Analysis of Spatial Patterns in Sorghum Downy Mildew with Morisita's Index of Dispersion

W. Schuh, R. A. Frederiksen, and M. J. Jeger

Graduate research assistant, professor, and associate professor, respectively. Department of Plant Pathology & Microbiology, Texas Agricultural Experiment Station, College Station 77843.

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ABSTRACT

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The distribution and spatial pattern of sorghum plants systemically infected with *Peronosclerospora sorghi* were determined by using Morisita's index of dispersion. A contiguous area of 1,300 m^2 was assessed for disease incidence twice during the growing season at two locations. Each assessment was done by using eight quadrat sizes (binary series from 1 m^2 to 128 m^2). The spatial pattern of diseased plants was clumped at each date and location as determined by the use of Morisita's index. Several clump sizes, which were in a hierarchical order, were detected at each location. Comparison of clump sizes between assessment dates showed a reduction in average clump size which was attributed to premature plant death. The distribution of the data could not be fitted to known probability distributions with statistical significance.

Peronosclerospora sorghi (Weston & Uppal) C. G. Shaw (18) causes an important disease on sorghum (*Sorghum bicolor* (L.) Moench) throughout the world (4). *P. sorghi* causes two principal types of symptoms arising from systemic infection and local lesions. Systemic infection is caused by oospores or conidia, which infect the plant during the first 4 wk after seed germination. Local lesions are caused by conidial infection which may lead to systemic infection or remain restricted to the leaves (9). There are two variations on the expression of systemic infection (3,8). This variation depends on when the pathogen invades the apical meristem of the plant; if the fungus invades the growing point during the early differentiation phase, systemic infection is visible from the 3rd- to 4th-leaf stage. This symptom variation is termed early season downy mildew. Plants with this infection type have a high mortality rate. Invasion of the growing point at a later stage of differentiation causes systemic infection from the 6th- to 8th-leaf stage. Such plants produce sterile heads and normally survive for the duration of the growing season. This symptom variation is termed late season downy mildew.

In most areas of the world, the pathogen is spread by conidia, which also form one type of primary inoculum. Oospores are found in most geographic areas, but generally have only a minor role in the disease cycle and subsequent yield loss. In Texas, however, oospores are very important in the disease cycle and in causing yield loss (2). Oospores are found in the leaves 6–8 wk after systemic infection and constitute the primary inoculum for the next growing season. The role of conidia in the disease development under Texas conditions is unclear. Sorghum downy mildew is controlled mostly by using resistant cultivars. Since *P. sorghi* is an obligate parasite, the efficiency of resistance screening and fungicide trials in the field in Texas is dependent on natural inoculum levels. Thus, knowledge of inoculum density and spatial patterns is necessary to correctly interpret data from resistance screening trials (19).

Assessment of the pattern of soilborne pathogens by the use of soil samples has several disadvantages, mainly the amount of time, labor, etc. needed for sampling, isolating, and counting the propagules; and there is also the concern that sampling procedures are valid, i.e., to what extent do counts obtained from soil core samples truly represent the pattern of the pathogen populations. This question was discussed by Nicot et al (13). As a complementary method, the use of susceptible plants as indicators seems advantageous. The disease reaction of highly susceptible cultivars, planted in dense stands, should give information about the spatial pattern of the underlying pathogen population, or more closely the infectious part of it. Correlation of these spatial patterns with patterns obtained by analyzing soil samples could be used to determine the validity of either method.

Several authors (13,20,22) have stressed the need for spatial analysis that goes further than fitting of the data to probability distributions. The objective of the study reported here was to analyze spatial data obtained from plants with sorghum downy mildew in naturally infested fields. Evaluation of the spatial pattern of the pathogen populations will be reported in a future paper. Morisita's index of dispersion (I_δ) (11,12,17) and related statistics are used to describe the spatial patterns.

