Population Fluctuations and the Numbers and Types of Propagules of Phytophthora parasitica That Occur in Irrigated Citrus Groves

A. L. LUTZ and J. A. MENGE, Department of Plant Pathology, University of California, Riverside 92521

ABSTRACT


Citrus trees were irrigated via furrows or low-volume emitters, and populations of Phytophthora parasitica from the rhizosphere were monitored over a period of 12 days after irrigation by soil dilution plate assay on a selective medium. The types of propagules present under long- and short-interval irrigation regimes were quantified from November through August. Populations of P. parasitica increased from 17 propagules per gram (ppg) the day before irrigation to 70 ppg 2 days after long-interval furrow irrigation. Populations ranged from 71 to 93 ppg with short-interval drip irrigation. Low levels of a unique propagule of P. parasitica that resembled a dark, multipapillate sporangium were observed consistently. Low numbers of viable oospores were observed throughout the year. In November and from May through August, sporangia and zoospore cysts accounted for the majority of the population. From December through April, chlamydospores were the most frequently observed propagules.

Root rot of citrus caused by Phytophthora parasitica Dastur reduces tree vigor and depresses yields (5). Quantification of Phytophthora in the field can help to determine the necessity for and the extent of control measures, since noticeable yield losses usually occur only when high populations of the fungus are present (19). Usually, populations of P. parasitica are determined after soil temperature rises in the spring, when both the fungus and the citrus feeder roots have resumed active growth (16). Populations of P. parasitica remain high during the summer months but decrease to nearly zero in the winter months (6).

Long periods of soil saturation are required for P. parasitica to be an effective root decay agent (23). The length of time that the soil is saturated will depend, in part, on the irrigation regime. Most California citrus growers currently use low-volume irrigation systems, but the time interval between irrigations in the summer can vary from 2 days to 2 wk. The extent to which the population fluctuates during an irrigation cycle is not known. Soil samples collected at various times after irrigation may yield different populations, making accurate interpretation of the actual Phytophthora population difficult.

Factors that govern the formation of propagules of P. parasitica have been studied extensively in vitro. The importance of each type of propagule for survival has been based mainly on the properties of propagules produced in the laboratory, and there is no information available concerning natural production of propagules of P. parasitica within citrus groves. Oospores are considered to have great survival value because of their thick walls, but their role in the life cycle of heterothallic Phytophthora species is uncertain because germination is difficult to induce in vitro and because they are seen rarely in host tissues (18, 26,28). Chlamydospores also have been considered to be responsible for long-term survival of some Phytophthora species (7,9,24,28). However, such studies have been conducted with species other than P. parasitica (28) or have been conducted using propagules produced in the laboratory (7,24).

Propagules of P. parasitica overwinter in citrus soils in a quiescent state and can be induced to germinate after heat treatment in the laboratory (14,15). It is not known if only one overwintering type of propagule responds to heat or if this
is a general response common to all propagules. Several authors (3,11) have suggested that oospores of some Phytophthora species may respond to heat treatment.

The objectives of this study were to monitor the changes in the population of P. parasitica in citrus soil and to determine the types of propagules that contribute to these fluctuations during irrigation cycles having long- and short-term intervals between irrigations. The propagules that overwinter in naturally infested citrus soils were also examined to gain a better understanding of the life cycle of this pathogen in vivo. A technique was developed to isolate and identify propagules from naturally infested soils in order to achieve these objectives.

MATERIALS AND METHODS

Sites within two citrus groves in California were selected. The Highland grove in San Bernardino County contained 78-year-old cv. Washington Navel orange (Citrus sinensis (L.) Osbeck) scions on sweet orange rootstock. The soil was a Greenfield sandy loam that contained 9.4% clay, 21.1% silt, and 69.5% sand and had an electrical conductivity of 0.68 dS·m⁻¹. The Stone Corral grove in Tulare County contained 25-year-old cv. Valencia orange scions on cv. Cleopatra mandarin (C. reshni Hort. ex Tan.) rootstocks. The soil was a San Joaquin loam (14.1% clay, 39.2% silt, and 46.7% sand) and had an electrical conductivity of 2.2 dS·m⁻¹.

