Sampling Citrus Orchards in Florida to Estimate Populations of *Phytophthora parasitica*

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ABSTRACT

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Soil sample collection and soil handling methods were evaluated and the number of samples needed for reliable estimation of populations of *Phytophthora parasitica* were determined for Florida citrus orchards. Propagule densities of *P. parasitica* in field soil measured by assay on a selective medium were consistent for up to 8 days after collection if soil was maintained moist at 21–24 C, but they were reduced drastically by air drying. Assay of soils removed from storage at 8 C or collected from the field in late winter yielded low propagule densities, but incubation of soils 1 to 2 days at room temperature maximized recovery of the fungus. Collection of soil samples beneath the tree canopy within the drip line gave consistent recovery of *P. parasitica*. Propagule densities were lower and more erratic outside of the canopy, and root and fungal propagule densities diminished sharply with increasing depth. Mean propagule densities in 100 single-core samples from each of four 4-ha blocks of trees were 11.2, 0.8, 2.0, and 4.7, and frequency distributions best fit a negative binomial distribution (chi squares of 20.1, 3.88, 14.71, and 9.89 and k values of 0.93, 0.14, 0.12, and 0.62, respectively). The number of single core per tree samples needed to estimate the population with a standard error-to-mean ratio of 0.25 varied from 19 for sites with 11.2 propagules/cm^3 to 149 for sites with 2.0 propagules/cm^3. In an experiment to evaluate among-tree and among-sample variability, the number of trees that needed to be sampled varied from 23 when one core per tree was collected to five trees when 10 cores per tree were collected. Thus, if populations are moderate to high (above 5 propagules/cm^3) and about 20–40 samples per 4-ha block are collected from the surface soil beneath tree canopies in an orchard and processed as indicated above, populations can be estimated reliably.

Phytophthora foot rot and gummosis of the trunk are well-recognized problems on citrus (6). Until recently, the impact of feeder root rot caused by *Phytophthora* spp. on tree growth and production has been unknown, although it is generally assumed that some loss occurred on the most susceptible rootstocks (6). Systemic fungicides now are available that control *Phytophthora* spp. and permit the determination of the effect of feeder root loss. Preliminary results of fungicide trials in California and Florida (12,14) indicate that populations of 10–20 propagules/cm^3 of *Phytophthora* spp. result in significant feeder root loss.

Previously, the enumeration of propagules of *Phytophthora* spp. in soil required the use of baiting techniques and most probable number methods, making population assays tedious and time consuming. The use of hymexazol (17), which inhibits *Pythium* spp. but allows growth of most species of *Phytophthora*, permitted development of selective media for enumeration of soil propagules of *Phytophthora* spp. (9,17). In citrus, selective media have been used to determine the effects of irrigation methods and fungicide treatments on soil populations of *Phytophthora* spp. (4,12,15). However, no attempt has been made to develop sampling protocols to estimate populations in citrus orchards to ascertain the effects of treatments on populations of these fungi or the effect of fungal populations on yield.

No information is available on the most appropriate sample sites and depths for collection of soil samples or on proper methods of handling samples once collected. Tsao (16) recommended that samples be collected in the root zone at least 3–5 cm deep under the canopy on the north or east side of the tree and that samples be stored for a minimum amount of time at moderate temperatures. Chee and Newhook (2) premoistened dry soil for 28 hr; Okaisabor (13) maintained soil samples at 20% moisture for 5 days; Ioannou and Grogan (7) incubated samples at field capacity for 7 days before assay, but the effects of these treatments on populations of the fungus were not determined. Tsao (16) warned against premoistening or wet sieving soils for quantitative studies because such treatments might alter propagule densities.

The purpose of this study was to determine the most appropriate sample sites and depths, evaluate methods of handling soil samples, and determine the number of samples needed to estimate soil populations of *Phytophthora* spp. in Florida citrus orchards.

