Techniques

# Increased Germination of Propagules of *Phytophthora parasitica* by Heating Citrus Soils Sampled During Winter

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**ABSTRACT**

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The influence of heat treatment on the germination of propagules of *Phytophthora parasitica* was examined in three citrus groves during 1986. Temperatures between 12 and 34 C and lengthened incubation time increased recovery of propagules from soil. This effect could be expressed in terms of accumulated heat units calculated as degree-days. Similar numbers of propagules germinated from soils exposed to different temperatures when the durations of heat treatments were adjusted to ensure that soils received equivalent numbers of heat units. Germination of propagules was correlated directly with accumulation of heat units from 0 to 150 degree-days but was correlated negatively with accumulation of heat units from 150 to 1,650 degree-days. Maximal stimulation of propagule germination occurred when soils had acquired 100–150 degree-days. Population densities at this time were up to 9.5 times greater than populations recovered from unheated control soils. Heat treatment of soils collected during the summer months yielded population densities up to twice as large as control populations. There was no evidence that heat caused active growth of the fungus in sieved soils. We have selected 32 C for 5 days as a routine treatment for winter soils before isolations for *P. parasitica* are attempted.

*Additional keyword*: root rot.

*Phytophthora parasitica* Dastur causes a root rot of citrus in California (1), which often leads to slow decline of the tree and substantial yield loss. The pathogen becomes active as soil temperatures increase in the spring, and populations reach their maximal levels during the summer months (2). Dilution assays of soils collected in winter yielded only 0–2 colony-forming units (cfu) per gram of rhizosphere soil, even when high population densities of *P. parasitica* had been present during the previous summer (2). Similar results have been reported for dieback of *Rhododendron* sp. caused by *P. parasitica* (4). Propagules of *P. parasitica* survived the winter in citrus groves in a quiescent

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state, and the fungus could be isolated only after soils had been incubated at 24–31 C before assay (7).

Many California citrus soils contain *P. parasitica*, but the populations must reach a certain density before yield losses can be measured, and it becomes economical to treat for the disease (9). Groves usually are sampled only during the summer months, when the fungus is active, to determine if this threshold density is exceeded. At this time, however, *P. parasitica* is very responsive to changes in soil moisture and populations can fluctuate substantially.

We propose that heat treatment of winter soils might yield more stable populations of *P. parasitica*, which in turn could be used to help estimate summer populations. Sampling in winter would allow the grower more time to initiate control practices before the population increases the following summer. These studies were designed to examine the influence of heat treatments on the recovery of propagules of *P. parasitica* from citrus soils throughout the year.

## MATERIALS AND METHODS

Soils in three California orange groves were selected for studying the effect of cumulative heat treatment on recovery of *P. parasitica*. Soil in the Highland grove in San Bernardino County is a Greenfield sandy loam containing 9.4% clay, 21.1% silt, and 69.5% sand, with an electrical conductivity of 0.68 mmhos/cm. The trees were 78-yr-old cultivar Washington Navel on sweet orange (*Citrus sinensis* (L.) Osbeck) rootstock. Trees in the Stone Corral grove in Tulare County were 25-yr-old cultivar Valencia orange on Cleopatra mandarin (*C. reshni* Hort. ex Tan.) rootstock. The soil is a San Joaquin loam containing 14.1% clay, 39.2% silt, and 46.7% sand, with an electrical conductivity of 2.2 mmhos/cm. Trees in the Corona grove in Riverside County were 40-yr-old cultivar Washington Navel on sweet orange rootstock. This soil is a Garretson sandy loam containing 11.0% clay, 27.4% silt, and 61.6% sand, with an electrical conductivity of 0.6 mmhos/cm. All three groves were irrigated by low-volume emitters placed in rows between the trees.

Soil cores were collected from the wetted edge of the irrigation zone closest to the north side of individual citrus trees. Cores were obtained with a 7.5-cm-diameter bucket auger to a depth of 30 cm. The top 8 cm of soil was discarded. Each sample was gathered from two cores per tree. Only soil adhering to the feeder roots (rhizosphere soil) was collected for each sample. One sample for one tree was considered a replicate throughout this study. Each sample was adjusted to a soil moisture content of 12–13% (w/w), sieved through a 2-mm-mesh screen, and divided into the appropriate number of subsamples for each experiment. Subsamples were placed in polyethylene bags, and bags were closed with rubber bands.

Accumulation of heat units, expressed in degree-days, occurred both artificially in the laboratory and naturally in the groves as soil temperature rose in the spring. A threshold temperature of 12 C was selected. Below 12 C, heat units did not accumulate (see Discussion). Unless otherwise indicated, subsamples in polyethylene bags were incubated at 32 C until the desired number of heat units had been accumulated. During each experiment, one subsample from each tree was stored at 9 C to serve as an unheated control. Placement of soils in the 32 C incubator was timed so that all samples could be assayed simultaneously. Soil samples were usually placed directly into incubators after sampling. Some winter soil samples were stored at 9 C for later use.

Calculation of degree-days in the laboratory was determined by the following formula: (incubation temperature $-$ 12) $\times$ number of days of incubation. This same formula was used to calculate accumulation of heat units within the grove; however, the average weekly soil temperature at a depth of 30 cm was used as the incubation temperature. When samples were collected in winter, the soil temperature was below 12 C, and all heat units were accumulated by incubation in the laboratory. The total number of heat units accumulated by soils collected during spring and summer was the sum of heat units accumulated in both the field and laboratory.

A soil dilution assay (13) was used to quantitate populations of *P. parasitica*. Ten grams of soil per subsample (dry weight basis) was placed in a 250-ml flask, and water was added to give a total volume of 100 ml. After swirling the flask by hand, 1 ml of solution was pipetted into each of four petri dishes to which molten pimaricin-vancomycin-PCNB with hymexazol (PVPH) selective medium (12) was added. Dilution plates were incubated at room temperature for 4 days, and the number of colony-forming units dry weight of rhizosphere soil was determined. Two subsamples per soil sample from each tree were analyzed in this manner and the results were combined.

Trees with measurable populations of *P. citrophthora* (R. E. Sm. & E. H. Sm.) Leonian in the rhizosphere soil were avoided for these tests to quantitate populations of *P. parasitica* more readily. Differentiation between *P. parasitica* and *P. citrophthora* usually was possible by the gross morphology of colonies on PVPH dilution plates (1). When differentiation between *Phytophthora* species was difficult, transfers from dilution plates were made to fresh PVPH and cornmeal agar plates for microscopic identification.