MATERIALS AND METHODS

Two fields, one a sandy loam soil (60% sand, 18% silt, and 22% clay, located at the Texas A&M Research Station in Beeville, TX) and the other a sandy clay loam soil (62% sand, 14% silt, and 24% clay, located about 34 km [21 miles] away at Skidmore, TX) with a documented history of sorghum downy mildew were surveyed in the spring and summer of 1984. A highly susceptible hybrid, Pioneer 8515, was planted at both locations. Early season downy mildew was assessed 18 April 1984 and 21 April 1984, while late season downy mildew was assessed 15 June 1984 and 29 June 1984 at Skidmore and Beeville, respectively. In both fields, a point was randomly chosen as a physical origin. Twenty-six rows to the right of the origin were marked at the same position to form a baseline. Starting at this baseline, the next 50 m in each row was subdivided into 1-m segments. In each segment, the number of plants showing systemic infection was recorded and percent infection was calculated. Thus, a total of 1,300 segments was assessed in each field for each observation date. Early season downy mildew and late season downy mildew readings were obtained for the same physical location. The row spacing was 96.5 cm giving an area of approximately 1 m^2 for each segment.

Disease incidence counts were combined into 10 frequency categories (Table 1). The Fortran program developed by Gates and Ethridge (5) was used to calculate the chi-square statistic for goodness of fit of the data to the Poisson, Poisson with zeros, Negative Binomial, Thomas Double Poisson, Neyman Type A, and Poisson-Binomial distributions (14).

Morisita's index of dispersion. To compute I_δ, the counts were arranged in a 50 by 26 entry matrix, and I_δ was computed according

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to the formula

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

in which x is disease incidence and n is the number of sampling units. A series of quadrats with successively doubled areas starting with 1 m^2 and ending at 128 m^2 was chosen. A total of 45 samples were taken for each quadrat size in each field/date combination by using a randomly chosen point on the matrix as the origin (lower left corner) of each quadrat. If a quadrat of any size, or part of it, was outside the physical range, it was deleted and a new origin was randomly chosen. This procedure was continued until 45 assessments were completed per quadrat size. For sizes where a square could not be formed due to the matrix structure (i.e., 2 m^2, 8 m^2, 32 m^2, 128 m^2), a rectangle was used (6,10). The longer side of the rectangle was randomly oriented to avoid bias. Values of I_δ correspond to three different interpretations of a distribution: $I_\delta < 1$ indicates a uniform, $I_\delta = 1$ a random, and $I_\delta > 1$ a clumped or "contagious" distribution.