MATERIALS AND METHODS

Orchard sites. Three mature orchards (more than 20 yr old) were used for sampling studies: an orchard near Ft. Pierce, FL, of Hamlin sweet orange (*Citrus sinensis* [L.] Osb.) on sour orange (*C. aurantium* L.) rootstock planted on a shallow sandy soil (Pineda sand) at a spacing of 8.2 × 6.1 m on single row beds about 1 m high to allow adequate drainage; an unbedded orchard near Lakeland, FL, of Hamlin sweet orange on sweet orange rootstock planted at a spacing of 8.2 × 8.2 m on a deep, well-drained sandy soil (Tavares fine sand); and an unbedded orchard near Bowling Green, FL, of Valencia sweet orange on sour orange rootstock planted at a spacing of 7.6 × 7.6 m on a shallow sandy soil (Candler fine sand).

Propagule densities. Populations of *Phytophthora parasitica* Dast. (*P. nicotianae* Breda de Haan var. *parasitica* (Dast.) Waterhouse) were estimated with the method of Kannwischer and Mitchell (9). Ten cubic centimeters of soil from each well-mixed sample was diluted in 90 ml of 0.25% agar and 1.0 ml was spread on each plate of a selective medium. The selective medium of Kannwischer and Mitchell (9) was used and modified by using 125 instead of 250 mg of ampicillin per liter and adding 25 mg of hymexazol per liter. Five to ten plates from each replicate soil sample were incubated for 3 days in the dark at 28 C. The number of colonies of *P. parasitica* per plate was counted, and the counts were averaged and expressed as propagules/cm^3.

Sample storage. Soil for storage experiments was collected from beneath several trees in the Lakeland or Bowling Green orchards, composited, and mixed thoroughly. Soils were assayed by plating on selective media on the day of collection and after 1–12 days of storage under various conditions (Tables 1 and 2). Three

replications of each treatment were used. Samples were stored under the following moisture conditions: as collected, soil stored in resealable plastic bags, soil moisture as collected in the field; air dried (about −1,500 centibars [cB]), soil spread out and air dried at room temperature (21 to 24 C) and ambient relative humidity; moistened (about −3 cB), soil placed in 350-ml Styrofoam cups 10.5 cm tall with holes in the bottom, saturated with water and allowed to drain freely; saturated (0 cB), soil placed in the Styrofoam cups and flooded with water; air dried, then moistened, soil air dried for 24 hr, then moistened as above. Soil samples were incubated at room temperature or in incubators at the temperatures indicated.

For the heat treatment experiment, soil from the Lakeland orchard was collected in the winter (February 1987), placed in the Styrofoam cups, and moistened as above. The cups were covered with plastic wrap and incubated at the indicated temperatures.

TABLE 1. Effect of soil storage under various temperature and moisture conditions on the recovery of *Phytophthora parasitica* from Tavares fine sand from a citrus orchard near Lakeland, FL

Collection date	Storage conditions[a]		Propagules/cm^3 soil[b]					
			Time (days)					
	Moisture	Temperature	0	1	2	5	8	12
April 1986	M	RT	42 ± 5.3	31	28	36	27	13
	Sat	RT		48	29	18	17	11
	AC	RT		28	13	9	7	7
	AC	4C		33	11	4	8	2
	AC	34C		17	5	3	2	1
	AD	RT		9	1	3	7	1
	AD-M	RT		4	7	17	6	3
		$LSD_{0.05}$		9.9	11.6	7.2	13.9	3.6
February 1986	M	RT	15 ± 2.1	44	46		29	
	Sat	RT		45	42		24	
	AC	RT		34	35		31	
	AD	RT		7	9		8	
	AD-M	RT		5	5		6	
		$LSD_{0.05}$		5.3	5.0		3.0	

[a] M = moistened to about −3 centibars (cB); RT = room temperature (21–23 C); SAT = saturated (0 cB); AC = as collected, maintained in a sealed plastic bag; AD = air dried (about −1,500 cB); AD-M = air dried, then moistened after 1 day.

