

Frequency Distribution Analyses of Lettuce Drop Caused by *Sclerotinia minor*

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ABSTRACT

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The location of lettuce plants with symptoms of lettuce drop, which is caused by *Sclerotinia minor*, was mapped in six lettuce fields 2-3 wk before harvest. Disease incidence ranged from 2.00 to 9.16%. Frequency distribution analyses were performed with different sizes and numbers of quadrats. Eight different frequency distributions were analyzed for goodness of fit to the data by the chi square (χ^2) goodness-of-fit test. Quadrat size and number of quadrats influenced the type of distribution model fit and the goodness of fit of the frequency distributions. In only one instance was the Poisson distribution fit ($\chi^2 P = 0.88$)—when Field 1 was

sampled with a quadrat size of 0.9 by 0.9 m. In all other cases, the χ^2 probability for the Poisson distribution was less than 0.01. Of the distributions tested, the negative binomial distribution was fit most often. Lloyd's index of mean patchiness ranged from 1.10 to 3.62, indicating various degrees of aggregation. Lloyd's index of mean patchiness is a better index to compare the degree of aggregation because it does not require a goodness-of-fit test, was not affected by the mean in this study, and was not affected significantly ($P \leq 0.05$) by quadrat size. The standard-runs test also indicated that the disease was not random.

Frequency distribution analysis is used by plant pathologists to describe spatial patterns of soilborne plant pathogens (6, 11, 16) and diseased plants (15, 20). By determination of the actual frequency distribution, more accurate sampling strategies and improved use of control measures may be possible (1, 22). However, the increased accuracy may be ineffective due to the overriding influence of other variables such as microclimate that are not described as well (6).

Frequency distribution analysis requires a specified unit area (a quadrat) where the density (number of individuals per unit area) is determined for each quadrat. Three major factors affect these studies: size of quadrat, size of individuals studied, and spatial pattern of individuals. Of these, quadrat size is the only factor that can be controlled by the investigator.

Quadrat size is best delineated by the species studied. For example, a wheat leaf may be the appropriate quadrat for determining the density and distribution of rust lesions. In this type of disease the pathogenic species is confined to discrete habitat quadrats. In contrast, crops and soilborne plant pathogens occupy undefined quadrats, except for the limits of the field. In these situations, quadrat dimensions are chosen intuitively or logistically. Intuitively, one would not sample a field of lettuce plants with 1 cm² quadrats to determine disease incidence, although that may be appropriate for sampling a soilborne pathogen's population. Logistics also play an important role and are affected by the mean density of the population under study. Sampling and analysis of large quadrats with hundreds of individuals may be much more difficult and time consuming than smaller quadrats with fewer individuals.

The objectives of this research were to determine the distribution of lettuce drop, to measure the effect that quadrat size has on frequency distribution analysis, and to explore other possible analytical techniques which may be more robust (not affected by slight changes in parameters) sampling systems.

MATERIALS AND METHODS

Maps of the location of all lettuce (*Lactuca sativa* L.) plants that exhibited symptoms of lettuce drop, which is caused by *Sclerotinia*

minor Jagger, were made for six fields in New Jersey 2-3 wk before harvest. All of the fields contained Romaine lettuce planted in four-row beds and spaced at 0.3 m within and between rows. The number of beds and length of beds varied for each field (Fig. 1).

Computer programs were developed that analyzed each entire field with four quadrat sizes, 0.3 × 3.0 m, 0.6 × 3.0 m, 0.9 × 3.0 m, or 1.2 × 3.0 m. The quadrat dimensions were chosen so that they contained one, two, three, or four rows of 10 plants each. Quadrat dimensions were also developed so that the mean number of diseased plants per quadrat was about 0.5 or 2.0. This allowed comparison of quadrat shapes and sizes with similar mean densities of diseased plants.

The Poisson, negative binomial, positive binomial, Thomas double Poisson, Neyman Type A, Poisson binomial, Poisson-with-zeros, and logarithmic-with-zeros distributions were analyzed for goodness of fit to the frequency data by the chi square (χ^2) test with a Fortran program developed by Gates and Ethridge (2). These distributions have been used to analyze several types of biotic and abiotic data sets, and represent a broad range of possible frequency data. If the χ^2 probability was greater than 0.05, the tested distribution was not rejected.