Several experiments were conducted to determine the effects of temperature and duration of heat treatment on germination of *P. parasitica* from soil. In the first experiment, eight randomly selected trees in the Highland grove were sampled in January 1984, and 30-g subsamples of soil from each tree were incubated for 3 days at 20, 24, 27, or 31 C before dilution-plate assay. This experiment was repeated with soil from the Corona grove, and the soil was incubated for 3 days at 20, 24, 27, 31, or 34 C. Seven trees in the Highland grove were sampled during the winter of 1985 in the second experiment, and subsamples from each tree were incubated at 12, 15, 18, 21, 27, 30, or 33 C until each had accumulated 50 degree-days. Subsamples incubated at 12 C did not accumulate heat units, and they remained at 12 C for the duration of the heat treatments (17 days).

Samples were taken from seven trees in the Stone Corral grove in February 1985 in the third experiment. Subsamples were incubated at 32 C for 14.7–96 h and allowed to accumulate 12–80 degree-days. Subsamples from each tree were incubated at 9 C for 96 h for the control. The experiment was repeated in 1986.

In the fourth experiment, soil samples were collected from seven trees in the Highland grove in early March 1987. At sampling, the soil temperature was 12 C at a depth of 30 cm. Subsamples from each tree were incubated at 18, 24, or 32 C for 3, 6, 8, or 10 days and at 12 C for 10 days only.

All of the above experiments were repeated at a minimum of two sites. Usually, results from only one of the sites are reported, although in every case results from all sites were similar.

The influence of accumulation of heat units on recovery of *P. parasitica* was examined from January through September 1986 in a separate experiment. The Highland grove was sampled nine times (January 5 and 29, March 24, April 14 and 29, May 27, June 20, July 24, and September 4) and the Stone Corral grove was sampled five times (February 26, May 20, June 17, July 23, and September 29) during this interval. Ten trees were sampled on each sampling date. Soil from each tree was divided into 30-g subsamples, and one subsample from each tree was incubated at 32 C for 2, 3, 4, 5, 6, or 7.5 days so that 40, 60, 80, 100, 120, or 150 degree-days accumulated, respectively. Data on population densities of *P. parasitica* were analyzed as mean ratios rather than actual numbers of colony-forming units recovered. Mean ratios were calculated at each sample date by dividing the colony-forming units per gram obtained after each heat treatment by the colony-forming units per gram obtained in the unheated control. During May (Highland grove) or June (Stone Corral grove) 1986, populations of *P. parasitica* began to increase in the field due to resumption of parasitic growth. The use of mean ratios allowed population increases due to heat treatment to be examined without interference from population increases due to reproduction of the fungus.

Seven trees in the Highland grove were sampled in February 1987 in an additional experiment. Subsamples from each tree

were incubated at 32 C and allowed to accumulate 150, 200, 250, 300, or 500 degree-days. Subsamples incubated at 9 C for the duration of the experiment served as a control. The experiment was repeated at the Stone Corral site with similar results.

Chlamydospores, which are thought to overwinter in citrus soils, were produced in the laboratory and chilled before heat treatments to determine if they responded to heat treatment. In the first experiment, hyphal mats grown in half-strength V8 broth were submerged in water for 3 wk at 18 C to produce chlamydospores (11). Hyphal mats were blended in an Omnimixer (Omni Corporation International, Waterbury, CT), centrifuged to remove mycelial debris (11), and added to nonsterile field soil at a rate of 100 chlamydospores per gram of soil after adjustments of soil moisture contents to 14% (w/w). Infested soil (10 g dry weight basis) was stored at 9 C in test tubes capped and sealed with Parafilm (American Can Co., Greenwich, CT). The moisture content was maintained at 14% by the addition of water every 2 wk. After 1, 2, and 3 mo, four replicate tubes were incubated at 32 C for 2 days before dilution plating. Control tubes were kept at 9 C. The experiment was repeated twice.

In a second experiment, 2 g of feeder-root segments 2 cm long were cut from greenhouse-grown Pineapple sweet orange (*C. sinensis*) seedlings and inoculated with a zoospore suspension. Root segments were incubated between sheets of moist filter paper in glass petri dishes for 6 days until root pieces were colonized. To induce chlamydospore production, roots were buried about 7.5 cm deep in tamped, moist (soil moisture content = 14%), nonsterile field soil in three replicate 500-ml glass jars. Jars were airtight except for a 5-mm bent-glass tube, which was covered with Parafilm to reduce moisture loss. The temperature of the soil in the jars was reduced from 24 to 9 C in a graded temperature series over a 2-mo period and was kept at 9 C for 3 mo. After heat treatment in a 32 C incubator for 5 days, decaying root pieces and adhering soil were blended in an Omnimixer for 1 min in 50 ml of water. The soil slurry was wet-sieved through nested 350-, 245- and 45-$\mu$m mesh sieves, and twice through a 15-$\mu$m mesh nylon screen (Tetko, Inc., Elmsford, NY). Soil that remained on the 15-$\mu$m screen was collected both times and centrifuged at 3,000 rpm (1,200 *g*) for 4 min. The supernatant was discarded, and the pellet was resuspended in water to give a total volume of 10 ml. Samples (0.1 ml) of the suspension from each replicate were mixed with molten PVPH in petri dishes. Colonies were counted after 4 days. The suspension (2 ml) also was pipetted onto microscope slides that had been dipped three times in PVPH medium that was modified by using 8.5 g of Difco cornmeal agar and 22.5 g of Difco Bacto-agar per liter of water (Difco Laboratories, Detroit, MI). Slides were placed in glass petri dishes lined with moist filter paper and incubated in a dark cabinet at room temperature. The soil was rinsed from the slides under a gentle stream of water 24 h later. Controls were not heat treated before sample preparation. Slides were examined microscopically for germinated propagules. The experiment was repeated once.

An experiment was conducted to examine the effect of soil dilution on propagule recovery. Soil collected from each of eight trees in the Stone Corral grove during the winter of 1987 was divided into two sets of three replicate subsamples. One set was assayed immediately; the second set was assayed after incubation at 32 C until 100 degree-days had been accumulated. A soil dilution plate assay was performed with 10, 5, or 1 g of soil per subsample (dry weight basis). These soils were placed in 250-ml flasks, and water was added to give a total volume of 100 ml per flask.

