Impact of Different Preplant Cultural Treatments on Survival of *Phytophthora nicotianae* in Soil

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ABSTRACT

Matheron, M. E., and Porchas, M. 2009. Impact of different preplant cultural treatments on survival of *Phytophthora nicotianae* in soil. Plant Dis. 93:43-50.

During the life of a citrus planting, the population of *Phytophthora* pathogens can build to significant levels in orchard soil. A study was initiated to examine the impact of some nonchemical cultural practices on survival of *P. nicotianae*, the most prevalent *Phytophthora* sp. in Arizona citrus groves, in soil formerly planted to citrus. In three trials over a 3-year period, *P. nicotianae* could not be detected at a depth of 10 cm after soil naturally infested with the pathogen was subjected to a dry summer fallow period of at least 31 days in the desert southwest region of Arizona. The mean temperature of soil at this depth during these trials ranged from 37 to 39°C. Furthermore, in two of these trials, after summer dry fallow periods of 38 and 45 days, the pathogen could not be detected at a depth of 15 to 20 cm and was detected in only one of 19 soil samples at a depth of 25 to 30 cm. In comparison, the pathogen was recovered from a high proportion of soil samples subjected to a dry winter fallow period or maintained in the greenhouse and planted with a seedling of citrus, alfalfa, or irrigated without the presence of any plant, where mean temperature of soil ranged from 15 to 30°C. In regions with a hot and dry summer climate, a dry summer fallow treatment of soil after removal of an existing citrus planting and before establishment of a new grove could provide a rapid and relatively inexpensive means of lowering the population of *P. nicotianae* to virtually nondetectable levels to at least a depth of 30 cm.

Phytophthora gummosis and root rot is an economically important disease of citrus trees (7). The cited causal agents of this disease in the United States are *Phytophthora citrophthora* (R.E. Sm. & E.H. Sm.) Leonian and *P. nicotianae* Breda de Haan (syn. *P. parasitica*). Both oomycete pathogens are cited as important citrus pathogens in Arizona (16) and California (8), whereas *P. nicotianae* alone is the primary pathogen on citrus in Florida (26) and Texas (24). Although both pathogens are present in Arizona citrus groves, *P. nicotianae* is much more prevalent than *P. citrophthora* (16).

Infection of citrus tissue usually occurs by zoospores, which are released from sporangia in the presence of free water (25). In addition to soil moisture, disease development and severity within a citrus planting can be affected by rootstock (17), seasonal differences in rootstock susceptibility to *P. citrophthora* and *P. nicotianae* (10,11), and soil temperature (12,13). Disease management within infested citrus plantings is achieved by avoiding overirrigation (27), facilitating soil drainage (27), and using systemic fungicides such as

fosetyl-Al (Aliette, Bayer CropSciences, Research Triangle Park, NC) and mefenoxam (Ridomil Gold, Syngenta Crop Protection, Inc., Greensboro, NC) (25).

During the life of a citrus planting, the population of *Phytophthora* pathogens can build to significant levels in orchard soil. When this orchard is removed and replanted, the residual population of *Phytophthora* remaining in soil can infect the new trees, resulting in suppressed growth and possible tree death, delayed onset of commercial fruit yields, and long-term decrease in yield and tree growth compared to trees growing in the absence of the pathogen. Utilization of fallow periods with or without a cover crop or soil fumigation are potential means of reducing the population of *Phytophthora*, so that newly planted trees can grow without the deleterious influence of these organisms.

In Arizona, several different methods are used by growers to prepare land for replanting of citrus trees. One approach involves total removal of old trees, including as many roots as possible, followed by rapid replanting without any additional treatment of soil. An alternative approach is to treat soil after tree and root removal with a soil fumigant such as metam sodium before replanting the orchard. Additional practices include leaving the soil fallow or planting the former orchard site to alfalfa (*Medicago sativa* L.) for 1 to 3 years before replanting with citrus. It is unknown how these different cultural practices affect the final population of *Phy-*

tophthora present in soil prior to establishment of another citrus planting. The objective of this research was to examine the impact of different nonchemical cultural practices on survival of *P. nicotianae*, the most prevalent *Phytophthora* sp. in Arizona citrus groves, in soil formerly planted to citrus (16).

MATERIALS AND METHODS

Survival of *P. nicotianae* in citrus orchard soil. For each of the five experiments described below, a 15-liter capacity container was filled with a sandy loam soil collected from within the drip line of each of 8 (experiment 1), 9 (experiment 2), or 10 (experiments 3 to 5) different trees within a mature lemon (*Citrus limon* (L.) N.L. Burm.) grove at least 20 years old in Yuma, AZ. The soil sample from each tree was thoroughly mixed and tested for the presence of *P. nicotianae* by adding 500 cm³ of soil into a container (13 cm wide × 23 cm long × 7 cm deep), then placing two ripe but green-colored unblemished pear fruit on the surface of the soil. Sufficient water was added to establish a 1- to 2-cm layer of free water at the soil surface. After incubation at 26°C for 48 h, the fruits were removed from the soil, washed in water, and incubated for an additional 48 to 96 h at 26°C. Firm brown lesions developed on pear fruits invaded by *P. nicotianae*. To confirm the identity of the pathogen, a small piece of tissue from the advancing margin of one or more lesions per pear fruit was placed on corn meal agar amended with pimaricin, ampicillin, rifampicin, and pentachloronitrobenzene (PARP) (5) and observed for mycelial growth characteristics of *P. nicotianae*.

