Optimizing Plot Size for Field Studies of Phymatotrichum Root Rot of Cotton

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ABSTRACT

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A study was conducted to determine the effect of plot size on the variance and mean percent disease incidence in field studies of Phymatotrichum root rot of cotton. The variance among sample areas (σ_ϵ^2) and within plots (σ^2), the coefficient of variation (CV), and the mean of percent disease were systematically evaluated. Measurements were made in 1983 at three locations in the Blackland Prairie that differed in respect to disease incidence. Plot sizes ranged from 5.5 to 88.0 m^2. The σ^2 and the respective CVs were reduced by increasing the sizes of the sampling area. Minimum plot sizes of 33 m^2 (ie, three rows × 16 m or two rows × 24 m) were necessary to estimate the true mean. However, when plot size was held constant, the σ^2 and associated CV was lower when plots had a greater number of adjacent rows compared to increased row length. This occurred irrespective of location or level of disease incidence. Because the σ^2 was not reduced further when plot size exceeded 33 m^2, we conclude that minimum plot areas of 33 m^2 (ie, three rows × 16 m) are required to give the best estimate of the true mean and to provide the lowest variance in field experiments of Phymatotrichum root rot on cotton.

Phymatotrichum omnivorum (Shear) Duggar [*Phymatotrichopsis omnivorum* (Duggar) Hennebert] is a serious soilborne pathogen that causes root rot on cotton (*Gossypium hirsutum* L.) in the southwestern United States and Mexico (7). Since the disease was first reported by Pammel (6), many field studies have been conducted on its ecology and epidemiology and on control methodologies. It has been a common practice to locate these experiments in areas where the pathogen is established and disease history is known.

The incidence and spread of the disease varies within fields and among years even in heavily infested soil (13). Accordingly, plot sizes, experimental designs, and sampling methods have been varied to minimize experimental error and increase the precision in estimating treatment effects (1,2,3,4,9,10). However, little information can be gleaned concerning the extent and nature of variation associated with plot size or sampling method in these studies. This restricts the design of experiments, a priori, that minimize plot size and experimental error in field studies on Phymatotrichum root rot of cotton. It is recognized that experimental error can be reduced by increasing plot size and altering the plot shape to account for biological parameters (12).

The objective of the study was to determine the optimal plot size beyond which effects on error variance and mean percent disease of Phymatotrichum root rot of cotton were negligible.

MATERIALS AND METHODS

The variation in incidence of *P. omnivorum* in field plots of cotton was systematically evaluated at three locations in the Blackland Prairie of Texas in 1983. At each site, the disease incidence of Phymatotrichum root rot among plants was greater than 80% at cotton harvest in years favorable to disease development. The soil at each site was classified as a Houston Black clay (Udic Pellusterts [fine, montmorrillonitic, thermic]). The sites were located 8 km south of Whitney, TX (S1), 3 km north of Hillsboro, TX (S2), and at the Blackland Research Center, Temple, TX (S3).

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Conventional cultural practices were maintained at each site. *Gossypium hirsutum* L. 'GP3774,' an early fruiting and high-yielding cultivar commonly grown in the area, was planted at each site in rows spaced 69 cm apart during mid-April. Plant densities at emergence ranged from 11 to 13 plants per square meter at each site. Fertilizer was applied preplant at rates of 60 kg $N \cdot ha^{-1}$ and 30 kg $P_2O_5 \cdot ha^{-1}$. Weed control was maintained with a preplant application of 125 kg $\cdot ha^{-1}$ trifluralin (*a*,*a*,*a*,-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) and mechanical cultivation when required.

Field areas dedicated to this study at S1 and S2 were a part of other experiments to evaluate the effects of tillage depth and timing on the incidence of Phymatotrichum root rot. These areas were chosen for similarity to S3 regarding soil type, disease history, and cultural practices. Treatments included three autumn plowing dates and two tillage depths (0 and 40 cm) with three replicates at S1 and two autumn plowing dates and three tillage depths (0, 30, and 40 cm) with three replicates at S2. In both cases, the 18 individual experimental units were 46 m long, 11 m wide, and arranged in a split plot, completely randomized design with plowing dates as the main plots and tillage depths as subplots. Tillage experiments were not superimposed on the field area at S3. Instead, the 1.1-ha area was treated uniformly using the conventional cultural practices outlined above.

