Soil Solarization and Thermal Death: A Logarithmic Relationship Between Time and Temperature for Four Soilborne Plant Pathogens

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ABSTRACT

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Temperatures of 37–50 C for different time periods were lethal to mycelia, spores, and resting structures of *Verticillium dahliae*, *Pythium ultimum*, and *Thielaviopsis basicola* on an agar medium. At 37 C, exposure times for an LD_{90} were 28.8, 25.8, 17.9, and 33.5 days, respectively, for *V. dahliae* (strains T9 and SS4), *P. ultimum*, and *T. basicola*. At 50 C, LD_{90} values were 23, 27, 33, and 68 min, respectively, for the same fungi. Field-produced propagules of *V. dahliae* in moist field soil also were killed when incubated at temperatures of 37–50 C for specific time periods. Temperatures of 39 and 50 C killed cultures of *Rhizoctonia solani* on agar medium in 14 days and 10 min, respectively. A linear relationship existed between logarithms of times required to kill 90% of the propagules when plotted against temperatures. This linear relationship was observed for populations of fungi in both agar and soil tests. These fungi were killed in field soils solarized for the necessary time periods. The exposure times and temperatures necessary to kill these fungi are useful for evaluating the progress of soil solarization under field conditions.

Additional key words: solar heating, mulching, polyethylene.

Heat treatment of soil has been used for many years to control soilborne plant pathogens in greenhouse plant culture (1). Steam heat treatment of field soil however, is not generally economically practical. Recently, soil solarization, the process of heating soils under transparent plastic tarps to temperatures lethal to soilborne pathogens, was reported to be successful in controlling several plant diseases (12,14,15,21).

In previous field studies by the authors, inoculum of *Verticilliium dahliae* Kleb., *Pythium ultimum* Trow., *Rhizoctonia solani* Kuehn, and *Thielaviopsis basicola* (Berk. & Br.) Ferr. was eliminated or greatly reduced to depths of 46 cm in soil solarized for 3–5 wk during the summer months (21). The solarization procedure increased soil temperatures to various levels depending on soil depth. Soil population densities of the plant pathogenic fungi monitored were greatly reduced at depths of 30–46 cm even though temperatures at these depths did not exceed 41 or 39 C, respectively. Katan et al (15) reported similar reductions in soilborne plant pathogenic fungi where soil temperatures did not exceed 41 C.

Extensive studies by many workers worldwide have shown that 30 min at 65 C will kill most of the important plant pathogens, insects, and weeds. Little information is available, however, concerning exposure times necessary to kill soilborne plant pathogens at temperatures below 45 C, which are often considered to be "sublethal." A few investigations with bacteria and fungi have shown that temperatures below 45 C can be lethal if maintained for long periods (4,13,17,23,24).

Reports on thermal death of plant pathogenic fungi deal mainly with temperatures near 50 C. Two-week-old microsclerotial cultures of *V. dahliae* on PDA were killed within 4 min at 55 C in tests by Miller and Stoddard (16), while Nelson and Wilhelm reported that 10 min at 50 C or 40 min at 47 C would kill moist microsclerotia of this fungus (18). *P. ultimum* was eradicated from diseased *Aloe* plants after 20-min hot water treatments at 46 C (2) and *R. solani* was injured by temperatures of 45 C or higher, with mycelia being killed within 5 min at 50 C (22). Chlamydospores of *T. basicola* lost viability when held at 40 C for 115 hr (13).

To determine the mechanisms involved in controlling soilborne

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fungi in solarized soil, information is needed on the effects of long-term exposures at temperatures below 45 C. The objective of this study was to determine the thermal sensitivity of *V. dahliae, P. ultimum*, *R. solani*, and *T. basicola* at elevated temperatures for different time periods on agar medium and in field soil. A brief report on part of this work has appeared (20).