Population fluctuations of P. para-
sitica were monitored under long-interval furrow and short-interval drip irrigation regimes within the Highland grove. Two rows of trees irrigated by drip were alternated with three rows of furrow-irrigated trees. Furrow-irrigated trees received water every 12–14 days at a rate of 20 L/min for a period of 24 hr. Trees under the drip system were irrigated for 4–8 hr three times a week depending on the evapotranspiration values calculated each week. There were three 4.2 L/hr emitters (Vortex emitters, Roberts Irrigation, San Marcos, CA) on both sides of each tree.

Nine single-tree replicates with similar foliar symptoms of root rot were selected. The replicates occurred randomly within three rows of furrow-irrigated trees and within two rows of drip-irrigated trees. The experiment was repeated three times with a different set of nine trees to prevent resampling areas previously disturbed by sampling. After completion of the population fluctuation studies, but before studies on the examination of propagule types, the grower switched from a furrow system to a sprinkler system. A sprinkler was positioned on either side of each tree in the three rows previously irrigated by furrows. Irrigation water was applied for 24 hr at 12- to 14-day intervals, as with furrow irrigation, and each sprinkler delivered water at a rate of 32 L/hr. The spray pattern of the sprinklers overlapped the area that had been wetted by the furrows.

Quantification of P. parasitica from rhizosphere soil. A minimum of two soil samples (23 cm deep, approximately 800 cm² per sample) were collected before noon from the north side of citrus trees, either along the upper edge of the furrow closest to the tree or from the perimeter of the area wetted by emitters or sprinklers, as previously described (15). The top 7.5 cm of soil was discarded because of variability due to wetting and drying. Only soil adhering to the feeder roots (rhizosphere soil) was collected; 100 g of rhizosphere soil was a minimum sample. The two samples from a single tree were combined and mixed. Furrow-irrigated trees were sampled the day before irrigation and nine times over the next 12 days. Drip-irrigated trees were sampled eight times over a 12-day period. Soil samples were sieved through a 2-mm screen, diluted 1:10 with water, and plated on eight plates of PVPH (cornmeal agar with 10 mg/L of pimarinin, 200 mg/L of vancomycin, 100 mg/L of PCNB [Terrachlor], and 50 mg/L of hymexazol [25]) as previously described (15). From previous work (6) it was evident that the site contained P. parasitica and almost no P. citrophthora (R.E. Sm. & E.H. Sm.) Leonardi during the summer months. Nevertheless, 100–200 colonies per experiment were routinely plated on separate agar plates for positive identification. No P. cit-

Fig. 1. Mean numbers of propagules of Phytophthora parasitica recovered from citrus rhizosphere soil and changes in the soil water matric potential in the rhizosphere soil over a 12-day period in a grove at Highland, California, with a 12-day interval between irrigations. Values are the means of three experiments, with nine single-tree replicates per treatment per experiment. Vertical lines represent ± standard error of the mean.
*Phytophthora* was found during the course of these experiments. The experiment was repeated three times during the summer months of 1983 and 1984 for the furrow-irrigated trees and three times during the summer months of 1984 and 1985 for the drip-irrigated trees. The mean population of *P. parasitica* for each sample date during the 12-day period was calculated for each irrigation system using all three replications of the experiment. A previously published moisture release curve (16) for the Highland grove was used.

**Examination of propagules from rhizosphere soil during long and short irrigation cycles**. The same rows of citrus trees within the Highland grove that had been sampled for populations of *P. parasitica* were sampled again for propagules that survived in rhizosphere soil during long-interval (sprinkler irrigated 24 hr, 12- to 14-day intervals) and short-interval (drip irrigated 4-8 hr, 2- to 3-day intervals per week) irrigation cycles. Samples were taken about 1 yr after the sprinklers had replaced the furrow irrigation system. Both sprinkler- and drip-irrigated trees were sampled eight times over a 12-day interval. Trees irrigated by sprinklers received water for 24 hr the day before sampling began. Drip-irrigated trees received water four times during this 12-day period. The types of propagules present on each sample date were quantified. Rhizosphere soil from six trees within each of the two irrigation systems was sampled (15). Samples from two trees similar in size, amount of foliage, foliage color, and fruit yield were combined within each irrigation treatment so that three soil samples per treatment were examined. The experiment was repeated three times during the summer of 1985, and the data were combined.