To estimate the optimum plot size, sampling areas (four adjacent rows, 32 m long) were selected within each experimental unit at S1 and S2. At S3, the 18 sampling areas were randomly chosen within the field. Each row within the sampling areas was then divided into four segments 8 m long providing 16 subplots to assess the disease severity. This was accomplished by counting the number of diseased and healthy plants within each subplot on 2, 3, and 4 August 1983 at S1, S2, and S3, respectively. Larger subplots were formed from the original 16 subplots by grouping various row segments and adjacent rows. This also simulated different sampling methods by yielding plots of equal size, but with different row number and length combinations. Pooled estimates of variance of subplots among the sampling areas (σ_ϵ^2) and within sample plots (σ^2) and associated means and coefficients of variation (CV) were calculated for the different subplot groupings at each site by using the respective analyses of variance shown in Table 1. This systematically removed the proportion of the variance attributed to the treatment effects (plowing dates and tillage depths) and provided an unbiased estimate of the variance for similar

treatments. Because no treatments were imposed at S3, the σ_ϵ^2 was calculated directly as the variance among the 18 individual experimental units.

RESULTS

The mean incidence of root rot varied with location and size of the individual subplots (Table 2). Disease levels were 22, 45, and 19% at S1, S2, and S3, respectively, when means were taken on the whole sampling area (4 rows × 32 m). By using the smallest subplots (1 row × 8 m), disease levels were underestimated at S1 by 36% from those obtained on the whole plots and overestimated by 20 and 58% at S2 and S3, respectively. However, subplot sizes greater than 33 m^2 (ie, 3 rows × 16 m or 2 rows × 24 m) closely represented the disease levels obtained on the whole sample plot (Fig. 1).

The variance among sample areas (σ_ϵ^2), variance within sample plots (σ^2), and coefficient of variation for each row length and row

TABLE 1. Analysis of variance[w] for field plot size optimization experiments on Phymatotrichum root rot of cotton at three field sites[x] in Texas

Source of variation	Degrees of freedom[y]	Expected mean squares[z]
I.		
Plowing date	a-1	$\sigma_\epsilon^2 + b\,\sigma_{AR}^2 + rb\theta_A^2$
Replication × plowing date	$a(r$-1)	$\sigma_\epsilon^2 + b\,\sigma_{AR}^2$
Tillage	b-1	$\sigma_\epsilon^2 + ra\,\sigma_B^2$
Tillage × plowing date	(a-1) (b-1)	$\sigma_\epsilon^2 + r\,\sigma_{AB}^2$
Tillage × replication/ plowing date	$a(b$-1) (r-1)	σ_ϵ^2
II.		
Plowing date	a-1	$\sigma^2 + sb\,\sigma_{AR}^2 + sbr\theta_A^2$
Replication × plowing date	$a(r$-1)	$\sigma^2 + sb\,\sigma_{AR}^2$
Tillage	b-1	$\sigma^2 + s\,\sigma_{ABR}^2 + sra\theta_B^2$
Tillage × plowing date	(a-1) (b-1)	$\sigma^2 + s\,\sigma_{ABR}^2 + sr\theta_{AB}^2$
Tillage × replication/ plowing date	$a(b$-1) (r-1)	$\sigma^2 + s\,\sigma_{ABR}^2$
Subplot error	$abr(s$-1)	σ^2
III.		
Plot	r-1	$\sigma^2 + S\sigma_R^2$
Subplot error	$r(s$-1)	σ^2

[w] I. The determination of the variance over sample areas (σ_ϵ^2) using different plot sizes and configurations at S1 and S2. II. The determination of the variance within sample plots (σ^2) using different plot sizes and configurations at S1 and S2. III. The determination of the variance within sample plots for different plot sizes and configurations at S3.

[x] Site 1 (S1) 8 km south of Whitney, TX; site 2 (S2) 3 km north of Hillsboro, TX; and site 3 (S3) Blackland Research Center, Temple, TX.

[y] If location = S1, then $a = 3$, $b = 2$, $r = 3$, and s = number of subplots; if location = S2, then $a = 2$, $b = 3$, $r = 2$, and s = number of subplots; and if location = S3, then $a = 18$ and s = number of subplots.

[z] Random and fixed factors are represented by σ and θ, respectively. *A, B,* and *R* = the portion of variance for plowing date, tillage, and replication, respectively.

