

Effect of Temperature and Moisture on Survival of *Phytophthora* in Citrus Grove Soil¹

Michael Matheron, Martin Porchas and Michael Maurer

Abstract

Before replanting a citrus grove in Arizona, different preplant cultural activities may be performed, such as immediate replanting of the new citrus grove, allowing soil to lay fallow for various lengths of time, or planting the site to alfalfa for one or more years before the new citrus grove is established. A study was conducted to compare the effect of these different cultural preplant practices on the survival of *Phytophthora* in citrus grove soils. In June, 1998, and July, 1999, a total of 18 soil samples were collected within mature lemon groves. Each initial bulk sample was pretested, found to contain *Phytophthora parasitica*, then thoroughly mixed and partitioned into 1-liter plastic containers, which were subjected to different environmental and cultural conditions. The soil in each 1-liter container was tested for the presence of *P. parasitica* 1 and 3.5 to 4 months later. All soil samples then were placed in the greenhouse and a 6-month-old *Citrus volkameriana* seedling was planted in soil samples not containing plants. Three 1-liter sub-samples from each of ten 7-liter volumes of soil incubated outside for three months were also planted to citrus in the greenhouse. The soil containing plants in the greenhouse was watered as needed for 3 months, then again tested for the presence of *Phytophthora*. Irrigating soil infested with *Phytophthora parasitica*, whether it was planted to a host (citrus) of the pathogen, planted to a non-host (alfalfa) of the pathogen, or not planted at all, did not lower the pathogen to nondetectable levels. *Phytophthora* became and remained nondetectable only in the soil samples that were not irrigated and subjected to mean temperatures of 35 to 37°C (94 to 98°F). On the other hand, the pathogen was detectable in some soil samples subjected to dryness at lower mean temperatures of 26 to 30°C (79 to 86°F) after a citrus seedling subsequently was grown in the soil for 3 months. A dry summer fallow period following removal of a citrus grove (including as much root material as possible) was the only cultural practice among those tested that reduced the level of *Phytophthora* to nondetectable levels in all soil samples tested.

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Introduction

Several different methods are used in Arizona to prepare land for replanting of citrus trees. One approach involves the total removal of old trees including as many roots as possible, followed by replanting without any additional treatment of soil.

An alternative procedure is to treat planting sites with a fumigant such as Vapam before planting trees. Additional practices include leaving the soil fallow for one or more years or planting the former grove site to alfalfa for 1-3 years before returning to citrus.

During the life of a citrus grove, the population of pathogens such as *Phytophthora* and the citrus nematode can increase dramatically. When a grove is removed and replanted, resident populations of these pathogens potentially could remain in the soil and attack the new trees, resulting in slow growth, delayed onset of commercial fruit yields, and long-term decrease in yield and tree growth compared to noninfected trees. Utilization of fallow periods with or without a cover crop or soil fumigation are potential means of reducing populations of soil pathogens so that newly planted trees can grow without the detrimental influences of these organisms. Populations of citrus nematodes in soil are known to drop dramatically in the absence of live citrus roots; however, the fate of *Phytophthora* after grove removal is not as well documented. The objective of this research was to examine the survival of *Phytophthora* in soil subjected to different temperature, moisture and cultural conditions.

Materials and Methods

1998 study. In June, 1998, an 8-liter volume of soil was collected from a total of eight different sites within a mature lemon grove on a sandy soil in Yuma or a lemon planting on a heavier soil in Mesa, AZ. Each sample was pretested and found to contain *Phytophthora parasitica*, then thoroughly mixed and partitioned into six 1-liter plastic containers. These sub-samples were treated as follows for 6 months: 1) soil not irrigated, maintained in the laboratory; 2) soil not irrigated, maintained outside exposed to full sunlight in a 1-liter container buried in soil so that the level of soil in the container was the same as that of the surrounding soil; 3) soil not irrigated, maintained in the greenhouse; 4) soil irrigated when treatments 5 and 6 received water, maintained in the greenhouse; 5) soil irrigated every 2-4 days as needed, maintained in the greenhouse with a 1-year-old *C. volkameriana* seedling growing in the soil; 6) soil irrigated every 2-4 days as needed, maintained in the greenhouse with a 1-year-old alfalfa plant growing in the soil. Temperature probes were placed in the soil at the 10-cm depth in the various locations and soil temperature was recorded hourly.

