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Loss of viability of *Dematophora necatrix* in solarized soils

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Abstract

To study the relationship between temperature regimes and loss of viability of *Dematophora necatrix* in soil, two field experiments were conducted to determine the effectiveness of soil solarization on reducing the population of *D. necatrix* colonizing avocado root segments buried at a depth of 15–60 cm. Increase of maximum hourly temperatures attributable to soil solarization reached, depending on depth, 6.7–4.6 °C in unshaded areas and 3.9–1.5 °C for shaded areas in the first experiment (starting in early June, 1995). The better environmental conditions in the second experiment (starting by mid-July, 1995) led to higher temperature increases (8.6–5.6 °C, depending on depth) when solarization was conducted in unshaded areas. One, 4, 5 and 6 weeks of solarization were required to eliminate the viability of *D. necatrix* at 15, 30, 45 and 60 cm depths in the first experiment, whereas only 8, 10, 15 and 22 days of solarization were needed for the loss of viability of *D. necatrix* at the same depths in the second experiment. In shaded areas, however, soil solarization attained significant effectiveness at 15 cm depth.

Regression analyses of fungal viability (ln-transformed data) over accumulated temperature–time showed best fits when the minimum threshold temperature was 30 °C. Although eradication of *D. necatrix* in soil can be achieved down to 60 cm depth in solarized plots, and at 15 cm depth in unsolarized unshaded plots, the accumulation of temperature–time appeared less effective in reducing inoculum viability in the latter.

Introduction

Avocado root rot caused by *Phytophthora cinnamomi* Rands and avocado white rot caused by*Dematophora necatrix* Hartig (teleomorph: *Rosellinia necatrix* Prill.) are the most important diseases caused by soil fungi in avocado (*Persea americana* Mill.) orchards in south Spain (López-Herrera , 1989). Avocado orchards occupy ca. 8000 ha in this area, with a production of 45000 t of avocado fruit in 1997 (Anonymous, 1998).

Soil solarization has been effective in reducing the population of *P. cinnamomi* infesting soil in Israel (Pinkas and Katan, 1984) and in controlling *Phytophthora* root rot and *Rosellinia* root rot in established orchards, with a subsequent improvement of tree growth (López-Herrera et al., 1997, 1998). The thermal sensitivity of these pathogens has been studied (Khan, 1959; Barbercheck and von Broembsen, 1986; Sztejnberg et al., 1987; Juárez-Palacios et al., 1991). The thermal death of the population of an organism depends on both the temperature and the exposure time, which are inversely related (Katan, 1996). In addition, sublethal temperatures are able to weaken fungal propagules, decreasing their ability to withstand further biotic and abiotic stresses (Pullman et al., 1981; Lifshitz et al., 1983).

The aim of this work was to determine a relationship between the fluctuating temperatures reached in solarized soil and the reduced viability of *D. necatrix* at different soil depths after several periods of solarization.

This would enable the rate of heat mortality of this pathogen under different temperature regimes to be forecast.

Materials and methods

Two field experiments (I and II) were conducted in bare wet soil (clay, pH 8.2, organic matter 1.9%) which had been rototilled and irrigated by microsprinklers until achieving field capacity over the entire 60 cm soil layer. Eight nylon nets containing ten segments of avocado roots naturally infected by *D. necatrix*, corresponding to eight sequential samplings, were buried in soil at each of four depths (15, 30, 45 and 60 cm) in each replicated plot. Four replicates per treatment (i.e. solarized and unsolarized, both in shaded and unshaded locations) were set up according to a split-plot complete randomized block design with the solarization factor as a subplot of the main plots which were shaded or unshaded. Shading in the plots was achieved by covering them with a black shading (80%) net suspended 2 m above the soil surface. Afterwards, subplots (4×4 m) to be solarized were tarped with 75 µm thick transparent polyethylene on 12 June, 1995 (Experiment I) or 4 July, 1995 (Experiment II). Analyses of variance were performed with the data of the percentage of segments with viable inoculum and mean values were compared by general linear contrasts using the software package Statistix 4.1 (Analytical software, Roseville, MN, USA).