MATERIALS AND METHODS

Field experiments. During 1978, field experiments were located at Davis and Shafter, CA. Individual plots 2.6 × 4.6 m were arranged in a randomized complete block design consisting of four treatments each with four replications. Plots were either nontarped or tarped with 25-μm (1-mil) transparent polyethylene plastic for 4 wk during May, June, or July. Tarps were placed on the soil by hand and firmly anchored in trenches along the sides of the plots. The soil underneath the plastic was irrigated with about 8 cm/ha of water. Nontarped plots were not irrigated in order to avoid the growth of weeds. Soil samples were collected as cores 2.5 cm in diameter from soil depths of 0–15, 15–30, and 30–46 cm in May prior to placement of the plastic and in July after the last tarps were removed. Soil temperatures were continuously recorded in a single replication of each treatment at depths of 5, 15, 30, and 46 cm.

Heat treatment of field soil. Soil was collected from the upper 20 cm of the soil profile in three California fields infested with *V. dahliae*. Samples were passed through a 6-mm-mesh screen and stored at 4 C. The location, soil type, population density of *V. dahliae* as propagules per gram of soil (p/g), and percent water content at field capacity, respectively, are shown for each field site: Five Points, Panoche clay loam, 144 p/g, 29.2%, Shafter, Hesperia fine sandy loam, 90 p/g, 10.2%, and Davis, Yolo clay loam, 339 p/g, 23.9%. Thirty grams of soil from each loction was placed in test tubes 12 mm in diameter. A predetermined amount of water was added to each tube to adjust soil water content to field capacity. Test tubes were capped with plastic tops, which retarded water loss but allowed the gas exchange. Tubes were allowed to equilibrate overnight and the next day were partially immersed in hot water temperature baths maintained at 50, 47, 45, or 42 C ± 0.2 C, or placed in a constant temperature incubator at 38.5 or 37.0 C. Temperatures in incubators were less precise and varied ± 0.4 C. Three tubes per soil type were treated for each temperature and exposure time. Soil from the same location and exposure time was removed from the test tubes, bulked, and assayed for *V. dahliae*.

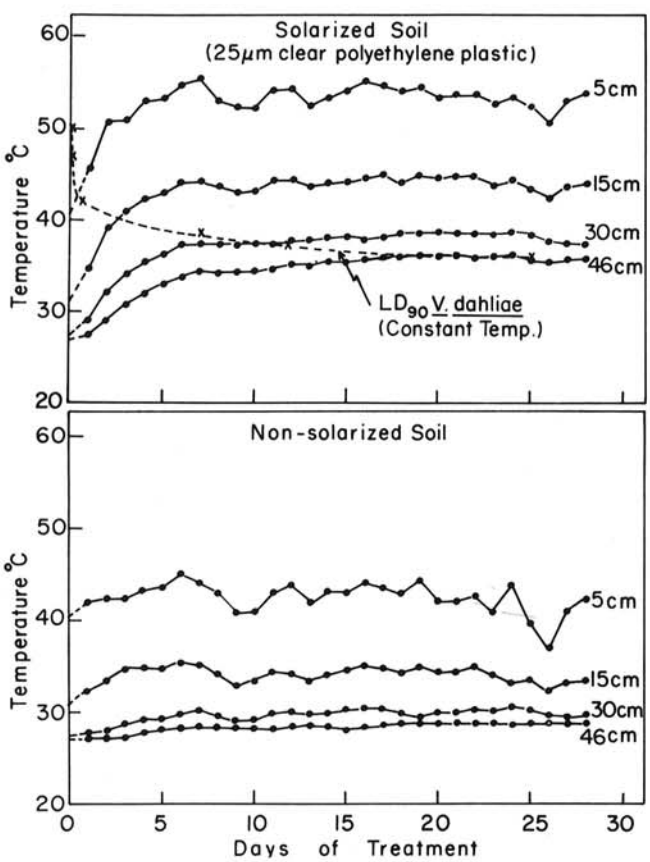

Fig. 1. Daily maximum soil temperatures in solarized (moist) and nonsolarized (continuously drying) soil for depths of 5, 15, 30, and 46 cm. Each value represents an average of four experiments during 1978. Experiments occurred at Shafter, CA, from 2 June–29 June and 29 June–27 July and at Davis, CA, from 3 June–1 July and 3 July–31 July. Time and temperature exposures necessary to kill 90% of the propagules of *Verticillium dahliae* in field soil with moisture at field capacity are shown on the dotted line.

