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Use of soil solarization to improve growth of eucalyptus forest nursery seedlings in Argentina

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Abstract. Damping-off and root rot are major diseases affecting seedlings of *Eucalyptus* species in forest nurseries in temperate regions in Argentina. The most common fungi associated with these diseases and affecting the vigor of the root system are *Fusarium* and *Pythium* species. Two forest nursery experiments were conducted in the province of Buenos Aires, Argentina, to determine the effect of soil solarization on growth of *Eucalyptus viminalis* seedlings and relate this effect to the presence of pathogenic and native ectomycorrhizae populations in roots and nutrient availability in soil. Changes in populations of soilborne pathogens were determined by a bioassay that relates their potential to induce disease. Changes in native ectomycorrhizae were assesed by measuring colonization levels in roots. Nutrient availability was determined by the amount of nitrates released by solarization. Solar heating decreased pathogenic and ectomycorrhizal inoculum potential and increased soil nitrates. Seedling growth in solarized seedbeds may be related to a low initial pathogenic population and/or to increases in nitrate availability. Solarization may induce soil suppressiveness against re-establishment of major seedling pathogens in treated soils.

Palabras clave: incremento nitratos en suelo, potencial de inóculo patógeno, población de ectomicorrizas nativas, damping-off

Resumen. El damping-off y la podredumbre de las raíces son las enfermedades más importantes que afectan a las plantas de differentes especies de *Eucalyptus* en viveros ubicados en las regiones templadas de la Argentina. Los hongos más comúnmente asociados con estas enfermedades y que afectan el vigor del sistema radicular son diferentes especies de *Fusarium* y *Pythium*. En dos viveros forestales localizados en la provincia de Buenos Aires se llevaron a cabo diferentes ensayos con el objeto de determinar el efecto de la solarización sobre el crecimiento de las plantas de *Eucalyptus viminalis* y paralelamente relacionar este efecto con la presencia de los patógenos, la población ectomicorrícica nativa en las raíces y la disponibilidad de nutrientes en el suelo. Los cambios en la población patógena fueron determinados a través de un ensayo biológico que relaciona la presencia de patógenos con la inducción a la enfermedad. Los cambios en la población ectomicorrícica nativa fueron evaluados mediante la medición del porcentaje de colonización en las raíces. La disponibilidad de los nutrientes se determinó a través de la cantidad de nitratos liberados después del tratamiento. La solarización disminuyó la presencia de patógenos, la población ectomicorrícica natural y produjo un incremento de los nitratos en el suelo. El crecimiento de las plántulas en los almácigos solarizados pudo estar relacionado con la disminución del potencial de inóculo patógeno y/o con el incremento en la disponibilidad de nitratos en el suelo. La solarización favoreció la supresión de suelos retardando la recolonizacion de los principales patógenos en los suelos tratados.

Introduction

In Argentina, seedling stem condition, size, foliage abundance and health are the main criteria used in judging seedling quality. Less attention is paid to characteristics such as root quality. However, once seedlings are shipped from the nursery and outplanted in the field the roots are affected by an array of complex environmental and biotic conditions (Chavasse, 1980).

Eucalyptus seedlings are one of the major exotic species planted in Argentina as they grow fast over a wide range of soil and climatic conditions. *E. grandis* and *E. saligna* are cultivated in the hot and humid region whereas *E. viminalis*, *E. globulus* and *E. camaldulensis* in the temperate region.

Apart from the correct choice of species for specific sites, reforestation depends upon good quality seedlings. Damping-off and root rot are major diseases affecting young forest nursery seedlings of many plant species around the world and poor nursery practices such as continuous cropping favor these diseases. They are often caused by species of soil-borne *Pythium*, *Fusarium* and *Rhizoctonia solani* (Sutherland and VanEerden, 1980). *Pythium* spp. and *Fusarium* spp. are the main pathogens responsible for severe damage in eucalypt seedlings in temperate regions and *Fusarium* spp. and *R. solani* are the major ones in areas of high temperature and humidity (Frezzi, 1947; Sharma and Mathew, 1990; Arentz, 1991; Salerno, 1999). Other pathogenic fungi affecting eucalyptus seedlings in the subtropical areas are *Cylindrocladium* species (Figueiredo and Namekata, 1967, Reis and Hodges, 1975). *Phytophthora cinnamomi* is sometimes a root parasite causing dieback in *E. marginata* especially in areas with abundant moisture (Campbell and Hendrix, 1972).

