



SOLARIZATION FOR THE CONTROL OF SOIL-BORNE PATHOGENS IN FOREST NURSERIES IN TEMPERATE CLIMATE

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ABSTRACT

Soil solarization was carried out at three eucalyptus forest nurseries in Argentina in order to evaluate an alternative method for controlling damping-off of seedlings. The experiments were performed at forest nurseries, located in a temperate climate (province of Buenos Aires), infected with *Fusarium* (mainly *F. oxysporum*) and *Pythium* species, using single and double layers of transparent polyethylene film during summer months (January and February). Effectiveness of the treatments was evaluated using a biological assay enabling assessment of soil inoculum potential.

Soil temperatures reached during solarization at the three nurseries were 44, 45 and 49°C, respectively. The soilborne pathogens were controlled within 4 weeks using a single layer in one of the nurseries and using a double layer in the two others. Although the temperatures generated in the soil were not very high, the results suggest that the antagonistic microflora present in the native virgin soils in Argentina and/or biological processes may have contributed to the control of these pathogens.

RIASSUNTO

LA SOLARIZZAZIONE PER LA DIFESA DAI PATOGENI DEL TERRENO NEI VIVAI DI PIANTE FORESTALI IN CLIMA TEMPERATO. La solarizzazione del terreno è stata eseguita in tre vivai di eucalipto in Argentina allo scopo di valutare un metodo alternativo per il controllo della moria dei semenzali. Gli esperimenti sono stati condotti in vivai siti in una zona a clima temperato (provincia di Buenos Aires), in terreni infetti da specie di *Fusarium* (principalmente *F. oxysporum*) e *Pythium*, usando film di polietilene trasparente a singolo o doppio strato durante i mesi estivi (Gennaio e Febbraio). L'efficacia dei trattamenti è stata valutata usando un saggio biologico che

permette la stima del potenziale di inoculo del terreno. Le temperature del suolo raggiunte nei tre vivai sono state rispettivamente di 44, 45 e 49°C. I patogeni tellurici sono stati controllati con quattro settimane di trattamenti usando un film a singolo strato in uno dei vivai ed uno a doppio strato negli altri due. Sebbene le temperature generate nel terreno non siano state molto alte, i risultati suggeriscono che la microflora antagonista presente nel terreno naturale argentino e/o i processi biologici possono aver contribuito al controllo di questi patogeni.

Key words: *Eucalyptus*, soil solarization, damping-off, *Fusarium*, *Pythium*.

INTRODUCTION

The genus *Eucalyptus* is one of the major exotic species planted in Argentina due to its fast growth and to the fact that this species grows over a wide range of soil and climatic conditions. The most important species include *E. grandis* and *E. saligna*, found in hot and humid regions, and *E. camaldulensis*, *E. viminalis* and *E. globulus* found in temperate areas of the province of Buenos Aires. Apart from the choice of species adapted to specific sites, reforestation depends upon good seedling stock. Soilborne nursery pests including weeds and plant pathogens are the major cause of decreasing quality and quantity of forest nursery production. Among the most widespread and destructive diseases of nursery tree seedlings, damping-off is the most important. It kills seedlings either before or immediately after emergence of the plant. Several fungi are responsible for damping-off but the most damaging agents are *Pythium* spp., *Fusarium* spp. and *Rhizoctonia solani* (Sutherland and VanEerden, 1980). According to Sampangi (1985) and Camporota and Perrin (1994) disease severity and occurrence of a particular causal agent vary greatly depending on soil characteristics and local conditions. Frezzi (1947), Sharma and Mathew (1990), and Arentz (1991) reported *Pythium* spp. and *Fusarium*

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spp. as the main pathogens responsible for severe damage in eucalypt seedlings in temperate regions, whereas in areas of high temperature and humidity *Fusarium* spp. and *R. solani* are the major pathogens.

