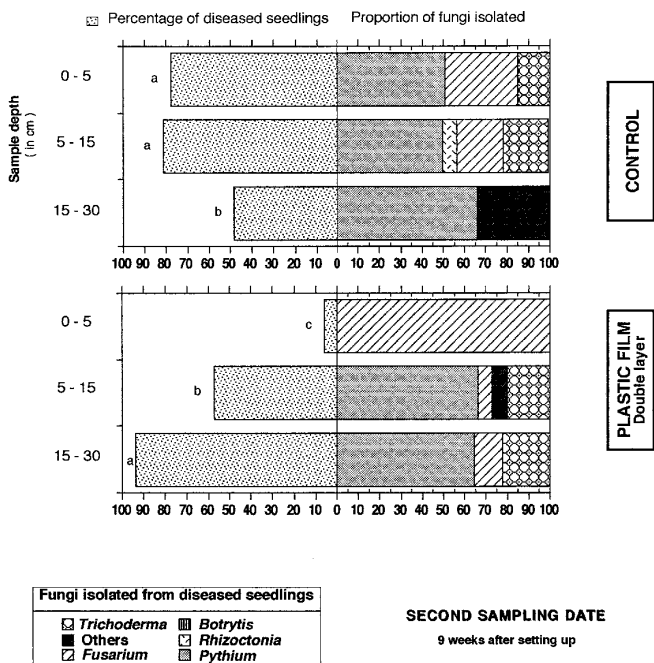[b] Number of periods/maximum number of consecutive days



**Fig. 5** Soil infectivity, expressed as seedling mortality and proportion of fungi isolated from damped-off seedlings according to the bioassay performed on soil samples collected after 9 weeks of treatment at the St. Laurent nursery. *Values followed by the same letter are not significantly different (LSD test at P=0,05)*

## Discussion

### Soil infectivity

At both nurseries, soil infectivity is very high and *Pythium, Fusarium, Rhizoctonia* and *Botrytis* species are the main soil-borne pathogens involved in plant disease. The only difference is related to the proportion of the main pathogens occurring at different depths.

At the St. Jean nursery, soil damping-off was mainly induced by *Pythium* spp. particularly *P. ultimum*. When *Pythium* was controlled or reduced by soil treatments, *Fusarium* spp., *Rhizoctonia solani* and *Botrytis cinerea* occurred but subsequent disease incidence only reached low levels. In the St. Laurent soil *Pythium* spp. were more prevalent near the soil surface while *R. solani* was responsi-

ble to a greater extent for disease at deeper soil layers. When *Pythium* was controlled, *Fusarium* spp. occurred but disease severity was very low.

### Comparative effect of soil treatments on soil infectivity

Fumigation was the most effective treatment in reducing soil infectivity even at 30 cm depth. Fungal reinfestation from deeper layers, adjacent infested beds or external sources occurred later when compared with both steamed and solarized plots.

The results reported here indicate that soil solarization can provide satisfactory control of damping-off under the climatic conditions prevailing in southern France. Four weeks of solar heating was effective in controlling or reducing *Pythium* spp. but only in the upper layer. The technique was successful in generating soil temperatures comparable to those reported under field conditions (Katan et al. 1976) (46–54 °C at 5 cm depth) which are known to be lethal or sublethal to many soil-borne fungi. Effectiveness of solarization is clearly related to soil temperatures attained during the process. Daily temperatures reached at the 5-cm depth in our study usually lasted several hours and were greater than or close to the thermal death temperature reported in the literature. *Pythium ultimum* does not survive in vitro after treatment at 46 °C for 20 min (Pullman et al. 1981 a). These lethal conditions have frequently been achieved during the first 4 weeks of treatment under both single and double layers of plastic film (3.75 h daily exposure on average and 5 periods of a maximum of 5 consecutive days) near the soil surface at the St. Jean nursery. These results are consistent with those reported in the literature (Stapleton and De Vay 1981; Baker and Cook 1974) and may explain the good control of the disease in the upper layer. Our results related to *Rhizoctonia solani* are not in agreement with those reported by some authors (Annesi and Motta 1994). *Rhizoctonia solani* is injured when the temperature reaches 45 °C or after 5 min at 50 °C (Pullman et al. 1981a) but *Pythium ultimum* is reported to survive greater heat dosages than *R. solani* (Pullman et al. 1981 b). *R. solani* was not controlled under a single layer of plastic at the St. Jean nursery in spite of temperatures above 45 °C sufficient to control *Pythium* spp. *Fusarium* spp. remained unaffected by solar heating of soil at both nurseries. The resistance of *Fusarium* spp. to temperatures above 46 °C is in agreement with the results of Croghan et al. (1984) and observations of Ben-Yephet et al. (1987), who consider *F. oxysporum* to be among the species of plant pathogens which are more tolerant to soil solarization.