In Argentina, Jauch (1943) has recorded *Cylindrocladium scoparium* on five species of *Eucalyptus* spp., Frezzi (1947) has reported *Pythium ultimum* and *Rhizoctonia solani* as the most important fungi, followed by *Fusarium* spp., *Phytophthora* spp. and *Sclerotium* spp. and Salerno et al. (1999) cited different *Fusarium* and *Pythium* species.

Seedlings are also dependant upon mycorrhizae for growth and survival as evidenced by the failure of nonmycorrhizal seedlings to survive when planted into soil lacking mycorrhizal fungi (Trappe and Strand, 1969). The presence and abundance of mycorrhizae have a major impact on root system health.

Soil fumigation especially with methyl bromide, is still a common nursery practice in Argentina, used to reduce damage from soilborne pathogens present either in the seedbeds or in containers, particularly fungi in the genus *Fusarium* (Williams, 1976). However methyl bromide also kills beneficial organisms (Munnecke et al., 1978; Ebben et al., 1983) such as mycorrhizal fungi and antagonists.

Following methyl bromide fumigation soil is soon reinvaded by microorganisms (Vaartaja, 1967; Danielson and Davey, 1969). However, a concern is the high number of asymptomatic seedlings with *Fusarium*-infected roots as they may suffer poor survival or growth, or both, when outplanted. A beneficial effect is that saprophytes often colonize fumigated soil at higher levels than pathogens (James and Gilligan, 1985).

Recent environmental concerns about using a toxic chemical has resulted in the search for alternative practices to methyl bromide soil fumigation (Fraedrich, 1993). One of the most promising alternatives is soil solarization which controls several pathogenic fungi (Pullmann et al., 1979; Katan, 1981; De Vay, 1991; Salerno et al., 1999). Solarization affects many soil microorganisms (Katan, 1987), but very little is known about its effects on mycorrhizal fungi. Soulas et al. (1997) reported that among soilborne microorganisms ectomycorrhizal fungi have low tolerance to soil solar heating.

Increased plant growth following solarization has also been observed (Chen and Katan, 1980). Many factors could contribute to this phenomenon, e.g. reduction in the number of soilborne pathogens, chemical factors including release of mineral nutrients and biological factors such as stimulation of beneficial organisms (Stapleton and De Vay, 1984). The increases in nutrient availability, particularly those tied up in the organic fraction (NO_3-N and P), are another advantage resulting from solarization and may provide the equivalent of a pre-plant fertilizer. These changes in solarized soils depend on soil type, organic matter, the extent of heating, amount of soil moisture during treatment and pathogen species.

The objectives of this study were to determine the effect of soil solarization on growth of *Eucalyptus viminalis* nursery seedlings and relate this effect to the presence of pathogenic and beneficial microorganisms on roots and nutrient availability in soils.

Materials and methods

Soil solarization experiments

This work was carried out over 6 weeks (summer months) at the Miramar and Saladillo forest nurseries. The forest nurseries were located at Saladillo (35°40′S latitude, 42 m asl) and Miramar (38°20′S latitude, 17 m asl) in the Province of Buenos Aires, Argentina. Miramar nursery is located about 2 km from the Atlantic Ocean coast. It is under the influence of a maritime climate, in which the diurnal temperature amplitude is not high. However, the area is submitted to rapid temperature fluctuations due to the ingressions of the air masses from different directions: cold and humid from the SE, cold and dry from the SW and hot from the N or NE. On the other hand, Saladillo nursery is located about 200 km from the coast so the maritime influence is less pronounced and amplitude is higher.

For solarization, the soil was watered to field capacity, and then covered with a single layer (SL) 50 μm thick transparent polyethylene film, placed either over the nursery bed flat against the soil or raised as a tunnel 30 cm high on metal supports (double layer, DL) according to Ben-Yephet et al. (1987). Untreated plots (control, C) were left uncovered. The plot area was 2,5 m^2 at Saladillo nursery and 5 m^2 at Miramar nursery. The experimental plots were arranged in a completely randomized block design with three replications. Maximum and minimum soil temperatures were recorded using soil thermometers placed at 5 cm depth under the double layer polyethylene film.