In order to control damping-off and weeds, pre-sowing fumigation with chemicals such as methyl bromide is used on a regular basis at most forest nurseries in Argentina. Alternatively, acidification with sulphuric acid, formaldehyde treatment and hand weeding are also carried out in some nurseries. Although widely used, soil chemical fumigation is becoming controversial in recent years because of concern about environmental contamination and groundwater pollution as well as disruption of the biological balance of microorganisms in the soil (James, 1989).

Solar heating of soil is a method for soil disinfection developed in Israel and tried and adapted in many countries since 1976 (Katan *et al.*, 1976). It is achieved by covering the soil with a transparent polyethylene film for one month or more during the hot season thereby heating the soil and killing the pathogens (De Vay, 1990). Soil is irrigated before mulching in order to increase thermal sensitivity of soilborne microflora and fauna as well as to improve heat conduction in the soil (Katan, 1984). The decrease of the pathogen population during solarization depends on soil temperatures reached during the process, soil moisture content and exposure time. For mesophilic organisms a temperature of about 37°C is critical. Exposure time critical to pathogens at about 37°C is reported to be from 2 to 4 weeks (De Vay, 1990). With increasing temperature less time is required to reach a lethal combination of time and temperature.

In addition to direct thermal death, the effect of sub-lethal heating results in delayed propagule germination and reduced growth rates. Biological control induced by the effect of sub-lethal heating has also been involved in the efficacy of solar heating (Katan *et al.*, 1984; Greenberger *et al.*, 1987).

Soil solarization was found to be effective in various regions of the world in many agricultural crops (Stapleton and DeVay, 1983; DeVay, 1990) but few data are available in forest nurseries (Old, 1981; Annesi and Motta, 1994; Le Bihan *et al.*, 1997).

In Argentina, the effectiveness of solarization has been tried in horticultural areas but this practice has not been applied in forest tree nurseries.

The purpose of this study was to evaluate solar heating of soil for effectiveness in controlling soilborne pathogens in three nurseries in the province of Buenos Aires, Argentina.

MATERIALS AND METHODS

Solarization experiments were carried out during summer months in three forest nurseries in the province of Buenos Aires, Argentina in 1995. The solarization treatment began on January 17th, 18th and 19th, in Saladillo, Tandil and Miramar nurseries respectively.

Geographical location of the nurseries and soil properties are described in Table 1. The standard method of growing eucalyptus seedlings varies in each nursery; consequently the experimental design was different (Table 2).

Table 1. Geographical location, climatic conditions and soil properties of forest nurseries in the province of Buenos Aires, Argentina.

	Saladillo	Tandil	Miramar
Latitude	35° 40' S	37° 00' S	38° 20' S
Elevation (m)	42	200	17
Mean annual rain	850 mm	800 mm	860 mm
Mean annual T°	16.0°C	15.0°C	14.0°C
Mean T° (January)	22.5°C	21.0°C	19.5°C
Soil texture	sandy loam	clay loam	sandy loam
Sand (%)	64	23	54
Silt (%)	27	43	35
Clay (%)	10	34	11
Organic matter content (%)	3.99	3.09	3.71
pH (paste)	7.5	5.6	8.2

Solarized plots were watered until a water film was observed on the surface, and then covered with a 80 mm thick transparent polyethylene film, placed either over the nursery bed lain flat against the soil (single layer) or/and raised as a tunnel 40 cm high over metal structures (double layer) (Table 2). Three replications of each treatment were done in a completely randomized block design. Soil temperatures were recorded daily with the appropriate soil thermometers placed at 5 cm depth (Table 3).

FIELD SAMPLING

In order to assess the effect of soil treatment on soil inoculum potential, samples were collected after 4 and 6 weeks of solarization. Soil samples consisted of 8-10 subsamples collected from either the individual plots or the containers at 0-5 cm depth. The subsamples were pooled together to provide one single sample for each individual plot. Soil samples were passed through a 4 mm sieve and then stored at 4°C prior to biological assays.