To compare the χ^2 values, they were standardized by removal of the effects of number of quadrats and degrees of freedom (equation 1). The value of D is a sample estimate of the true distance between the population proportions and those proportions predicted by some proposed probability model, based on the noncentral χ^2 distribution (17). The estimate of D is

$$D = \chi^2/n - df/n \quad (1)$$

in which n = number of quadrats and df = degrees of freedom with the standard error of D being

$$2\sqrt{(D/n)}. \quad (2)$$

Because the sample sizes (n , number of quadrats) were unequal, due to the size of the quadrat and size of the field, the GT2 method described by Sokal and Rohlf (19) was used to compare the estimated values of D .

The frequency data were also analyzed by using Lloyd's (9) formulas for indices of mean crowding and patchiness:

$$*m = 1/n \sum_{i=0}^n X_i$$

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and

$$P = \bar{m}/m.$$

in which \bar{m} = mean crowding, n = total number of individuals, X_i = number of co-occupants in i th unit, P = mean patchiness, and m = the sample mean.

Mean patchiness, P , can be derived from the equation

$$P = [m + (v/m - 1)]/m$$

in which m = the sample mean and v = the sample variance (14).

To determine the effect of sample size on Lloyd's index of patchiness, each field was randomly sampled 10 times with 75 quadrats each, using quadrat sizes of 0.3×3.0 m, 0.6×3.0 m, 0.9×3.0 m, 1.2×3.0 m, or 1.5×3.0 m. A mixed model analysis of variance was used to account for the replications within each field.

RESULTS

Symptoms of lettuce drop occurred on 6.22% of the 5,940 plants in Field 1 (Fig. 1). All of the distributions tested had a χ^2 probability greater than 0.05 at least once, depending upon the size of the quadrat (Table 1). The Poisson or logarithmic-with-zeros

