Comparisons of Spatial Patterns of Oospores of *Peronosclerospora sorghi* in the Soil and of Sorghum Plants with Systemic Downy Mildew

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ABSTRACT

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The spatial pattern of sorghum systemically infected with downy mildew and of soil-residing oospores of *Peronosclerospora sorghi* was assessed using Morisita's index of dispersion. A binary series of quadrat sizes, starting at 1 m^2 and ending at 128 m^2 for infected plants and at 512 m^2 for the oospores, respectively, formed the basis for the computation. The pattern was clumped ($P = 0.01$) for oospores and infected plants at all quadrat sizes except the 32-m^2 and 64-m^2 quadrat size for the oospore pattern. The behavior of the index was similar for both populations. The use of susceptible plants to assess spatial patterns of oospores of *P. sorghi* instead of direct sampling by soil cores is indicated.

Sorghum downy mildew, caused by *Peronosclerospora sorghi* (Weston & Uppal) C. G. Shaw (10), is an important disease of sorghum (*Sorghum bicolor* (L.) Moench) throughout the tropical and subtropical regions of the world (2). Control methods rely mostly on the use of resistant cultivars and, to a lesser degree, on seed treatment (1). In Texas, soilborne oospores are the major source of inoculum, and yield loss is directly related to oospore-initiated infection (1). Field screening methods for the identification of disease resistance rely, at present, on natural oospore populations for their effectiveness. The actual inoculum density of the soil in screening nurseries is routinely assessed by taking soil samples and isolating and counting oospores, but this procedure is time and labor intensive. Questions have been raised about the validity of propagule counts obtained by soil sampling (7), and spatial patterns derived from soil samples may prove particularly misleading for *P. sorghi* since the presence of viable and nonviable oospores confounds propagule counts. Comparison of spatial patterns of oospores with those of systemically infected sorghum plants, however, should help clarify this problem. Because an infected plant is the best indicator of an infectious oospore assuming favorable environment, the spatial patterns of these plants should match the spatial pattern of the infectious oospores. Furthermore, such comparisons should determine the method of assessment of disease potential (oospore or infected plant distribution) that is more reliable for field screening. The purposes of this study were to determine the spatial pattern of the oospores, compare it with that of systemically infected plants, and complement studies already reported (8).

MATERIALS AND METHODS

Soil samples were collected in February 1984 near Beeville, TX, in a binary series of 10 quadrat sizes. The soil was a sandy loam (60% sand, 18% silt, and 22% clay). For quadrat sizes up to 8 m^2, 10 quadrats per size class were placed randomly throughout the sample area (approximately 2 acre). In each quadrat, nine soil-core samples of 3-cm diameter and 15-cm depth were taken at regularly

spaced points on a grid within each quadrat and bulked for each replicate quadrat. For the remaining quadrat sizes (16 m^2–512 m^2), seven quadrats per size class were placed randomly in the sampling area. In each quadrat, 16 soil-core samples of the same dimensions as for the smaller quadrats were collected and bulked separately for each replicate quadrat. The number of soil-core samples was raised to 16 in an attempt to increase the validity of the propagule counts with the larger quadrat sizes. Soil was mixed thoroughly before subsampling. Fifty grams of dry soil was subsampled from each bulked soil sample. A total of 82 50-g soil samples were processed as follows. Soil particles were dispersed in 100 ml of sterile H_2O and 1 ml of Tween 80 using a magnetic stirrer, and then centrifuged at 550 *g* for 10 min. The supernatant was decanted and saved. The pellet then was resuspended in a saturated sucrose solution (specific gravity = 1.47 g/ml) and centrifuged at 550 *g* for 40 min. The supernatant was again decanted and saved. The centrifugation in sucrose was repeated five times. The combined supernatants were then filtered through a 20-μm-mesh nylon screen. Oospores trapped on this filter were resuspended in about 100–250 ml of sterile H_2O to facilitate counting. The exact amount of H_2O used for resuspension was recorded. Three 2-ml subsamples were taken, dropped on gridded filter paper, and the number of oospores was counted. The mixture was stirred while samples were drawn. The mean of the three subsamples was multiplied with the volume used for resuspension and divided by 50 to determine the number of oospores per gram of soil.

Data on the pattern of systemically infected plants were obtained by adding values for early season downy mildew and late season downy mildew (Beeville location) described previously by Schuh et al (8). This addition was valid because both disease symptoms are the result of oospore-initiated infection, are easily separable and identifiable, and were assessed at the same location. For the quadrat sizes up to 8 m^2, 10 replicates per quadrat size were sampled from the 45 samples per quadrat size (8); for sizes 16–128 m^2, there were seven subsamples. The number of replicates was set at 10 and 7 to achieve equal power per quadrat size in testing for departures from random distribution as compared with the oospore samples. The two largest quadrat sizes were not included in the analysis because of the boundary problems incurred, i.e., these quadrat sizes could not be projected into the sampling area without consistently extending over its borders. The area in which the spatial distribution of diseased plants was assessed was contained within the area sampled for oospore distribution.