In Experiment I, inoculum samples were withdrawn at weekly intervals during the 8 week period of the solarization. An additional set of inoculum samples was kept for 8 weeks under optimal incubation conditions in the laboratory. According to the results obtained, the second experiment was established to further relate loss of inoculum viability and duration of solarization. Thermistors (model 107, Campbell Scientific) connected to a data logger were placed, for both experiments, at the same depths in each of the treatments considered. Soil temperatures were recorded at 5 min intervals and averaged for each hour. The viability of *D. necatrix* in the root samples was determined by the active mycelial growth of the fungus on the root segments incubated for 7 days at 22–24 °C in a moist chamber. The percentage of root segments showing mycelial growth was averaged for the four replications.

In Experiment II, temperature–time accumulated from the commencement of solarization until sampling, using 16 minimum temperature thresholds from 20 to 35 °C, were calculated by the equation $DH = \sum_i (T_h - T_t)_i$ where DH = temperature–time (degree–hours); T_h = hourly temperature; T_t = minimum threshold temperature; and i = each hour of the period considered in which $T_h > T_t$ (López-Herrera et al., 1994). Ordinary least squares regression analyses of the ln of the viability of *D. necatrix* over accumulated temperature (degree–hours) was determined for the different depths of burial in solarized and unsolarized plots.

Results

Experiment I

Maximum soil temperatures in unshaded solarized plots reached 39.3 and 33.2 °C at 15 and 60 cm depth respectively. These were 6.7 and 4.6 °C higher than in unsolarized plots. However, maximum temperatures in shaded solarized plots, ranging 31.7–27.4 °C, were only 3.9–1.5 °C higher than reached in the plots not solarized (Figure 1).

Results for infected-root segments sampled at 3, 6 and 8 weeks are given in Figure 2. The viability of *D. necatrix* in avocado root samples after 3 weeks burial differed significantly ($P < 0.001$) between treatments, locations and depths, as well as in their interactions. The viability of *D. necatrix* in samples buried at 15, 30, 45 and 60 cm in unshaded solarized plots was nil after 1, 4, 5 and 6 weeks of solarization, respectively. In contrast, fungal viability was 12.5–80% in unshaded unsolarized plots after 8 weeks of burial, depending on depths. The differences between treatments, locations and depths were statistically significant ($P < 0.01$) after 6–8 weeks of burial, as was the interaction treatments × locations ($P < 0.001$). Soil solarization had a small effect on shaded areas regardless of the depth of burial. Thus, viability of *D. necatrix* was over 60% in both unsolarized and solarized plots except at 15 cm depth in soil solarized for 8 weeks (Figure 2). Inoculum kept under laboratory conditions showed a high viability (95%) after 8 weeks incubation.

Experiment II

The maximum soil temperature in unshaded solarized plots reached 39.8–33.1 °C depending on depth. Temperature increases attributable to soil solarization were 8.6–5.6 °C. Maximum temperatures at 15 cm depth in shaded plots were 31.7 and 27.8 °C for solarized and