Although temperatures of up to 46 °C were attained at the St. Laurent nursery, *Pythium* spp. was not controlled after 7 weeks. The decline in the viability of soil-borne microorganisms during solarization depends on both the soil temperature and exposure time, which are inversely related. At St. Laurent soil temperatures exceeded 45 °C only twice, and lasting only 2 h on average. Temperatures recorded near the soil surface fluctuated within the range

of sublethal temperatures for damping-off fungi. Under such conditions the treatment needs more time to have a detrimental effect on *Pythium* spp. It was shown that solar heating had a pronounced effect on disease severity after only 9 weeks at the St. Laurent nursery.

As soil temperatures become cooler with increasing depth, pathogen control decreases. The levels reached at the 15-cm depth at the St. Jean nursery (up to 42/44 °C) are often considered to be sublethal. These marginal temperatures affected the survival of *Pythium* spp. at the St. Jean nursery, resulting in a slight decrease in disease incidence after 4 weeks. But reinfestation could not be avoided after 7 weeks. This indicates that sublethal temperatures have not been maintained long enough to become lethal (De Vay 1990).

Although plastic films were partially (double layer) or totally (single layer) torn off by the last sampling date, a significant decrease in disease severity occurred. This reduction was concomitant with very frequent isolations of *Trichoderma* spp. The association of *Trichoderma* spp. with a reduction in damping-off incidence was also observed in steamed and control plots. The above considerations strongly suggested that bio-control processes involving *Trichoderma* spp. are induced or enhanced after solarization. These results are consistent with those obtained by various authors. Stapleton and De Vay (1984) consider that among the microorganisms which survive the solarization process and contribute to the suppressiveness of soil, *Trichoderma* sp. and *Talaromyces flavus* are representative of the main fungal antagonists which inhibit the development of pathogenic fungi.

Conclusions

Soil solarization provides a suitable method for control of *Pythium* spp., causing damping-off in forest nurseries under the climatic conditions prevailing in France. Since the suppressiveness of the upper layer of soil lasted several weeks after solarization, the best benefits of solar heating would be expected for an autumn-sown tree seedling crop suitable for a limited number of species in a few places located in the southern part of Europe. Since the method failed to control pathogens in the deeper layers, it is unlikely to control root diseases. Moreover, soil solarization does not destroy some beneficial microorganisms including biological control agents such as mycorrhizal fungi (Stapleton and De Vay 1981) and consequently contributes significantly to the principles of sustainable production. Although direct thermal effects on pathogens are probably the major factor involved in the control of soil-borne disease, soil solarization may affect other components involved in plant disease such as surrounding microorganisms and the physicochemical soil environment. These changes are related to induced suppressiveness from which long-term effects can be expected and further investigations are needed.

Although significant reduction in survival of *Pythium* species could be discerned near the soil surface, survival still occurred at deeper layers. This degree of control would have no ecological significance because of the risk of mingling microbial populations between different depths if the nursey beds are cultivated before sowing, except if biological control is working well.

Within the pathogens involved in damping-off disease in forest nurseries, some of them are less heat sensitive than others such as *Fusarium* species. Their control requires optimal conditions of temperature and moisture during solarization, or involves additional practices such as organic amendments. The use of broader continuous tarp will likely increase the effectiveness of soil solarization as emphasized by Annesi and Motta (1994).

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