[b] Initial propagule densities are the mean plus or minus the standard deviation for three replicate determinations made at the time of sample collection from bulked soil used to establish the treatments. ND = not determined.

TABLE 2. Effect of soil storage under various temperature and moisture conditions on the recovery of *Phytophthora parasitica* from Chandler fine sand from a citrus orchard in Bowling Green, FL

Collection date	Storage conditions[a]		Propagules/cm^3 soil[b]					
			Time (days)					
	Moisture	Temperature	0	1	2	3	5	8
November 1984	M	RT	ND	23	20	24	24	
	AC	RT		17	15	9	14	
	AD	RT		7	4	1	4	
	AD-M	RT		5	7	7	8	
		$LSD_{0.05}$		7.9	5.2	9.0	5.8	
December 1984	M	RT	10 ± 0.6	8		11		12
	Sat	RT		7		23		13
	AD	RT		2		0		1
		$LSD_{0.05}$		4.5		4.5		4.6

[a] M = moistened to about −3 centibars (cB); RT = room temperature (21–23 C); Sat = saturated (0 cB); AC = as collected, maintained in a sealed plastic bag; AD = air dried (about −1,500 cB); AD-M = air dried, then moistened after 1 day.

[b] Initial propagule densities are the mean plus or minus the standard deviation for three replicate determinations made at the time of sample collection from bulked soil used to establish the treatments. ND = not determined.

Four replications per treatment were used in this experiment (Table 3).

Sample site and depth. These experiments were conducted in a bedded (Ft. Pierce) and in a nonbedded (Lakeland) orchard. Samples were collected at five or six sites around the tree as indicated in Table 4 to determine the effect of location on the propagule density of *P. parasitica*. An auger, 18.5 cm deep by 8.0 cm in diameter (930 cm^3 total volume), was used to collect the samples, and a single core was collected at each of the five or six sampling sites from each of 10 replicate trees. In addition, all feeder roots (those less than 2 mm in diameter) in each core were collected and oven dried at 45 C to a constant weight. Root density was expressed as milligrams dry weight per cubic centimeter of sample volume.

To determine the effect of depth on propagule and root densities, single-core samples were collected from depths of 0 to 23 cm, 23 to 46 cm, 46 to 69 cm, and 69 to 92 cm from inside the drip line of each of eight replicate trees in the Ft. Pierce and Lakeland orchards. Propagule counts and root densities were determined as above.

Sampling intensity. To determine the optimum number of samples needed to estimate the population of *P. parasitica*, 100 cores taken with the auger described above were collected from four blocks of approximately 4 ha each in two locations in the Lakeland orchard and in one location in the Bowling Green and Ft. Pierce orchards. One core per tree was collected inside the drip line from 100 trees selected at random within each block. In addition, an area of the Lakeland orchard severely affected by Phytophthora crown and root rot was selected for further sampling to determine

TABLE 3. Effect of temperature of incubation of soil and plates on the recovery of *Phytophthora parasitica* from a Tavares fine sand from a citrus orchard near Lakeland, FL, in February 1987

Soil temperature (C)[a]	Plate incubation temperature (C)	Propagules/cm^3 soil				
		Soil incubation time (days)				
		0	1	2	4	8
28	28	4 ± 3.2[b]	16	8	21	13
34	28		15	10	19	11
8	20		0	0	0	0
8	28		10	3	6	2
8 (2 days), 28[c]	28		9	1	15	9
	$LSD_{0.05}$		6.8	7.2	7.1	6.0

[a] Soil moistened to about −3 centibars in all treatments; all treatments replicated four times.

[b] Initial propagule density plus or minus the standard deviation of four replicate determinations.

[c] Soil held at 8 C until day 2, then transferred to 28 C.