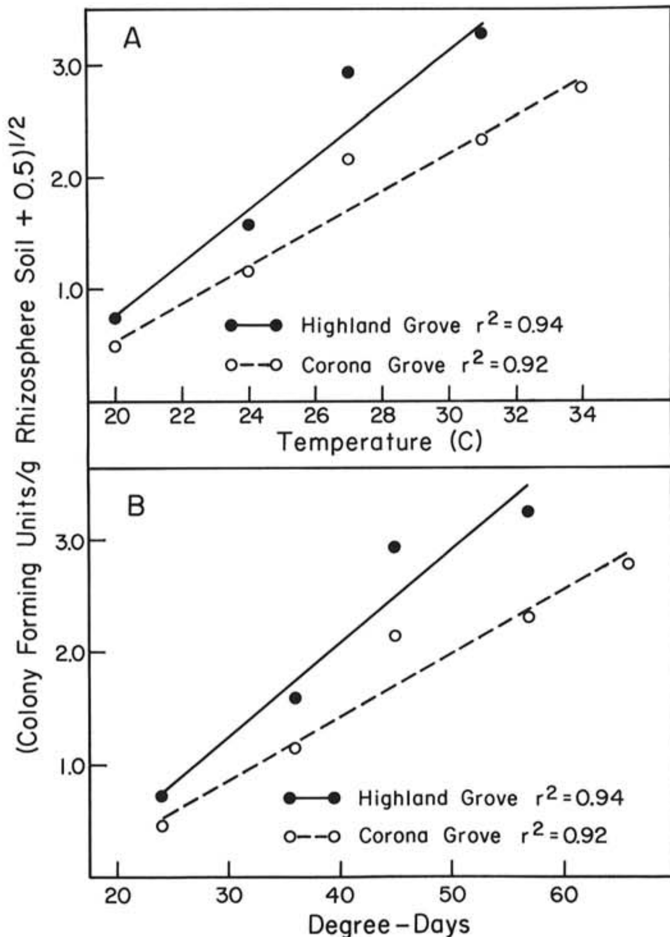


**Fig. 1.** The influence of **A,** temperature and **B,** accumulated heat units (expressed as degree-days) on colony-forming units of *Phytophthora parasitica* recovered from citrus rhizosphere soil during winter from two groves after heat treatment for 3 days.

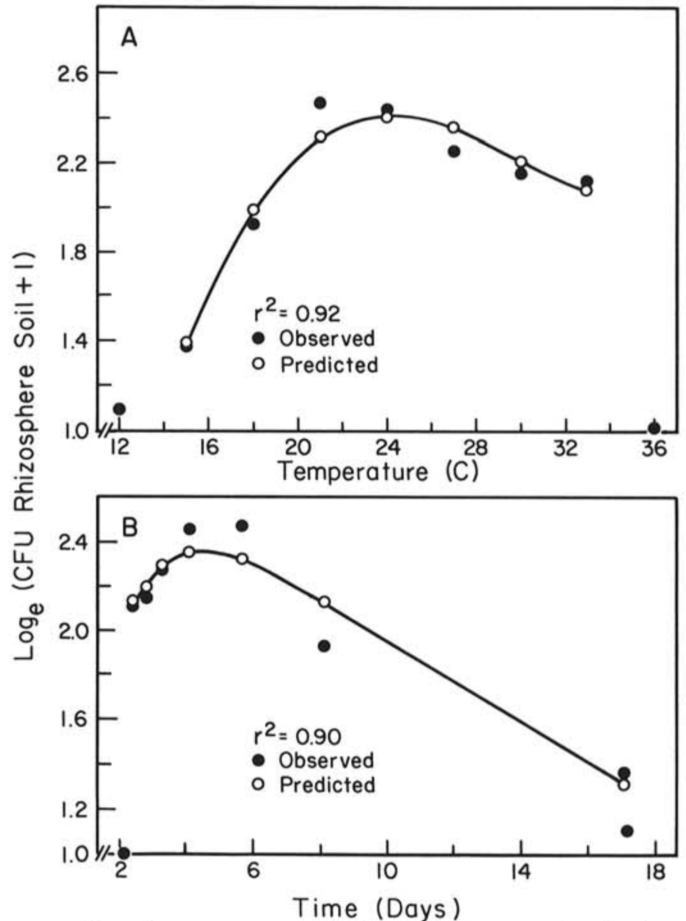


**Fig. 2.** Effect of **A,** temperature and **B,** incubation time on colony-forming units (cfu) of *Phytophthora parasitica* per gram of soil recovered from citrus rhizosphere soil sampled in winter. Predicted values were generated by the model $Y = a + b_1X + b_2X^2$, in which $Y = \log_e(\text{cfu/g} + 1)$ and $X = \log_e(\text{temperature})$ or $\log_e(\text{time})$. Values obtained after incubation at 12 and 36 C were not included in the analysis.

The assay to quantify the population density of *P. parasitica* is described above. The experiment was repeated twice.

Samples were collected during January 1986 from the Highland grove to quantitate bacterial and fungal populations in soils before and after heat treatments. Soil samples from each of six trees were divided into two subsamples. One subsample was assayed immediately; the other subsample was incubated at 32 C until 100 degree-days had been accumulated before assay. Samples from appropriate serial dilutions were spread uniformly onto five plates each of nutrient agar and *Pseudomonas* F agar (Difco) plus 100 μg/ml of cycloheximide for quantitation of bacteria (KBC), and PVP (13) and modified potato-dextrose agar (PDA) for quantitation of fungi. The PDA was half-strength Difco PDA to which an additional 7.5 g of agar per liter of water was added before autoclaving. Streptomycin sulfate (250 μg/ml) was dissolved in 10 ml of sterile water and added after the medium had cooled to 50 C. Plates were incubated at room temperature in the dark, and the number of colony-forming units per gram of soil was determined after 2 days for bacteria and 3 days for fungi. The experiment was repeated in February with similar results.

An experiment was conducted to determine the effect of heat on reproduction of *P. parasitica* from survival structures. Sieved, nonsterile field soil samples from the Stone Corral and the Highland groves were amended with 100 chlamydospores per gram of soil. The soil was mixed thoroughly after the soil moisture content had been adjusted to 12% (w/w). Each of the two soils was infested with chlamydospores from isolates of *P. parasitica* that originally had been isolated from the parent soil. Chlamydospores were grown and harvested by the methods of Tsao (11). Treatments consisted of infested and uninfested samples that had been held at 9 C for 1 day (no heat), or had been allowed to accumulate 40 or 100 degree-days in the laboratory. Each treatment for each soil type was replicated three times.