Table 2. Standard method of growing eucalypt seedlings and experimental design.

	Saladillo	Tandil	Miramar
Substrate	natural soil	natural soil	natural soil
Method of growing eucalypt seedlings	sowing in beds (bare-root)	sowing in containers (300 cm ³)	sowing in beds (bare-root)
Plot size (m)	2.5 x 1	200 containers 1 x 0.5	5 x 1
Treatments	uncovered single layer double layer	uncovered double layer double layer	uncovered single layer
Temperature record depth	5 cm	5 cm	5 cm
Sampling dates	15/2 1/3	16/2 2/3	17/2 3/3
Exposure time to solar heating	4 weeks 6 weeks	4 weeks 6 weeks	4 weeks 6 weeks

Table 3. Total number of days at maximum temperature recorded at 5 cm depth after 4 weeks (4w) and 6 weeks (6w) of solarization in the three nurseries under the double layer film.

Temperatures	Saladillo 4w/6w	Tandil 4w/6w	Miramar 4w/6w
40-42°C	10/11	6/9	2/3
42-44°C	17/19	4/4	3/3
44-46°C	3/3	4/4	2/2
46-48°C	–	3/3	1/1
48-49°C	–	2/2	–

Bioassay for estimation of soil inoculum potential.

The effect of solarization on soilborne pathogens was assayed using a standard bioassay described by Bouhot (1975a; 1975b), and adapted for eucalyptus seedlings and performed at the greenhouse, that estimates soil inoculum potential.

Soil inoculum potential defines the ability of a soil to induce disease caused by soilborne pathogens. The method is based on inoculation of 7-8 day-old seedlings of *E. viminalis*, corresponding to the period of greatest susceptibility to damping-off, with the test soil. *E. viminalis* was chosen as a bait plant. Seeds were surface disinfested with sodium hypochlorite (1%) for 10 minutes rinsed three times in sterile water and plated for germination. Ten seedlings were transplanted into plastic pots containing a mixture of perlite-vermiculite (50:50). The collar of eucalyptus seedlings was then inoculated by cov-

ering the substrate with a 1 cm layer of the soil sample.

Damping-off occurrence was monitored daily during one month and disease incidence 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 dipping in 30% H₂O₂ for 30 seconds, rinsed three times in distilled sterile water and plated on malt agar acidified with citric acid (250 ppm) for isolation and identification of pathogenic fungi. Colonies of *Fusarium* species were transferred to potato-dextrose agar (PDA). Each single spore culture was incubated 7-14 days at 22°C under fluorescent lamps supplemented with UV light with a 12 h photoperiod. The cultures were examined microscopically and identified according to the system of Booth (1971) and Nelson *et al.*, (1983). Molecular techniques (PCR/RFLP) were used for the identification of *Pythium* species (Mugnier and Grosjean, 1995).

Field assay to estimate the control of diseases in nurseries. In order to assess the effectiveness of soil solarization on seedling quality, seeds of *E. viminalis* were sown at the three nurseries in the solarized plots and/or in the containers and in the untreated control ones. Three months later seedlings were lifted and 20 subsamples for each treatment were monitored randomly to evaluate the sanitary condition using a stereoscopic microscope.

Seedlings with necrotic symptoms were surface disinfected and fragments of roots and collar were plated separately on PDA to determine the presence of soilborne pathogens.

Statistical analysis. Data taken as percentage from the bioassay for soil inoculum potential 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 STATGRAPHICS program.

RESULTS

Temperatures reached during the process are shown in Table 3 indicating the total number of days with maximum temperatures recorded after 4 and 6 weeks of solarization.

At Saladillo nursery, maximum soil temperatures reached during this period at 5 cm depth were 44°C under the double layer film.

At Tandil nursery, maximum soil temperatures reached in the plastic containers at 5 cm depth using a double layer film were 49°C. These temperatures were reached 2 days after setting up.