Soil samples (a composite of 5 or 6 subsamples) from the control plot soils at Miramar and Saladillo were sampled at the beginning of the experiment and analyzed for: pH (paste), soil texture (Bouyoucos method), organic carbon-organic matter (Walkley-Black method) and nitrates (phenoldisulphonic acid method) (Black, 1965).

At Miramar the sandy loam (USDA texture class) soil had a pH of 8.3 and contained 3.71% organic carbon, 6.4% organic matter and the nitrate content was 10 mg Kg^{-1}. At Saladillo soil pH was 7.5, the soil was a sandy loam with 4% organic carbon, 6.9% organic matter and the nitrates content was 43.2 mg Kg^{-1}.

Changes in populations of soilborne pathogenic fungi in the solarized plots were previously evaluated by determining soil inoculum potential (Salerno et al., 1999) using a modified standard bioassay (Bouhot, 1975a, 1975b; Le Bihan et al., 1997) adapted for *Eucalyptus viminalis* seedlings (Salerno et al., 1999). Soil inoculum potential relates to the potential for soilborne pathogens to induce disease. At Saladillo nursery, the untreated (non-solarized) seedbeds had a soil inoculum potential of 40% and the main fungi isolated from killed seedlings were: *Pythium* spp., *Fusarium oxysporum* Schlechtend.:Fr and *F. equiseti* (Corda) Sacc. At Miramar nursery the untreated seedbeds had a soil inoculum potential of 89%. The main pathogens isolated were *Pythium* spp. and *F. oxysporum*, *F. equiseti* and *F. solani* (Mart.) Sacc. At the end of the solarization treatment the solarized substrates at both nurseries had a soil inoculum potential of 0% (Salerno et al., 1999).

Plant growth response after solarization

Immediately following removal of the polyethylene film, seeds of *E. viminalis*, were direct sown in the seedbeds (300–500 plants/m^2). Three months after seed sowing five subsamples of ten seedlings (50 plants per treatment) were harvested from each treatment (C, SL, DL). The oven dry weight (80 °C for 72 hours) of the shoots (foliage and stem) and leaf area (cm^2/pl) were determined. The latter was determined using a portable leaf area meter (Model LI-3000, Li-Cor).

Presence of soilborne pathogens in E. viminalis root systems

The roots of 3-month-old-plants, from solarized and control seedbeds (SL, DL, C) were assessed for pathogenic fungi. Pieces (4 mm) of root tips and seedling root collar were washed in water, surface sterilized with 30% H_2O_2 and incubated on 2% potato dextrose agar (PDA) to determine presence of *Fusarium* and *Pythium* species. The *Fusarium* isolates were identified according to the system of Booth (1971) and Nelson et al. (1983). Morphology of reproductive structures, growth rates and colony morphology were used to identify the *Pythium* isolates (Frezzi, 1956; Van der Plaats-Niterink, 1981).

Greenhouse assays

Soil samples were collected after 45 days of solarization (end of the experiment) from solarized and the untreated plots to determine, (i) indigenous ectomycorrhizae present and (ii) nitrogen availability. Five to eight subsamples (5 cm depth) were combined into one 3 kg-sample per plot. The soil samples for ectomycorrhizae were passed through a 4 mm sieve and then stored at 4 °C until processed. The samples for nitrate analysis were stored at 4 °C after collection and processed immediately.

Effect of solarization on ectomycorrhizae survival

The effect of solar heating on survival of native ectomycorrhizae was determined by a bioassay that measures colonization levels of *E. viminalis* roots. The method is based on the visual estimation of the percentage of short, ectomycorrhizal root (Grand and Harvey, 1982). *Eucalyptus viminalis* seeds were sown on autoclaved vermiculite and grown in the greenhouse for 1 month. Ten seedlings were then transplanted to plastic containers (250 cm^3), previously filled with soil taken at 5 cm depth from either the solarized or control plots (Soulas et al., 1997). Mycorrhizal development was assessed 3 months after transplanting the plants (grown in the greenhouse under ambient conditions). The plants were carefully removed from the containers and their roots were washed clean with water. The roots were then arbitrarily divided into upper, middle and lower root sections. For each section, 100 root apices were examined, using a stereoscopic microscope, and the mycorrhizal root apices were recorded for each section. The survival of native ectomycorrhizae was calculated as the mean percent mycorrhizal roots in each treatment.