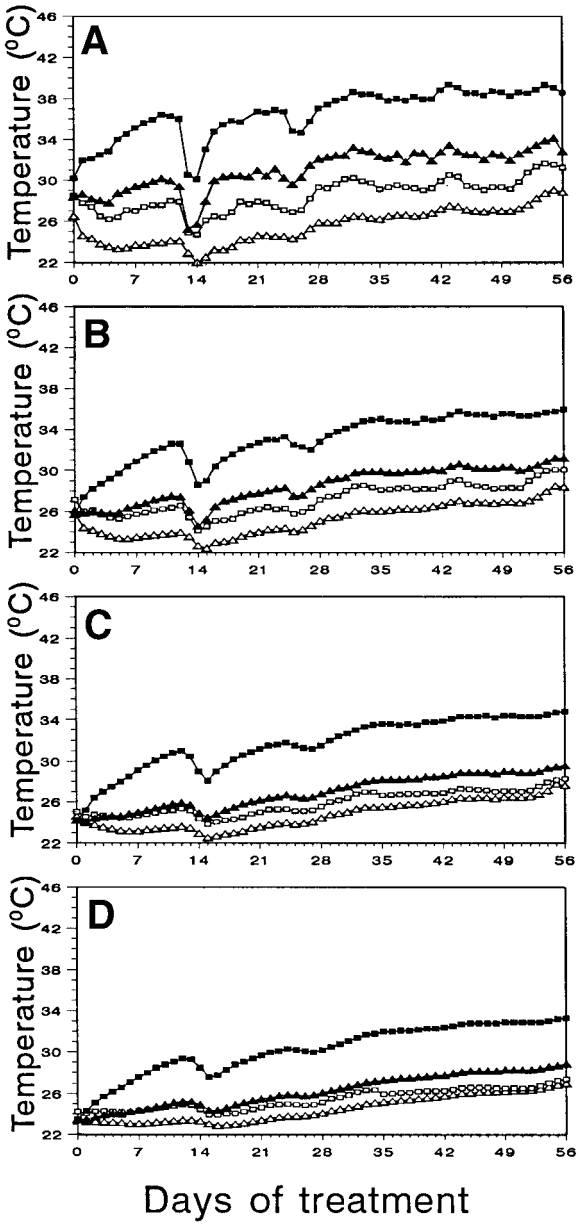

Figure 1. Time-course of maximum soil temperature in different treatments and locations (■ = solarized unshaded, □ = solarized shaded, ▲ = unsolarized unshaded and Δ = unsolarized shaded) and depths (A = 15 cm, B = 30 cm, C = 45 cm and D = 60 cm) in bare soil (Experiment I, 12 June–7 August, 1995).

unsolarized treatments, respectively. At deeper layers, soil temperatures were lower and temperature increases were much lower (4–2.5 °C) than for unshaded plots (Figure 3).

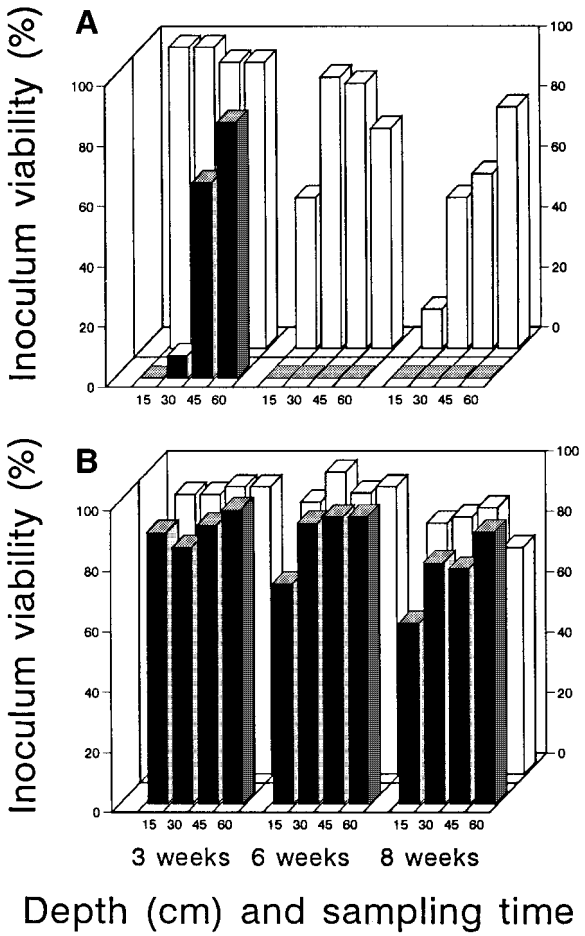

Figure 2. Effect of different treatments (■ = solarized and □ = unsolarized) on the viability of *Dematophora necatrix* in bare soil after three, six and eight weeks of treatment in unshaded (A) and shaded (B) locations at different soil depths (Experiment I, 12 June–7 August, 1995).

Viability of *D. necatrix* in unshaded plots was nil after 8, 10, 15 and 22 days of solarization at 15, 30, 45 and 60 cm, respectively. Only after 6 weeks was fungal viability reduced to 2.5% in root samples buried at 15 cm depth in unshaded unsolarized plots. However, viability at deeper layers was higher than 60% after the same period. After 8 weeks, the viability of *D. necatrix* in shaded solarized plots was reduced to 40% at 15 cm depth, whereas it was more than 85% for root samples buried at 30–60 cm depth. Similar values were observed in shaded unsolarized plots, except for samples at 15 cm depth, which had a viability of 70%.