At Miramar nursery, maximum temperatures were 46°C under the double layer film.

Assessment of soil inoculum potential (SIP) at 0-5 cm depth. The results are presented in Fig. 1 and in Fig. 2.

Saladillo nursery. Four weeks after solarization, SIP in non solarized plots was 30%. The fungi isolated from diseased seedlings were mainly *P. torulosum* (66.6%). Other fungi isolated were *Penicillium* sp. *Rhizopus* sp. and *Alternaria* sp. (33.3%).

Solarization had significantly ($P < 0.05$) reduced the percentage of damped-off seedlings with a single or double layer film after 4 weeks of treatment. Soil assays showed no significant differences between the solarized plots using either a single or a double layer.

After 6 weeks of solarization, SIP was 40%. The main pathogens isolated from diseased seedlings were *Fusarium* and *Pythium* species. Among *Fusarium* species, *F. oxysporum* was the main one involved (76.2%) and *F. equiseti* was less frequently isolated (4.7%). Among *Pythium* species, *P. torulosum* was the most prominent one (14.2%). Other fungi corresponded to 4.7%. The assays showed also significant reductions ($P < 0.05$) in SIP in solarized plots compared to untreated control plots. No significant difference was apparent between the plots under either the single or the double layer film at 5 cm depth.

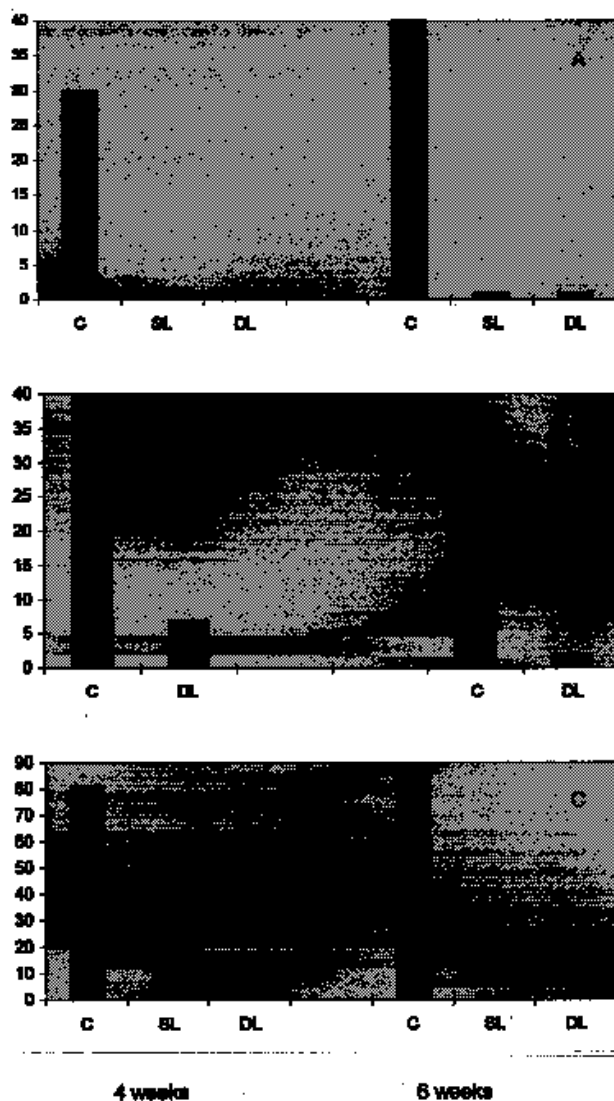


Fig. 1. Soil inoculum potential at the three nurseries expressed as seedling mortality (%) in control (C) plots compared to single layer (SL) and double layer (DL) after 4 and 6 weeks solarization treatment. **A:** Saladillo nursery; **B:** Tandil nursery; **C:** Miramar nursery.