To quantify the germinated sporangia from each treatment, a 35-g sample of rhizosphere soil was brought to a total volume of 100 ml with water and shaken on a rotary shaker at 300 rpm for 1 min. The sporangia either resulted from germinated chlamydospores, which were used as inoculum, or they were indigenous in the soil. The suspension was decanted through a 250-μm-mesh sieve, and the washings were centrifuged for 4 min at 3,200 rpm (1,500 *g*). The pellet was resuspended in water to give a total volume of 30 ml. Samples (1 ml) were spread over three replicate half-strength PVPH plates. After 24–36 h, agar surfaces were rinsed with a gentle stream of water and examined microscopically for germinated propagules. To ascertain that germinated sporangia were *P. parasitica*, a small agar block containing the propagule was transferred from the dilution plate to a fresh PVPH plate. After 4 days, the plates were examined for growth. *P. parasitica* was distinguished from *P. citrophthora* primarily by colony morphology.

For most experiments, data were analyzed by linear regression, relating time of incubation or temperature to colony-forming units (Linear Regression, UCR Plant Path. Stat Pack, Borland Inc. Riverside, CA, 1985). Simple models such as $Y = a + b_1X + b_2X^2$, in which $Y = \log_e(\text{cfu/g} + 1)$ and $X = \log_e(\text{temperature})$ or $\log_e(\text{time})$ were compared with existing data. The combined effect of temperature and time was examined with multiple regression analysis and the model $Y = a + b_1X_1 + b_2X_2$, in which $Y$ = cfu/g of rhizosphere soil, $X_1$ = incubation temperature, and $X_2$ = incubation time. The model was expanded to include the interaction of incubation time and incubation temperature ($b_3X_1X_2$) as a third independent variable. In many of the laboratory experiments, standard deviations and standard errors were generated for the means. Colony-forming units recovered from soil frequently were transformed by logs or square roots to stabilize the variance.

## RESULTS

Recovery of *P. parasitica* from the Highland and Corona groves during winter was correlated directly with both incubation temperature and the accumulated number of heat units. This was evident when the mean colony-forming units from rhizosphere soil were transformed by $(\text{cfu} + 0.5)^{1/2}$ and were plotted against temperature or heat units (Fig. 1). The relationships were significant ($P = 0.05$) over the ranges of 20–31 C (24–57 degree-days) in the Highland grove and 20–34 C (24–66 degree-days) in the Corona grove.

In the second experiment, both length of incubation time and temperature were varied to hold the number of accumulated heat units constant at 50 degree-days. The colony-forming units were analyzed with the model $Y = a + b_1X + b_2X^2$, in which $Y = \log_e(\text{cfu/g} + 1)$ and $X = \log_e(\text{temperature})$ or $\log_e(\text{time})$. The

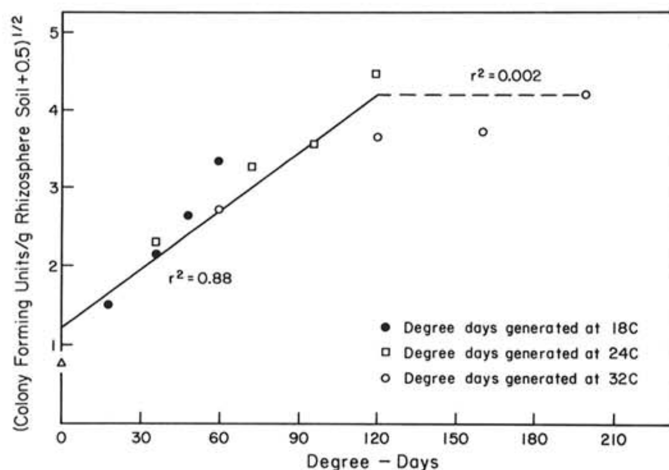


**Fig. 3.** Influence of accumulation of heat units on colony-forming units of *Phytophthora parasitica* recovered from citrus rhizosphere soil sampled in winter from the Stone Corral grove. Heat units were accumulated by incubation of soils at 32 C for various lengths of time. Linear regression analysis was calculated with all data from 0 to 120 degree-days, inclusive. A separate linear regression was calculated for all data with 120–200 degree-days, inclusive, and is shown as a dashed line. The slope was not significantly different from zero.

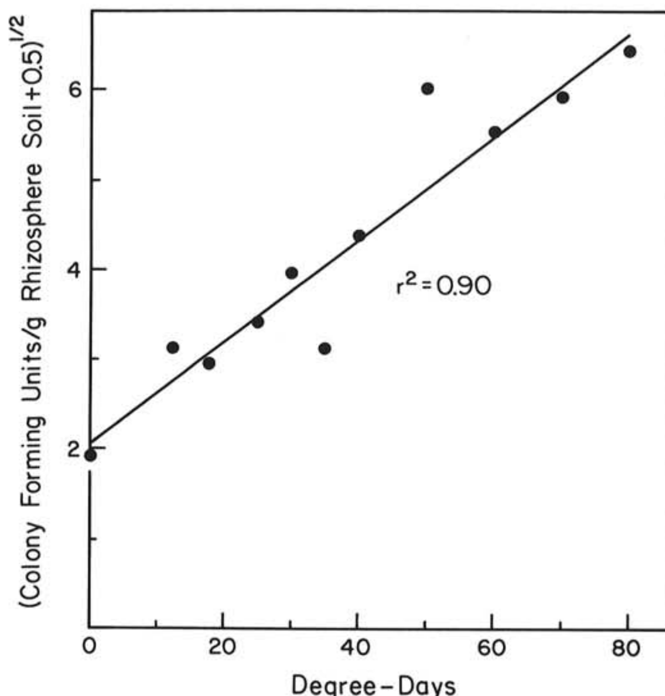


**Fig. 4.** Influence of accumulation of heat units on colony-forming units of *Phytophthora parasitica* recovered from citrus rhizosphere soil sampled in winter from the Stone Corral grove. Heat units were accumulated by incubation of soils at 32 C for various lengths of time.

relationships were significant ($P = 0.05$) for both temperature and time ($r^2 = 0.92$ and 0.90, respectively) (Fig. 2). Temperatures between 21 and 33 C, inclusive, were considered acceptable for use in heat treatments because of the similarity in colony-forming units recorded. Temperatures from 12 to 36 C were tested, although data from incubation at 12 and 36 C were not included in the analysis. No heat units accumulated at 12 C, the threshold temperature, and colony-forming units recorded were very low, not significantly different from those recorded at the storage temperature of 9 C (Fig. 2). Incubation at 36 C inhibited germination of propagules. Incubation times longer than 8 days did not stimulate germination of propagules compared with unheated controls. However, limited data were collected between 8 and 18 days. Heat treatments at 21–33 C for incubation periods of 2–8 days were more effective in increasing propagule germination than longer heat treatments at lower temperatures.