Soil assays for *V. dahliae*. Soil samples were air dried for 6–10 wk and assayed by the dry soil plating method of Butterfield and DeVay (5) by using an Anderson Air Sampler (Anderson Samplers Inc., Atlanta, GA 30336). Five 100-mg samples of soil were impacted onto a semiselective sodium polypectate medium and plates were incubated for 14 days at room temperature (20–24 C). Plates were rinsed with water and examined with the aid of a stereo dissecting microscope for microsclerotial colonies of *V. dahliae*. As few as two p/g of soil could be detected with this technique.

Growth of test fungi on agar at elevated temperatures. Fungi were incubated on potato-dextrose agar (PDA) at various temperatures to determine the maximum temperature at which growth would occur. Five-millimeter-diameter disks cut from the margins of rapidly growing PDA cultures of *V. dahliae* (T9 defoliating isolate and SS4, nondefoliating isolate), *P. ultimum* (F-367), *R. solani* (F-366, Anastomosis Group IV) and *T. basicola* (F-364) were placed on petri plates containing 20 ml of PDA. Duplicate plates for each fungus were incubated in constant temperature incubators maintained within 0.4 C of selected temperatures. Visible growth was noted after 14 days.

Preparation of agar cultures before heat treatment. The cultures used for heat treatment were grown in glass petri plates containing 20 ml of PDA and were incubated at room temperature (20–24 C) for 3–4 wk. In the case of *V. dahliae*, 0.1 ml of conidial suspension (5×10^6 conidia per milliliter) was spread over the agar surface. Cultures of *R. solani*, *P. ultimum*, and *T. basicola* were started from mycelial disks cut from the advancing margins of growing cultures. After incubation, the cultures contained the propagules expected in field soil: *V. dahliae*—mycelia, conidia, and microsclerotia; *R. solani*—mycelia, runner hyphae, and moniloid cells; *P. ultimum*—mycelia, sporangia, and oospores; and *T. basicola*—mycelia, endoconidia, and chlamydospores.

Heat treatments and viability tests of test fungi on agar. Three- to 4-wk-old PDA cultures in petri plates were heat treated in constant temperature water baths maintained at 50, 47, 45, and 42 C ± 0.2 C. Prior to immersion the petri plate tops were removed and the halves containing the cultures were placed in sterile plastic bags. The bags were evacuated and sealed allowing the plastic to contact the culture surface thus preventing contamination during the hot water treatment. For temperature treatments at 39 and 37 C, culture plates (with tops) were placed in platic bags in constant temperature incubators for various time periods.

After heat treatment, 50 disks 5-mm in diameter, were cut from the center of each plate with a cork borer. Ten disks of *V. dahliae* and *T. basicola* were placed on each of five PDA plates. These were rated after 21 days for visible growth, indicating survival. Similarly, disks from *P. ultimum* and *R. solani* were transferred to five plates; however, to restrict growth from surviving propagules, the 5-mm-diameter disks were placed on larger disks of fresh PDA (12 mm in diameter). Plates were examined daily for 21 days and those disks with growing mycelia were removed. Experiments for each organism, temperature and exposure time were repeated three to four times providing a total of 150–200 5-mm-diameter disks for each test.

RESULTS

Field experiments. At both the Shafter and Davis locations, soil temperatures were higher in tarped than in nontarped soil at all depths monitored (Table 1). The average maximum soil

temperatures in tarped (moist) and nontarped (drying) soil during June and July at Shafter and Davis are shown in Fig. 1 for soil depths of 5, 15, 30, and 46 cm. Daily maximum temperatures occurred for approximately 2 hr at 5 and 15 cm and for longer periods at depths of 30 and 46 cm. Additional soil and air temperature and average daily solar radiation data are not reproduced here but are available (19). Plots tarped for 4 wk during May, June, or July provided soil heated within a wide range of elevated temperatures depending on the soil depth. Differences in inoculum density of *V. dahliae* and the associated maximum soil temperatures are shown in Table 1. At Shafter, the lowest soil temperatures that reduced populations of *V. dahliae* occurred in May between soil depths of 15 and 30 cm. Temperatures at these soil depths reached 43 and 35 C, respectively. At Davis, *V. dahliae* was eradicated in soil exposed to maximum temperatures of 39 and 37 C which developed at soil depths of 30 and 46 cm, respectively, in plots tarped during June. These results indicated that *V. dahliae* was killed in moist soil at temperatures as low as 35–37 C and suggested that thermal inactivation was responsible for the death of pathogenic fungi below depths of 30 cm.