Soil was sieved through a 2-mm screen to remove roots and small stones. A 75-g sample (dry weight basis) was mixed for 1 min in 100 ml of sterile distilled water in a Waring blender at low speed. The soil slurry was wet-sieved through nested 350-, 245-, and 45-μm sieves and twice through a 15-μm nylon mesh screen (Teto, Inc., Elmsford, NY). The soil that remained on the 15-μm screen was collected both times and centrifuged at 3,000 rpm for 4 min. The supernatant was discarded, and the pellet was resuspended in water to give a total volume of 20 ml. Then, 2 ml of the resulting suspension was pipetted onto microscope slides that had been dipped three times in modified PVPH (25) medium (8.5 g/L of Difco cornmeal agar and 22.5 g/L of Difco Bacto agar). Three slides from each soil sample were placed in individual glass petri dishes (90 mm diameter) lined with moist filter paper and incubated in a dark cabinet at room temperature. The slides were examined microscopically 24-36 hr later after the soil was rinsed off under a gentle stream of water from a squeeze bottle. Slides that could not be examined immediately were kept moist and stored at 5°C for an additional 1-2 days.

For determination of the species of individual propagules that had germinated on the slides, a small block of agar containing the propagule was transferred to a plate of PVPH and examined after 4 days. Propagules always were plated onto fresh medium and allowed to grow before they were identified positively as *P. parasitica*. The percentage of the total population contributed by each type of propagule was determined after counting the total number of propagules per sample on all three slides and calculating the mean of the three samples. About 100-200 propagules were identified positively as *P. parasitica* for each sample date. Approximately 20% of the colonies originally examined were traced back to propagules hidden in organic debris or did not grow after transfer to PVPH medium and could not be identified. These colonies were excluded from the data.

At the same time that the slides were prepared, 0.1 ml of the soil slurry was added to each of eight petri dishes. Molten (55°C) PVPH medium was added, and the contents were swirled to distribute the soil uniformly. The population of *P. parasitica* was determined after incubating the plates at room temperature for 4 days. All data were expressed as the mean of three soil samples per irrigation treatment.

**Examination of propagules from rhizosphere soil throughout the year**. The types of propagules in the *P. parasitica* population were also determined monthly during most of the year in two orange groves. In the Highland grove, rhizosphere soil from six drip-irrigated trees was sampled monthly from November 1985 through August 1986, except that no samples were taken during January. Rhizosphere soil from six trees in the Stone Corral grove were sampled during November 1985 and in May, June, and July 1986. Because *P. parasitica* is dormant in soil samples from November to June, propagules had to be heat-shocked to effect germination (6,14,15). Samples collected from November through June were incubated at 32°C for 5 days to stimulate germination of quiescent propagules before soils were wet-sieved (14). Virtually no propagules could be found without heat-shocking during this period. About

![Fig. 2. Mean numbers of propagules of *Phytophthora parasitica* recovered from citrus rhizosphere soil and changes in soil water matric potential in the rhizosphere soil over a 12-day period in a grove at Highland, California, with a 2- to 3-day interval between irrigations. Values are the means of three experiments, with nine single-tree replicates per treatment per experiment. Vertical lines represent ± standard error of the mean.](image-url)

Plant Disease/February 1991 175
40-60 propagules were identified positively as *P. parasitica* at each sample date during the winter and spring months, and about 100-200 propagules per sample date were identified during the summer months. Approximately 35% of the colonies originally examined during the winter months were traced back to propagules hidden in organic debris or did not grow after transfer to PVPH medium and could not be identified. These isolations were excluded from the data.