In the third experiment, germination of propagules was correlated significantly with accumulation of heat units from 0 to 120 degree-days (Fig. 3). Maximal germination of propagules was achieved when soils had accumulated 120 degree-days. Data acquired beyond 120 degree-days were analyzed separately. There was no correlation between the numbers of germinated propagules and accumulation of heat units from 120 to 200 degree-days.

The highly significant ($P = 0.001$) linear correlation between the numbers of germinated propagules and accumulation of heat units also was evident in a separate experiment conducted with soil samples collected in the winter of 1986 from the Stone Corral grove (Fig. 4).

In the fourth experiment, germination of propagules was correlated directly with both length of heat treatment and incubation temperature (Figs. 5 and 6, respectively). Numbers of colony-forming units recovered from individual trees were transformed by $(\text{cfu/g} + 0.5)^{1/2}$ before analyses. The linear relationship between the number of germinated propagules and the number of days of heat treatment at 18, 24, and 32 C was highly significant ($P = 0.001$). Temperatures from 12 to 32 C were correlated directly with the number of germinated propagules from soil after 0, 3,

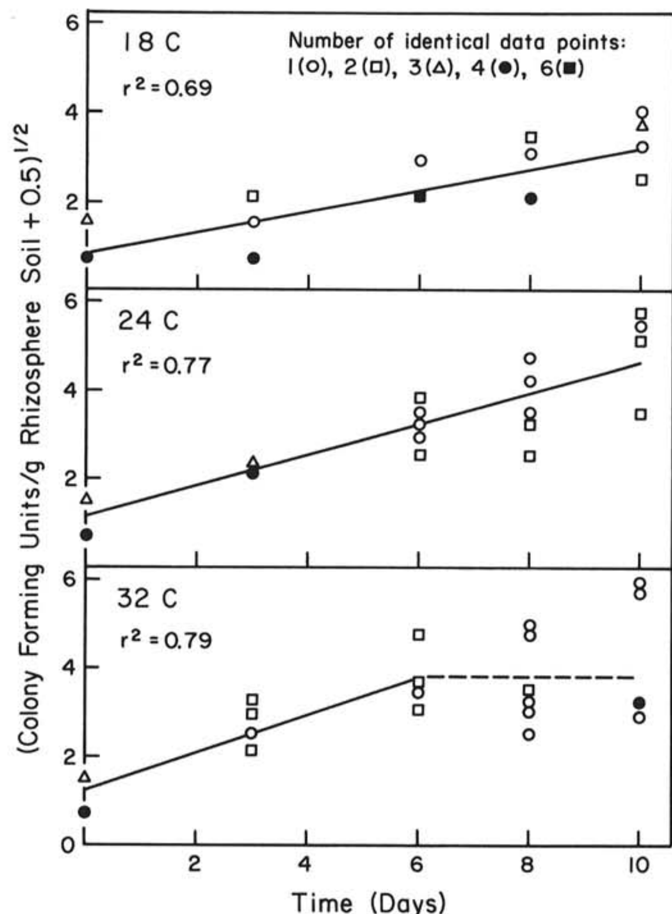


**Fig. 5.** The influence of incubation time on colony-forming units per gram of soil of *Phytophthora parasitica* recovered from citrus rhizosphere soil that was sampled in winter and incubated at three temperatures. The number of germinated propagules after 8 and 10 days of incubation at 32 C were not included in the regression analysis.

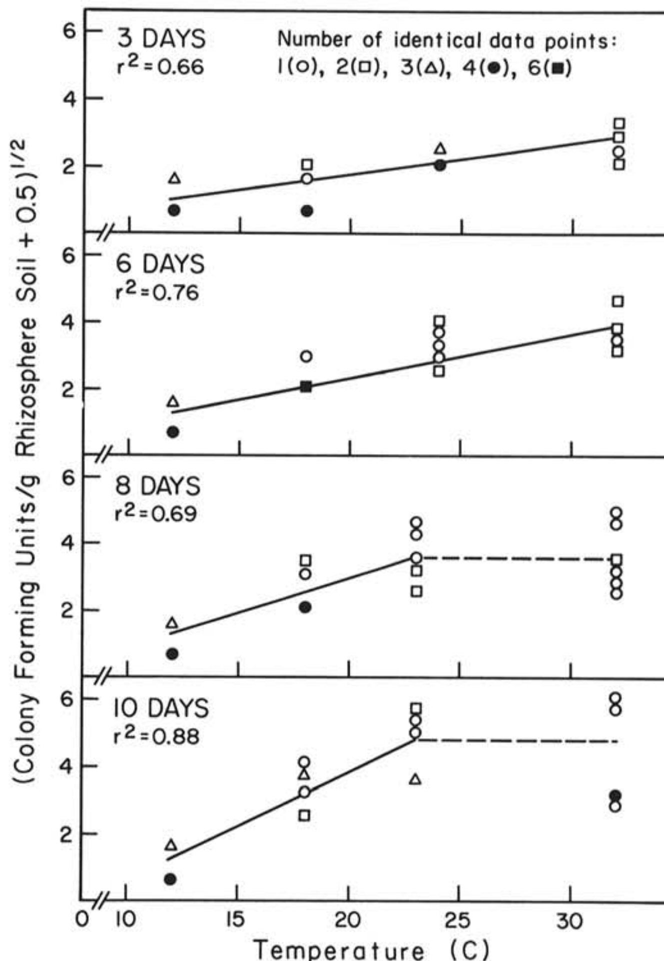


**Fig. 6.** The influence of incubation temperature on colony-forming units per gram of soil of *Phytophthora parasitica* recovered from citrus rhizosphere soil that was sampled in winter and incubated for 3, 6, 8, or 10 days. The numbers of germinated propagules after 8 and 10 days of incubation at 32 C were not included in the regression analyses.

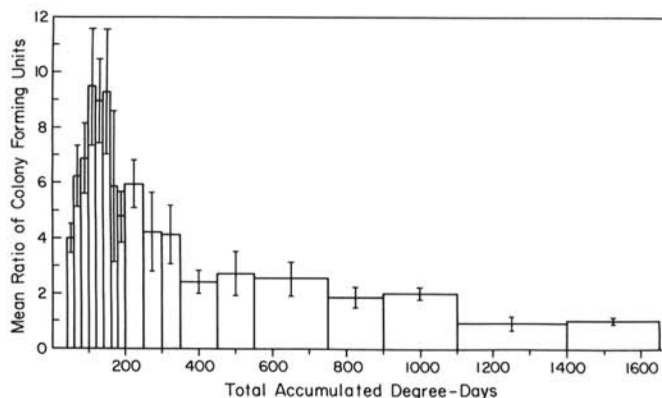


**Fig 7.** Mean ratios of population densities of *Phytophthora parasitica* recovered from citrus rhizosphere soil from two groves as affected by accumulation of heat units. Mean ratios equal the actual colony-forming units per gram of rhizosphere soil obtained after each heat treatment, divided by the colony-forming units recorded from the unheated controls at each sampling date. The height of each bar in the histogram is the average of the mean ratios within that interval of heat unit accumulation. The length of the vertical bar is twice the standard error.