TABLE 4. Effect of sample site location on the recovery of *Phytophthora parasitica* and root densities in a nonbedded orchard (Lakeland) and in an orchard planted on single row beds (Ft. Pierce)

Sample site	Propagules/cm^3 soil[a]	Root density (mg dry wt/cm^3 soil)[a]
Nonbedded orchard		
Near trunk, under canopy	99	1.29
Inside drip line, between rows	80	1.20
Outside drip line, between rows	13	0.21
Row middle	10	0.48
Inside drip line, in the row	68	1.13
$LSD_{0.05}$	22	0.25
Bedded orchard		
Near trunk, under canopy	21	0.79
Inside drip line, between rows	36	0.68
Outside drip line, between rows	25	0.76
One meter down bed, between rows	13	0.81
Between trees, in the row	15	0.54
Row middle	0	0.00
$LSD_{0.05}$	14	0.27

[a] Ten single-tree replications were used in each orchard.

the effect of tree decline on fungal populations and root densities. Single cores were collected and assayed separately from trees that were apparently healthy or suffering from mild, moderate, or severe decline.

Goodness-of-fit of data to the Poisson, negative binomial, Thomas double Poisson, Neyman type A, Poisson with zeros, logarithm with zeros, and positive binomial distributions was determined using a Fortran program (5).

The number of samples needed to achieve a reliable estimate of the mean in each of four locations was calculated using the formula of Elliott (3) for populations fitting a negative binomial distribution:

$$n = (t^2/D^2)\ 1/\bar{x} + 1/k \qquad (1)$$

where n = the number of samples; t = Student's t for $n-1$ degrees of freedom at the 95% confidence level; D = index of precision (one-half of the 95% confidence interval); $\bar{x}$ = mean of the population; k = the k parameter of the negative binomial distribution.

An index of precision of 0.5 at $P \leqslant 0.05$ was considered to be adequate for current purposes because precise threshold levels for populations of *P. parasitica* have not been determined.

To compare the variability attributable to among-tree differences and the variability due to among-sample differences, 15 single trees were selected at random within an area of 220 apparently healthy trees of the Lakeland orchard. Eight single-core samples at the eight cardinal compass directions around the circumference of the trees within the drip line were collected and assayed as above for propagule and root densities. Analysis of variance was conducted to determine whether sampling location around the tree or the tree selected in the orchard significantly affected propagule densities. Equation 4 of McSorley and Parrado (11) was used to determine the number of samples per tree needed given the number of trees to be sampled and assumes a standard error-to-mean ratio of 0.25:

$$0.0625\,\bar{\bar{y}}^2 = (N - n/N)\ Sb^2/n + Sw^2/mn \qquad (2)$$

where $\bar{\bar{y}}$ = the overall mean; N = the number of trees in the block; Sb^2 = variance among trees; Sw^2 = variance among samples; n = number of trees; m = number of cores per tree.

RESULTS

Sample storage. Little or no decline in the measured propagule densities occurred during 8 days of sample storage in any of the four experiments when the soil was maintained moist at room temperature (Tables 1 and 2). By day 12 in the Tavares fine sand collected April 1986 (Table 1), the propagule density had declined substantially. When the soil was maintained in a saturated condition, measured densities declined slowly in the Tavares fine sand collected in February and April 1986 but showed a sharp rise on day 3 in the Candler fine sand collected in February 1984. The measured propagule densities remained at the initial level for a time if samples were kept at the soil moisture as collected from the field but gradually declined. If samples were air dried, densities dropped drastically and did not increase after restoring soil moisture.

