Thermal Inactivation of *Phytophthora nicotianae*

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ABSTRACT

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Phytophthora nicotianae was added to pasteurized soil at the rate of 500 laboratory-produced chlamydospores per gram of soil and exposed to temperatures ranging from 35 to 53°C for 20 days. The time required to reduce soil populations to residual levels (0.2 propagule per gram of soil or less) decreased with increasing temperatures. Addition of cabbage residue to the soil reduced the time required to inactivate chlamydo-

spores. Temperature regimes were established to simulate daily temperature changes observed in the field, with a high temperature of 47°C for 3 h/day, and were good estimators of the efficacy of soil solarization for the control of *P. nicotianae* in soil. Cabbage amendment reduced the time required to inactivate chlamydospores of *P. nicotianae* and its effect was more pronounced at lower temperature regimes.

Additional keywords: *Brassica oleracea*, intermittent heat, nonchemical control, organic amendment.

Studies of thermal inactivation of pathogens can yield important information on survival of specific types of propagules, and when simulating field conditions, can serve as the basis for estimation of the time needed for soil solarization to effectively control soil-borne plant pathogens (2,18,21). Juarez-Palacios et al. (13) found that determination of the heat sensitivity of isolates of *Phytophthora* spp. in the laboratory closely reflected their inactivation in solarized soil and indicated the possible use of soil solarization for management of those pathogens. These authors found that an isolate of *P. megasperma* tolerant to high temperature survived exposure for 30 min at 45°C in a heat sensitivity study; however, *P. cinnamomi* and a low-temperature isolate of *P. megasperma* did not survive exposure at 45°C for 20 min. Bollen (5) noted that different types of propagules have different sensitivities to heat. Oospores of *P. capsici* were more thermotolerant than mycelium from either mating type of this pathogen; oospores survived at 50°C for 30 min, but mycelium was eliminated at 42.5 to 45°C for 30 min.

The use of heat to inactivate spores of microorganisms in diverse media began as early as 1920 (4). Soon after that the advantages of the logarithmic transformation for the analysis of the data were realized (3,4,24). The logarithmic transformation indicates that a constant proportion of the spore population is killed per unit of time and serves the purpose of linearizing the data. Simple linear regressions could be used for the analysis of the effect of time on the survival of spores at each temperature employed; coefficients for lethal dosages (LD₅₀ or LD₉₀) could be interpolated from the data. Pullman et al. (21) demonstrated that the logarithmic relationship between time and temperature on the survival of four soilborne plant pathogens was maintained at temperatures below 50°C. The exposure time required to reduce the

population by 90% (LD₉₀) for oospores of *Pythium ultimum* increased as the temperature decreased from 50 to 37°C. The study was performed with soil moisture adjusted to field capacity and closely reflects common temperature profiles observed in soil solarization tests.

One of the first attempts to control plant pathogens by exposure to intermittent heat was that of Grooshevoy et al. (12). The authors demonstrated that exposing chlamydospores of *Thielaviopsis basicola* or sclerotia of a *Rhizoctonia* sp. or *Sclerotinia sclerotiorum* for 3 h/day at 45°C for 5 days was sufficient to prevent germination of the propagules. However, in that study no attempts were made to simulate diurnal temperature fluctuations. Porter (19) evaluated the effect of intermittent heat on the control of sclerotia of *S. sclerotiorum* in soil and found continuous heat more effective than intermittent heat for reducing the number of viable sclerotia when the infested soil was heated for 6 h/day over 14 days to temperatures ranging from 30 to 45°C. In a similar study with several pathogens, Porter and Merriman (20) observed that the tolerance to heat exposure varied among different genera of plant pathogens; however, the type of fungal propagules used for this study was not determined. Due to the labor-intensive nature of these studies and the demand for equipment, very little research has been done on the quantitative relationship of intermittent heat to the inactivation of spores of plant pathogens.

Studies of thermal inactivation can be complemented to simulate other conditions in the field, such as the addition of organic amendments to the soil prior to solarization (10,11,22,23). Cabbage residue associated with solarization was used successfully to control cabbage yellows caused by *Fusarium oxysporum* f. sp. *conglutinans* (22,23). The addition of cabbage alone was only partially effective for the control of cabbage yellows, but when cabbage residue was heated in solarization there was significant control of the pathogen.