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

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

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

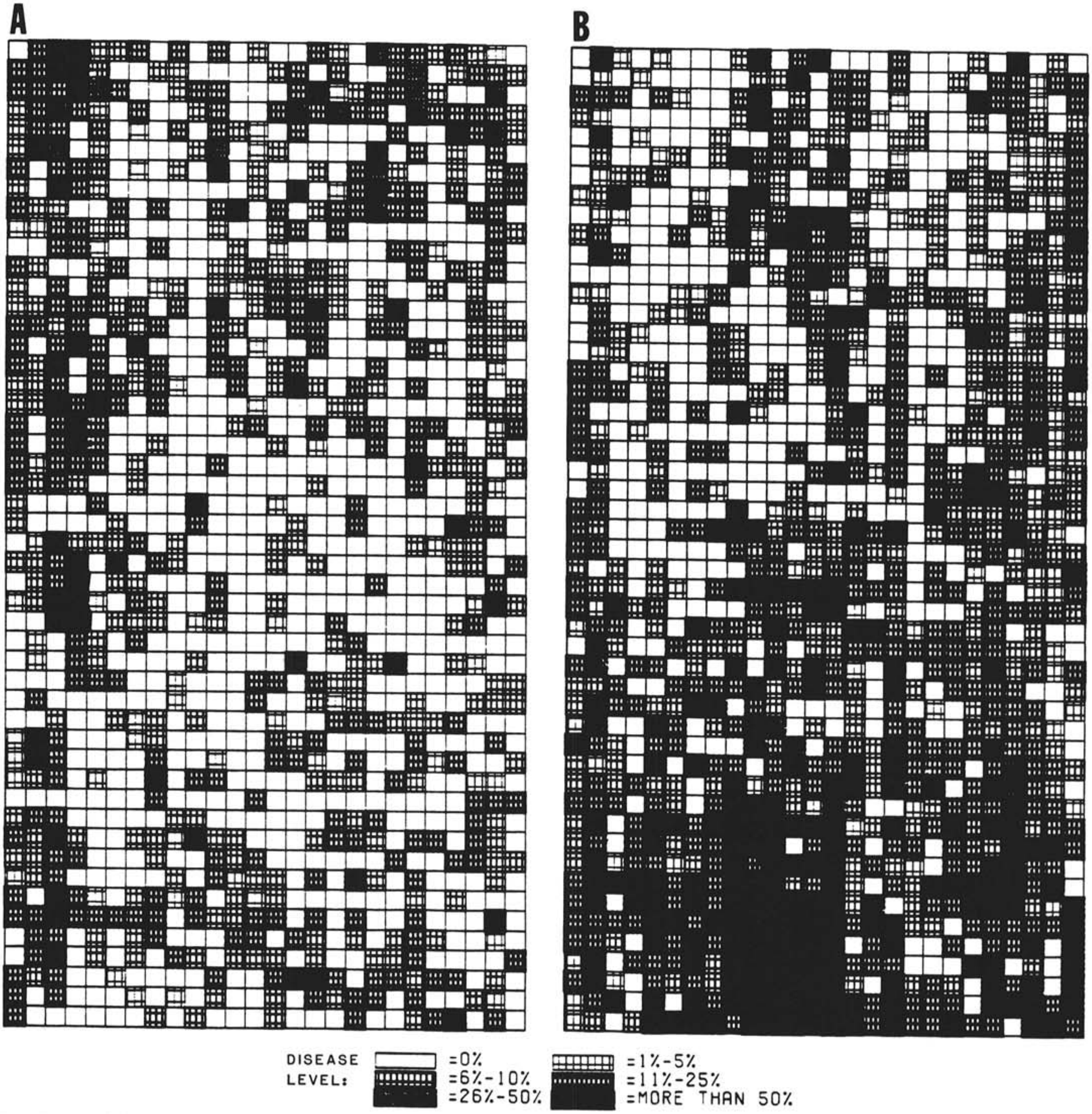

Fig. 1. Pattern of six classes of disease incidence for sorghum downy mildew (*Peronosclerospora sorghi*) in an area of 1,300 m^2 in Texas. **A,** Beeville location **B,** Skidmore location.

RESULTS

The average disease incidence for early and late season downy mildew assessment was 6.9 and 7.1% for the Beeville location and 14.5 and 15.7% for the Skidmore location, respectively. The actual pattern of early season downy mildew at Beeville for six levels of disease incidence is illustrated in Fig 1A. The average plant density for each 1 m segment of row was 13.4 plants with a standard error of ±0.11. Of the 1,300 assessed segments, 660 were free of disease. The actual distribution of early season downy mildew at Skidmore is illustrated in Fig. 1B. The number of disease-free segments was 398. The average number of plants was 16.1 plants per square meter with a standard error of ±0.12. The values for late season downy mildew at Skidmore and Beeville were 15.7 plants per square meter and 11.6 plants per square meter, with standards errors of ±0.08 and ±0.12, respectively The distribution of the disease incidences at each site are tabulated in Table 1.

The goodness of fit of each probability distribution was rejected ($P = 0.01$). When comparing the expected and observed frequencies for the 10 categories, however, a reasonable qualitative fit was given by the Neyman Type A distribution for Beeville and the Negative Binomial distribution and Neyman Type A for Skidmore data (Fig. 2A and B).