Four 1-liter subsamples of soil from each bulk sample collected from a citrus tree became samples of soil subjected to one of the following cultural conditions: (i) planted with a *Citrus volkameriana* Tan. & Pasq. seedling, maintained in the greenhouse, and irrigated as needed; (ii) planted with an alfalfa seedling, maintained in the greenhouse, and irrigated as needed; (iii) maintained in the greenhouse and irrigated at the same time as soil containing citrus or alfalfa plants; and (iv) soil maintained outside without irrigation in full sun within a 1-liter capacity container composed of white plastic (11 cm diameter × 13 cm deep) placed in the field so that the upper lip was 1 cm higher than the surrounding soil surface and the soil within the container. In all but experiment 1, a 7-liter

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Accepted for publication 22 September 2008.

capacity container composed of black plastic and containing drain holes in the bottom (15 cm × 15 cm × 38 cm deep) also was placed in the field and filled with soil collected from citrus trees so that the upper lip of the container also was 1 cm higher than the surrounding soil surface and the soil within the container. The purpose of maintaining soil in these different environmental conditions was to simulate what could happen after a citrus planting is removed, including rapid replanting of another citrus planting, planting the field to alfalfa, or subjecting the land to an extended period of dry fallow. In three experiments, the soil samples were subjected to these cultural conditions during the summer, in comparison with two experiments which were initiated in the winter. All citrus and alfalfa seedlings used in this study were started and maintained in a peat-based potting mix (Aqua Guard, St. Louis, MO) in a greenhouse before being transplanted into citrus orchard soil.

After the treatment phase of each experiment, where soil samples had been subjected to the various environmental conditions for 104 to 177 days (Table 1) depending on the experiment, all samples in 1-liter containers not yet in the greenhouse were placed there, and a 2-month-old *C. jambhiri* Lush. (rough lemon) seedling was planted into each container in experiment 1, while 6-month-old seedlings of *C. volkameriana* were used in experiments 2 to 5. The alfalfa and citrus plants previously growing in some of the soil samples were removed and replaced with these citrus seedlings as well. For soil within each 7-liter container, a 1-liter volume was collected from the top (5 to 10 cm depth), middle (15 to 20 cm depth), and bottom (25 to 30 cm depth) of each container and planted with a citrus seedling. All plants were watered as needed and maintained in the greenhouse until each experiment was terminated, which ranged from 85 to 182 days (Table 1). This incubation period with the citrus host was designed to allow time for multiplication of propagules of *P. nicotianae* that may have been below detectable levels in soil subjected to a dry summer fallow period.

The sandy loam soil in each container was tested for the presence of *P. nicotianae* once during the soil treatment phase (from 31 to 104 days after soil treatments were initiated, depending on the experiment)

and again at the conclusion of each experiment after the incubation period with the citrus host. For each replicate soil sample, a 50-g amount was placed into a 500 ml capacity container (10 g of soil placed into each of two 500 ml containers for experiment 1). An unblemished green but ripe pear fruit was placed in each container along with enough tap water to half submerge the fruit. Depending on fruit size, 265 to 300 ml of water was added to each container. After a 48-h incubation period on the laboratory bench at 26°C, pear fruits were removed from the water and soil mixture and placed on the laboratory bench for an additional 5 days. One or more lesions appearing on a pear fruit and caused by *P. nicotianae* was considered positive detection of the pathogen. Data recorded included the number of replicate soil samples per treatment from which *P. nicotianae* was recovered with the pear bait assay and the mean number of lesions per pear fruit bait. Temperature of soil samples as well as rainfall during these trials also was recorded. Additionally, soil temperatures during the summer were collected at a depth of 10 cm from a mature citrus grove, an established alfalfa field, and a nonplanted field in Yuma.

***P. nicotianae* detection limit for pear fruit bioassay.** Laboratory experiments were conducted to estimate the sensitivity of the pear fruit baiting technique for detecting zoospores of *P. nicotianae*. An isolate of *P. nicotianae* recovered from a citrus grove in Yuma was used in these trials. Four 5-mm-diameter agar disks containing mycelium from an actively growing 3-day-old culture of *P. nicotianae* on V8 juice agar (14) maintained at 24°C in darkness were placed into each of six plastic petri dishes (60 mm diameter). Seven milliliters of a nonsterile soil extract then was added to each dish. Nonsterile soil extract was prepared by mixing 15 g of a sandy loam orchard soil in 1 liter of distilled water with a magnetic stirrer for 8 h at 25 ± 2°C. After an additional 16-h incubation period without stirring, the coarse soil particles settled to the bottom of the container and the remaining suspension was decanted and used as the soil extract. Petri dishes containing mycelial disks and soil extract were then incubated at 26°C in darkness for 4 days. The resulting sporangia that were produced were induced to release zoospores by chilling at

4°C for 30 min. After rewarming at 25°C for 15 min, the zoospore suspension in the six petri dishes was decanted into a 50 ml beaker. Four containers (21 cm long × 11 cm wide × 7 cm deep) each containing 660 ml of tap water were placed in an incubator set at 22°C. When the temperature of the water in each container reached 22°C, four different amounts of the zoospore suspension were added and mixed into the water in each of the four containers. Three small green but mature Bartlett pear fruits were placed into each container, resulting in fruits half-immersed in the water. After a 48-h incubation period at 22°C, pear fruits were removed from the zoospore suspension and incubated on the lab bench at 26 to 27°C for 2 days, at which time the number of lesions on each fruit caused by *P. nicotianae* was recorded. To determine the concentration of the original zoospore suspension before dispersal to the four containers, 0.5 ml of the suspension was added to 99 ml of water. After thorough mixing, a 0.5-ml aliquot was distributed evenly over the surface within each of four 100-mm-diameter plastic petri dishes containing corn meal agar amended with vancomycin-pimaricin-pentachloronitrobenzene medium (19). These petri dishes, with lids removed, were maintained in a laminar-flow hood for 5 min to remove free moisture from the agar surface. Petri dishes then were incubated in darkness for 48 h at 24°C, after which the number of pathogen colonies developing from zoospores was determined. This value was used in turn to determine the concentration of the original zoospore suspension as well as the quantity of zoospores within each container with pear fruits. This trial was conducted three times.