Phytophthora nicotianae (Breda de Haan; synonym, *P. parasitica* Dastur) is an important soilborne plant pathogen with a wide host range (1). Diseases caused by this pathogen, such as seedling damping off, root rot, blight, and fruit rot have limited the production of several important crops (9,16). *P. nicotianae* can survive in soil for long periods of time in the absence of its host

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plants and when weather conditions are not favorable for disease initiation and development (9). Thus, the main strategies used by growers to reduce losses due to this pathogen, especially at the early stages of plant development, are preplant soil fumigation with methyl bromide and postplant application of fungicides. Although soil solarization has been used to reduce populations of *P. nicotianae*, disease management was not always observed (6,8). Therefore, thermal inactivation studies could indicate which adjustments are needed for the efficient use of solarization as an alternative for the control of this important soilborne plant pathogen.

Due to the demands on time and equipment, thermal inactivation studies very seldom explore extensive combinations of both time and temperature ranges. Thus, the full benefits that can be derived from such experiments are often not realized. The objectives of this study are to evaluate the effects of constant and pulsing temperatures and cabbage amendment on the survival of *P. nicotianae*.

MATERIALS AND METHODS

Production of chlamydospore inoculum. Chlamydospores of *P. nicotianae* were produced in V8-juice broth according to the procedures outlined by Mitchell and Kannwischer-Mitchell (17). The isolate of *P. nicotianae* used (Pn21) was originally isolated from periwinkle in South Florida and was maintained in the collection of *Phytophthora* spp. of the Plant Pathology Department at the University of Florida (Gainesville). The total number of chlamydospores in the suspension was estimated with a hemacytometer, and the suspension was used immediately to infest pasteurized soil. Soil was collected from a field previously cropped to tomato and tobacco in Decatur County, Georgia. Inoculum was adjusted to a density of 500 chlamydospores per g of soil. Thirty grams of infested soil was dispensed in a borosilicate glass test tube (25 × 150 mm) that was loosely closed with a plastic cap to allow exchange of air.

Effect of constant temperature on the inactivation of chlamydospores. A set of test tubes was placed in each of eight water baths held at the following constant temperatures with circulation heaters: 25, 35, 38, 41, 44, 47, 50, and $53 \pm 0.02^\circ\text{C}$. The time of exposure at each temperature was 5, 10, 15, 30, 45, 60, 75, 90, 105, and 120 min or 2, 4, 8, 12, 16, 20, 24, 48, 96, 192, 288, 384, and 480 h. Three tubes were removed at each time interval and a portion of the soil (15 g) from each tube was diluted with soft agar (2.5 g of Difco agar per liter of deionized water) and plated on a medium selective for pythiaceous fungi (PARP; 17). The soil overlay was removed from the plates after 48 h by gently washing the agar surface with tap water. The total number of colonies that formed on PARP after 72 h of incubation was recorded as an estimate of the number of chlamydospores that survived the heat treatment. The remaining 15 g of soil was baited with three 3-day-old 'Solar Set' tomato seedlings and incubated in a growth chamber at 27°C for 3 days. The seedlings were rinsed twice in sterile water, blotted dry, and plated on PARP to evaluate the colonization of tissues by *P. nicotianae*. The number of seedlings with fungal colonization was recorded. The experiment with exposure times ranging from 5 to 120 min was repeated once, and the experiment with exposure times ranging from 2 to 480 h was repeated twice.

The effect of cabbage amendments on the thermal inactivation of chlamydospores. In order to determine the effect of cabbage amendments on the inactivation of chlamydospores of *P. nicotianae*, experiments similar to those described previously were conducted with temperatures at 35, 38, and $41 \pm 0.02^\circ\text{C}$, and time periods of 2, 4, 8, 12, 16, 20, 24, 48, 96, 192, 288, 384, and 480 h. Leaves of cabbage (*Brassica oleracea* var. *capitata*) were air dried in a greenhouse and ground in a Wiley mill with a 20-mesh screen. Pasteurized soil was amended with dry, ground cabbage leaves at 0, 0.125, 0.25, and 0.5% (wt/wt) and infested with 500 chlamydospores per g of soil. Thirty grams of amended soil

infested with chlamydospores was dispensed in a borosilicate glass test tube (25 × 150 mm). The tube was sealed with a gas-impermeable plastic film (Bromotec film; Lawson Mardon Packaging, Workington, Cumbria, U.K.), and a plastic cap was placed over the tube to reduce the exchange of air and trap the volatiles released by heating the cabbage-amended soil. The test tubes were placed in water baths at constant temperatures with circulation heaters, three tubes were removed at each time interval, and the soil was processed as previously described.