6, 8, and 10 days of heat treatment. Data collected at 32 C after 8 and 10 days of heat treatment were excluded from the analyses, because the accumulation of heat units in these treatments had exceeded the number of degree-days optimal for germination of propagules.

The combined effect of temperature and time was examined with the model $Y = a + b_1X_1 + b_2X_2$, in which $Y$ = cfu/g of rhizosphere soil, $X_1$ = incubation temperature, and $X_2$ = incubation time; the model was highly significant ($P = 0.01$, $R^2 = 0.79$). When the model was expanded to include the interaction of incubation time and incubation temperature ($b_3X_1X_2$) as a third independent variable, the model again was highly significant ($P = 0.01$) and the $R^2$ was increased to 0.95. Multiple regression analysis calculated with either time and heat units or temperature and heat units as independent variables demonstrated that neither time nor temperature alone added significantly to this relationship beyond their contributions to the calculation of degree-days.

When both Highland and Stone Corral groves were sampled from January through September 1986, and soil samples were heated in the laboratory, maximal recovery of colony-forming units was achieved when soils had accumulated 100–150 degree-days (Fig. 7). Mean ratios of colony-forming units beyond this level were no longer correlated directly with accumulation of heat units. Population densities recovered from heat-treated winter soils were up to 9.5 times greater than population densities recovered from unheated soils. Stimulation of propagule germination by artificial heat diminished as additional heat units accumulated in the field, during 150–1,650 degree-days. Data were separated into two sections; 150 degree-days was chosen as the point of division for the purpose of analysis. Within the first section (0–150 degree-days), only data from trees sampled in January and February were used; within this section, accumulation of heat units occurred entirely within the laboratory. In the second section (150–1,650 degree-days), accumulation of heat units occurred mainly in the field. The mean ratios were correlated positively with degree-day accumulation in the first section (Fig. 8). Mean ratios transformed by 10 $\log_{10}$(mean ratio + 1) were correlated negatively with degree-day accumulation in the second section (Fig. 9). Accumulation of 0–500 degree-days in a separate experiment in the laboratory confirmed that the effect of heat diminished after 150 degree-days (Fig. 10).

Storage of unheated controls at 9 C for the duration of experiments did not affect propagule germination significantly. Similar numbers of propagules germinated from samples that were assayed the same day of sampling and from samples that were incubated at 9 C for 7.5 days.

Chlamydospores that were produced in the absence of host tissue did not become quiescent after 3 mo of chilling. Heat treatment did not stimulate germination, and there was no difference in the number of chlamydospores per gram of soil recovered from heated and unheated samples. Propagules produced within root tissue became quiescent after citrus root pieces infested with this fungus were buried in jars of moist field soil and chilled. *P. parasitica* was isolated from the decaying root pieces and surrounding soil only after heat treatment. Both sporangia and chlamydospores were isolated, and no oospores were seen. Fewer degree-days were used in the preparation of chlamydospores in artificial media than were used in the preparation of

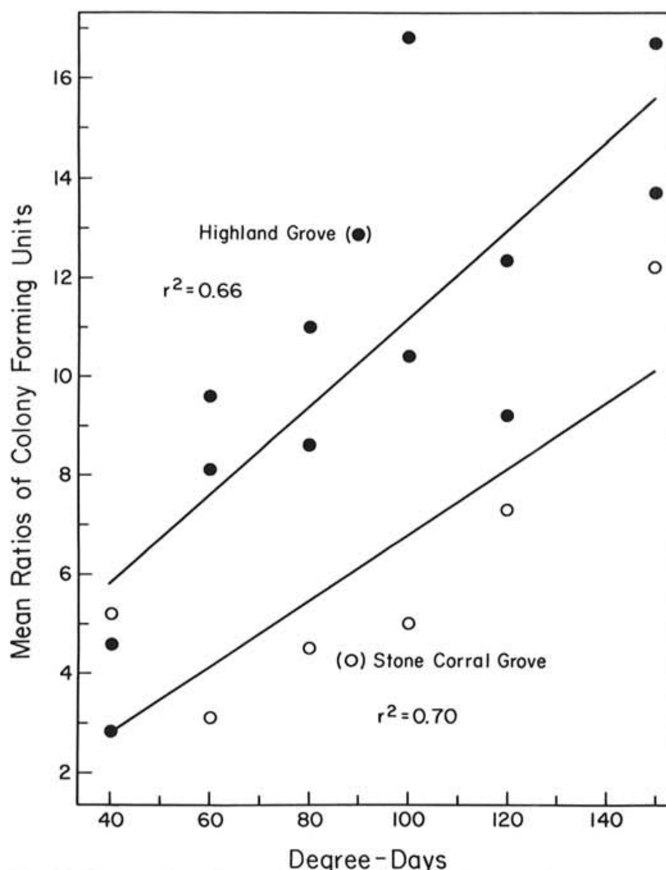


**Fig. 8.** Mean ratios of population densities of *Phytophthora parasitica* recovered from citrus rhizosphere soil in January and February 1986, after accumulation of 0–150 degree-days in the laboratory. The Highland grove was sampled twice and the Stone Corral grove was sampled once. Each data point is the mean value from 10 trees. Mean ratios equal colony-forming units per gram of rhizosphere soil obtained after each heat treatment, divided by colony-forming units recorded from the unheated control at each sampling date.