Analysis of data. CoStat statistical software (CoHort Software, Monterey, CA) was used to perform analysis of variance with the GLM procedure. Means were compared and separated at *P* = 0.05 according to Fisher's protected least significant difference (LSD). Linear regression analysis was performed by using the SigmaPlot software package (Systat Software, Inc., San Jose, CA) to show the relationship of zoospore concentration to the number of zoospore infections detected on a pear fruit, and to predict the limit of zoospore detection by the pear fruit bait assay.

Table 1. Experimental parameters for studies evaluating survival of *Phytophthora nicotianae* in citrus orchard soil

Experiment number	Duration of soil samples exposure to various cultural conditions		Duration of time after soil exposure to various cultural conditions until termination of trial	
	Dates	Total days	Dates	Total days
1	19 Jun to 13 Dec 1998	177	14 Dec 1998 to 8 Mar 1999	85
2	5 Jul to 4 Nov 1999	122	5 Nov 1999 to 21 Apr 2000	158
3	24 Jul to 30 Nov 2000	129	1 Dec 2000 to 9 Mar 2001	98
4	30 Nov 1999 to 21 Mar 2000	104	22 Mar to 17 Jul 2000	118
5	4 Dec 2000 to 28 Mar 2001	115	29 Mar to 27 Sep 2001	182

RESULTS

Survival of *P. nicotianae* in citrus orchard soil. For the three trials initiated in the summer (Table 1), *P. nicotianae* was not recovered after a period of 31 to 45 days from any of the soil samples main-

tained outside within a 1-liter capacity container in full sunlight and not irrigated (Tables 2 to 4). Total rainfall recorded during the time that these soil samples were maintained outside was 57.9, 15.2, and 30.0 mm for experiments 1, 2, and 3,

respectively. The mean temperature of the soil at a depth of 10 cm during this time ranged from 37 to 39°C for the three experiments. In comparison, the pathogen was recovered from a high proportion of soil samples within same-sized containers

Table 2. Detection of *Phytophthora nicotianae* in citrus grove soil after subjection to different temperature, moisture, and cultural conditions from June through December 1998 (experiment 1)

Soil treatment ^w	31 Days after initiation of soil cultural treatments ^x		Mean soil temperature (°C) during 31-day period ^y	85 Days after termination of soil cultural treatments ^x	
	Samples with <i>P. nicotianae</i> detected	Mean no. of lesions on pear bait		Samples with <i>P. nicotianae</i> detected	Mean no. of lesions on pear bait
Soil in 1-liter container in greenhouse with <i>Citrus volkameriana</i> seedling, irrigated as needed	5 a ^z	1.2	28	3 ab	0.9
Soil in 1-liter container in greenhouse with alfalfa plant, irrigated as needed	5 a	2.4	28	4 a	1.0
Soil in 1-liter container in greenhouse, irrigated at the same time as soil containing citrus and alfalfa plants	1 b	0.1	28	3 ab	5.2
Soil in 1-liter container, in full sun, not irrigated	0 b	0	37	0 b	0

^w Soil was collected from within the drip line of eight different trees within an established lemon grove. After soil from each tree was tested and found to contain *P. nicotianae*, four 1-liter aliquots of soil from each bulk sample became samples subjected to the listed cultural conditions. Soil samples were subjected to these conditions for 177 days (19 Jun to 13 Dec 1998), then placed in the greenhouse, and a 2-month-old *C. jambhiri* (rough lemon) seedling was planted into each 1-liter container. The *C. volkameriana* and alfalfa plants previously growing in some of the soil samples were removed and replaced with rough lemon seedlings as well. All plants were watered as needed until the trial was terminated 8 Mar 1999. Total rainfall from 19 Jun to 13 Dec was 57.9 mm.

^x Soil samples were tested for the presence of *P. nicotianae* 31 days after soil treatments were initiated (22 Jul 1998) and again 85 days after termination of the soil treatments and planting of rough lemon seedlings (8 Mar 1999). For each replicate soil sample, a 10-g amount was placed into each of two 500-ml containers. An unblemished green but ripe pear fruit was placed in each container along with enough tap water to half submerge the fruit. After a 48-h incubation period on the laboratory bench at 26°C, pear fruits were removed from the water and soil mixture and placed on the laboratory bench for an additional 5 days. One or more lesions appearing on a pear fruit and caused by *P. nicotianae* was considered positive detection of the pathogen. Each value is either the number of the eight replicate soil samples per treatment from which *P. nicotianae* was recovered with the pear-bait assay or the mean number of lesions per pear-fruit bait.

^y Mean soil temperature (°C) at a depth of 10 cm during this 31-day period.

^z Numbers in each column followed by a different letter are significantly different ($P = 0.05$) according to the Fisher's protected LSD.