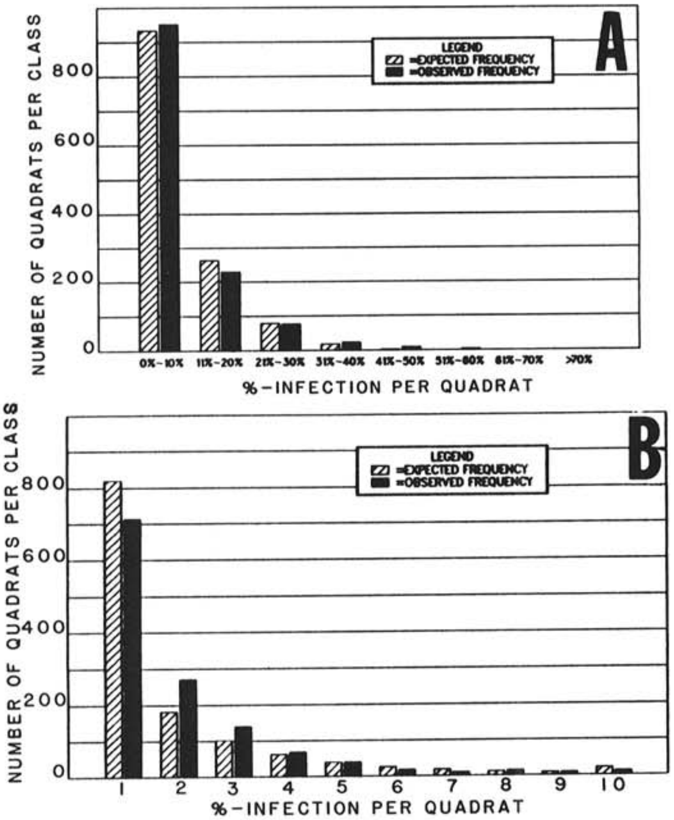

Fig. 2. Comparison of expected and observed frequencies of sorghum downy mildew incidence at two Texas locations. **A,** Neyman type A distribution, Beeville. **B,** Negative binomial distribution, Skidmore. Incidence classes 1,2,...9,10 = 0%–10%, 11%–20% ... 81%–90%, and 91%–100%, respectively.

***I_δ*.** For the early season downy mildew assessment at the Beeville location, I_δ was greatest (2.43) at the 1 m^2 quadrat size and decreased steadily with larger quadrat sizes. It reached a minimum of 1.1 at the 128 m^2 quadrat size. For late season downy mildew at Beeville, I_δ was also greatest (3.66) at the 1 m^2 quadrat size. It decreased to a minimum (1.18) at 64 m^2 and then increased slightly to 1.23 at the 128 m^2 quadrat size. When tested according to the formula given by Morisita (11), the distribution of the data departed from random expectation for all tested quadrat sizes at $P = 0.01$. The intraclump distribution was random, as indicated by comparing the shape of the curve with standard curves for different distributions (11).

The shape of the I_δ curve was similar for early season downy mildew and late season downy mildew at the Beeville location. It is typical for a pattern with small clumps, where the intraclump pattern is at random (11). In other words, the Beeville population (and the curve shape) were typical for populations arising from point sources. These point sources, or in this case, centers of high disease incidence, were surrounded by areas with lower disease levels. The further the distance to the point source, the lower the diseases incidences. Thus, small quadrats have larger I_δ values and larger quadrats smaller ones; this happens because calculations for large quadrats average areas with high and low incidence.

The shape of the I_δ curve for the Skidmore location differed from that of the Beeville location (Fig. 3B). Early season downy mildew at Skidmore had the largest value (2.43) at the 1 m^2 quadrat size. The curve slowly decreased until it reached a minimum of 1.16 at the 16 m^2 size. It had a secondary peak at the 32 m^2 size with 1.71 and then decreases again to an I_δ of 1.23 at the 128 m^2 size. Late season downy mildew had its global maximum (2.19) at the 1 m^2 quadrat size and a local maximum (1.59) at the 16 m^2 quadrat size; the minimum of 1.1 was observed at the 128 m^2 quadrat size. All I_δ values are significantly different from random expectations at the 1% level. In general, I_δ values for the Skidmore location were higher than the values for Beeville, for the same quadrat size, with the exception of the 1 m^2 area. The intraclump distribution was interpreted as partly random or uniform when compared with the standard curves (11). The I_δ curve for early season downy mildew at Skidmore had two distinct phases. Up to the 16 m^2 quadrat size, the curve could be interpreted as a population arising from point sources with random intraclump dispersion. From the 16 m^2 to the 128 m^2 quadrat size, the curve could be interpreted as a population with large clumps, where the intraclump dispersion is uniform. A population with less pronounced point sources organized in clumps rather than having a tendency towards random arrangement (as in the Beeville location) could exhibit such bimodal behavior. When translated to the left one quadrat size, the curve for late season downy mildew was similar to that for early season downy mildew.