If soil moisture was maintained as collected from the field, the measured propagule densities were generally somewhat lower if samples were stored at 4 C or at 34 C than if stored at room temperature (Table 1, April 1986). Similar results were obtained in a separate experiment (data not shown). However, if samples were collected in late winter when soil temperatures were low, the propagule density measured at the time of sample collection was lower than that measured after an incubation period of 1 or 2 days (Table 1, February 1986). When soil collected in late winter from the Lakeland orchard was incubated after moistening under various conditions, temperature had a significant effect on the measured densities (Table 3). If plates were incubated at 28 C, storage of the soil sample at 28 C for 1 day was sufficient to raise the measured density from 4 to 16 propagules/cm^3. Soil storage at 34 C gave similar results. If soil was stored at 8 C and the plates incubated at 20 C, no propagules were detected. However, if soil that was stored at 8 C for 2 days was then placed at 28 C for the remainder of the experiment, the number of propagules detected increased. Increasing the plate incubation temperature improved detection, but only moving the soil samples to 28 C maximized the number of propagules of *P. parasitica* detected. Response of propagule counts to temperature was similar in other experiments (data not shown).

Sample site and depth. Of the sample sites tested, those located beneath or near the tree canopy yielded the highest propagule densities in the bedded and nonbedded orchards (Table 4). Locations outside of the canopy had lower propagule densities, and root densities were also low.

Propagule densities of *P. parasitica* and root densities decreased sharply with depth in the bedded and in the nonbedded orchard (Fig. 1). A higher percentage of the roots was found in the surface 23 cm in the nonbedded than in the bedded orchard.

Relationship to root density. Data collected from the sample intensity experiments were expressed on the basis of propagules per unit volume of soil and on the basis of propagules per gram root dry weight (Table 5). The standard-deviation-to-mean ratio was high for data expressed on the basis of soil volume, but this value was not reduced when data were expressed on a root-weight basis. From the sample site and depth studies, it is obvious that propagules of *P. parasitica* are few or absent in samples that contain few or no feeder roots (Table 4, Fig. 1). However, there was no correlation between root densities and propagule densities when samples were collected from beneath the canopy of

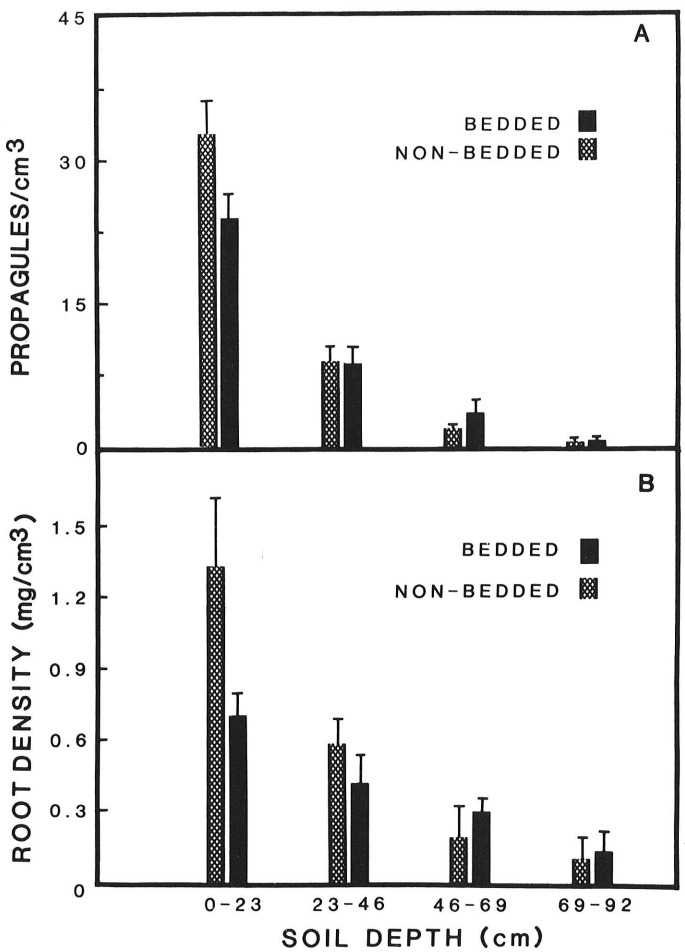

Fig. 1. Effect of sampling depth on the recovery of *Phytophthora parasitica* and on root densities in a nonbedded orchard (Lakeland) and an orchard on single row beds (Ft. Pierce). Eight single-tree replications were used at each depth in each orchard. Bars indicate the standard error of the mean.