**RESULTS**

Quantification of *P. parasitica* from rhizosphere soil. The amount of fluctuation in the population of *P. parasitica* was influenced by the time interval between irrigations. Results from the three experiments at different times of the year were very similar and were averaged. The number of *P. parasitica* propagules per gram of rhizosphere soil (ppg) recovered from the long-interval furrow-irrigated treatment (irrigated every 12 days) increased from 17 ppg the day before irrigation to 70 ppg 3 days after irrigation (Fig. 1). The number declined after 3 days postirrigation to 32 ppg and remained fairly stable thereafter. Soil water matrix potential from the rhizosphere soil ranged from −110 cb before irrigation to −7 cb shortly after irrigation. The population of *P. parasitica* remained fairly stable in the short-interval drip-irrigated treatments over the 12-day period when the time interval between irrigations was 1-2 days (Fig. 2). The matric potential of the rhizosphere soil varied from −26 to −6 cb, and the population ranged from 71 to 93 ppg.

Examination of propagules from rhizosphere soil during long- and short-interval irrigation cycles. Changes in the populations of sporangia and zoospore cysts accounted for most of the population fluctuations in the long-interval irrigation cycle. These two propagule types (Fig. 3 A,B,H) accounted for 84-90% of all propagules during the first 4 days after irrigation (Fig. 4). The number of sporangia rose from 17/g of soil the day after irrigation to 42/g of soil 2 days after irrigation. By the seventh day, the portion of the total population contributed by sporangia plus zoospore cysts dropped to 58%, with only seven sporangia per gram of soil. Zoospore cysts were isolated only during the first 5 days after irrigation, but sporangia continued to account for 41-65% of the total population during the remainder of the drying interval.

The portion of the population contributed by chlamydospores (Fig. 3G) increased from 6 to 36% 1 wk after irrigation (Fig. 4). This increase appeared to be due to a reduction in the number of other types of propagules (namely, sporangia and zoospore cysts) rather than to an actual increase in the number of chlamydospores. The number of chlamydospores per gram of soil ranged from one to six and did not appear to be influenced by the irrigation. The population of dark-walled sporangia (Fig. 3C,D) and zoospores (Fig. 3E,F), which were present in the soil at very low numbers throughout the 12-day period, was not altered substantially by wetting and drying of the soil.

Sporangia plus zoospore cysts accounted for 91-97% of the total population during the 12-day period of short-interval drip irrigation. The number of sporangia plus zoospore cysts ranged from 54 to 83 per gram of rhizosphere soil (Fig. 5). The numbers of chlamydospores, oospores, and dark-walled sporangia per gram of soil remained low and were similar to the numbers found under the long-interval irrigation regime.

A unique propagule was isolated at low levels from rhizosphere soil from both groves throughout the year. It resembled a sporangium except that it was darker and often multipapillate, which
The shape (Fig. 3C,D) of the propagules formed of *P. parasitica* when transferred to fresh PVPH. We called this propagule, which often formed within plant debris, a dark-walled sporangium.

**Examination of propagules from rhizosphere soil throughout the year.** Populations of sporangia and chlamydospores fluctuated substantially during the year in both groves. Within the Highland grove, sporangia and zoospore cysts accounted for the majority of the population from May through November, and their contribution reached 91% in August (Fig. 6A). The predominant propagule from December through April was the chlamydospore, which comprised up to 74% of the total population in February and 45% in April (Fig. 6B). Dark-walled sporangia and oospores composed a much smaller and less variable percentage of the population. Although these two types of propagules accounted for a larger percentage of the total population during the winter months because of the lower overall population of *P. parasitica*, the actual number of propagules per gram of soil remained fairly constant (Fig. 7). There were zero to three dark-walled sporangia and one to six oospores per gram of soil during the year. Data from the Stone Corral grove were similar and confirmed the results seen in the Highland grove.