Fungal viability of samples buried at 15 and 60 cm depth in unshaded plots was gradually reduced to very

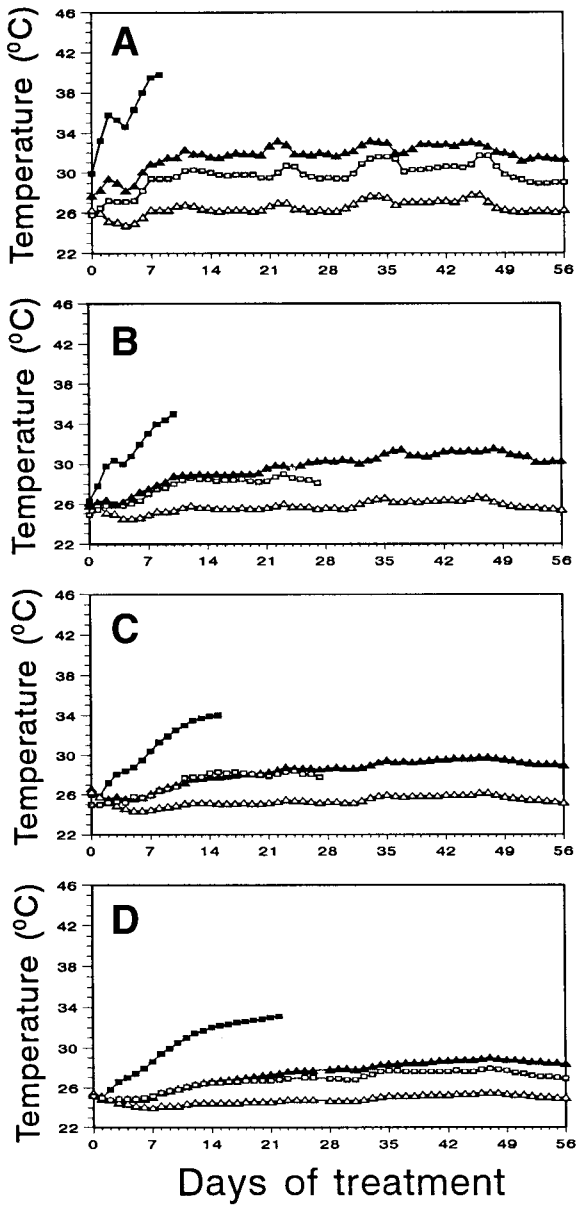

Figure 3. Time-course of maximum soil temperature in different treatments and locations (■ = solarized unshaded, □ = solarized shaded, ▲ = unsolarized unshaded and Δ = unsolarized shaded) and depths (A = 15 cm, B = 30 cm, C = 45 cm and D = 60 cm) in bare soil (Experiment II, 4 July–29 August, 1995).

low levels in solarized plots, as well as at 15 cm depth in unsolarized plots; a gradual reduction to moderate levels was observed in samples at 15 cm depth in solarized shaded plots. In both cases, the longer the burial periods, the scarcer was the mycelial growth on the root segments incubated in moist chamber. These reductions in viability levels allowed regression analyses of fungal viability over accumulated temperature–time. In the other cases, the sudden decrease in or the maintenance of fungal viability at high levels did not allow for regression analyses of the data.

Regression equations after ln transformation of fungal viability for those cases in which there was statistical significance ($P < 0.02$) are given in Table 1. The corresponding curves are shown in Figure 4. The best fits were always obtained with a temperature threshold of 30 °C. In contrast to the other treatments, a delay in the onset of viability loss of *D. necatrix* was observed in samples at 15 cm depth in unshaded areas of unsolarized plots and at 60 cm depth in unshaded solarized areas. At 15 cm depth, the accumulated temperature–time required for inoculum inactivation was higher for unsolarized than for solarized plots, and shading of these solarized plots slightly reduced the decrease in fungal viability (Table 1, Figure 4).

Discussion

Reductions in fungal soilborne inocula in established orchards by means of soil solarization requires the consideration of two critical factors. Infections can take place in very deep layers of soil, where the root system is developing and where root pathogens are active, and in the areas shaded by the tree's canopy soil temperature is always lower, possibly insufficient to achieve pathogen eradication. These facts are not frequently taken into account in most studies on soil solarization which are primarily applied to annual crops. Soil solarization has been successfully used to control *Verticillium* wilt in established orchards of pistachio, avocado, almond and olive trees (Ashworth and Gaona, 1982; Shabi et al., 1987; Tjamos et al., 1991), as well as in apple orchards affected by *R. necatrix* (Freeman et al., 1990). Although control of *Dematophora* root rot in established avocado trees by means of soil solarization has been studied (López-Herrera et al., 1998), a determination of soil temperature–fungal inactivation relationships was needed.