Effect of solar heating on nutrient availability
To determine the amount of soil nutrients released by solarization, soil samples were analyzed for available nitrogen. The amount of nitrates were determined colorimetrically by the phenoldisulfonic acid method and were expressed as $mg.kg^{-1}$.

Statistical analyses

The data were subjected to analysis of variance and the treatment means were compared using LSD ($P < 0.05$). To correct for heterogenity of variance the percentage data for the presence of ectomycorrhizae were arcsin-transformed prior to analysis. All analyses were performed using the STATGRAPHICS program (7.0).

Results

At Saladillo nursery, maximum soil temperatures were as high as 44 °C at 5 cm depth under the double layer polyethylene film. These temperatures were reached during three days between the third and fourth weeks after the beginning of the experiment (Figure 1a). At Miramar nursery, the maximal soil temperature was 45 °C and it was reached after three weeks during one day (Figure 1b). Soil temperatures under the single layer film were not recorded. Moreover, Ben-Yephet et al. (1987) cited differences of 3 °C reached under a single layer or double layer of clear plastic film.

Effect of solar heating on plant growth response
At Saladillo nursery, the dry weight of stems and foliage of *E. viminalis* seedlings increased significantly ($P < 0.05$) when grown in solarized soils

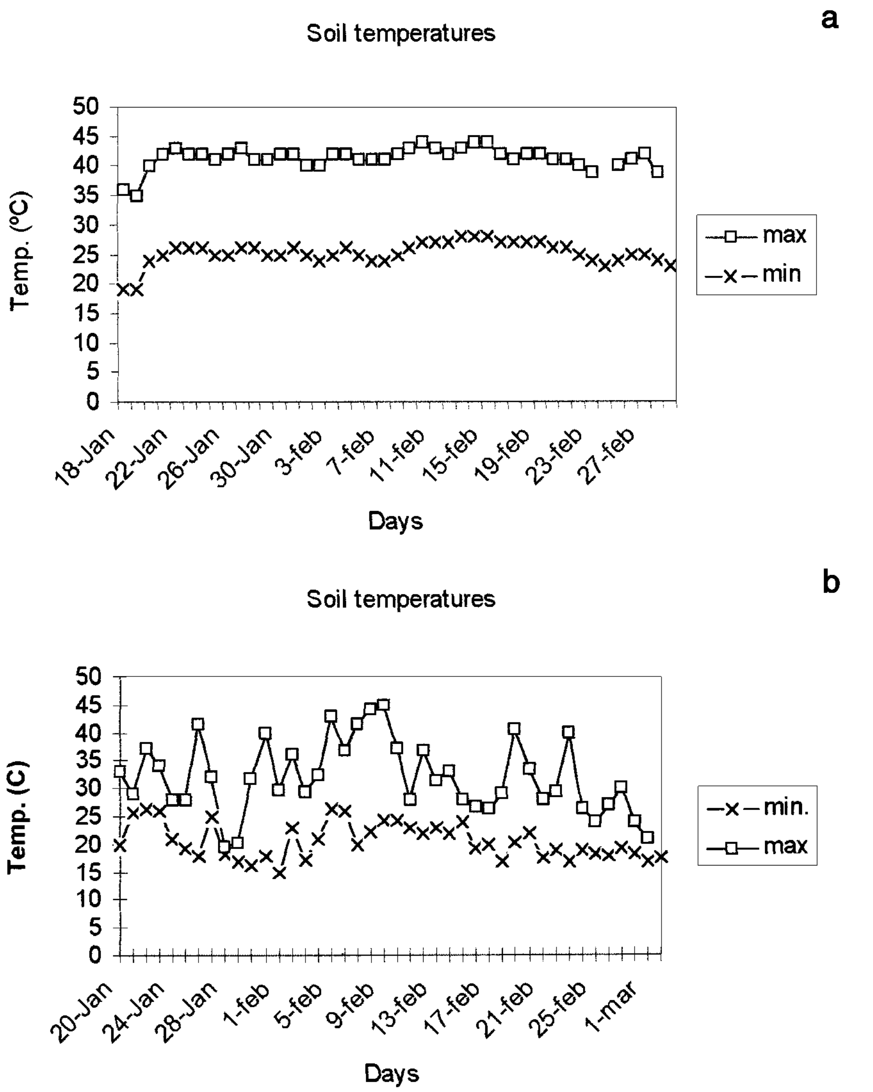

Figure 1. Maximum **and minimum** soil temperatures reached during solarization treatment at 5 cm depth under the double layer polyethylene film at (*a*) Saladillo nursery and (*b*) Miramar nursery.