Tandil nursery. Four weeks after solarization treatment, SIP in control plots was 40%. *F. oxysporum* was the main pathogen isolated from diseased seedlings (71.4%). Other fungi isolated from diseased seedlings were *F. solani* (7.2%), *P. torulosum* (14.2%) and *P. ultimum* (7.1%).

Solarization treatments using a double layer film showed significant ($P < 0.05$) reductions in damped-off seedlings to 7% in the plastic containers at 5 cm depth after 4 weeks compared to the untreated control plots.

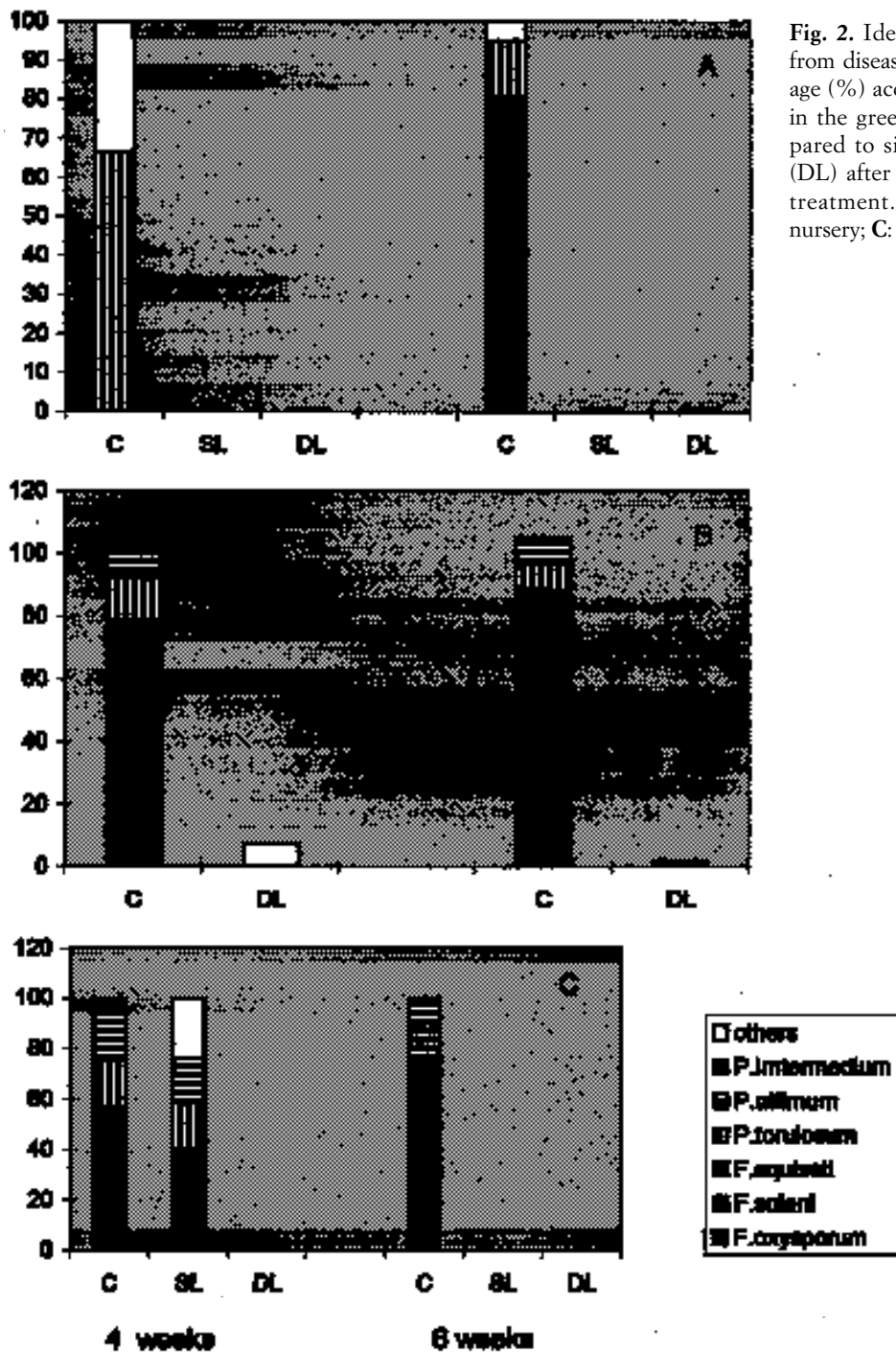


Fig. 2. Identification of main fungi isolated from diseased seedlings expressed in percentage (%) according to the bioassays performed in the greenhouse in control (C) plots compared to single layer (SL) and double layer (DL) after 4 weeks and 6 weeks solarization treatment. A: Saladillo nursery; B: Tandil nursery; C: Miramar nursery.

After 6 weeks of soil solarization, SIP was 34%; The fungi isolated from diseased seedlings were *F. oxysporum* (83.3%), *P. ultimum* (8.3%) and *P. torulosum* (8.3%); the soil assays from the two treatments (solarized and untreated control) were also significantly different ($P < 0.05$) at the same sampling depth ranges.

Miramar nursery. The experiment carried out at this nursery showed that SIP in control plots was very high. After 4 weeks of solarization, the percentage of damped-off seedlings in the untreated plots was 81%. *Fusarium* species were the main pathogens involved. *F. oxysporum* was the most predominant (34.8%) while *F. equiseti* (13%) and *F. solani* (8.7%) were also pre-

sent. Damping-off was also induced by *Pythium* species among which *P. ultimum* (24%) and *P. torulosum* (16%) were the main species responsible for the disease. *P. intermedium* was also isolated. There were significant differences ($P < 0.05$) between the control plots and the solarized plots using either a single or a double plastic film after 4 weeks of treatment while there were no significant differences between samples from the single or the double layer plots.