At 33 (July 22, 1998) and 108 (October 6, 1998) days after the initiation of this study, a 10-gram sample of soil was removed from each container and tested for the presence of *Phytophthora* by the following procedure. Each 10-gram sample of soil was placed in a 0.5-liter container to which was added one unblemished pear fruit and enough water to submerge the bottom half of the pear in water, then incubated for 48 hr at 25-27°C in the laboratory. Each pear then was removed from each container and maintained for an additional 5 days in the laboratory. Detection of *Phytophthora* was considered positive when one or more firm brown lesions developed on the pear fruit. Confirmation that these lesions were caused by the pathogen was achieved by plating a small piece of the fruit lesion onto agar medium and identifying the fungus emerging from the tissue as *Phytophthora parasitica*.

At six months (December 13, 1998) after the initiation of this study, all soil samples were placed in the greenhouse and a 2-month-old rough lemon seedling was planted in each container. Alfalfa and citrus plants initially planted in some of the soil samples were removed and replaced with rough lemon seedlings as well. All plants were watered as needed and the soil in each container again was tested for *Phytophthora* on March 8, 1999. Soil temperature continued to be monitored until the study was terminated.

1999 study. The study was repeated in 1999 with the following changes from 1998. In July, 1999, ten 16-liter volumes of soil were collected within a mature lemon grove on a sandy soil in Yuma. After pretesting to assure the presence of *Phytophthora parasitica*, each sample was thoroughly mixed, then distributed into five 1-liter plastic containers and for 4 months was treated as in the 1998 study, with the following changes: (a) the treatment involving nonirrigated soil maintained in the laboratory was not repeated in 1999; (b) alfalfa was seeded into the appropriate treatment rather than transplanting a 1-year-old plant; (c) a treatment not present in the 1998 study involved the placement and maintenance of soil outside exposed to full sunlight in a 7-liter container (6 inches in width and length at the top and 16 inches deep) buried in soil so

that the level of soil in the container was the same as that of the surrounding soil.

At 45 (August 19, 1999) and 99 (October 12, 1999) days after the initiation of the study, a 50-gram sample of soil was removed from each container (except for the 7-liter containers) and tested for the presence of *Phytophthora* using the technique described for the 1998 study. At 132 days (November, 1999) after the initiation of the study, all soil samples in 1-liter containers were placed in the greenhouse and a 6-month-old *Citrus volkameriana* seedling was planted in all soil samples not already containing citrus or alfalfa plants. For soil in each 7-liter container, the top liter of soil was removed and discarded. The 2nd, 4th, and 6th liter volumes of soil to be removed from each 7-liter container were saved and a citrus seedling was planted in each of these soil samples. Soil samples already containing citrus or alfalfa plants were not disturbed. Seven months (February 15, 2000) after initiating this study, the soil in each container again was tested for the presence of *Phytophthora*.

In both years, soil populations of *Phytophthora* at the original collection sites were assayed and compared to values for the soil subjected to the different environmental conditions of these studies. Soil temperatures were monitored as described for the 1998 study. For the soil within the buried 7-liter containers, soil temperature was recorded at the 10 cm and 30 cm depth.

Results and Discussion

The objective of this research was to examine the survival of *Phytophthora* in soil subjected to different temperature, moisture and cultural conditions, with the hope of finding environmental conditions that would reduce the level of this pathogen to nondetectable levels. Irrigating soil infested with *Phytophthora parasitica*, whether it was planted to a host (citrus) of the pathogen, planted to a non-host (alfalfa) of the pathogen, or not planted at all, did not lower the pathogen to nondetectable levels (Tables 1 and 3). On the other hand, *Phytophthora* could not be recovered from soil that was not irrigated for 3.5 to 4 months. On further examination, when these nonirrigated soil samples were subsequently planted to citrus and irrigated for 3 months, *Phytophthora* remained nondetectable only in the soil samples that were not irrigated and subjected to mean temperatures of 35 to 37°C (94 to 98°F) (Tables 1 to 4).

In comparison, the pathogen was detected in some soil samples subjected to dryness at lower mean temperatures of 26 to 30°C (79 to 86°F) after a citrus seedling subsequently was grown in the soil for 3 months. The soil samples from which *Phytophthora* could not be detected, even after a citrus seedling was subsequently grown in the soil for 3 months, were subjected to a dry summer fallow treatment by placement in the field in Yuma. These samples did receive some moisture in the form of rainfall. The following rainfall amounts (in inches) were recorded during this study in 1998: Jul, 0.06; Aug, 0.30; Sep, 1.84; Oct, 0.00; and 1999: Jul, 0.36; Aug, 0.04; Sep, 0.20; Oct, 0.00.