Heat-treated field soil. Changes in inoculum density of *V. dahliae* in field soil heated at constant temperatures are shown in Fig. 2A. All temperatures produced sigmoidal death curves when percent survival was plotted against time. Curves were drawn with the aid of a computer-assisted program that calculated the third-degree polynomial equation for a curvilinear regression. Exposure times

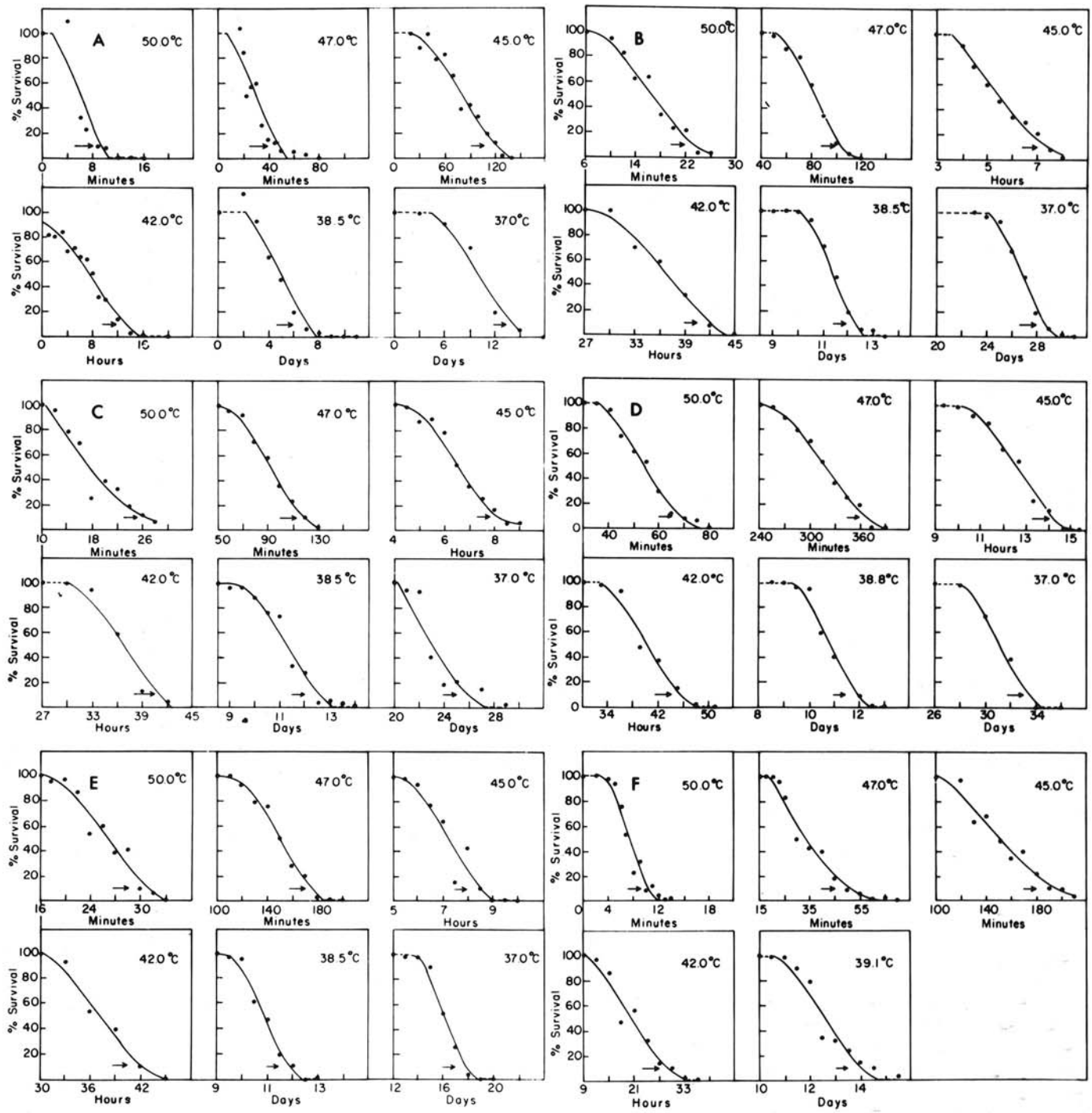

Fig. 2. Curvilinear regressions of percentage survival of fungal propagules in field soil and on potato-dextrose agar in relation to temperature and time of exposure. The arrows show the exposure times required to kill 90% of the propagules for **A,** *Verticillium dahliae* in natural field soil with moisture at field capacity, **B,** *V. dahliae* T9 defoliating isolate on PDA, **C,** *V. dahliae* - SS4 nondefoliating isolate on PDA, **D,** *Thielaviopsis basicola* on PDA, **E,** *Pythium ultimum* on PDA, and **F,** *Rhizoctonia solani* on PDA.

TABLE 1. Survival of *Verticillium dahliae* propagules and maximum soil temperatures at several depths near Shafter and Davis, CA during soil solarization experiments conducted in 1978