TABLE 5. Relationship of propagule densities of *Phytophthora parasitica* to citrus root densities in apparently healthy and declining citrus trees

Orchard				Propagules/ cm^3		Root density (mg/cm^3)	Propagules/ mg root		Simple correlation coefficient (r)
Location	Sampling date	Tree condition	No. of samples	$\bar{x}$[a]	Sd $\bar{x}$[a]	$\bar{x}$	$\bar{x}$	Sd $\bar{x}$	Propagules vs. root weight
Lakeland	May 1986	H	100	11.2	1.1	1.07	50.3	1.3	+0.05
Ft. Pierce	Jan. 1986	H	100	0.8	2.6	0.54	1.7	5.4	+0.03
Bowling Green	Oct. 1986	H	100	2.0	3.3	1.22	2.6	1.4	+0.04
Lakeland (healthy)[b]	Nov. 1986	H	100	4.7	1.5	0.50	18.5	1.5	+0.04
Lakeland (decline)[b]	Nov. 1986	H	25	11.5	0.8	0.81	17.5	1.2	+0.23
		Mi	22	15.2	1.6	0.58	31.8	1.4	+0.58[c]
		Mo–S	14	20.0	0.9	0.53	42.2	0.9	+0.41

[a] $\bar{x}$ = over all mean; Sd $\bar{x}$ = standard-deviation-to-mean ratio.
[b] Trees apparently healthy (H) or suffering from mild (Mi), moderate (mo), or severe (S) decline due to Phytophthora crown or scaffold root rot.
[c] Correlation coefficient significant at $P \leq 0.01$.

TABLE 6. Means and distributions of propagule densities of *Phytophthora parasitica* in four Florida citrus orchards and the number of samples needed to estimate the population

Orchard					
Location	Sampling date	$\bar{x}$[a]	χ^{2}[b]	k[c]	No. of samples needed[a]
Lakeland	May 1986	11.2	20.10	0.93	19
Ft. Pierce	Jan. 1986	0.8	3.88	0.14	134
Bowling Green	Oct. 1986	2.0	14.71	0.12	149
Lakeland	Nov. 1986	4.7	9.89	0.62	29

[a] Overall mean propagules/cm^3.
[b] Chi-squared test of fit to a negative binomial distribution; all values significant at $P \geq 0.05$.
[c] k value for the negative binomial distribution.
[d] Number of samples needed to estimate the mean assuming a 95% confidence interval of ± 50% of the mean using equation 1.

apparently healthy trees at a uniform depth (Table 5). However, if samples were collected from an area of an orchard where trees were declining, root density decreased and propagule density increased as the severity of the tree decline increased (Table 5). In this case, the correlation between root and propagule densities was positive but was significant only with trees in mild decline.

Sampling optimization. When the frequency distribution was analyzed for the four sites sampled, the negative binomial distribution provided the best fit for the data in all cases with chi-squared values of 3.88 to 20.1 (Table 6). All distributions also fit a Neyman Type A distribution, but chi-squared values were higher in every case. A significant fit at $P \geq 0.05$ was obtained from one or two of the orchards with the Poisson, Thomas double Poisson, Poisson with zero, and logarithm with zeros, but chi-square values were always higher than with the negative binomial distribution. The observed and expected frequencies for the orchards with the highest and lowest propagule densities are shown in Figure 2. The k values for the negative binomial distribution ranged from 0.12 to 0.93, indicating a high degree of aggregation of propagules especially where densities were low. The estimated number of samples from equation 1 ranged from 19 to 149 depending on propagule density.