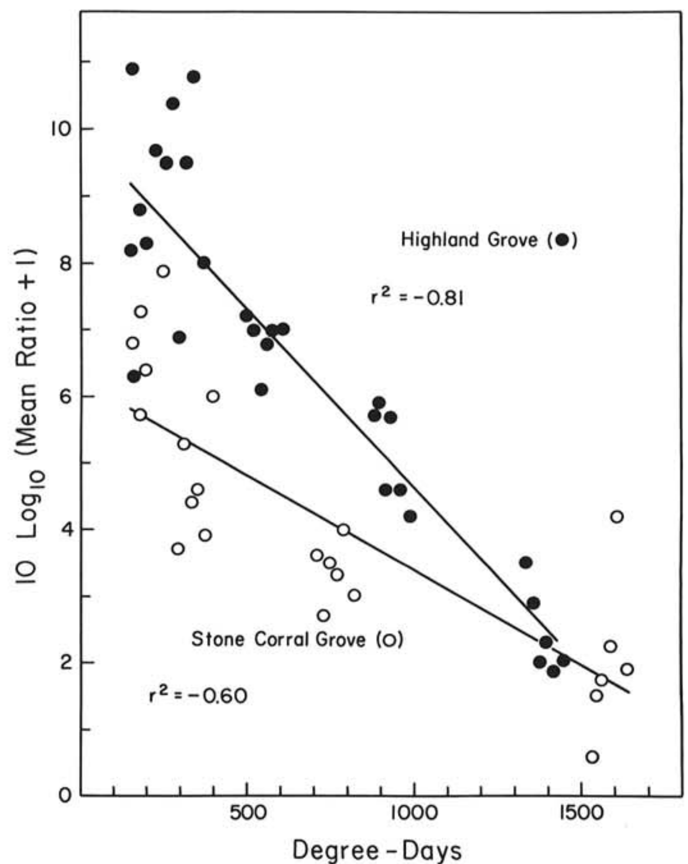


**Fig. 9.** Transformed mean ratios of population densities of *Phytophthora parasitica* recovered from citrus rhizosphere soil during March through September 1986, after accumulation of 150–1,650 degree-days in the field and laboratory. The Highland grove was sampled seven times and the Stone Corral grove was sampled four times. Each data point is the mean value from 10 trees. Mean ratios equal colony-forming units per gram of rhizosphere soil obtained after each heat treatment, divided by the colony-forming units recorded from the unheated control at each sampling date.

chlamydospores in root tissue.

The numbers of colony-forming units of *P. parasitica* recovered from soil dilutions were correlated negatively ($P = 0.001$) with the number of grams of soil per 100 ml of water over the range of 1–10 g of soil. This relationship existed both before and after soils had accumulated heat units in the laboratory.

The number of bacteria or fungi recovered before and after soils had accumulated 100 degree-days in the laboratory did not differ significantly on any of the media tested (Table 1). A similar experiment was run with controls stored at 9 C while other samples accumulated heat units. There were no significant differences between these treatments.

Accumulation of heat units did not appear to stimulate production of sporangia or zoospore release by *P. parasitica* in soil (Table 2). The increase in numbers of sporangia that occurred in uninfested Stone Corral soil after 120 degree-days most likely was due to germination of existing sporangia rather than to active fungal growth and reproduction from hyphae or chlamydospores. Sporangia from uninfested soils that germinated after heat treatments were dark and appeared to be old.

## DISCUSSION

*P. parasitica* survives in citrus rhizosphere soils at much higher population densities during the winter than had been recognized previously (2). Both incubation temperatures between 12 and 34 C and lengthened incubation time stimulated germination of propagules. The significant interaction term (temperature × time) in the model used to predict propagule germination justified the combination of these two variables into a single term for heat units, which was defined as degree-days. Degree-days are the interaction of temperature × time, with the incorporation of a threshold temperature below which degree-days do not accumulate. Similar numbers of propagules of *P. parasitica* germinated from soils exposed to different temperatures when the durations of heat treatments were adjusted so that soils received the same number of heat units. Heat had the greatest impact on stimulation of propagule germination during the accumulation of the first 150 degree-days. An accumulation of this magnitude occurred in the field during mid- through late May in the Highland grove and early to mid-June in the Stone Corral grove, which was located about 275 miles north of Highland. This corresponds to the time of year when the fungus first becomes active and population densities begin to increase in each grove (2). The benefits of heat treatment were reduced when more than 150 degree-days had accumulated.

Accumulation of heat units was not considered to occur below 12 C. This temperature was used because soils in California citrus-growing regions rarely exceed 12 C in the winter at a 30-cm depth. *P. parasitica* is not active when soils are this cool, as evidenced by the difficulty of isolating it during the winter even when the same soil had a high population density during the preceding summer. Additionally, radial growth on solid media in the laboratory is very slow at or below 12 C (1). Growth of citrus feeder roots also is reduced greatly during winter (8), and Girton (3) demonstrated that citrus roots virtually ceased to elongate at 12 C.

Although heat stimulated propagule germination during the first 150 degree-days, variability was highest at this time as well. This could be explained in part if the propagules of *P. parasitica* that composed the winter populations differed widely in their levels of maturity. Older propagules would have required only a few heat units to reach maturation, whereas newly formed propagules would have required more heat units to reach maturation. As the soil continued to warm, propagules that remained dormant would have had a lower heat requirement. This, then, would explain why heat-unit accumulation had a less marked impact on propagule germination as the summer progressed. It is possible that several groups of propagules exist that are physiologically different, as determined by the surrounding environment at the time of formation. The optimal number of heat units required to stimulate germination might differ between propagule groups, thus, increasing variability.

Maximal population densities of *P. parasitica* obtained from winter soils after heat treatment in the laboratory often were similar to those obtained during July through September when no additional heat was added in vitro. This seemed unusual because active growth and reproduction by the fungus during the summer would be expected to result in a much higher popu-

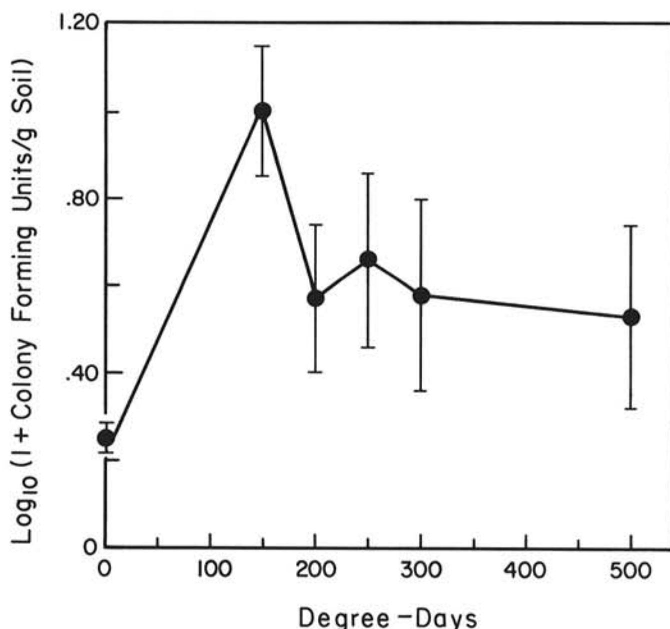


**Fig. 10.** Transformed colony-forming units of *Phytophthora parasitica* recovered per gram of citrus rhizosphere soil in February 1987, after accumulation of 0–500 degree-days in the laboratory. Vertical bar represents ± standard deviation.