Location, treatment, and date[a]	Prop./g soil 0–15 cm	Max. Soil temperature[b] 15 cm	Prop./g soil 15–30 cm	Max. Soil temperature[b] 30 cm	Prop./g soil 30–46 cm	Max. Soil temperature[b] 46 cm
Shafter - 1978						
Nontarped	5.5	32, 36, 38	6.0	29, 31, 34	···	28, 30, 33
Tarped: 5 May–2 June	1.0	43	0.5	35	···	32
Tarped: 2 June–29 June	0.5	45	0	38	···	33
Tarped: 29 June–27 July	0	47	0	41	···	37
Davis - 1978						
Nontarped	120	38, 37, 37	27	29, 29, 31	18	27, 27, 29
Tarped: 12 May–9 June	1	48	0	41	0	37
Tarped: 3 June–1 July	0	46	0	39	0	37
Tarped: 3 July–31 July	0	48	0	41	0	39

[a]Clear 25-μm polyethylene tarps were placed on the soil near the beginning of May, June, or July and were left in place for 4 wk.
[b]The three temperatures listed for nontarped soil are for the same time periods as tarped soil during May, June, and July, respectively. Air temperatures did not exceed 41 and 39 C, respectively, at the Shafter and Davis locations.

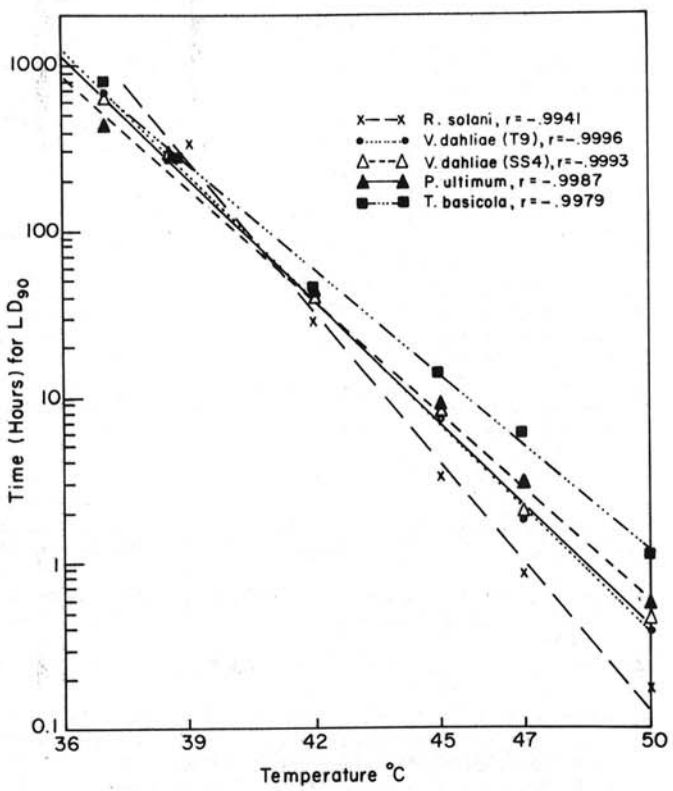

Fig. 3. Time and temperature exposures required to kill 90% of the propagules of *Verticillium dahliae* present in three field soils with moisture at field capacity.