with single and double layer polyethylene films compared to untreated soil. Leaf area was significantly ($P < 0.05$) greater for plants grown in treated soils. Also, there were significant differences ($P < 0.05$) in both parameters between single and double layer polyethylene film (Figure 2a and 2b).

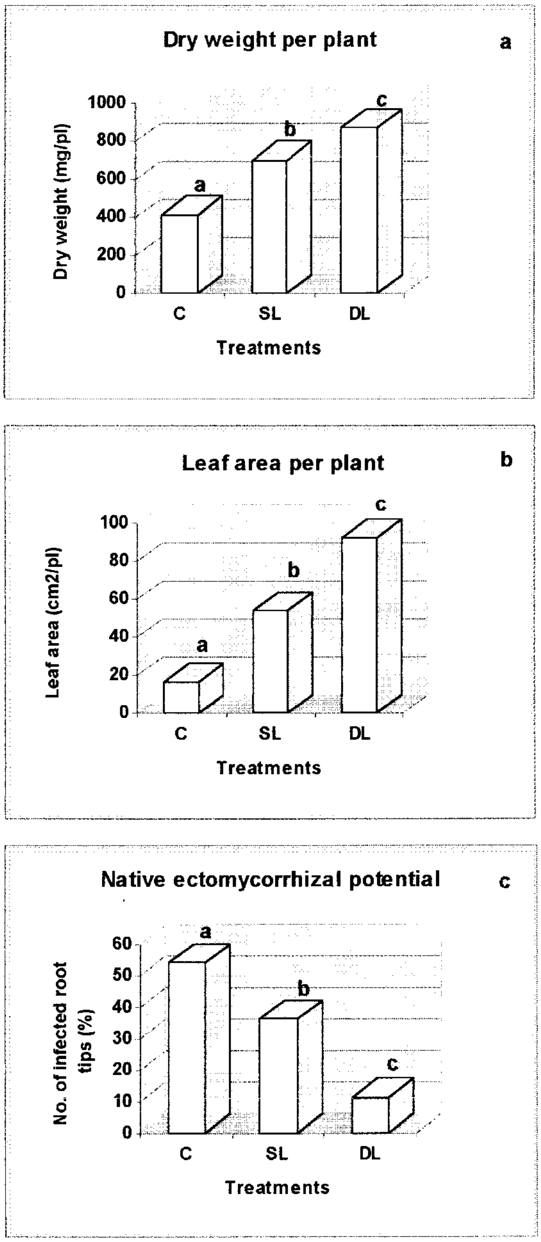

Figure 2. Effect of soil solarization at the **Saladillo** nursery on growth of *Eucalyptus viminalis* seedlings: (*a, b*) seedling growth expressed as dry-weight (mg/pl) and leaf area (cm^2/pl) per plant and (*c*) survival of native ectomycorrhizae expressed as the mean percent mycorrhizal roots Within parameters columns topped by the same letter are not significantly diifferent ($P < 0.05$). C: untreated seedbeds; SL: single layer plastic cover treatment; DL: double layer plastic cover treatment.

At Miramar nursery, seedling stem plus foliage weight and leaf surface area of plants from the solarized plots were significantly ($P < 0.05$) greater than plants from control plots. Both parameters were also significantly different ($P < 0.05$) between treatments (Figure 3a and 3b).

Soilborne pathogens in E. viminalis roots

At both nurseries necrotic lesions were observed on the roots of the three month old-*E. viminalis* seedlings grown in control soils. At Saladillo, *Pythium* species were isolated from roots while roots from the Miramar nursery yielded *F. oxysporum* and *F. solani*.