TABLE 1. Effect of heat treatment on bacterial and fungal population densities from citrus rhizosphere soil sampled in winter

| Medium[b] | Colony-forming units per gram of soil[a] | |
|---|---|---|
| | No heat | 100 Degree-days[c] |
| KBC | $6.15 \times 10^7$ ($\pm 1.89 \times 10^7$) | $7.97 \times 10^7$ ($\pm 1.66 \times 10^7$) |
| NA | $5.60 \times 10^7$ ($\pm 9.19 \times 10^6$) | $5.08 \times 10^7$ ($\pm 5.67 \times 10^6$) |
| PDA-S | $4.14 \times 10^4$ ($\pm 3.64 \times 10^3$) | $3.60 \times 10^4$ ($\pm 5.81 \times 10^3$) |
| PVP | $2.27 \times 10^2$ ($\pm 2.59 \times 10^1$) | $2.63 \times 10^2$ ($\pm 5.43 \times 10^1$) |

[a] Data are means ± standard error of six replicates.
[b] KBC = *Pseudomonas* F agar plus 100 μg/ml of cycloheximide; NA = nutrient agar; PDA-S = half-strength potato-dextrose agar plus 7.5 g of agar and 250 μg/ml of streptomycin sulfate; PVP = corn meal agar plus 10 μg/ml of pimaricin, 100 μg/ml of PCNB, 200 μg/ml of vancomycin. All media are with 15 g of agar in 1 L of water. Bacteria were assayed on KBC and NA; fungi were assayed on PDA-S and PVP.
[c] Degree-days are the interaction of temperature × time.

TABLE 2. The influence of heat-unit accumulation on the numbers of sporangia that germinated from two citrus soils naturally and artificially infested with *Phytophthora parasitica*

| Treatment | Number of sporangia per 10 g of field soil[a] | | | |
|---|---|---|---|---|
| | Infested[b] | Uninfested | Infested[b] | Uninfested |
| No heat | 8.9 ± 0.7 | 3.3 ± 3.4 | 2.9 ± 2.9 | 0.8 ± 0.8 |
| 40 Degree-days[c] | 2.9 ± 1.4 | 7.0 ± 1.0 | 4.1 ± 0.5 | 2.0 ± 2.0 |
| 120 Degree-days | 7.8 ± 1.7 | 4.3 ± 0.5 | 4.3 ± 2.5 | 10.1 ± 1.3 |

[a] Data are means of three replicates ± standard error.
[b] Soil was infested artificially with 100 chlamydospores per gram of soil.
[c] Degree-days are the interaction of temperature × time.

lation density than quiescent winter population densities. Propagule germination often doubled after summer soils received an additional 40–60 degree-days in the laboratory. Longer heat treatments usually had no effect or decreased propagule germination. This suggests that a certain percentage of the population was immature or dormant, even in late summer, and was not recovered during routine assays. In the summer, however, heat units are accumulated much more quickly. An alternate explanation is that the effect of heat units accumulated in the laboratory differed somewhat from accumulation of heat units in the field. Soils in the laboratory were exposed to a constant high temperature as opposed to a gradually increasing temperature in the field.

Heat treatment reduced the soil moisture content a maximum of 1%. This change in moisture content was not considered a factor in stimulation of propagule germination, because soil moisture content from 9 to 14% (soil matric water potential = −0.07 to −0.01 MPa) did not have a significant impact on germination of propagules from soils collected during winter before or after heat treatment in previous experiments (7).

Dormancy of fungal propagules usually is believed to be quiescence and the result of adverse environmental factors that delay maturation. Another theory is that dormancy is constitutive and the result of an innate property within the propagule (10). Mechanisms that have been tested to explain environmental dormancy, or quiescence, include lack of available nutrients to spores due to microbial competition and the presence of inhibitory substances in soil (5,10). It does not appear that quiescence of *P. parasitica* can be explained primarily by a lack of exogenous nutrients due to competition with other microorganisms. Presumably, competition for nutrients would not be important when 1:10 soil dilution plates are prepared, yet recovery of propagules from winter soils remained low unless soil was heated before dilution plating. Because heat treatments did not alter the size of bacterial and fungal populations quantitated on nutrient agar, KBC, PVP, and PDA, heat probably does not increase nutrient availability by substantially reducing overall microbial competition. Because quantitation of these organisms before and after heat treatments only considered the sum of all organisms that grew on each medium, it is likely that individual species were stimulated or inhibited, but these effects were nullified by the overall population. It is possible that inhibitory compounds within the soil environment may complicate the quiescence of propagules of *P. parasitica*, because changing the soil dilution factor from 1:10 to 1:100 increased propagule germination even without heat treatment. Heat may inactivate inhibitory factors before plating on media. However, even after dilution increased propagule germination, heat stimulated germination proportionally as much as it did without dilution. This would indicate that two different mechanisms may be involved in breaking quiescence of propagules of *P. parasitica*.

Most researchers who have studied dormancy of propagules of *Phytophthora* spp. have addressed constitutive dormancy (14,15) and have focused primarily on oospores. It is unlikely that oospores alone are responsible for such high overwintering populations of *P. parasitica*. Germinated sporangia, chlamydospores, and oospores all have been recovered from soils that were collected in winter and subjected to heat treatments (6). Therefore, the dormant state in which *P. parasitica* overwinters in irrigated citrus groves probably encompasses many types of propagules. When unheated soil samples were examined, very few propagules of any type germinated.

The environment in which propagules are associated at the time of formation may determine whether or not they become quiescent and respond to heat. Propagules formed on laboratory media did not become quiescent despite aging for 3 mo in nonsterile field soil. Differences in nutrition may, in part, determine whether or not dormant propagules are produced (11). This illustrates the need for caution when interpreting data from propagules produced on media. The environment also will influence the response of propagules isolated from the field to heat treatment, because these propagules may develop within soil or within root tissues that later decay.

Heating winter soils in the laboratory can be used to increase propagule germination of *P. parasitica* from citrus groves. Winter populations are more stable, because the fungus is dormant during this time. Winter sampling and exposure of soil to a given number of heat units may serve to extend the time of year when populations of *P. parasitica* can be quantitated. It also may allow researchers to quantitate propagule numbers without interference from fluctuations in population due to irrigation.

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