Table 3. Detection of *Phytophthora nicotianae* in citrus grove soil after subjection to different temperature, moisture, and cultural conditions from July through November 1999 (experiment 2)

Soil treatment ^w	45 Days after initiation of soil treatments ^x		Mean soil temperature (°C) during 45-day period ^y	158 Days after termination of soil treatments ^x	
	Samples with <i>P. nicotianae</i> detected	Mean no. of lesions on pear bait		Samples with <i>P. nicotianae</i> detected	Mean no. of lesions on pear bait
Soil in 1-liter container in greenhouse with <i>Citrus volkameriana</i> seedling, irrigated as needed	7 a ^z	5.1	28	8 a	18.6
Soil in 1-liter container in greenhouse with alfalfa plant, irrigated as needed	8 a	3.1	28	9 a	19.1
Soil in 1-liter container in greenhouse, irrigated at the same time as soil containing citrus and alfalfa plants	8 a	11.1	28	8 a	20.8
Soil in 1-liter container, in full sun, not irrigated	0 b	0	38	0 b	0
Soil in 7-liter container, in full sun, not irrigated (soil at 5 to 10 cm depth)	N.D.	N.D.	38	0 b	0
Soil in 7-liter container, in full sun, not irrigated (soil at 15 to 20 cm depth)	N.D.	N.D.	N.D.	0 b	0
Soil in 7-liter container, in full sun, not irrigated (soil at 25 to 30 cm depth)	N.D.	N.D.	36	1 b	0.2

^w Soil was collected from within the drip line of nine different trees within an established lemon grove. After soil from each tree was tested and found to contain *P. nicotianae*, 1- or 7-liter aliquots of soil from each bulk sample became samples subjected to the listed cultural conditions. Soil samples were subjected to these conditions for 122 days (5 Jul to 4 Nov 1999), then placed in the greenhouse, and a 6-month-old *C. volkameriana* seedling was planted into each 1-liter container. The *C. volkameriana* and alfalfa plants previously growing in some of the soil samples were removed and replaced with 6-month-old *C. volkameriana* seedlings as well. All plants were watered as needed until the trial was terminated 21 Apr 2000. Rainfall from 5 Jul to 4 Nov was 15.2 mm.

^x Soil samples were tested for the presence of *P. nicotianae* 45 days after soil treatments were initiated (19 Aug 1999) and again 158 days after termination of the soil treatments and planting of *C. volkameriana* seedlings (21 Apr 2000). For each replicate soil sample, a 50-g amount was placed into a 500-ml container. An unblemished green but ripe pear fruit was placed in each container along with enough tap water to half submerge the fruit. After a 48-h incubation period on the laboratory bench at 26°C, pear fruits were removed from the water and soil mixture and placed on the laboratory bench for an additional 5 days. One or more lesions appearing on a pear fruit and caused by *P. nicotianae* was considered positive detection of the pathogen. Each value is either the number of the nine replicate soil samples per treatment from which *P. nicotianae* was recovered with the pear-bait assay or the mean number of lesions per pear-fruit bait. N.D. = not determined.

^y Mean soil temperature (°C) at a depth of 10 cm during this 45-day period.

^z Numbers in each column followed by a different letter are significantly different ($P = 0.05$) according to the Fisher's protected LSD.

maintained in the greenhouse and planted with a seedling of *C. volkameriana* or alfalfa during the same time periods in all three trials (Tables 2 to 4). Recovery of *P. nicotianae* from irrigated soil in the greenhouse not planted to citrus or alfalfa was variable, ranging from one of eight, eight of nine, and nine of 10 samples for experiments 1, 2, and 3, respectively. The mean temperature of soil planted to citrus, alfalfa, or irrigated without the presence of any plant for these trials ranged from 28 to 30°C.

The mean soil temperature at a depth of 10 cm during the summer months for soil samples maintained outside in 1-liter containers (11 cm diameter × 13 cm deep) and not irrigated (experiments 1 to 3) ranged from 36.0 to 37.8°C (Table 5). The total hours during this time that soil temperatures at a depth of 10 cm were greater than or equal to 35.0°C ranged from 951 to 1,132. The mean soil temperature at a depth of 30 cm during the summer months for soil samples maintained outside in 7-liter capacity containers (15 cm × 15 cm × 38 cm deep) and not irrigated (experiments 2 and 3) ranged from 34.0 to 35.0°C (Table 5). Total hours that soil temperatures at a depth of 30 cm were greater than or equal to 35.0°C ranged from 570 to 800. Soil samples placed outside and not irrigated were maintained in this condition for 177, 122, and 129 days for experiments 1, 2, and 3, respectively. Soil samples for these

experiments then were moved into the greenhouse, planted to citrus, maintained for an additional 85, 158, and 98 days, respectively, then tested for the presence of *P. nicotianae*. The pathogen was not recovered from any of the 27 soil samples that were maintained outside in 1-liter containers or the 19 soil samples in 7-liter capacity containers at soil depths of 5 to 10 cm and 15 to 20 cm (Tables 2 to 4). The pathogen was recovered from one of 19 soil samples maintained outside at a depth of 25 to 30 cm. In comparison, *P. nicotianae* was recovered from a high proportion of soil samples initially planted to citrus, alfalfa, or irrigated without the presence of any plant for 177, 122, and 129 days, with mean soil temperatures ranging from 28 to 30°C, then planted to citrus for an additional 85, 158, and 98 days, respectively. For comparison, the mean summer temperatures recorded for soil at a depth of 10 cm within a mature citrus grove, an established alfalfa field, and a nonplanted field in Yuma were 30.9, 31.1, and 36.9°C, respectively.

For the two trials initiated in the winter (Table 1), *P. nicotianae* was recovered after a period of 104 and 115 days from 70 and 40% of the respective soil samples maintained outside within a 1-liter capacity container in full sunlight and not irrigated (Tables 6 and 7). Total rainfall recorded during the time that these soil samples were maintained outside was 10.9

and 74.7 mm for experiments 4 and 5, respectively. The mean temperature of the soil at a depth of 10 cm during this time was 18 and 15°C for the respective experiments. Additionally, the pathogen was recovered from 80 to 100% of soil samples planted to citrus, alfalfa, or irrigated without the presence of any plant (Tables 6 and 7). The mean temperature of soil planted to citrus, alfalfa, or irrigated without the presence of any plant for these trials ranged from 18 to 20°C.