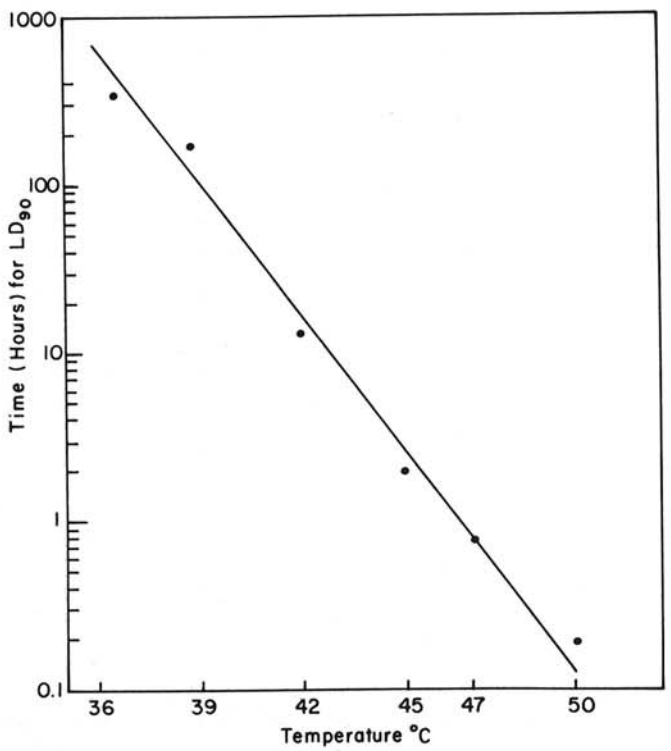

Fig. 4. Time and temperature exposures required to kill 90% of the fungal propagules of *Rhizoctonia solani, Verticillium dahliae* (T9 and SS4), *Pythium ultimum,* and *Thielaviopsis basicola* on potato-dextrose agar.

at which 90% of the population was killed were plotted on a logarithmic scale against temperature in a fashion similar to previous workers (4,10,17). A linear regression analysis of the data points showed a highly significant correlation coefficient (Fig. 3). Similar plots can be made for any LD percentage desired. The LD_{90} exposure times as related to temperatures in solarized soil are plotted in Fig. 1

Growth of test fungi on agar at elevated temperatures. All fungi showed visible growth at 30 and 33 C, while at 36 C only *R. solani* grew. None of the fungi grew at 39 C. In view of these data, thermal death tests were conducted at temperatures between 37–50 C for *V. dahliae* (two isolates), *P. ultimum*, and *T. basicola*. *R. solani* was treated at temperatures between 39–50 C.

Heat treatments and viability tests of test fungi on agar. Responses of test fungi on agar to elevated temperatures are shown in Fig. 2B–F. All fungi again had sigmoidal death curves when plotted as precent survival vs exposure time. Thermal death curves were drawn by curvilinear regression. Exposure times at each temperature necessary to kill 90% of the agar propagules (LD_{90}) were plotted on a logarithmic scale against temperature. Linear regression analysis of the data points again showed highly significant correlation coefficients (Fig. 4). Slope values of the regressions for the fungi were in a narrow range between −0.30330 and −0.21321. When compared with sublethal exposures at high temperatures, lower temperatures caused greater delay times before propagules on agar disks began to produce growing hyphae. For example, propagules of *P. ultimum* on agar heated at 37 C for 13 days did not grow for 6–7 days after treatment. Once growth began, however, it continued at a normal rate. In comparison, after exposure to 50 C for 18 min, growth began after 3–4 days and proceeded at a normal rate. *R. solani* generally had the shortest recovery times, followed by *P. ultimum, V. dahliae* (T9 and SS4) and *T. basicola.*