After 6 weeks of solarization, SIP was 89%. Main fungi isolated from diseased seedlings were *F. oxysporum* (65%), *F. equiseti* (10%), *F. solani* (10%) and *P. ultimum* (22.5%). Damped-off seedlings were also significantly reduced ($P < 0.05$) in each of the solarized treatments compared to the untreated one. No significant differences were observed between the two solarized treatments.

At this site, while using a single layer film, only *P. intermedium*, *F. solani* and *F. equiseti* were controlled after 4 weeks of treatment. Moreover, the control of *P. ultimum*, *P. torulosum* and *F. oxysporum* was achieved after 6 weeks of treatment.

Control of diseases in the nurseries. At the end of the growing season (3 months after the treatment), the eucalyptus plants grown in the control plots in the Saladillo nursery showed necrotic symptoms; in contrast, seedlings grown in the solarized plots did not show any necrotic symptoms. No pathogenic fungi were isolated from the roots and collars of the plants taken from the solarized plots. On the other hand, roots taken from the untreated control plots were colonized by *Pythium* spp.

In the Tandil nursery, the plants grown in the untreated containers showed necrotic symptoms meanwhile the plants taken from the solarized containers did not show root infections. No pathogens could be detected in the root samples throughout the treated containers. In contrast, root segments sampled from the untreated containers were colonized by *F. oxysporum*.

At the Miramar nursery, 25% of the seedlings monitored from solarized beds either using a single or a double layer film showed root infections. The pathogen isolated from the roots fragments was *F. oxysporum* (100%). Infection of the root tissue from plants tested in the control beds was by *F. oxysporum* (53%) and *F. solani* (47%).

DISCUSSION

From an etiological point of view, the incidence of damping-off at the three nurseries induced mainly by species of *Fusarium* and *Pythium* is in accordance with

other surveys of damping-off carried out by several authors around the world (Sutherland and Van Eerden 1980; Perrin and Sampangi 1986).