The effect of cycling temperatures and cabbage amendments on the thermal inactivation of chlamydospores. A critical analysis of the effects of daily temperature fluctuation, as observed during soil solarization, on the survival of the pathogen was performed. Based on an analysis of published data from North Florida (6), two temperatures, representative of average temperature profiles at 10 and 25 cm of soil depth, and duration of daily exposure were selected. The daily regimes selected were 5 h at 41°C and 8 h at 35°C , each coupled with a baseline temperature of 25°C for the rest of the day. These temperatures were maintained with circulation heaters in water baths. After preliminary tests indicated a high percentage of the spores survived the heat treatment, a third temperature at 44°C for 1.5 h/day was added. The duration of the daily exposure at 44°C was based on the results of previous tests with constant temperature and on the analysis of temperature profiles from field experiments in 1994 (6).

Pasteurized and infested soil was adjusted to a final moisture of 6% with sterile water. Half of the infested soil was amended with dry, ground cabbage leaves at 0.125% (wt/wt). This concentration of cabbage was selected because no phytotoxic effects were observed on tomato seedlings and it matched the amount of cabbage residue applied in the solarization tests in North Florida (7). Thirty grams of soil was dispensed in a test tube that was sealed with a piece of the gas-impermeable plastic film and a plastic cap.

The test tubes were placed in water baths at 25°C until the temperature of the soil and the water were equilibrated, and cycling was initiated. Temperatures were monitored with thermocouples connected to a datalogger (CR10; Campbell Scientific Inc., Logan, UT). Three tubes were removed after 1, 2, and 3 days and every 3 days thereafter for a period of 24 days. Part of the soil (15 g) was diluted with soft agar and plated on PARP within 24 h, as previously described. The number of colonies was recorded. The remaining soil was used in a plant disease assay.

At the end of the thermocycling experiment, all remaining samples were transferred to tripour plastic beakers (50 ml), and one 1-month-old tomato seedling was transplanted in the soil in each beaker. Vermiculite (10 ml) was poured on the top of the soil to prevent the roots from desiccating. All plants were kept in a growth chamber at 27°C for 30 days. As plants died, the root systems were rinsed in water, surface disinfested for 30 s in 70% ethanol, rinsed twice in sterile water, and plated on PARP. At the end of the experiment, all plants were removed and the root systems were plated on PARP for determination of infection.

The use of average temperatures achieved during soil solarization for the temperature cycling experiments were not sufficient to completely inactivate chlamydospores of *P. nicotianae*; therefore, an additional experiment was conducted with temperature regimes achieved during an optimum solarization day at the North Florida Research and Education Center at Quincy in 1995 (7). Temperature regimes were at 47°C for 3 h/day, 44°C for 5 h/day, and 35°C for 8 h/day. Throughout this experiment, a baseline temperature of 25°C was maintained for the remainder of the day. The duration of each experiment was 15 days, and the experiment was repeated once.

The procedures for production of chlamydospores, treatment of soil, and estimation of soil populations of *P. nicotianae* were the same as described previously. At the end of the temperature cycling experiments, a plant disease assay was performed for 4 weeks with 1-month-old tomato seedlings, as described previously.

Statistical analysis. The results of each experiment were analyzed individually. When statistical analysis of the residuals indicated that the results could be pooled due to the lack of variation, a final analysis was done with the pooled data.

The response surfaces were analyzed according to the procedure PROC NLIN (SAS Institute, Cary, NC; release 6.11). Modeling was done with temperatures above 38°C to better establish end points of the surfaces. Survival data were transformed using $\ln(\text{ppg} + 1)$ prior to analysis. The analyses of the effect of temperature cycling and cabbage amendment on the survival of chlamydospores of *P. nicotianae* were performed using Statgraphics Plus (Version 2.1, Manugistics Inc., Rockville, MD) for the convenience of comparing several linearization models at once.

RESULTS

Effect of constant temperature on the inactivation of chlamydospores. *P. nicotianae* was consistently recovered throughout the 2-h experiment at temperatures below 47°C (Fig. 1A). At 44°C, a sharp decrease in recovery was observed after 30 min, but 10% of the initial population of 500 propagules per gram (ppg) of soil survived to the end of the experiment. After 75 min of heat treatment at 47°C, population levels dropped below 1 ppg of soil, and after 2 h populations declined to levels undetectable by the procedure used to quantify the inoculum in the soil. At 50 and 53°C populations were very low after only 5 min of exposure.