Soil samples placed outside and not irrigated were maintained in this condition for 104 and 115 days for experiments 4 and 5, respectively. These soil samples then were moved into the greenhouse, planted to citrus, and maintained for an additional 118 and 182 days, respectively, at which time the soil samples were tested for the presence of *P. nicotianae*. The pathogen was recovered from 70 to 100% of soil samples that were maintained outside in either 1-liter containers or 7-liter capacity containers at soil depths of 5 to 30 cm (Tables 6 and 7).

For trials initiated in the summer, the mean number of lesions recorded on pear fruit incubated with soil samples initially planted to citrus, alfalfa, or irrigated without the presence of any plant ranged from 0.1 to 2.4, 3.1 to 11.1, and 26.7 to 37.6 when tested during the treatment phase of experiments 1 to 3, respectively (Tables 2 to 4). When soil samples within the same

Table 4. Detection of *Phytophthora nicotianae* in citrus grove soil after subjection to different temperature, moisture, and cultural conditions from July through November 2000 (experiment 3)

Soil treatment ^w	38 Days after initiation of soil treatments ^x		Mean soil temperature (°C) during 38-day period ^y	98 Days after termination of soil treatments ^x	
	Samples with <i>P. nicotianae</i> detected	Mean no. of lesions on pear bait		Samples with <i>P. nicotianae</i> detected	Mean no. of lesions on pear bait
Soil in 1-liter container in greenhouse with <i>Citrus volkameriana</i> seedling, irrigated as needed	9 a ^z	37.6	30	8 a	3.9
Soil in 1-liter container in greenhouse with alfalfa plant, irrigated as needed	9 a	26.7	30	9 a	9.9
Soil in 1-liter container in greenhouse, irrigated at the same time as soil containing citrus and alfalfa plants	9 a	30.6	30	8 a	14.3
Soil in 1-liter container, in full sun, not irrigated	0 b	0	39	0 b	0
Soil in 7-liter container, in full sun, not irrigated (soil at 5 to 10 cm depth)	N.D.	N.D.	39	0 b	0
Soil in 7-liter container, in full sun, not irrigated (soil at 15 to 20 cm depth)	N.D.	N.D.	N.D.	0 b	0
Soil in 7-liter container, in full sun, not irrigated (soil at 25 to 30 cm depth)	N.D.	N.D.	36	0 b	0

^w Soil was collected from within the drip line of 10 different trees within an established lemon grove. After the soil from each tree was tested and found to contain *P. nicotianae*, 1- or 7-liter aliquots of soil from each bulk sample became samples subjected to the listed cultural conditions. Soil samples were subjected to these conditions for 129 days (24 Jul to 30 Nov 2000), then placed in the greenhouse, and a 6-month-old *C. volkameriana* seedling was planted into each 1-liter container. The *C. volkameriana* and alfalfa plants previously growing in some of the soil samples were removed and replaced with 6-month-old *C. volkameriana* seedlings as well. All plants were watered as needed until the trial was terminated 9 Mar 2001. Rainfall from 24 Jul to 30 Nov was 30.0 mm.

^x Soil samples were tested for the presence of *P. nicotianae* 38 days after soil treatments were initiated (31 Aug 2000) and again 98 days after termination of the soil treatments and planting of *C. volkameriana* seedlings (9 Mar 2001). For each replicate soil sample, a 50-g amount was placed into a 500-ml container. An unblemished green but ripe pear fruit was placed in each container along with enough tap water to half submerge the fruit. After a 48-h incubation period on the laboratory bench at 26°C, pear fruits were removed from the water and soil mixture and placed on the laboratory bench for an additional 5 days. One or more lesions appearing on a pear fruit and caused by *P. nicotianae* was considered positive detection of the pathogen. Each value is either the number of the 10 replicate soil samples per treatment from which *P. nicotianae* was recovered with the pear-bait assay or the mean number of lesions per pear-fruit bait. N.D. = not determined.

^y Mean soil temperature (°C) at a depth of 10 cm during this 38-day period.

^z Numbers in each column followed by a different letter are significantly different ($P = 0.05$) according to the Fisher's protected LSD.

respective experiments were tested again at the conclusion of each experiment, the mean number of lesions per pear fruit ranged from 0.9 to 5.2, 18.6 to 20.8, and 3.9 to 14.3. For the two trials initiated in the winter, the mean number of lesions per

pear fruit incubated with soil samples initially planted to citrus, alfalfa, or irrigated without the presence of any plant ranged from 4.2 to 6.8 and 11.1 to 13.1 when tested during the treatment phase and 2.2 to 17.1 and 7.7 to 9.9 when tested at the

conclusion of experiments 4 and 5, respectively (Tables 6 and 7). The mean number of lesions from pear fruit incubated with soil initially subjected to a dry fallow period during the winter ranged from 2.9 to 8.6 and 4.7 to 8.0 when tested at the con-

Table 5. Comparison of soil temperature of citrus grove soil containing *Phytophthora nicotianae* and subjected to different cultural conditions with subsequent detection of the pathogen