TABLE 2. The effect of plot size on the mean and range of percent disease incidence of Phymatotrichum root rot on cotton at each of three locations

Plot size		Location					
		S1		S2		S3	
Length[z]	Number of adjacent rows	$\bar{X}$	range	$\bar{X}$	range	$\bar{X}$	range
8	1	14	0–52	54	0–98	30	0–83
8	2	15	0–41	49	0–95	28	0–79
16	1	15	0–61	53	6–95	22	0–69
8	3	18	0–59	43	2–89	27	0–76
24	1	17	0–68	54	9–95	23	0–62
8	4	19	2–64	42	14–87	24	2–67
32	1	18	0–70	54	8–95	23	0–67
16	2	15	0–51	49	8–95	21	0–59
16	3	18	2–63	44	9–91	21	1–53
24	2	17	0–54	49	10–96	21	0–50
16	4	20	1–65	44	9–85	19	1–46
32	2	19	0–59	49	10–96	21	0–47
24	3	20	2–59	44	10–92	20	1–49
24	4	20	2–62	44	10–86	18	2–42
32	3	21	5–62	44	10–93	20	2–45
32	4	22	6–64	45	11–88	19	2–39

[z] Length of plots (meters).

TABLE 3. The effect of plot size on the variance among sample plots (σ_ϵ^2) and coefficient of variation (CV) of percent disease incidence of Phymatotrichum root rot on cotton at three locations

Plot size			Location					
			S1		S2		S3	
Length[y]	Number of adjacent rows	Subplots[z]	σ_ϵ^2	CV	σ_ϵ^2	CV	σ_ϵ^2	CV
8	1	1	381	144	930	57	807	94
8	2	1	216	101	815	58	512	85
16	1	1	543	152	843	55	532	103
8	3	1	405	110	603	56	417	77
24	1	1	546	134	641	47	420	89
8	4	1	452	114	567	56	298	73
32	1	1	501	122	506	43	412	89
16	2	1	316	115	1076	66	364	88
16	3	1	370	103	779	62	238	72
24	2	1	370	111	927	62	270	78
16	4	1	393	99	736	61	171	67
32	2	1	374	103	808	58	248	76
24	3	1	393	101	751	45	190	69
24	4	1	429	101	677	58	140	64
32	3	1	388	93	679	59	181	66
32	4	1	428	93	614	55	141	63

[y] Length of plots (meters).

[z] Number of subsamples per plot.

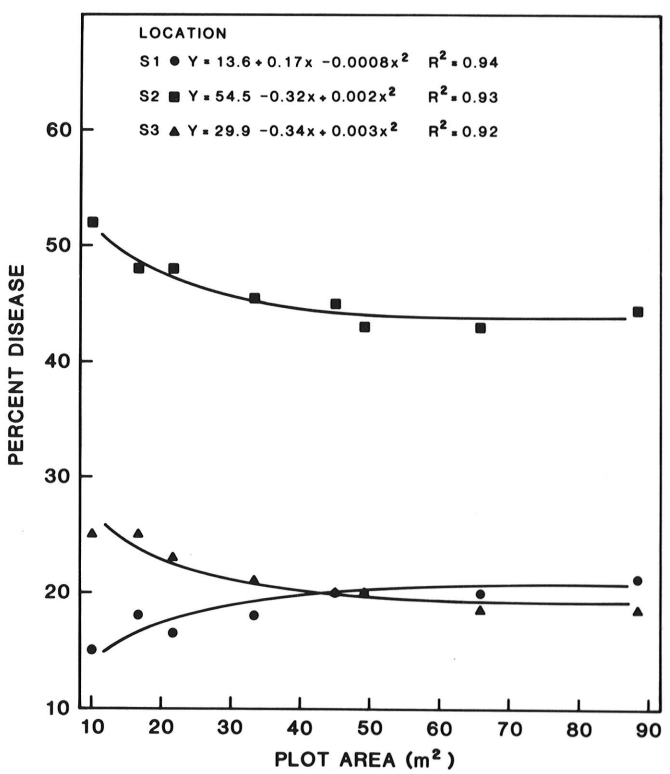

Fig. 1. The effect of plot area (square meters) on percent disease incidence of Phymatotrichum root rot on cotton at three locations.