In the experiment where the among-tree and the among-sample variabilities were compared, the overall mean was 8.8 propagules/cm^3, and analysis of variance indicated that there was no significant effect of individual tree or individual sample site around the circumference of the tree on propagule or on root densities. Likewise, in the sample site and depth studies (Table 4, Fig. 1), individual tree was not a significant factor in the analysis of variance in those experiments. Applying equation 2 to these data, then $0.0625\ (8.8)^2 = (220 - n/220)(18.92/n + 92.42/mn)$, and the relationship of the number of trees sampled (n) to the number of cores per tree (m) is $n = 3.81 + 18.56/m$. Thus, if 1, 2, 3, 4, 5, or 10 cores per tree are collected, then 22, 13, 10, 8, 7, and 5 trees, respectively, must be sampled.

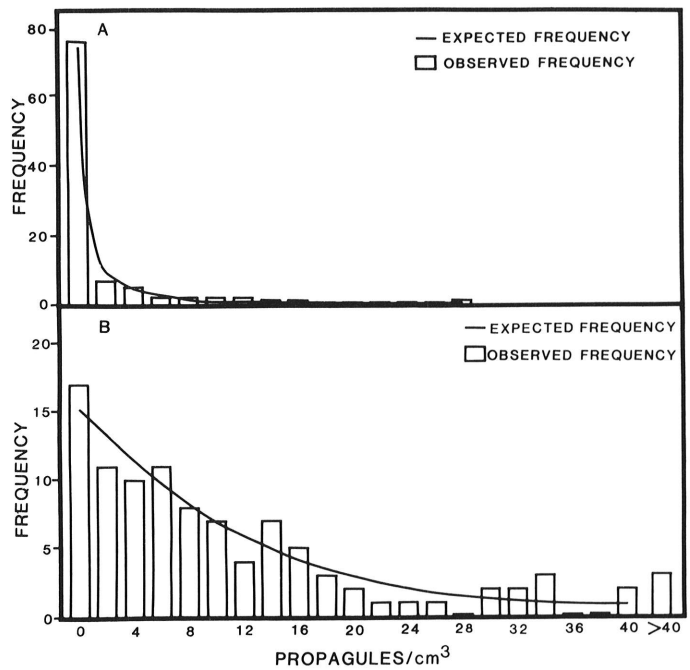

Fig. 2. Frequency distributions of propagule densities of *Phytophthora parasitica* in the **A,** Ft. Pierce and the **B,** Lakeland orchards. Data best fit a negative binomial distribution: mean = 11.2 propagules/cm^3, chi squared = 20.1, and $k = 0.93$ for the Lakeland orchard; mean = 0.8 propagules/cm^3, chi squared = 3.88, and $k = 0.14$ for the Ft. Pierce site.

DISCUSSION

The moisture and temperature of the soil sample during storage had important effects on the measured propagule densities of *P. parasitica*. Drying the soil reduced propagule numbers drastically and remoistening did not increase the measured densities. Air drying may have caused the loss of zoospores, mycelial fragments, or other fragile propagules. Saturation of soil samples often had little effect, but in one experiment, measured density rose sharply, probably indicating that zoospores had been released. Although Tsao (16) did not recommend premoistening soils for later assay for fear of artificially increasing the observed number of propagules, this appears to be the best method to maintain propagule densities of *P. parasitica* at the level observed at the time of sample collection. In contrast to our results, Jeffers and Aldwinckle (8) found that air drying, then remoistening soils from apple orchards enhanced recovery of *P. cactorum* using baiting techniques. The difference may be due to different survival structures between the two species. The homothallic *P. cactorum* would be more likely to survive as oospores, whereas *P. parasitica* probably survives mostly as chlamydospores (10).