Soil treatment and depth of temperature measurement ^y	Mean summer soil temp. (°C) ^z	Total hours that soil was within a temperature range (°C) of						Total h ≥35°C	Samples with <i>P. nicotianae</i> detected
		35.0-37.9	38.0-40.9	41.0-43.9	44.0-46.9	47.0-49.9	50.0-52.9		
Soil in 1-liter container, full sun, not irrigated. (experiment 1)	37.8	397	292	205	192	46	0	1,132	0
Soil in 1-liter container, full sun, not irrigated. (experiment 2)	37.0	339	350	333	171	0	0	1,193	0
Soil in 1-liter container, full sun, not irrigated. (experiment 3)	36.0	396	298	151	106	0	0	951	0
Soil in 7-liter container, full sun, not irrigated. (experiment 3)	34.0	762	38	0	0	0	0	800	0
Soil in 7-liter container, full sun, not irrigated. (experiment 2)	35.0	570	0	0	0	0	0	570	1
Soil in 1-liter container in greenhouse with <i>Citrus volkameriana</i> seedling or alfalfa, irrigated as needed. (experiment 1)	29.2	185	67	0	0	0	0	252	3.5
Soil in 1-liter container in greenhouse with <i>Citrus volkameriana</i> seedling or alfalfa, irrigated as needed. (experiment 3)	29.0	94	46	0	0	0	0	140	8.5
Soil in 1-liter container in greenhouse with <i>Citrus volkameriana</i> seedling or alfalfa, irrigated as needed. (experiment 2)	28.0	109	19	3	0	0	0	131	8.5

^y Soil temperature was measured at a depth of 10 and 30 cm for soil in 1- and 7-liter containers, respectively.

^z Mean temperature during the summer months (June to September) in which each experiment was conducted. For experiment 1, 2, and 3, these periods of time were 19 Jun through 30 Sep, 5 Jul through 30 Sep, and 24 Jul through 30 Sep, respectively.

Table 6. Detection of *Phytophthora nicotianae* in citrus grove soil after subsection to different temperature, moisture, and cultural conditions from November 1999 through March 2000 (experiment 4)

Soil treatment ^w	104 Days after initiation of soil treatments ^x		Mean soil temperature (°C) during the 104-day period ^y	118 Days after termination of soil treatments ^x	
	Samples with <i>P. nicotianae</i> detected	Mean no. of lesions on pear bait		Samples with <i>P. nicotianae</i> detected	Mean no. of lesions on pear bait
Soil in 1-liter container in greenhouse with <i>Citrus volkameriana</i> seedling, irrigated as needed	9 a ^z	6.8	20	10 a	17.1
Soil in 1-liter container in greenhouse with alfalfa plant, irrigated as needed	8 a	6.2	20	8 ab	7.5
Soil in 1-liter container in greenhouse, irrigated at the same time as soil containing citrus and alfalfa plants	8 a	4.2	20	8 ab	2.2
Soil in 1-liter container, in full sun, not irrigated	7 a	1.6	18	7 ab	8.6
Soil in 7-liter container, in full sun, not irrigated (soil at 5 to 10 cm depth)	N.D.	N.D.	18	8 ab	2.9
Soil in 7-liter container, in full sun, not irrigated (soil at 15 to 20 cm depth)	N.D.	N.D.	N.D.	8 ab	8.3
Soil in 7-liter container, in full sun, not irrigated (soil at 25 to 30 cm depth)	N.D.	N.D.	18	9 a	5.3

^w Soil was collected from within the drip line of 10 different trees within an established lemon grove. After the soil from each tree was tested and found to contain *P. nicotianae*, 1- or 7-liter aliquots of soil from each bulk sample became samples subjected to the listed cultural conditions. Soil samples were subjected to these conditions for 104 days (30 Nov 1999 to 21 Mar 2000), then placed in the greenhouse, and a 6-month-old *C. volkameriana* seedling was planted into each 1-liter container. The *C. volkameriana* and alfalfa plants previously growing in some of the soil samples were removed and replaced with 6-month-old *C. volkameriana* seedlings as well. All plants were watered as needed until the trial was terminated 17 Jul 2000. Rainfall from 30 Nov 1999 to 21 Mar 2000 was 10.9 mm.

^x Soil samples were tested for the presence of *P. nicotianae* 104 days after soil treatments were initiated (14 Mar 2000) and again 118 days after termination of the soil treatments and planting of *C. volkameriana* seedlings (17 Jul 2000). For each replicate soil sample, a 50-g amount was placed into a 500-ml container. An unblemished green but ripe pear fruit was placed in each container along with enough tap water to half submerge the fruit. After a 48 h incubation period on the laboratory bench at 26°C, pear fruits were removed from the water and soil mixture and placed on the laboratory bench for an additional 5 days. One or more lesions appearing on a pear fruit and caused by *P. nicotianae* was considered positive detection of the pathogen. Each value is either the number of the 10 replicate soil samples per treatment from which *P. nicotianae* was recovered with the pear-bait assay or the mean number of lesions per pear-fruit bait. N.D. = not determined.

^y Mean soil temperature (°C) at a depth of 10 cm during this 104-day period.

^z Numbers in each column followed by a different letter are significantly different ($P = 0.05$) according to the Fisher's protected LSD.

clusion of experiments 4 and 5, respectively.

***P. nicotianae* detection limit for pear fruit bioassay.** The final concentration of zoospores per milliliter of water in the four containers for the first trial was 67.00, 6.70, 3.30, and 0.70; for the second trial was 360, 36, 18.0, and 3.6; and for the third trial was 3.10, 0.63, 0.031, and 0.003. The mean number of lesions on the three pear fruits exposed to each of these concentrations of zoospores ranged from 0 for a concentration of 0.003 zoospores per ml of water to so many infection points that individual spots could not be discerned at a concentration of 360 zoospores per ml of water. Regression analysis (Fig. 1) revealed a significant linear relationship between the $\log_{10}$ values of zoospore concentration and the number of lesions caused by *P. nicotianae* that developed on pear fruits ($Y = 14.67 \log_{10} X + 0.643$, $r^2 = 0.756$, $P = 0.005$) when comparing concentrations of zoospores that caused on average at least one but less than 40 lesions per pear fruit. The predicted limit of detection of zoospores from the linear regression line was less than one zoospores per ml of water.