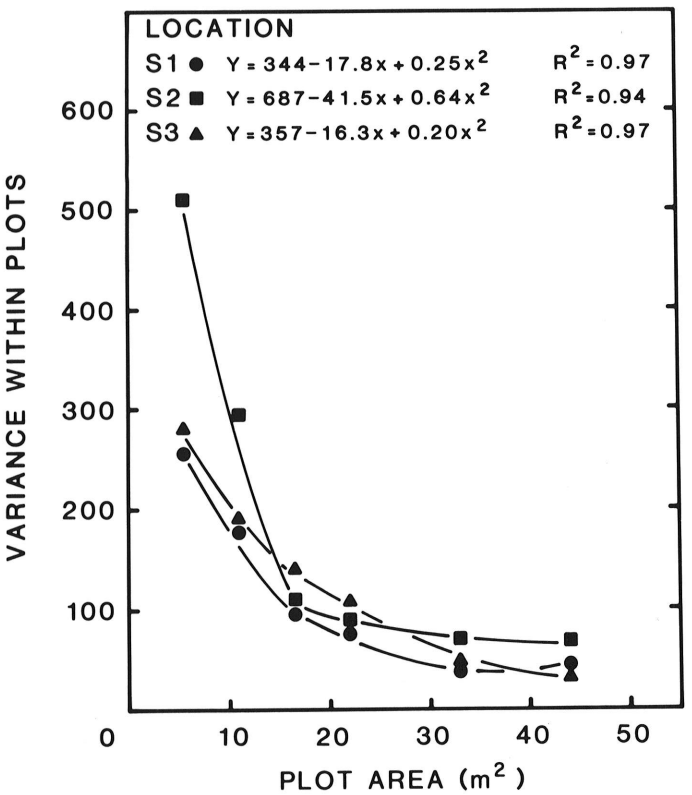

Fig. 2. The effect of plot area (square meters) on the variance within sample plots (σ^2) for the disease incidence of Phymatotrichum root rot on cotton.

combination are shown in Tables 3 and 4. Only at S3 were σ_ϵ^2 and the respective CV lowered as plot size increased (Table 3). Consistent trends between σ_ϵ^2 and plot size were not apparent at S1 and S2. However, the σ^2 and respective CV were lowered at each location as plot size increased (Table 4). In fact, when the plot size was held constant, σ^2 and the CVs were lower for the subplots that had a greater number of adjacent rows compared to those with increased row lengths. Given this consideration, the σ^2 was not further reduced when plot size exceeded 33 m^2 (Fig. 2).

DISCUSSION

The coefficients of variation obtained in this study demonstrate the extreme variability of Phymatotrichum root rot in cotton found in field areas with previously high levels of the disease. However, the error variance and CV could be reduced by increasing the size of the sampling area. This was clearly shown by the variance among the subplots (σ_ϵ^2) at S3, an area not confounded with extraneous treatment effects (Table 3). Because of possible confounding treatment effects and insufficient replicates, the σ_ϵ^2 and coefficient of variation were not reduced by increasing plot size at S1 or S2.

Our study showed that sampling areas of 33 m^2 (ie, 3 rows × 16 m or 2 rows × 24 m) closely estimated the true mean and produced the lowest variance within experimental units. This finding was consistent over three locations with disease incidence ranging from 19 to 45%. Apparently, it is not necessary to use extremely large sampling areas (9) to reliably estimate the true mean and minimize the error variance. Small sampling areas (12 m^2) normally used by plant breeders to evaluate genetic progency do not give adequate estimates of the true mean and give the high error variances in this instance. The probability of finding significant treatment differences in field experiments on Phymatotrichum root rot of cotton is evidently restricted using such small areas.

One interesting point that emerged during the study was the effect of sampling methods on the variance within sample plots (σ^2). Results showed that irrespective of location or lack of disease, the variance was lower when the subplot unit size was increased by

TABLE 4. The effect of subplot size on the variance within sample plots (σ^2) and coefficient of variation (CV) of percent disease incidence of Phymatotrichum root rot on cotton at three locations

Plot size			Location					
			S1		S2		S3	
Length[y]	Number of adjacent rows	Subplots[z]	σ^2	CV	σ^2	CV	σ^2	CV
8	1	16	261	74	513	51	284	93
8	2	8	180	61	292	38	186	72
16	1	8	184	62	459	48	189	73
8	3	4	94	46	110	23	142	60
24	1	4	157	62	444	48	151	65
8	4	4	82	41	97	22	104	55
32	1	4	148	55	396	45	147	64
16	2	4	119	49	252	36	113	57
16	3	2	43	30	66	18	43	32
24	2	2	102	46	273	37	115	58
16	4	2	41	28	66	18	31	30
32	2	2	104	45	251	36	103	54

[y] Length of plots (meters).
[z] Number of subsamples per plot.

increasing the number of rows rather than row length. This infers that the disease tends to be concentrated on plants within a row as compared to across rows and that the disease spreads from plant-to-plant. Therefore, to most closely estimate the true mean and minimize the error variance for field studies on Phymatotrichum root rot of cotton, large plots (33 m^2 or more) should be used and, whenever possible, plot sizes should be increased by increasing the number of adjacent rows as compared to row length.