The time course of temperature at 60 cm did not differ greatly from that at 45 cm depth (Figures 1, 3). However, temperatures were higher for Experiment II (Figure 3) than for Experiment I (Figure 1), since the solarization period in the latter started 23 days earlier, i.e. in June, 1995, when temperatures were not optimal

Table 1. Regression equations of *Dematophora necatrix* viability[a] over accumulated temperature–time using temperature threshold of 30 °C (Experiment II)

Treatment	Depth (cm)	Regression equation[b]	r^2	P
Solarized unshaded	15	$\ln V = 4.51 - 0.006DH$	0.87	0.006
Unsolarized unshaded	15	$\ln V = 5.13 - 0.005DH$	0.94	0.0001
Solarized shaded	15	$\ln V = 4.50 - 0.008DH$	0.96	0.0001
Solarized unshaded	60	$\ln V = 5.12 - 0.008DH$	0.96	0.02

[a]Determined in naturally infected avocado root segments buried at given locations and depths.
[b]V = viability (%) DH = temperature–time (degree–hours).

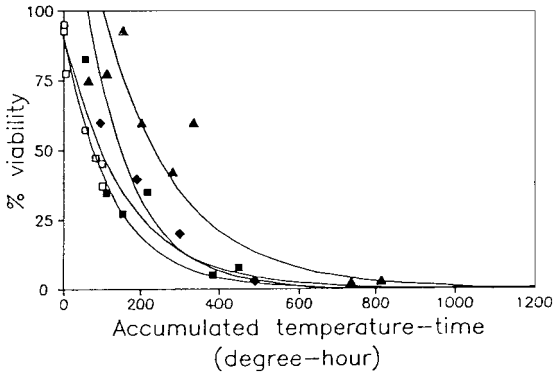

Figure 4. Effect of accumulated temperature–time (threshold temperature 30 °C) on the viability of *Dematophora necatrix* in naturally infected avocado root samples buried in soil at different depths and with different treatments (■ = solarized unshaded 15 cm, □ = solarized shaded 15 cm, ♦ = solarized unshaded 60 cm, and ▲ = unsolarized unshaded 15 cm) (Experiment II, 4 July–29 August, 1995).

for solarization. Consequently, 4–6 weeks of solarization were required in the unshaded solarized locations of Experiment I to achieve the eradication of inoculum at depths of 30–60 cm (Figure 2), whereas in Experiment II this was achieved within 10–22 days. These results confirm the partial reduction of the populations of several soil microorganisms in shaded areas of solarized plots as compared to the unshaded ones (Stapleton and DeVay, 1984), for which correlative increased growth responses were also reported. Moreover, in Experiment I, inoculum viability was not affected in any of the samples located in the shaded areas of plots solarized for as long as 8 weeks (Figure 2), whereas a significant reduction in the inoculum viability was observed for samples buried at 15 cm depth in shaded solarized plots of Experiment II. Therefore, solarization must be conducted during optimal periods and conditions in order to attain maximum benefits.

Heat inactivation of *R. necatrix* depends on temperature, exposure time and inoculum quality (Sztejnberg et al., 1987). Our results suggest that the loss of inoculum viability in naturally infected roots could be related to accumulated temperature–time, a minimum threshold temperature of 30 °C being adequate for regression fitting. The exponential nature of the relationship between accumulated temperature–time and viability of *D. necatrix* found in our study (Figure 4) agrees with a previous report (Katan, 1996).

The accumulation of degree–hours required for there to commence a decrease in *D. necatrix* viability was higher for the treatments with lower temperatures i.e. upper layer of unsolarized plots and deeper layer of solarized plot (Table 1, Figure 4). These results, along with the weakening effect observed on fungal mycelium, in Experiment II, could be explained by the accumulated temperature–time being less effective in the case of long exposures at temperatures relatively close to the threshold temperature than in short exposures at higher temperatures. This delay in inoculum inactivation is consistent with the requirement of long periods of solarization (8 weeks) to achieve eradicative control at deeper layers, where we have frequently observed viable mycelium of *D. necatrix*.

The higher temperature–time accumulation required for loss of fungal viability in unsolarized unshaded, as compared to solarized unshaded or shaded plots at 15 cm depth, would suggest an effect of anaerobic conditions in polyethylene-mulched plots, possibly related to confinement of volatile compounds, additional to the thermal effect, in agreement with previous suggestions (Stapleton and DeVay, 1984).

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