As suggested by Tsao (16), temperatures at or near room

temperature appear to be optimal for storage of samples for subsequent assay. As Lutz and Menge (10) found in California, low temperatures apparently induced dormancy of some propagules of *P. parasitica*. In our studies, storage of soil at 8 C reduced the measured propagule densities to 0 if plates were incubated at 20 C and significantly reduced densities even when the plates were incubated at 28 C. Because soil temperatures in Florida dropped to low levels (12–14 C) only in late winter in 1986, this was only an occasional problem in field sampling (Table 1, February 1986). However, in California soil, temperatures of 9–12 C caused propagule densities to decline to near zero for the entire winter (4). When low soil temperatures occurred in our study, a 1- to 2-day incubation of the samples at room temperature was sufficient to overcome the dormancy if plates were incubated at 28 C. Thus, the optimum method of sample handling in Florida is to moisten the soil and incubate it at room temperature for at least 2 days. Samples can be held for up to 8 days without affecting the measured propagule density, but densities declined significantly by 12 days (Table 1). This method of sample handling should be evaluated independently for areas with fine-textured soil because all of the current work was done with sandy soils.

Samples should be collected from the surface 23-cm depth of soil beneath the tree canopy to maximize the recovery and the consistency of propagule counts of *P. parasitica*. Samples collected near the trunk or inside the drip line, whether in the row or between rows, yielded consistent results. There were no significant differences between samples collected at various locations around the circumference of the canopy if they were collected within the drip line of the tree. However, outside of the canopy, soil temperature and moisture extremes and cultural practices such as disking may drastically affect the measured propagule densities. The lack of roots in row middles and at greater depths diminishes the chances of recovering the fungus.

Propagule densities are probably most conveniently expressed on a per unit volume of soil basis. Menge (12) expressed densities on the basis of propagules per gram dry weight of rhizosphere soil. However, in the sandy soils of Florida, we found it nearly impossible to collect rhizosphere soil, and thus all of our data is expressed on the basis of bulk soil. Because the moisture-holding capacity of these soils is low, the propagule counts do not change appreciably whether expressed on a volume or dry-weight basis. Data also could be expressed on the basis of the feeder root dry weight if samples were collected in similar locations, but we saw no particular advantage to that system.

Propagule densities appeared to increase with the severity of tree decline especially if expressed on the basis of propagules per milligram of root (Table 5). In contrast, Benson (1) found that propagule densities of *P. cinnamomi* on azaleas decreased sharply on severely declining or dead plants. Although the canopies of the citrus trees sampled (Table 5) were in moderate to severe decline, root densities were still relatively high, which probably allowed multiplication of the fungus. In contrast to the situation with *P. cinnamomi* on azaleas and other crops, death of mature citrus trees as a result of feeder root rot would not be expected. Thus, citrus trees even though in decline probably continue to produce sufficient feeder roots to serve as a substrate for *P. parasitica*.

The number of samples needed to accurately estimate the population varies with the population mean. Using the formula of Elliott (3), the calculated number of samples needed per 4-ha site ranged from 19 for orchards with high populations to 149 for those with low populations. Probably about 20–40 samples per site would be sufficient where populations are moderate to high. We believe larger areas could be sampled with the same number of cores as long as the rootstock, soil conditions, and cultural practices were uniform. On the other hand, where conditions vary in an orchard, it may be necessary to sample more intensively. Even though the variability would be much higher in orchards with low populations, little information would be lost. For example, if the true mean population is 1.0 propagules/cm^3, it is probably not important if the mean is estimated at 0.0 or 2.0 propagules/cm^3, i.e., an error of 100%.

The system we have developed gives an acceptably accurate estimate of populations of *P. parasitica* while minimizing the labor involved. However, the biological significance of these propagule densities to citrus trees remains to be established. Preliminary data from fungicide trials in Florida indicate that root densities increase following fungicide treatment in orchards where average annual propagule densities have been 10 propagules/cm^3 or above (14). The effect of rootstock cultivar on propagule density and the biological significance of a given propagule density on a specific tolerant or susceptible rootstock is presently unknown.

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