The decline in survival of the chlamydospores of *P. nicotianae* in soil exposed to constant heat above 38°C for 5 to 120 min was determined by

$$\ln(\text{ppg} + 1) = (6.2 - 0.00108 \times \text{time}^2)e^{-0.02208 \times \text{temperature}}$$

Model selection was based on comparison of observed vs predicted values, residual error vs time, residual error vs temperature, and on the coefficient of determination ($R^2_{\text{adj}} = 0.8234$).

The use of tomato seedlings as bait for the detection of survival of *P. nicotianae* indicated that survival was affected by the heat treatment only after 90% of the initial population of chlamydospores had been inactivated, which occurred generally only after exposure to 47°C or higher (Fig. 1B). Detection of survival by the seedlings was not as sensitive as detection by the soil dilution procedure. For example, at 53°C no seedlings were infected after heat treatment following the 3-day incubation period (Fig. 1B); in contrast, the soil dilution procedure indicated that 0.1 ppg of soil was still alive after 15 min of treatment (Fig. 1A).

Survival of chlamydospores exposed to 35°C for 480 h demonstrated that the pathogen can survive for a long period (Fig. 2A). Populations declined to levels below 1 ppg of soil when temperatures were maintained at 38°C for more than 288 h. After 96 h of exposure at 41°C or 48 h at 44°C, no propagules were recovered from the soil. Exposure at 47°C reduced the populations to residual levels after 4 h. The decline in survival of the chlamydospores of *P. nicotianae* in soil exposed to constant temperatures above 38°C for 2 to 480 h was determined by

$$\ln(\text{ppg} + 1) = 6.2e^{(-0.00759 \times \text{time} - 0.0138 \times \text{temperature})}$$

The coefficient of determination (R^2_{adj}) was 0.6773.

Generally, the use of the tomato seedling assay provided results similar to the soil dilution plating procedure when residual inoculum levels were greater than 0.1 ppg of soil (Fig. 2B). Baiting the soil with seedlings allowed detection of the fungus in two cases that were not detected by soil plating, but failed to detect the pathogen in three other instances that were detected by the plating procedure. All tomato seedlings were colonized by *P. nicotianae* when more than 50 ppg of soil was recovered in the soil dilution plates with PARP; in one case, 100% infection was observed when 10 ppg of soil was recovered.

The effect of cabbage amendments on the thermal inactivation of chlamydospores. Addition of dried, ground cabbage leaves changed the response of chlamydospores to heat (Fig. 3). As concentration of cabbage increased, thermal inactivation of chlamydospores also increased. Thus, less time was required to inactivate chlamydospores in cabbage-amended soil.

At 35°C, chlamydospores survived in the soil for 480 h when no cabbage amendment was present (Fig. 3A). The addition of 0.125% cabbage to the soil reduced the population to residual levels after 192 h (Fig. 3B). Ninety-six hours was required with 0.25% cabbage to reduce population counts below 1 ppg of soil (Fig. 3C); similar population reductions were attained after 48 h when 0.5% cabbage was added to the soil (Fig. 3D). A similar

Fig. 1. Effect of temperature (35 to 53°C) and time (5 to 120 min) on the survival of chlamydospores of *Phytophthora nicotianae*. **A**, Propagules per gram of soil (ppg) detected on a selective medium. **B**, Percentage of tomato seedling baits colonized by the pathogen.

trend in population reduction was obtained with the other two temperatures. The decline in survival of the chlamydospores of *P. nicotianae* in soil amended with cabbage residue and exposed to constant temperature was best described by the linear-power model (Table 1).

Detection of survival of *P. nicotianae* with tomato seedlings as baits followed similar trends as those observed by the soil dilution plating procedure (Figs. 3 and 4). As the number of propagules recovered in the soil plates decreased from 500 to 100 ppg of soil, the percentage of colonized seedlings was relatively constant. Further decreases in the propagule counts were generally reflected in a decrease in the percentage of colonized seedlings. Of the

41 cases in which no propagules were detected by the soil dilution plating procedure, survival of the pathogen was detected in 17 cases by the baiting technique.