At Saladillo nursery the roots of seedlings from the solarized seedbeds did not show necrotic symptoms and no pathogens were isolated. However, at Miramar nursery, the roots of seedlings from the solarized seedbeds in both the single and double polyethylene film layer treatment were necrotic and *F. oxysporum* was isolated from roots.

Greenhouse assays

Effect of solar heating on ectomycorrhizae survival

At Saladillo nursery, *E. viminalis* seedlings grown in the untreated control soils had 54% of their short roots with mycorrhizae. Solarization decreased the abundance of ectomycorrhizae, i.e. seedlings from the single layer treatment had 36% of the short roots with mycorrhizae. This decreased to 10% in the double layer treatment (Figure 2c).

At Miramar nursery, the percentage of mycorrhizal short roots was 48% in control soils. Solar heating reduced the abundance of mycorrhizal short roots to 33% in the plants grown with the solarized soil with single layer and to 1% with double layer (Figure 3c).

Effect of solar heating on nutrient availability

Compared to the controls, nitrates increased significantly in solarized plots in both the single or double layer plastic cover treatment. At Saladillo, the amount of available nitrates was 43.2 $mg.kg^{-1}$ in control plots, 156.3 $mg.kg^{-1}$ in single layer and 208.5 $mg.kg^{-1}$ in double layer plastic cover treatments. At Miramar, the amounts were 10, 125.6 and 102.8 $mg.kg^{-1}$ respectively.

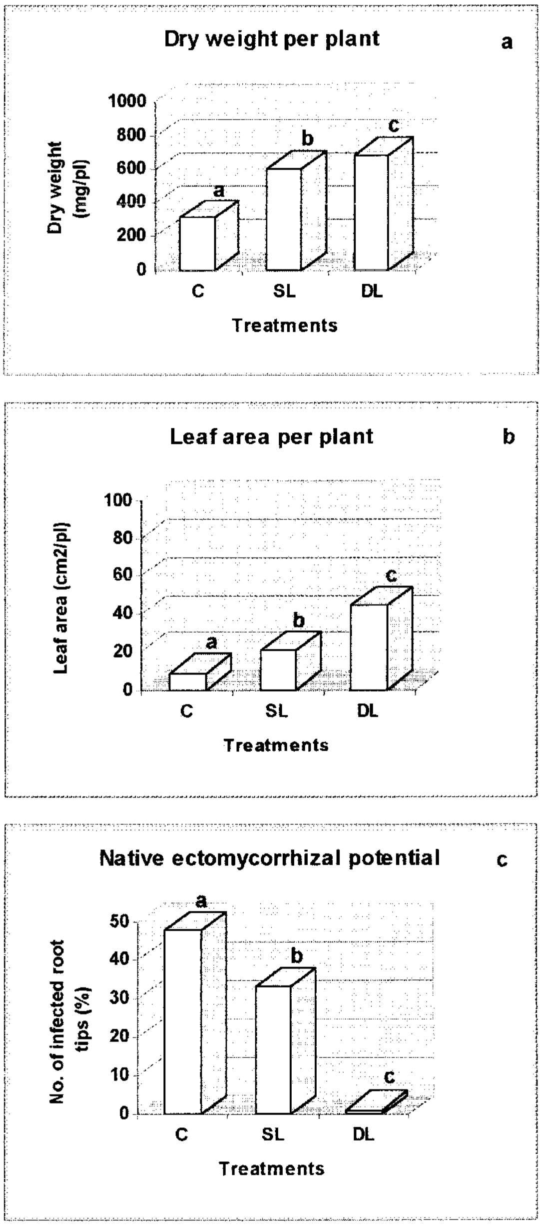

Figure 3. Effect of soil solarization at the **Miramar** nursery on growth of *Eucalyptus viminalis* seedlings: (*a, b*) seedling growth expressed as dry-weight (mg/pl) and leaf area (cm^2/pl) per plant and (*c*) survival of native ectomycorrhizae expressed as the mean percent mycorrhizal roots. Within parameters columns topped by the same letter are not significantly diifferent ($P < 0.05$). C: untreated seedbeds; SL: single layer plastic cover treatment; DL: double layer plastic cover treatment.