**DISCUSSION** Rapid changes occurred in the population of *P. parasitica* when a 12-day interval existed between irrigations. This illustrates one of the difficulties of assessing population levels accurately in citrus groves. Because low populations (fewer than 15–20 ppg of rhizosphere soil on susceptible rootstocks) do not cause detectable yield losses (19), populations of the fungus must be quantitated to determine the contribution of this pathogen to tree decline and yield loss within a particular grove. Populations of *P. parasitica* in the Highland grove varied between 20 and 70 ppg over the 12-day irrigation cycle. Therefore, it will be necessary to use care in selecting the proper time for soil sampling in groves with long-interval irrigation. Propagule quantitation fluctuated less and soil matric potential was less variable when a 2- to 3-day drying period existed between irrigations. Therefore, soil sampling in drip- or minisprinkler-irrigated groves should require less precise timing and less interpretation.

Changes in the populations of sporangia and zoospore cysts accounted for the majority of the fluctuation in the population of *P. parasitica* when the interval between irrigations was long. The numbers of these propagules recovered throughout the 12-day interval were correlated with changes in soil moisture. Release of zoospores from sporangia requires contact with free water (4), thus limiting zoospore release to the time of irrigation and shortly thereafter. Zoo-

**Fig. 4.** Changes in the populations of individual types of propagules of *Phytophthora parasitica* and changes in the soil matric potential after long-interval (12 days between irrigations) irrigation on day 0. Percentages of the total population contributed by each type of propagule (solid bars) as well as numbers of each type of propagule per gram of citrus rhizosphere soil (slashed bars) were calculated using the mean of three samples. (A) Zoospore cysts (shaded upper portion of bars; no shading indicates no contribution by zoospores) and sporangia (lower portion of bars), (B) chlamydospores, (C) dark-walled sporangia, (D) oospores, and (E) soil matric potential (*---*). Vertical lines represent ± standard error of the mean.

**Fig. 5.** Changes in the populations of individual types of propagules of *Phytophthora parasitica* and changes in the soil matric potential during a 12-day period of short-interval (2–3 days) irrigation; I = days when trees were irrigated. Percentages of the total population contributed by each type of propagule (solid bars) and numbers of each type of propagule per gram of citrus rhizosphere soil (slashed bars) were calculated using the mean of three samples. (A) Zoospore cysts (shaded upper portion of bars) and sporangia (lower portion of bars), (B) chlamydospores, (C) dark-walled sporangia, (D) oospores, and (E) soil matric potential (*---*). Vertical lines represent ± standard error of the mean.
spores were released by isolates of *P. megasperma* Drechs. and *P. cryptogea* Pethyr. & Lafferty when the soil water matric potential was held at values greater than −10 mb for 1 hr (17). Sporangia are usually produced maximally at soil water matric potential values slightly lower than those optimal for zoospore release. An isolate of *P. parasitica* from a citrus grove produced the greatest numbers of sporangia when mycelial mats were held at soil water matric potential values of −5 or −10 cb, although numerous sporangia were produced at all matric potentials tested between −5 and −70 cb (6). An isolate of *P. parasitica* from tomato produced abundant sporangia when mycelium or infected pieces of tomato fruit were incubated in soil at −2.5 to −30 cb (8). Soil irrigated at 2- to 3-day intervals remained at soil water matric potential values conducive to production and indirect germination of sporangia for a longer period of time than did soil irrigated with 12 days between irrigations.

Release of zoospores from sporangia that survive the 12 days between long-interval irrigations as well as production of new sporangia from mycelium in infected root tissue are most likely responsible for the rapid increase in numbers of propagules after irrigation. If chlamydospores or other resistant propagules germinated and produced sporangia after irrigation, their numbers in soil would be expected to decline, at least temporarily, as sporangial populations increased, but this did not occur.