***IC*.** According to Morisita (11), a peak of the plotted *IC* occurs when quadrat size and clump size are equal. At the Beeville location (Fig. 4A), the curve for early season downy mildew was unimodal and had a maximum at the 16 m^2 quadrat size, the curve for late season downy mildew was bimodal with peaks at the 2 m^2 and the 64 m^2 quadrat sizes. The early season downy mildew curve had a positive slope at the larger quadrat sizes. The peak on the *IC* curve for early season downy mildew indicated an average clump size of 16 m^2. The positive slope of the curve at the two largest quadrat

TABLE 1. Number of quadrats in the sample ($n = 1,300$), belonging to 10 classes of disease incidence for sorghum downy mildew from two locations planted to Pioneer 8515 hybrid sorghum in Texas

Location	Symptom type	Incidence classes 0–10%	11–20%	21–30%	31–40%	41–50%	51–60%	61–70%	71–80%	81–90%	91–100%
Beeville	ESDM[a]	953	231	77	23	10	4	1	0	0	1
	LSDM	963	194	84	34	21	3	0	0	0	1
Skidmore	ESDM	714	271	141	68	42	18	10	15	9	13
	LSDM	641	320	161	61	47	25	15	19	9	2

[a] ESDM = early season downy mildew, and LSDM = late season downy mildew.

sizes raised the possibility of another *IC* peak at quadrat sizes larger than the ones tested. The *IC* curve for late season downy mildew had two peaks, indicating an average clump size of 2 m^2 These clumps again were organized in clumps of 64 m^2 At the Skidmore location (Fig. 4b), the index of clump size curve for the early season downy mildew had peaks at the 4 and 16 m^2 quadrat sizes. The maxima for late season downy mildew were at the 2, 8, and 32 m^2 quadrat sizes. Both curves had positive slopes at the two largest quadrat sizes. The smallest clump size for early season downy mildew was thus 4 m^2. These clumps were organized in clumps of 16 m^2. The response at the largest quadrat size indicated the possibility of another peak outside the tested range. Thus, compared to the Beeville location, an additional clump was present which could explain the bimodal behavior of the I_δ curve for early season downy mildew. The *IC*-curve for late season downy mildew showed three clump sizes, which were organized in a strong hierarchical fashion.

DISCUSSION

A reduction in the number of infected plants can be detected with time at both locations, although the consequences of the reduction differ due to different disease incidence levels and distributional patterns in the two fields. This reduction in incidence explains the higher I_δ values for late season downy mildew for the small quadrat sizes at the Beeville location. Plants infected very early in their development have a high mortality rate. This premature plant death would show different effects dependent on the characteristic size of the clumps.

Small clumps, consisting of one to three systemically infected plants, have a high probability of mortality whereas large clumps (10–20 systemically infected plants) tend to decrease in disease incidence rather than in clump size. This would cause the pattern of sorghum downy mildew at the Beeville location to become more polarized, i.e., to have more quadrats with no systemically infected plants and fewer quadrats with low disease incidences. A quantitative description of this process is outlined by Ripley (16). Further evidence of this reduction effect is given by comparing the *IC* curves for early season downy mildew and late season downy mildew. The change in average clump size can be interpreted as the result of a reduction process, with large clumps (16 m^2) being reduced to small clumps (2 m^2), and the suspected clumps outside the tested range for early season downy mildew falling into the tested range, manifested at a lower quadrat size (64 m^2). Another indication of the reduction effect is the decrease of the average number of plants per 1 m^2 from 13.4 to 11.7 and the increase of disease free-segments from 660 to 731.

A similar, though less drastic, effect can be seen for the Skidmore location. The change in average clump size can be explained (as in Beeville) by the premature death of systemically infected plants, i.e., a 4-m^2 clump was reduced to a 2-m^2 clump, the 16-m^2 clump to an 8-m^2 clump, and so on. Further evidence of this is the reduction, although slight, of average plant number per square meter from 16.1 to 15.7. Clearly, the thinning process is less drastic at Skidmore as can be seen by the lesser reduction in clump size (from a 16-m^2 clump to an 8-m^2 clump at the Skidmore location compared to 16 m^2 clumps to 2 m^2 at the Beeville location), and the relatively stable I_δ values. As mentioned before, this could be related to the larger clump sizes at the Skidmore location. Systemically infected plants are at a competitive disadvantage compared to healthy neighbors for water, light, etc because they are stunted and, due to chlorosis, possess a reduced leaf area for photosynthesis. The probability of a neighboring plant being healthy is higher in small clumps than in