Estimating optimal plot size is an important first step in epidemiological studies of root rot on cotton. Apparently, this is the first systematic attempt. An awareness of the spatial variability of disease is essential to design experiments and efficient sampling procedures (11), the proper interpretation of field data (5), and develop accurate population dynamic models (8). The variance within plots, mean relationship for each plot size, appears to follow Taylor's law (14) in this present study. We are now extending these studies to examine the spatial heterogeneity and dynamics of Phymatotrichum root rot.

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Ecology and Epidemiology

Influence of Matric and Osmotic Water Potentials and Soil pH on the Activity of Giant Vampyrellid Amoebae

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ABSTRACT

Homma, Y., and Cook, R. J. 1985. Influence of matric and osmotic water potentials and soil pH on the activity of giant vampyrellid amoebae. Phytopathology 75:243-246.

Perforation of conidia of *Cochliobolus sativus* by giant vampyrellid amoebae did not occur at soil matric water potentials higher (wetter) than −10 to −50 mbars or lower (drier) than −200 to −250 mbars. Perforation activity commenced with air entry into the soil, which occurred at about −50 mbars (or slightly drier) in both a silt and clay loam and at about −10 and −25 mbars in the same two soils, respectively, mixed (1:1, v/v) with coarse sand. In liquid culture, amoebae were highly active in 10% soil (Palouse silt loam) extract and in distilled water (0 bars), indicating that the flooding is not directly limiting to their activity. Perforation activity also tended to cease at lower matric potentials in the loams (−200 to −250 mbars) than in the loam-sand blends (−150 mbars), either because too few water-filled pores remained in the soils and blends at these matric potentials, or because the amoebae can no longer maintain adequate turgor at these matric potentials. In soils from 183 fields in Japan, perforation of conidia of *C. miyabeanus* was maximal in soils at pH 6.5–7.0 and at electrical conductivity (EC) values for salinity in the range from 400 to 800 μmho/cm, and it was nil in soils at pH 4.0 and having EC values >1,200 μmho/cm (= −426 mbars osmotic potential). In liquid culture, the trophozoites remained active in distilled water adjusted to about −450 mbars osmotic potential with KCl, and in 10% soil extract solution adjusted to −750 to −800 mbars osmotic potential with either KCl or $NaNO_3$. The different lower limits of osmotic potential for perforation activity in 10% soil extract than in distilled water probably indicate the lower limits at which the trophozoites can maintain turgor in a saline environment. Turgor may be lost and the trophozoites may therefore cease activity at even higher water potentials in nonsaline soils.

Additional key words: antagonists, biological control, soilborne plant pathogens.

Giant vampyrellid amoebae cause perforations and annular depressions (partial perforations) in the walls of pigmented spores (1,9–11) and hyphae (7,8) of plant pathogenic fungi. However, the effectiveness of these organisms as agents of biological control will depend on their ability to move in soil from one pathogen propagule to another. As shown for nematodes (12), aquatic fungi (5), and bacteria (6), the ability to move from site to site within the soil matrix depends on the size and continuity of water-filled pores. Amoebae can move through a Nuclepore filter provided the openings are 5.0 μm or greater (9); in soil, pores of this size and larger drain at a matric potential of about −600 mbars. In addition, amoebae in their trophozoite state may be more sensitive than bacteria or fungi to salts in soil; without a rigid cell wall, the trophozoite would be unable to maintain a high turgor (pressure) potential and hence also may be less able to make the internal osmotic adjustments necessary for maintenance of a turgor when moving between saline and nonsaline environments. Finally, amoebae, being strictly aerobic, are likely to become inactive in water-saturated soil if oxygen diffusion through the water-filled pores is too slow to meet their needs.

If amoebae are to be used as agents of biological control, either introduced or managed as residents in the soil (2), then the environmental parameters under which they function must be known. This work was undertaken to determine the influence of various physical and chemical properties of soils on the perforation activity of amoebae. A preliminary report has been published (3).

MATERIALS AND METHODS

Quantification of perforation activity. Perforation activity was quantified by counting the holes produced by amoebae in conidia of either *Cochliobolus sativus* (Ito et Kurib.) Drechs. ex Dast. or *C. miyabeanus* (Ito et Kurib.) Drechs. ex Dast. The conidia were collected dry from 2-wk-old cultures grown on potato-dextrose

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