A Fortran program (3) was used to test the goodness-of-fit of data to seven probability distributions. Morisita's (5,6) index of dispersion and index of clump size was computed by using the procedure described by Schuh et al (10). The index of dispersion is calculated according to the formula

$$I_\delta = [(\Sigma(x^2) - \Sigma x)/((\Sigma x)^2 - \Sigma x)]n \quad (1)$$

where x is the number of systemically infected plants/oospores per gram of soil for each replicate, and n is the number of replicates. This was done for each quadrat size separately.

The following formula was used to compute the average clump size. If the smallest area (in square meters) is y, the index of clumping (IC) is computed

$$IC = (I_\delta \text{ for quadrat of area } y)/(I_\delta \text{ for quadrat of area } 2y) \quad (2)$$

and is plotted against area of $2y$. This procedure was repeated for each pair of quadrat sizes in the whole series. The quadrat size at which the plotted IC is at maximum determines the clump size.

RESULTS

The index of dispersion (Fig. 1) describing the spatial pattern of systemically infected plants had the largest index values at the smallest quadrat sizes. The maximum was at the 1-m^2 quadrat size with an index value of 1.91. The curve declined with larger quadrat sizes, reaching a minimum of 1.11 at the 64-m^2 quadrat size. Subsequently, the curve rose again to an index value of 1.14 at the 128-m^2 quadrat size. All index values constituted a statistically significant departure ($P = 0.01$) from a random pattern when tested according to Morisita (5) by comparison of F_0 with the value of $F^{(q-1)}(\alpha)$. F is computed through

$$F_0 = I_\delta(n-1) + q - N/q - 1, \quad (3)$$

where q = number of samples, N = number of oospores, and I_δ the index for a specific quadrat size.

The index of dispersion describing the spatial pattern of the oospores had a local maximum at the 1-m^2 and 2-m quadrat sizes ($I_\delta = 1.5$). It then declined to a local minimum at the 64-m^2 quadrat size ($I_\delta = 1.02$) and increased again to another local maximum at the 256-m^2 quadrat size ($I_\delta = 1.82$). The curve had a negative slope toward the largest quadrat size ($I_\delta = 1.45$). All index values except at the 32- and 64-m^2 quadrat sizes represented a statistically significant ($P = 0.01$) departure from random patterns.

The index of clump size (Fig. 2) for the systemically infected plants had a local maxima at the 2- and 8-m^2 quadrat sizes. The lowest index value was found at the largest quadrat size (128 m^2) at 0.97. The index of clump size for the oospore pattern had a first local maximum at the 4-m^2 quadrat size. After a series of small fluctuations, it had a local minimum at the 256-m^2 quadrat size (index value = 0.77), followed by another local maximum at the 512-m^2 quadrat size. The number of oospores per gram of soil as

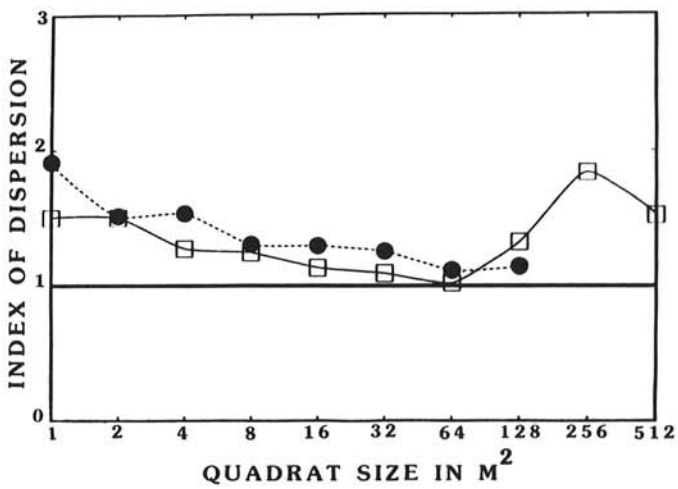

Fig. 1. Morisita's index of dispersion plotted against a binary series of quadrat sizes. Dotted line, systemically infected plants (sorghum downy mildew); solid line, oospores (*Peronosclerospora sorghi*).