Quantification of the different types of *Phytophthora* propagules in rhizosphere soil provided a conservative estimate of the actual number of each type of propagule. The number of zoospore cysts counted was probably much lower than the number actually present in soil, since the 15-μm nylon mesh screen used to prepare the samples undoubtedly allowed many zoospore cysts to escape. When the slides were first layered with the soil slurry, free water was available for several hours. Sometimes sporangia on the slides germinated indirectly and released zoospores into the soil slurry. This situation was easy to detect microscopically because the cysts usually remained clumped near the sporangium. Cysts in this situation were not counted. Zoospore counts primarily served to indicate when zoospores were present in the soil, and the accuracy of such counts is debatable.

One limitation of the procedure that concentrated a soil slurry on a slide was that only the propagules germinating on the slides were detected. A second limitation was that propagules that formed within intact host tissue were excluded from the count, since large root pieces were removed during sample preparation. Consequently, propagules that formed deep within host tissues were less likely to be isolated than propagules that formed predominantly along the edges of root fragments and in outer cortical tissues. Examination of stained root segments showed that sporangia frequently were seen along the edges of root tissues, whereas oospores were found primarily along the stellar tissues. This has been observed with other pythiaceous fungi (2). It is probable, however, that many propagules isolated from soil were originally formed within host tissue that later deteriorated.

Although techniques used in this study did not allow good recovery of propagules occupying host tissue, these propagules may not contribute to disease severity until the tissue has been de-
Propagules of *Phytophthora* may be protected by host tissue (26) and, by remaining dormant until the tissue deteriorates, may survive better than those free in the soil. Oospores of many species of *Pythium* are endogenously dormant when first produced within the host (22), and thick-walled dormant spores are converted to thin-walled germinable spores after exposure to non-sterile soil extract (13), which presumably would occur after tissue deterioration. Dormancy in host tissue could be an ecological advantage, since the pathogen would not be in competition with saprophytes in the decayed host material. Recovery of *P. parasitica* from dead and decomposing citrus roots is usually poor. Propagules of *P. parasitica* germinating within decomposing tissue most likely would be subject to intensive competition and may not survive, since the fungus is known to be a poor saprophyte (24).

We have demonstrated the existence of viable oospores of *P. parasitica* in citrus soil throughout the year. The role of oospores in diseases caused by *P. parasitica*, as with other heterothallic *Phytophthora* species, remains uncertain (10,26). Oospores of *P. parasitica* var. *nicotianae* have never been documented in nature in association with the black shank disease of tobacco (17). They are formed when opposite mating types are paired on a suitable medium, but they rarely germinate (10). Examination of more than 100 tomato root rot isolates of *P. parasitica* showed that they were all of the A2 mating type (9). Compatibility studies with field isolates of *P. parasitica* showed that the A1 compatibility type predominated. Of 60 isolates tested, 56 were A1, 3 were A2, and 1 produced oospores when paired with either mating type (14). Despite this skewed distribution of compatibility types, oospores were isolated consistently from two groves throughout the year. This suggests that production of oospores was stimulated by some factor in the environment other than the opposite mating type. Soil microorganisms, other *Phytophthora* species, and host root extract have been demonstrated to stimulate oospore production in many heterothallic *Phytophthora* species (1,20-22,27).

Our results with *P. parasitica* on citrus support the concept that the chlamydospore is the most important means of long-term survival (10,26). The chlamydospore was the predominant propagule from December through April. Germinating chlamydospores were probably responsible for the initial increase in inoculum in the spring, since the actual number of chlamydospores per gram of soil and the percentage of the population contributed by chlamydospores began to decrease at the same time as the sporangium population increased. Sporangia usually have not been considered to be an important overwintering structure for *P. parasitica* (7,12). Sporangia were, however, isolated from citrus soils throughout the year. Although some sporangia could have been produced in soil during the winter, it seems likely that these propagules survived most of the winter in a quiescent state, since most sporangia were isolated only after the soil had been heated to 32 C (14,15). We have found a new dark, multipapillate, distorted sporangium that we call a dark-walled sporangium because they appear dark. It is possible that these propagules are better survivors than normal sporangia. Although chlamydospores made up the majority of the overwintering population, at least 16% of the total wintering propagule population was composed of sporangia, dark-walled sporangia, and oospores.

**LITERATURE CITED**