What are the practical implications of this study. First of all, these research findings show that a dry summer fallow period in Arizona (with mean soil temperatures of 35 to 37°C) following removal of a citrus planting (including as much root material as possible) appears to be the only cultural practice among those tested that can reduce the population of *Phytophthora parasitica* to nondetectable levels and maintain this status for at least 3 months after replanting to citrus. Secondly, recovery of *Phytophthora* is still possible in soil subjected to dry fallow at lower temperatures of 26 to 30°C. Finally, the pathogen can be readily recovered from infested soil at least 9 months after planting to alfalfa, suggesting that planting of this crop between plantings of citrus may not lead to a rapid decline in populations of *Phytophthora parasitica*.

Table 1. Detection of *Phytophthora parasitica* in citrus grove soil after placement in different environmental conditions. 1998 study.

Soil Treatment From Jun through Dec. 1998*	Number of soil samples in which <i>P. parasitica</i> was detected **		
	Jul. 1998	Oct. 1998	Mar. 1999
Soil from the original collection site	8	--	8
Soil planted with citrus seedling, maintained in greenhouse, irrigated	4	4	3
Soil planted with alfalfa, maintained in the greenhouse, irrigated	5	4	4
Bare soil, maintained in the greenhouse, irrigated	1	3	3
Bare soil maintained in the greenhouse, not irrigated	0	0	3
Bare soil, maintained in the laboratory, not irrigated	1	0	1
Bare soil, maintained outside, not irrigated	0	0	0

* These treatments were in place from June 19 through December 13, 1998. On this December date, all soil samples were placed in the greenhouse and a 2-month-old rough lemon seedling was planted in each container. Alfalfa and citrus seedlings initially planted in some of the soil samples were removed and replaced with rough lemon seedlings as well. All plants were watered as needed and the soil in each container again was tested for *Phytophthora* on March 8, 1999.

** Soil samples were tested for the presence of *P. parasitica* on Jul 22 and Oct 6, 1998 and Mar 8, 1999.

Table 2. Soil temperatures from June 22 to October 6, 1998.

Soil location	Soil temperature (°C) at the 4-inch depth		
	Minimum	Maximum	Mean
In laboratory – Not irrigated	25	27	26
In greenhouse – Not irrigated	18	42	30
Soil outside in full sun – Not irrigated	24	49	37
In greenhouse with citrus seedling – Irrigated	17	42	30
Under citrus tree canopy – Yuma, AZ	24	33	29
Under citrus tree canopy – Mesa, AZ	20	31	27

Table 3. Detection of *Phytophthora parasitica* in citrus grove soil after placement in different environmental conditions. 1999 study.

Soil Treatment From Jul through Nov. 1998*	Number of soil samples in which <i>P. parasitica</i> was detected **		
	Aug. 1999	Oct. 1999	Feb. 2000
Soil from the original collection site	9	7	3
Soil planted with citrus seedling, maintained in greenhouse, irrigated	8	8	4
Soil planted with alfalfa, maintained in the greenhouse, irrigated	9	7	5
Bare soil, maintained in the greenhouse, irrigated	9	10	5
Bare soil maintained in the greenhouse, not irrigated	0	0	1
Bare soil, maintained outside, not irrigated (1-qt container)	--	--	0
Bare soil, maintained outside, not irrigated (top of 7-qt container)	--	--	0
Bare soil, maintained outside, not irrigated (middle of 7-qt container)	--	--	0
Bare soil, maintained outside, not irrigated (bottom of 7-qt container)	--	--	0

* These treatments were in place from July 5 through November 4, 1999. On this November date, all soil samples in 1-liter containers were placed in the greenhouse and a 6-month-old *Citrus volkameriana* seedling was planted in all soil samples not already containing citrus or alfalfa plants. For soil in each 7-liter container, the top liter of soil was removed and discarded. The 2nd, 4th, and 6th liter volumes of soil to be removed from each 7-liter container were saved and a citrus seedling was planted in each of these soil samples. Soil samples already containing citrus or alfalfa plants were not changed. All plants were watered as needed and the soil in each container again was tested for *Phytophthora* on February 15, 2000.

** Soil samples were tested for the presence of *P. parasitica* on Aug 19 and Oct 12, 1999 and Feb 15, 2000.

Table 4. Soil temperatures from July 5 to October 12, 1999.

Soil location	Soil temperature (°C) at the 4-inch depth		
	Minimum	Maximum	Mean
In greenhouse – Not irrigated	18	43	29
Soil outside in full sun – Not irrigated (10 cm depth)	23	47	36
Soil outside in full sun – Not irrigated (30 cm depth)	28	38	35
In greenhouse with citrus seedling – Irrigated	18	43	29
Under citrus tree canopy – Yuma, AZ	23	32	28