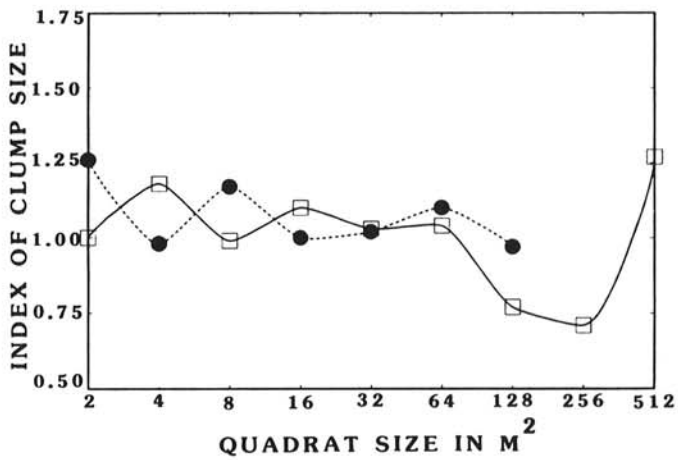

Fig. 2. Index of clump size plotted against a binary series of quadrat sizes. Dotted line, systemically infected plants (sorghum downy mildew); solid line, oospores (*Peronosclerospora sorghi*).

determined by soil sampling ranged from 0.7 to 56. The majority of the sample values was below 20 oospores per gram of soil (Table 1).

DISCUSSION

The shape of the index of dispersion curves (oospores and systemically infected plants) is typical for population patterns arising from point sources (4). The index values for the pattern of systemically infected plants are very similar to those described by Schuh et al (8). Differences may arise through the use of a combined data set as compared with two separate ones used in that study. Because both disease symptoms are caused by oospores produced in previous years, a combined data set more accurately represents the overall pattern of plants systemically infected by oospores.

When the shapes of the two curves are compared, index values for the spatial patterns of oospores are lower than those for the infected plants over the range of comparable quadrat sizes besides the 128-m^2 quadrat size. No method exists to test whether there is a statistically significant difference between index values. This is the major disadvantage of Morisita's index. In addition to questions concerning the use of soil samples as indicators of propagule patterns (7), several biological mechanisms could account for this difference. There is a high probability that the oospores collected through soil core sampling may in part, or entirely, consist of nonviable oospores. At present, there is no fast, reliable method for testing the viability and infectivity of oospores, and these oospores can remain in the soil for many years without showing visual signs of deterioration. Because the sampling procedure recovers oospore that were produced over several years during which spread has occurred, the pattern of oospore clumps could be expected to be more random than aggregated. This is substantiated by the observation that all soil samples contained at least one oospore, whereas in more than 50% of the quadrats in the original study for early and late season downy mildew assessment, no systemically infected plants were observed (8). Secondly, when the oospore density falls below a critical level, the probability of a susceptible roots being located within the competence zone of a viable oospore becomes small. This critical oospore density appears to be approximately 20 oospores per gram of soil when 1-yr-old oospores are used (9). The majority of mean oospore densities obtained through soil sampling were below 20 oospores per gram of soil. This, again, could cause the pattern of the oospores to appear less clumped as compared with that of infected plants; only oospore densities above that minimum would be recognized as such through plant reactions causing clumps of systemically infected plants to be smaller than oospore clumps and the disease-free area to be larger.

TABLE 1. Mean number of oospores per gram of soil (*Peronosclerospora sorghi*) for 10 quadrat sizes

Quadrat size[a]	Mean oospore number
1	22.0[b]
2	36.3[b]
4	28.6[b]
8	14.2[b]
16	5.4[c]
32	7.2[c]
64	5.2[c]
128	6.0[c]
256	6.0[c]
512	3.2[c]

[a] Quadrat size in square meters.
[b] Average of 10 replicates.
[c] Average of seven replicates.

Comparison of the indices of clump size between oospore and plant data sets is difficult because of problems associated with infectivity and the reduced probability of infection at low oospore densities. Oospore clumps below a certain minimum density would be recognized as clumps by the index for the oospore population, but not by the index for the plant population. Nevertheless, the general trend of the index curve is similar when comparing the range of index values. This similarity could be caused by the restricted dispersion of the pathogen, which would cause newly formed oospores to be deposited close to the location where the oospores were produced in the previous year. Mechanisms leading to this type of dispersal are described by Schuh et al (8).

The rise in index values at the 128-, 256-, and 512-m^2 quadrat sizes brings out a very important point in this type of study. Results obtained by sampling can depend on the size of the sampling area. Odvody, when assessing bulked samples taken from a sub-area of the same field, found mean values of 94 oospores per gram of soil (G. N. Odvody, *personal communication*). There is the strong possibility that the spatial patterns of the oospore population or of the plant population when tested at even larger quadrat sizes than in this study would produce different results. This has important implications for field screening trials where knowledge of the inoculum density and pattern is important for the evaluation of test results. More than one quadrat size must be assessed before inferences can be made.

The question as to which of two assessment methods (infected plants or oospores) should be used to obtain information about the inoculum density/disease potential of a field used for resistance screening, when the indication of inoculum presence is binomial can be answered. The distribution patterns were very similar in this study. Considering that in a favorable environment plant roots readily differentiate infective and noninfective propagules, and that assessment of plant spatial pattern using larger quadrat sizes is easier, the use of systemically infected plants seems the more logical and effective choice.

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