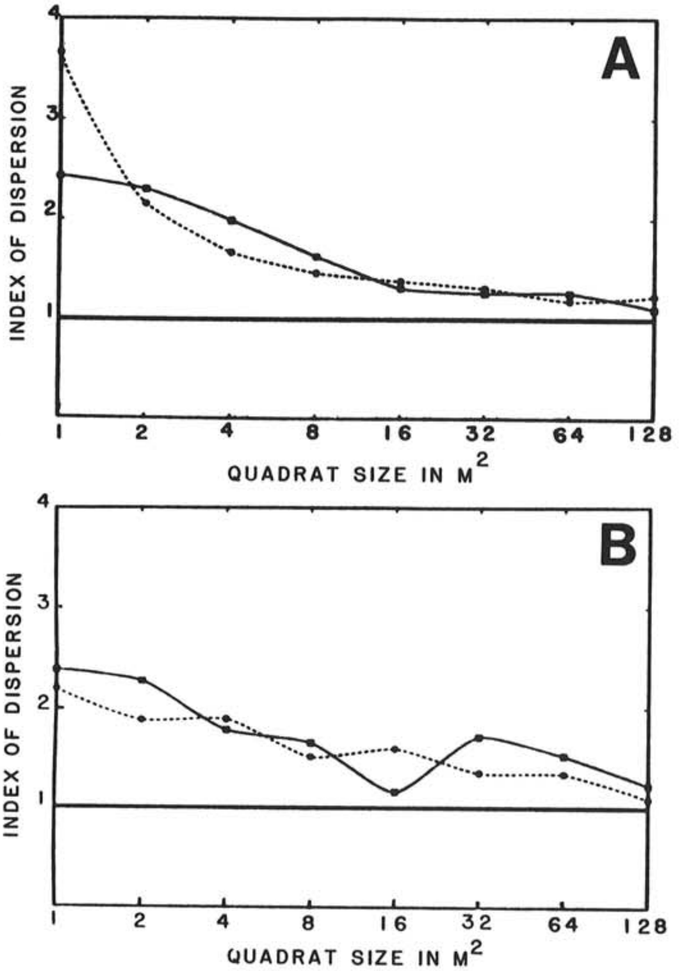

Fig. 3. Morisita's index of dispersion plotted against a binary series of quadrat sizes used to analyze the incidence of sorghum downy mildew at two Texas locations. Solid line, early season downy mildew; dotted line, late season downy mildew. **A,** Beeville and **B,** Skidmore. Value of 1 indicates a random pattern.

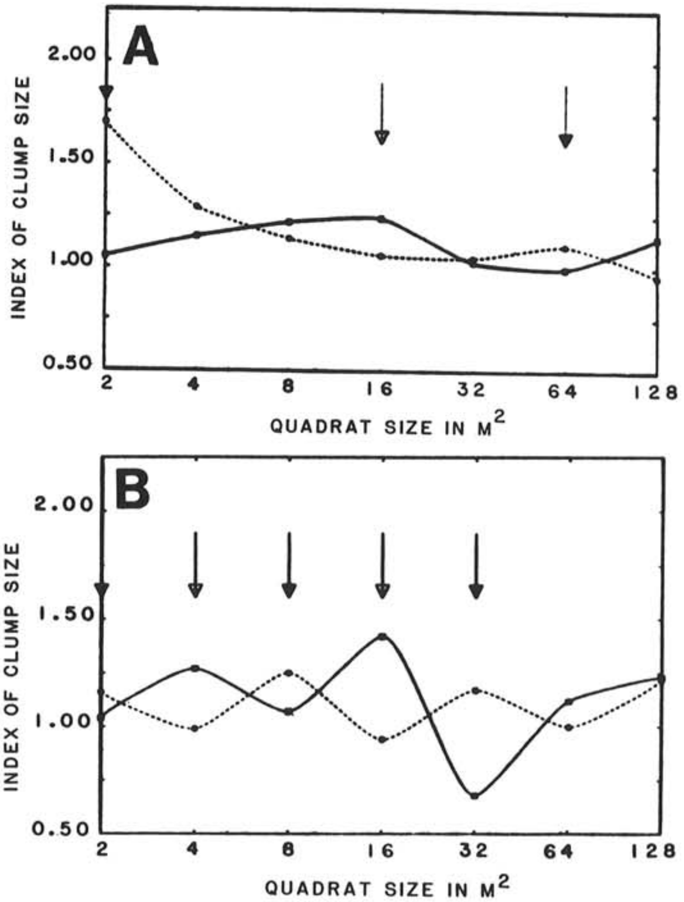

Fig. 4. Index of clump size in sorghum downy mildew (caused by *Peronosclerospora sorghi*) at two Texas locations plotted against a binary series of quadrat sizes. Solid line, early season downy mildew; dotted line, late season downy mildew. **A,** Beeville and **B,** Skidmore.