DISCUSSION

The objective of this research was to test the effect of different cultural practices on the survival of *P. nicotianae* in soil formerly planted to citrus, with the goal of

identifying the most effective means of reducing the population of this pathogen to nondetectable levels. In three trials over a 3-year period, *P. nicotianae* was not detected at a depth of 10 cm after subjecting soil naturally infested with the pathogen to a summer dry fallow period of at least 31 days in the desert southwest region of Arizona. The mean temperature of soil at this depth for the three trials ranged from 37 to 39°C, which was similar to the mean summer temperature of 37°C recorded at the same depth in a nonplanted field in Yuma. Furthermore, in two of these trials, where soil was subjected to a summer dry fallow for 38 and 45 days, the pathogen could not be detected at a depth of 15 to 20 cm and was detected in only one of 19 soil samples at a depth of 25 to 30 cm. The mean maximum and minimum daily air temperatures were 41 and 26°C, respectively, during July and August in the region where these trials were conducted. The abundance of high temperatures during the summer facilitates the heating of soil to levels attainable only by soil solarization with plastic in other areas with more moderate climates. Although not experimentally addressed, deep tillage of soil after approximately 1 month to move soil below a depth of 30 cm to a shallower depth may increase the efficiency of the overall summer fallow treatment at depths at or below 30 cm.

In contrast to the dry summer fallow treatment, a dry winter fallow treatment

did not reduce the population of the pathogen to nondetectable levels. The mean temperature of soil at a depth of 10 cm during the treatment period for the two trials initiated in the winter was 18 and 20°C. Furthermore, growing alfalfa in soil containing *P. nicotianae* from 104 to 177 days during any time of the year did not lead to reduced detection of the pathogen compared to soil containing citrus, which would be considered the control in this study. In the three greenhouse trials initiated in the summer as well as in a mature citrus grove and alfalfa field, the mean summer temperature of soil at a depth of

Fig. 1. Relationship of concentration of *Phytophthora nicotianae* zoospores in incubation water to number of infection lesions detected on pear fruit bait.

Table 7. Detection of *Phytophthora nicotianae* in citrus grove soil after subjection to different temperature, moisture, and cultural conditions from December 2000 through March 2001 (experiment 5)

Soil treatment ^w	115 Days after initiation of soil treatments ^x		Mean soil temperature (°C) during the 115-day period ^y	182 Days after termination of soil treatments ^x	
	Samples with <i>P. nicotianae</i> detected	Mean no. of lesions on pear bait		Samples with <i>P. nicotianae</i> detected	Mean no. of lesions on pear bait
Soil in 1-liter container in greenhouse with <i>Citrus volkameriana</i> seedling, irrigated as needed	10 a ^z	11.5	18	9 a	8.0
Soil in 1-liter container in greenhouse with alfalfa plant, irrigated as needed	10 a	13.1	18	8 a	9.9
Soil in 1-liter container in greenhouse, irrigated at the same time as soil containing citrus and alfalfa plants	10 a	11.1	18	10 a	7.7
Soil in 1-liter container, in full sun, not irrigated	4 b	1.1	15	8 a	4.7
Soil in 7-liter container, in full sun, not irrigated (soil at 5 to 10 cm depth)	N.D.	N.D.	15	9 a	7.9
Soil in 7-liter container, in full sun, not irrigated (soil at 15 to 20 cm depth)	N.D.	N.D.	N.D.	9 a	6.2
Soil in 7-liter container, in full sun, not irrigated (soil at 25 to 30 cm depth)	N.D.	N.D.	16	10 a	8.0

^w Soil was collected from within the drip line of 10 different trees within an established lemon grove. After the soil from each tree was tested and found to contain *P. nicotianae*, 1- or 7-liter aliquots of soil from each bulk sample became samples subjected to the listed cultural conditions. Soil samples were subjected to these conditions for 115 days (4 Dec 2000 to 28 Mar 2001), then placed in the greenhouse, and a 6-month-old *C. volkameriana* seedling was planted into each 1-liter container. The *C. volkameriana* and alfalfa plants previously growing in some of the soil samples were removed and replaced with 6-month-old *C. volkameriana* seedlings as well. All plants were watered as needed until the trial was terminated 27 Sep 2001. Rainfall from 4 Dec 2000 to 28 Mar 2001 was 74.7 mm.

^x Soil samples were tested for the presence of *P. nicotianae* 115 days after soil treatments were initiated (28 Mar 2001) and again 182 days after termination of the soil treatments and planting of *C. volkameriana* seedlings (27 Sep 2001). For each replicate soil sample, a 50-g amount was placed into a 500-ml container. An unblemished green but ripe pear fruit was placed in each container along with enough tap water to half submerge the fruit. After a 48-h incubation period on the laboratory bench at 26°C, pear fruits were removed from the water and soil mixture and placed on the laboratory bench for an additional 5 days. One or more lesions appearing on a pear fruit and caused by *P. nicotianae* was considered positive detection of the pathogen. Each value is either the number of the 10 replicate soil samples per treatment from which *P. nicotianae* was recovered with the pear-bait assay or the mean number of lesions per pear-fruit bait. N.D. = not determined.

^y Mean soil temperature (°C) at a depth of 10 cm during this 115-day period.

^z Numbers in each column followed by a different letter are significantly different ($P = 0.05$) according to the Fisher's protected LSD.