The results show that soil solarization can decrease SIP within 4 weeks using a double layer film in the climatic conditions found at the three nurseries located in the province of Buenos Aires. Maximum soil temperatures reached in the solarized plots at the three nurseries under the double layer polyethylene film were within the range of temperatures mentioned by several authors who reported good control of soilborne pathogens (Katan, 1986; De Vay, 1990; Gonzalez Torres *et al.*, 1992).

The single layer was also effective at eliminating the main pathogens involved in damping-off. Even though soil temperatures under the single layer film were not recorded. Ben-Yephet *et al.* (1987) cited differences of 3°C reached under a single layer or double layer of clear plastic film.

We should also consider that the plots used in our study were too narrow or too small and solarization is less effective with small plots due to the border effect. Hence, it might be possible to improve the results by using larger plots.

Control of *Pythium* species is in accordance with previous reports (Old, 1981; Pullman *et al.*, 1981; Barbercheck and von Broembsen, 1986; Juarez-Palacios *et al.*, 1991; Le Bihan *et al.*, 1997) that have shown that *Pythium* species are particularly susceptible to high soil temperatures. Duff and Connelly (1993) cited temperatures of 41-46°C over a period of 2-6 weeks for the control of these species.

Solarization was also effective in controlling *Fusarium* species in the present experiments even though these species are reported to be among the most resistant pathogens under laboratory conditions (Bollen, 1983), Ben-Yephet *et al.* (1987) consider *F. oxysporum* to be the species of plant pathogens which are more tolerant to soil solarization. De Vay (1990) reported good control of *F. oxysporum* f.sp. *vasinfectum* with maximal temperatures around 50°C at 5 cm depth. Failure in other situations (Old, 1981) indicates that there might be differences in lethal temperatures of different species of *Fusarium* or *formae speciales* as reported by Bollen (1983). Differences in the results with *F. oxysporum* in studies carried out in Australian nurseries by Old (1981) and by the same authors in French nurseries (Le Bihan *et al.*, 1997) may be explained by the variation in the temperature sensitivity of isolates. The temperatures attained in this study were not very high compared to those attained in the studies above mentioned. The control of *Fusarium* species achieved during our trials in Argentina suggests that factors other than soil temperatures may have contributed to the loss of viability of

this pathogen. A wide spectrum of beneficial thermophilic and thermotolerant fungi, bacteria and actinomycetes antagonistic to pathogens found in the rhizosphere of Argentine native field soils could have been involved in the induced suppressiveness phenomenon. This has also been cited by Chen *et al.* (1991) and Duff and Barnaart (1992) who suggested that bacteria antagonistic to *F. oxysporum* may be implicated in controlling these species in solarized soils. On the other hand, Barbercheck and von Broembsen (1986) and Kaewruang *et al.* (1989) suggested that ethylene, carbon disulphide and carbon dioxide could be implicated in the control of these pathogens. As field soils in these nurseries have a relatively high organic matter content, volatile gases could have been alternatively implicated.

Solarization was also effective for the control of root necrosis of the plants grown in the treated plots and containers in the Saladillo and Tandil nurseries respectively. The benefits of solarization are even better because the method of growing eucalypt seedlings in these nurseries allows an autumn-sown tree seedling-crop. Moreover, at the Miramar nursery, the solarization treatment did not work as well as in the other nurseries and the first symptoms of root necrosis appeared 3 months later. The presence of *F. oxysporum* may have been due to fungus recolonization from the subsoil or due to too high a pathogen inoculum present in the soil at this experimental site. Alternatively, the high pH value at this nursery may have played a major role. At high pH values several nutrients become unavailable and seedlings are weakened by nutritional problems and rendered more susceptible to fungal attacks as stated by Sharma *et al.* (1984).

Finally, our results show that soil solarization appears to be a suitable treatment for disinfecting soil nursery beds and containers under the climatic conditions found at the three nurseries located in Buenos Aires, Argentina

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