Discussion

Our finding that seedling weight and leaf surface area increase after soil solarization using a single or double polyethylene film layer agrees with earlier results (Chen and Katan, 1980; Stapleton and De Vay, 1982). The double layer treatment was the most effective at both nurseries. According to Stapleton and De Vay (1982), increased plant growth following soil solarization indicates that the treatment is successful.

Our results showed that the solar treatment was significantly better than the control treatments and the roots of seedlings grown in the solarized seedbeds at the two nurseries were relatively free from infection regardless of soil properties and the initial soil inoculum potential of the untreated control plots near the soil surface.

Increases in plant growth following solar heating may be related to soils that had initially a low soil inoculum potential and did not yield major root pathogens (*Pythium* and *Fusarium* species) at the two nurseries. Even though soil temperatures were not very high, maximum soil temperatures reached during the process were critical to both pathogens (Salerno et al., 1999). *Pythium* species are particularly susceptible to temperatures of 41 °C–46 °C reached over periods of 2–6 weeks. On the other hand, *Fusarium* species require higher lethal temperatures (Old, 1981) but factors other than soil temperatures may contribute to the loss of viability of this pathogen (Salerno et al. 1999).

Our study shows that nitrogen availability increased following soil solarization. As solarized soils contained increased amounts of available nitrogen, the increased growth of seedlings at both nurseries following solarization may result either from the reductions in *Pythium* and *Fusarium* populations and/or from the high nitrogen content of the soil. In fact, the effects of the two factors are confounded and their separation is not possible. According to Stapleton et al. (1985) when mineral nutrition is the limiting factor for plant growth, increases in growth result from control of less limiting factors such as soilborne diseases, only occur after fertilization. Stapleton and De Vay (1984) believed that increased plant growth following soil solarization resulted from not only the increased availability of some soil nutrients, plus the reduction in numbers of soilborne pathogens, but may also be due to population shifts in favor of beneficial soil microorganisms (antagonists), especially when crops are planted shortly after the plastic film is removed.

There was also a significant effect of solar heating on colonization levels of native ectomycorrhizae after 6 weeks of treatment at 5 cm depth in the seedbeds under the double layer film. Therefore, the mycorrhizal status of the soils at the time of sowing was very low. These results agree with those of Soulas et al. (1997) who reported that temperatures higher than 45 °C suppress ectomycorrhizal infectivity. Numerous plant pathogens, such as species of *Pythium* and *Fusarium* or certain endomycorrhizal fungi may survive higher heat temperatures (De Vay, 1991).

Regarding ectomycorrhizal fungi, Harley and Smith (1984) stated that these symbionts generally have negligible competitive saprophytic ability and consequently, the reinfestation from deeper layers occurs later than with most soil-borne fungi. In this way soil solarization allows controlled mycorrhization with selected ectomycorrhizal strains as was demonstrated by Soulas et al. (1997).

Mycorrhizal fungi and pathogens, were highly reduced or killed by solarization at 0–5 cm. Even though *Eucalyptus* seedlings roots go by far below 5 cm in three months, the feeder roots, which are those particularly specialized for absorption and normally mycorrhizal, are most abundant in the upper 10 cm of soil (Campbell and Hendrix, 1972). The conditions under which root growth takes place determine the number and nature of the feeder roots; under optimum soil conditions, the extent of fedder root tissue is much greater than that developing under less favorable conditions. The feeder roots are susceptible to damage by pathogenic organisms and the loss of a relatively large number of them reduces nutrient uptake (Campbell and Hendrix, 1972).

At Miramar nursery, there was some evidence of root disease following soil solarization using either the single or double layer polyethylene films. *Fusarium oxysporum* was isolated from root tips showing necrotic symptoms, probably because this fungus reinvaded treated soils. At Saladillo nursery, no pathogenic fungi were isolated from asymptomatic roots from solarized plots. In the untreated plots, fungi detected on necrotic root tips were *F. oxysporum* and *F. solani* in Miramar and *Pythium* spp. in Saladillo. This suggests that decreased plant growth in control plots may be related to root infections by these fungi. Feeder root losses may severely affect the plant's ability to absorb nutrients needed to produce food reserves.

Bassett (1969) reported that attacks by pathogenic fungi may not be apparent on stock sent out for planting but affected plants may later show severely reduced growth.

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