The effect of cycling temperatures and cabbage amendments on the thermal inactivation of chlamydospores. Generally, the use of pulsing temperatures increased the time required to inactivate the chlamydospores of *P. nicotianae* in comparison with the constant temperature treatment (Figs. 3 and 5A and B). After 24 days at 35°C for 8 h/day, 40% of the initial infestation level of spores (500 chlamydospores per g of soil) was recovered from the nonamended soil in the selective medium. Addition of 0.125% cabbage to the soil reduced the population to levels below 10% after 18 days. The effect of cabbage was evident from the beginning of the experiment. A similar trend in population reduction was obtained with the other two temperature regimes.

Exposure to temperature regimes simulating optimum solarization periods reduced the populations to very low levels (Figs. 5A and B). After 5 h/day at 44°C for 15 days the population was reduced to 0.4 ppg of soil. Cabbage amendment reduced populations to the same levels after 6 days. Three days of exposure at 47°C for 3 h/day was required to reduce populations to levels below 1 ppg of soil; cabbage amendment populations were reduced to levels below 1 ppg of soil after 2 days. Populations were reduced to levels below detection only at the two highest temperature regimes (44°C for 5 h/day and 47°C for 3 h/day) after 9 days of treatment. Soil amendment with cabbage had a greater impact at lower temperatures, because the heat alone was not sufficient to inactivate the chlamydospores.

The reduction in survival of *P. nicotianae* was modeled using linear regression. The model selected for the comparisons among temperature regimes and cabbage amendment was $\ln(Y + 1) = a + b \times X$, in which Y is the number of propagules recovered per gram of soil and X is the number of days of exposure to the temperature regime. A lack of fit of the model to the data was observed at 35°C, regardless of the cabbage amendment (Table 2).

The plant disease assay verifying the pathogenicity of the surviving populations of *P. nicotianae* indicated that temperature regimes below 44°C for 5 h/day did not reduce the colonization of the tomato seedling root systems (Fig. 5C and D). At 44°C for 5 h, colonization was reduced in the soil amended with cabbage after 3 days. After 3 days of heat treatment at 47°C for 3 h/day, no colonization of seedlings was observed. Mortality of the seedlings was observed in the lower temperature regimes (Fig. 5E and F); however, when the soil was treated at 44°C for 5 h/day, no seedlings died, except after 2 or 3 days in soil amended with cabbage. No seedlings died when the soil was heated to 47°C for 3 h/day.

DISCUSSION

In the present study, the effects of time and temperature on the survival of *P. nicotianae* were evaluated over a series of temperatures and times (35 to 53°C for 5 to 120 min and 35 to 47°C for 2 to 480 h), in which a response surface approach allowed the simultaneous analysis of the effects of both time and temperature on the survival of the pathogen. The models that best described survival of *P. nicotianae* were linear-exponential or exponential.

The analysis of the effect of temperature regimes on the survival of *P. nicotianae* indicates that a single model is inadequate to describe the changes in survival over time as temperature increased. At the base temperature regime of 35°C for 8 h/day, none of the models accurately described survival, which was affected little by the temperature regime. As temperature increased, the change in survival was best explained by an exponential model, a model of square root of either time or survival, a logarithmic model of time, and a model of the reciprocal of time. The changes in spore survival that resulted in these models may have occurred as constant numbers of spores died per unit time at lower temperatures, constant proportion of the population was killed per unit time

Fig. 2. Effect of temperature (35 to 53°C) and time (2 to 480 h) on the survival of chlamydospores of *Phytophthora nicotianae*. **A**, Propagules per gram of soil (ppg) detected on a selective medium. **B**, Percentage of tomato seedling baits colonized by the pathogen.

at intermediate temperatures, and larger proportions of populations were inactivated early during exposure to the highest temperature.

The use of tomato seedlings as baits to detect survival of *P. nicotianae* in the treated soil demonstrated that this method can be as sensitive as the soil dilution plating procedure, except when residual populations of less than 1 ppg of soil occur. The rationale for using the baiting test was that heat treatment at temperatures that did not eliminate the pathogen from the soil could have affected the ability of the surviving population to infect and colonize a susceptible host or to germinate in the presence of an external stimulus, such as root exudates. However,

it is clear from these tests that when *P. nicotianae* is present in the soil, even at residual levels, infection of a tomato seedling can occur. The baiting technique has several disadvantages: the population of the pathogen is not quantified, extensive amounts of time and space may be required to grow the seedlings and incubate the baited soil, and the soil samples may be cross contaminated due to handling procedures.