10 cm ranged from 28 to 31°C. Soil temperatures in this range have been shown to be ineffective in significantly reducing the population of *P. nicotianae* in soil (1). The effect of a 1- to 3-year rotation to alfalfa between citrus plantings was not assessed in this trial; therefore, the effect of this cultural practice on the population of *P. nicotianae* is unknown. Alfalfa is not a recognized host for *P. nicotianae* in the United States, although the pathogen was associated with a root rot on lucerne in Australia (22).

Temperatures achieved during soil solarization have been sufficient to inactivate propagules of *P. nicotianae* (1,18), *P. cinnamomi* (4,20), and *P. capsici* (3) as well as reduce the incidence of diseases caused by these pathogens. Differences in methods of solarization, experimental design, and presentation of data make direct comparisons of studies difficult; however, maximum daily temperatures of 40°C or higher at a soil depth of 5 cm during the 1- to 2-month solarization periods for a major portion of the solarization period were necessary to achieve significant inactivation of these pathogens. Coelho et al. (1) found that the time required to reduce soil populations of *P. nicotianae* to residual levels decreased with increasing temperatures. Similar findings occurred in the present study, where *P. nicotianae* was not recovered from any soil sample originally containing this pathogen that was maintained in full sun, not irrigated, and subjected to temperatures ranging from 35.0 to 40.9°C for 800 h compared to the same result for soil subjected to temperatures ranging from 41.0 to 46.9°C for at least 257 h.

The effectiveness of soil solarization is a function of the thermal dose (21), which is derived from the interaction of temperature, exposure time, thermal sensitivity of the organism, and the chemical, physical, and biological characteristics of the soil, including moisture content (23). The same parameters affect the thermal dose in the summer fallow treatment, which was essentially soil solarization without plastic. A plastic covering over soil to be solarized is used to suppress the loss of heat and moisture from soil (6). In the dry summer fallow treatment, total rainfall amounts of 56, 10, and 29 mm were recorded during the summer days that these soil samples were maintained outside during 1998, 1999, and 2000, respectively. Soil moisture during the summer fallow treatment was not recorded in this study; however, in earlier work on the effect of dry soil on viability of sclerotia of *Sclerotinia minor* and *S. sclerotiorum* in the desert in Yuma, water potential levels ≤ -100 MPa were recorded for nonirrigated soil during the summers of 1996 to 1998 in nonirrigated soil (15). The findings of the current study suggest that sufficiently high temperature, but not high soil moisture, is required to inactivate *P. nicotianae*.

The identity and quantity of the different types of *P. nicotianae* propagules present in soil were not assessed in this study; however, Lutz and Menge (9) reported that the types of propagules of *P. nicotianae* (*P. parasitica*) that occur in irrigated California citrus groves include sporangia, zoospore cysts, chlamydospores, and oospores. Of these, chlamydospores and oospores are considered long-term survival propagules in soil, and chlamydospores were the predominant long-term survival propagule (9).

Each experiment conducted in this study consisted of a soil treatment phase followed by an incubation period with the citrus host. The period of incubation with the citrus host was included to allow pathogen propagules that may exist at nondetectable levels after the summer fallow period an opportunity to multiply in the presence of citrus to levels that would be detected in a subsequent assay. During the treatment phase and at the end of the incubation period with the citrus host, a pear fruit bioassay was used to detect the presence of the pathogen in each soil sample. Plants and plant parts have been used to detect or in some cases quantify disease severity due to *P. nicotianae* and other *Phytophthora* spp. (3,4,16,18,20). In the current study, pear fruits were incubated with various known concentrations of zoospores to determine the approximate detection limit of this assay for *P. nicotianae* in soil. The limit of detection indicated by the linear regression of the data was less than one zoospore per ml of water.

Using the detection limit for zoospores determined by the pear fruit bioassay in the laboratory to ascertain the detection limit for propagules of *P. nicotianae* present in soil samples cannot be accurately accomplished due to the following factors. Firstly, in soil subjected to a dry period, the presumptive surviving propagules would be chlamydospores and perhaps oospores if present (9). Detection of these propagules would only occur if they produced sporangia, which in turn liberated zoospores that infected the pear fruit bait within the 48-h period that the pear fruit was incubated with soil. Furthermore, indirect detection of these propagules would be affected by the number of zoospores produced from a sporangium originating from a chlamydospore or an oospore in soil. Finally, irrigated soil supporting growth of citrus or other hosts of *P. nicotianae* may contain encysted zoospores and sporangia in addition to chlamydospores and oospores, further complicating determination of the detection limit of *P. nicotianae* in these situations. As noted by Erwin and Ribeiro (2), an extended baiting period may overestimate inoculum density, as the primary inoculum, which probably exists as oospores or chlamydospores, will produce sporangia and subsequently many zoo-

spores, each of which is a colony-forming unit. Due to the noted complications in using the pear fruit bioassay as a measure of inoculum density of *P. nicotianae* in soil, only the data on positive or negative detection of the pathogen were considered relevant in this study, not the quantitative number of lesions recorded from pear fruits incubated with soil.

In regions with a hot and dry summer climate, the findings of this study suggest that a dry summer fallow treatment of soil after removal of an existing citrus planting and before establishment of a new citrus grove would provide a rapid and relatively inexpensive means of reducing the population of *P. nicotianae* to virtually nondetectable levels, at least to a depth of 30 cm. Traditional soil solarization could be employed in areas where summer temperatures are not high enough to heat soil to a sufficient degree without the use of a plastic cover. If found to be efficacious in future studies, crop rotation could be a longer term approach to significantly reducing the population of this pathogen; however, care would have to be taken to avoid planting any of the many known hosts of *P. nicotianae* (2). A long-term period of crop rotation with plants that are not hosts for *P. nicotianae* was not tested in this study.

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