large clumps for a randomly selected infected plant in the clump. Therefore, infected plants would be subjected to a higher stress in smaller clumps as compared to larger ones, which would cause a higher rate of plant death in small clumps, consequently a greater reduction effect. A decrease of quadrats with no infection from 398 to 276 at the Skidmore location seems to contradict the evidence for a thinning process; but when comparing the number of quadrats with less than 10% infection, we observed an increase from 316 to 365 quadrats. Thus, we have a thinning effect, on the whole, which is counteracted by an increase in quadrats with very low infection levels (possibly due to conidial spread).

The Neyman Type A and Negative Binomial distributions gave good qualitative descriptions of the observed data at the Beeville and Skidmore locations, respectively, even though not significant at $P = 0.01$. The Neyman Type A distribution has often been used to describe the dispersion of insect larvae hatched from randomly distributed clumps of eggs (1,15) when spread is limited by the maximum distance the larvae can crawl. Some analogies could be drawn here for the Beeville location. As shown by the I_δ curve, sorghum downy mildew arises from point sources or clumps. The spread of sorghum downy mildew is limited due to oospores falling to the ground more or less vertically due to their release as clumps of spores still attached to leaf debris; secondly, plants are plowed under (in this situation the disease nursery) with a proportion of the total oospores still within the leaf tissue. Thus, the spread is limited to a certain arbitrary distance. For the Skidmore location, the Negative Binomial distribution seemed to fit the data better. Again, the lack of fit can be partially explained by the nonrandom distribution of the clumps. This can also be seen when interpreting the IC data; the clumps are organized in several hierachical levels and the curve has a positive slope at the largest quadrat sizes. Whether conidia play an important role in disease spread cannot, as yet, be answered definitively. The shrinking clump sizes at both locations does suggest that conidia did not significantly contribute to the epidemiology of sorghum downy mildew. This is confirmed by the increase in the number of disease-free quadrats at Beeville. In Skidmore, on the other hand, there was an increase in the number of quadrats with infected plants, mainly low incidence levels. This increase could be caused by conidia. The lesser spread at the Beeville location, assuming both fields were exposed to similar environmental conditions, can be explained by the lower disease incidences and therefore a smaller quantity of conidia produced.

Methods for assessing spatial pattern can be divided into three categories: methods of autocorrelation (22,23), nearest-neighbor methods (15), and indices of dispersion. Comparison of autocorrelation methods with Morisita's index has been discussed in detail (15). A disadvantage of the autocorrelation method is the demand for a contiguous sampling area. The number of samples multiplied by area represented by each sample results in the total sampling area. Accordingly, as any single sample can only represent an area of a certain size with validity, the total sampling area is limited.

The third category of methods includes various indices of dispersion; statistical properties of which have been described by Pielou (15) and Goodall and West (7). The independence of the index I_δ from the sample mean has been disputed by Taylor (24). Additionally, Taylor (24) questioned the value of this property. Morisita's index has several advantages over other indices. By choosing a series of quadrat sizes, the index avoids the problems associated with obtaining data from one quadrat size (effect of quadrat size on index value) (15), and permits the description of "intensity and grain." These quadrats can be placed randomly in the field, so that an area considerably larger than that of the combined sample areas can be assessed. The shape of the curve also gives information about the intra-clump distribution. The determination of the average clump size and their heirarchical structure is another useful property. Even though a direct interpretation of the average clump size is hard to realize in the field, the values can nonetheless be useful in detecting population increase with time, or comparing patterns between or among fields. The use of plant reactions to determine propagule distribution seems advantageous for several reasons. Roots of susceptible plants sample a volume of soil much more accurately and thoroughly than do soil core samples. Roots also discriminate between infectious and noninfectious propagules and, since the control of disease is a major objective in plant pathology, this ability is very useful for obligate parasites (such as *P. sorghi*) where the distribution of infectious propagules is otherwise confounded with the distribution of noninfectious propagules. Furthermore, the shorter period of time required for assessment and evaluation of disease incidence enables the survey of larger areas.

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