Amending infested soils with dried, ground cabbage leaves and exposing it to heat treatment generally reduced survival of *P. nicotianae* in the present study. The effectiveness of cabbage amendment can be observed in two ways: (i) at a given tempera-

Fig. 3. Effect of temperature, cabbage amendment, and time on the survival of chlamydospores of *Phytophthora nicotianae*. Propagules per gram of soil (ppg) detected on a selective medium after heat treatment in soil amended with A, 0% cabbage; B, 0.125% cabbage; C, 0.25% cabbage; and D, 0.5% cabbage.

TABLE 1. Models of the thermal inactivation of chlamydospores of *Phytophthora nicotianae* as a function of constant temperature between 35 and 41°C, time of exposure from 2 to 480 h, and cabbage amendment concentrations

Cabbage %	Equation ^z	R ²
0	$\ln(\text{ppg} + 1) = (6.2 - 0.0141 \times \text{Time})e^{-0.0063 \times \text{Temp}}$ a	0.5062
0.125	$\ln(\text{ppg} + 1) = (6.2 - 0.0181 \times \text{Time})e^{-0.0191 \times \text{Temp}}$ b	0.5474
0.25	$\ln(\text{ppg} + 1) = (6.2 - 0.0183 \times \text{Time})e^{-0.0232 \times \text{Temp}}$ b	0.6124
0.5	$\ln(\text{ppg} + 1) = (6.2 - 0.0187 \times \text{Time})e^{-0.0282 \times \text{Temp}}$ c	0.6928

^z Equations followed by the same letter do not differ statistically ($P \leq 0.05$); ppg = propagules per gram of soil.

ture, less time is required to achieve the same reduction in population as cabbage concentration increases; and (ii) with increasing concentrations of cabbage amendment, lower temperatures are required to achieve the same reduction in populations of the pathogen. In soil amended with 0.5% cabbage, populations of *P. nicotianae* were eliminated after 24 h at 41°C, 48 h at 38°C, and 96 h at 35°C. These findings contrast with the results obtained in field work where no additional reductions in populations of *P. nicotianae* were achieved with cabbage amendments (8). Such a contrast can be explained by the different environments and procedures used in the two experiments. Cabbage was air dried and ground to a fine powder and uniformly mixed with the soil for the thermal inactivation tests. The field trials were performed with

Fig. 4. Percentage of tomato seedling baits colonized by *Phytophthora nicotianae* after heat treatment in soil amended with A, 0% cabbage; B, 0.125% cabbage; C, 0.25% cabbage; and D, 0.5% cabbage.

coarsely chopped cabbage, dried on the soil beds, and disked into the soil, resulting in large pieces of cabbage unevenly distributed in the soil that may have prevented a uniform release of volatile compounds upon heating.

The use of cabbage amendment without additional soil heating was effective for the control of *Aphanomyces euteiches* (14) but provided inconsistent control of *F. oxysporum* f. sp. *conglutinans* (22,23). Heating cabbage-amended soils induces the volatilization

Fig. 5. The effect of temperature regime, cabbage amendment, and time on the survival of chlamydospores of *Phytophthora nicotianae*. Temperature regimes that simulated solarization consisted of temperatures increased daily to 35°C (●), 41°C (▽), 44°C (■), 44°C (◇), or 47°C (▲) for 8, 5, 1.5, 5, or 3 h, respectively; the remainder of each day was maintained at 25°C. **A and B,** Propagules per gram (ppg) of soil detected on a selective medium. **C and D,** Percentage of tomato seedlings colonized by *P. nicotianae* after 1 month growing in infested soil. **E and F,** Percentage of tomato seedling mortality after incubation for 1 month in soil amended with cabbage and infested with *P. nicotianae*.

of certain soil volatile compounds, such as methanol, isothiocyanates, and aldehydes, which could be directly correlated to the inactivation of the spores of *Pythium ultimum* and sclerotia of *Sclerotium rolfsii* (10). The effect of cabbage amendment is directly proportional to its concentration in the soil (23). Increasing the concentration of cabbage from 0.25 to 2% reduced the population of *F. oxysporum* f. sp. *conglutinans* to undetectable levels in 12 days instead of the 30 days required at the lower concentration. Other crucifers, such as kale (*B. oleracea* var. *viridis*) and mustard (*B. nigra*) were also effective for the control of *F. oxysporum* f. sp. *conglutinans*. Another factor that may determine the effectiveness of the crucifer amendment is the concentration of glucosinolates present in the crop (15).