DISCUSSION

In the present study of *V. dahliae*, death of structures on agar disks correlated well with the death of propagules found in field soil. Because each agar disk consisted of abundant fungal structures, apparently all structures had to die or else mycelial growth would occur. Temperature-time exposures which killed 95–100% of the individual propagules in soil resulted in the lack of growth from a few agar disks. As the exposure times were increased, growth from additional disks failed to develop. Therefore, the data collected for soil inoculum reflects the depth of individual propagules while that from the agar disks represents the death of populations of propagules. All agar cultures tested contained fungal survival structures normally present in field soil.

Sigmoidal curves described the relationship between death rate as a function of exposure time at each temperature. Smith in 1923 (23) produced similar thermal death curves in tests with condidia of *Botrytis cinerea*. He also observed a linear relationship when the logarithms of the times taken to kill 50% of the spores were plotted against the reciprocals of the corresponding temperatures. In this study, similar linear relationships were observed when the exposure times resulting in an LD_{90} were plotted on a logarithmic scale against temperature. These relationships predicted the time necessary to kill 90% of the propagules at temperatures within the range of 37–50 C. Other workers also have observed or discussed linear relationships between logarithms of thermal death times and temperatures for nematodes (10), bacteria (4,11), viruses (8,9,25), and fungi (17).

The fungi studied differed in their responses to heat. Although *R. solani* was the only fungus that grew at 36 C, it was the first to lose viability at temperatures above 39 C. At 37 C, strain T9 of *V. dahliae* was less temperature-sensitive than was strain SS4. This was not surprising because T9 germinates at slightly higher temperatures than SS4 (27). At temperatures above 37 C, however, SS4 was able to survive longer exposures than T9. *P. ultimum* survived greater heat dosages than *R. solani* or *V. dahliae*, while *T. basicola* required the longest exposure times for cell death. These results are in agreement with measurements of population densities in solarized soils where the inoculum density of *R. solani* declined faster than *V. dahliae, Pythium* spp., and *T. basicola* (21).

Sublethal temperatures caused delays in germination, which varied with temperature and duration of exposure. Germination delays were the longest when fungi were exposed to 37 and 39 C. The longer a propagule was heated and still survived, the longer it required to germinate. This delay indicates that heat damage accumulates gradually to a point beyond which the propagule cannot recover. However, if heat treatments are stopped before this point, recovery may occur. A "partially viable" propagule may recover and resume its normal course of development if given optimal conditions and a sufficient amount of time. Previous workers have observed similar heat effects on *Armillaria mellea* (17), *B. cinerea* (23), and *Bacillus botulinus* (26). In the soil environment, however, "partially viable" propagules would not be expected to recover due to surrounding microbial antagonists and additional stress factors.

Sublethal heating probably decreases the ability of a propagule to withstand further stress and cause plant disease. In previous studies on the control of *V. dahliae* by soil solarization, surviving propagules cased less disease than similar inoculum densities of nonheated propagules (19). Microsclerotia of *V. dahliae* are multicellular and can produce many germ tubes upon germination (3,7). Heating probably results in the death of individual cells, which in turn decreases the inoculum potential of a microsclerotium. In addition, sublethal heating may increase the sensitivity of fungal propagules to antagonistic microflora as was shown with *A. mellea* (17). Soilborne propagules of *R. solani* and chlamydospores of *T. basicola* are also multicellular and probably decline in inoculum potential similarly to *V. dahliae*.

The mechanisms of heat inactivation in fungi are not clearly understood but may involve enzyme inactivation, phase changes in fatty acids and membrane components, and the slow turnover of heat-sensitive proteins (6). Although direct thermal effects are probably the major factors involved in the control of soilborne plant pathogens by soil solarization, the possible role of biological control mechanisms at temperatures below 37 C should be investigated.

Data generated in this study on the temperature sensitivity of fungi correlate well with their inactivation in soils solar heated under plastic tarps (12,15,20). Thus, the thermal death curves presented may provide a useful indicator for evaluating the control of the investigated soilborne propagules in solarized field soil.

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