One major benefit of the use of intermittent heat in thermal inactivation studies is the provision of estimates of the effectiveness of soil solarization to control plant pathogens. However, cycling temperatures have not been employed routinely, possibly due to the compounded difficulties of establishing appropriate temperature regimes, the daily requirement of adjusting each temperature, and the longer time required to reach desirable control of the organism under study.

Wicks (26) analyzed the effect of intermittent heat on the survival of mycelium of *P. cambivora*. None of the isolates tested survived after 1 day at a regime of 45°C for 6 h/day and 20°C for 18 h/day. The response of the isolates was variable and inconclusive at 40 and 35°C for 6 h/day for 4 days. However, mycelium is not the most likely survival structure of *P. cambivora* in the soil.

Tjamos and Fravel (25) evaluated intermittent heat on the survival of a suspension of microsclerotia of *Verticillium dahliae* over 4 days. After 4 days at the highest temperature regime, less than 1% of the sclerotia germinated. In their study the use of sclerotium suspensions in water negated the insulating effect of air pockets in the soil, and the duration of the high temperature in each regime was longer than that which would normally occur under field conditions.

A more comprehensive study was done by Porter and Merriman (20) with several soilborne plant pathogens. Survival of each pathogen depended on the heat sensitivity of the type of propagule being evaluated. For example, *V. dahliae* did not survive for 15 days when the high temperature was above 40°C; in contrast, *Pythium irregulare* was recovered at 5×10^4 ppg of soil at 50°C at the end of the experiment.

Gamliel and Stapleton (10) selected two temperature regimes similar to those found in the San Joaquin Valley in California to evaluate the effectiveness of the regimes and cabbage amendment on the control of *Pythium ultimum* and *S. rolfsii*. Both pathogens were eliminated after 4 days in the cabbage-amended soil at tem-

perature regimes of 38 or 45°C for 4 h/day plus 20 h at 30°C; however, propagules of these pathogens could be recovered from the nonamended soil after 4 days at the same temperature regimes.

Comparisons between studies are further complicated due to the difference in length of the experiments, amplitude between low and high temperatures in each regime, and duration of the high temperature. If an in vitro experiment is compared with soil solarization in the field, then the duration of the in vitro study should be similar to the solarization trial and the temperature regimes should simulate those observed during solarization.

In the present study, populations of *P. nicotianae* decreased to residual levels after 15 days only when temperature regimes simulating optimum solarization conditions (47°C for 3 h/day) were used; under these circumstances no infection of tomato seedlings was observed after as little as 3 days of heat treatment. The use of 44°C for 5 h/day reduced populations to levels below 1 ppg of soil. However, colonization of tomato seedlings exposed to this regime was observed throughout the experiment. The use of temperature regimes simulating average field temperature regimes reduced the populations to at least 40% of the initial infestation level, but all seedlings were colonized at these regimes.

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TABLE 2. Regression coefficients of the linear regressions of temperature regimes and survival of chlamydospores of *Phytophthora nicotianae*^a

Temp (°C) ^y	Cabbage %	Parameters		R ²	P value ^z
		Slope	Intercept		
35 (8)	0	-0.01203	5.717	0.0267	0.062
35 (8)	0.125	-0.05508	4.808	0.2800	0.001
41 (5)	0	-0.09034	6.049	0.6679	0.001
41 (5)	0.125	-0.05091	3.812	0.2195	0.001
44 (1.5)	0	-0.07680	4.631	0.7264	0.001
44 (1.5)	0.125	-0.04426	3.454	0.2856	0.001
44 (5)	0	-0.26132	3.886	0.8684	0.001
44 (5)	0.125	-0.21618	2.531	0.5631	0.001
47 (3)	0	-0.05692	0.718	0.2422	0.001
47 (3)	0.125	-0.04026	0.455	0.2272	0.001

^a The model used for the analysis was $\ln(\text{ppg} + 1) = a + b \times \text{days}$.

^y Temperature regimes that simulated solarization consisted of temperatures increased daily to 35, 41, 44, 44, and 47°C for (length of time in parenthesis in hours) 8, 5, 1.5, 5, and 3 h, respectively; the remainder of each day was maintained at 25°C.

^z Significance associated to the coefficient of determination (R²).

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