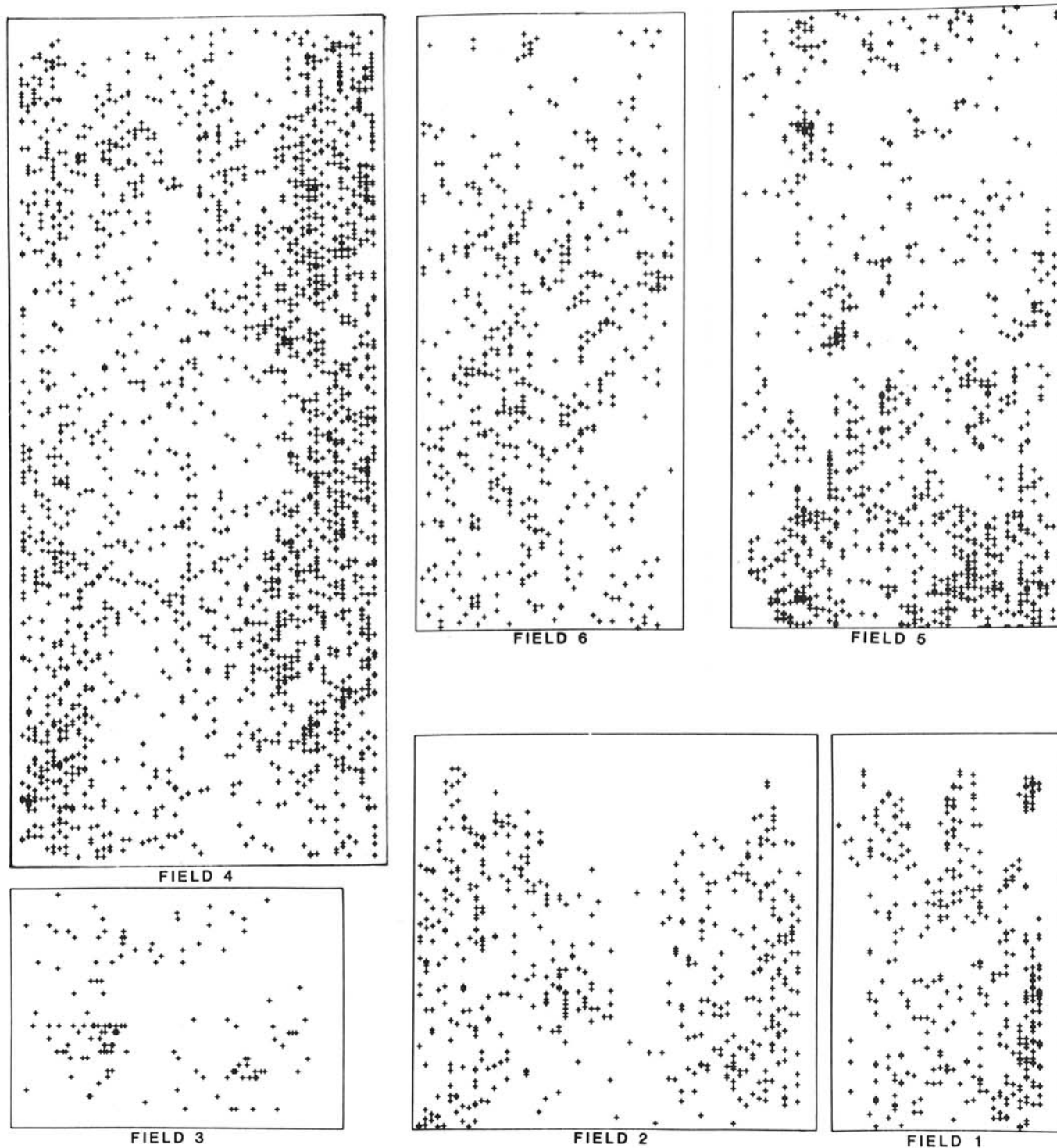


Fig. 1. Map of location of lettuce plants with symptoms of lettuce drop in six fields. Scale is 350 to 1 on the horizontal and 1,000 to 1 on the vertical.

distributions did not fit the frequency data when quadrat dimensions were 0.3 × 3.0 m, 0.6 × 3.0 m, 0.9 × 3.0 m, or 2.1 × 1.8 m. When quadrat size was 0.9 × 0.9 m, none of the distributions tested were rejected (Figs. 2 and 3). This was the only case in the entire study for which the Poisson distribution was not rejected. When quadrat dimensions were 1.2 × 3.0 m, the Poisson, positive binomial, Poisson-with-zeros, and logarithmic-with-zeros distributions were not fit.

After the χ^2 goodness-of-fit values were standardized according to equation 1, it was possible to compare the goodness of fit ($P = 0.05$) of the data to the different distributions. When the quadrat dimensions were 0.3 × 3.0 m, the χ^2 value for the Poisson distribution was different from the other distributions (Table 2). When the quadrat dimensions were 0.6 × 3.0, the χ^2 value from the positive binomial was different from the others. When the quadrat dimensions were 0.9 × 3.0 m, both the Poisson distribution and the logarithmic-with-zeros, χ^2 values were different from the other values but not different from each other. When quadrat dimensions were 1.2 × 3.0 m, the χ^2 value for the Poisson distribution was different from all other values; the χ^2 values from the logarithmic-with-zeros, Poisson-with-zeros, Poisson binomial, and positive binomial distributions were different from the other values but not different among themselves. When quadrat dimensions of 0.9 × 0.9 m were used, all of the values were the same. When a quadrat dimension of 2.1 × 1.8 m was used, the positive binomial and

Neyman Type A χ^2 values were different from the other values. Similar varying results were obtained from the other fields.

Of the 12,100 lettuce plants in Field 2, 4.08% had symptoms of lettuce drop (Fig. 1). All of the distributions but the Poisson were fit with a χ^2 probability greater than 0.05, depending upon the size of the quadrat. At the same time, each distribution was rejected ($P = 0.05$) at least once with particular quadrat sizes. When quadrat size was 0.3 × 3.0 m or 0.6 × 3.0 m, the Poisson, positive binomial, Poisson-with-zeros, and logarithmic-with-zeros distributions were not fit at the probability level of 0.05. If the quadrat size was 0.9 × 3.0 m, the Poisson, negative binomial, positive binomial, Poisson-with-zeros, and logarithmic-with-zeros had χ^2 probability values less than 0.05. With quadrat size of 1.2 × 1.2 m the Poisson, Thomas double Poisson, and logarithmic-with-zeros distributions were not fit. When the quadrat size was 2.1 × 2.4 m, only the Thomas double Poisson distribution was fit at greater than 0.05.

In Field 3, 2.00% of the 6,552 lettuce plants had symptoms of lettuce drop. The Poisson, positive binomial, and Poisson-with-zeros distributions did not have a χ^2 probability value greater than 0.05, regardless of the quadrat size used. The negative binomial and the logarithmic-with-zeros distributions did not have a probability value less than 0.05, regardless of the quadrat size.

Symptoms occurred on 9.16% of the 23,360 lettuce plants in Field 4. The Poisson, positive binomial, and Poisson-with-zeros distributions gave χ^2 probability values less than 0.05 for all of the

TABLE 1. Goodness of fit as determined by chi square probability for frequency distributions of lettuce drop in six fields utilizing different quadrat sizes

Field	Quadrat size	Model ^a								
		Poisson	NB	PB	TDP	NTA	PoB	PwZ	LwZ	P
1	0.3 × 3.0 m	0.01	0.62	0.61	0.66	0.88	0.90	0.61	0.02	1.55
	0.6 × 3.0 m	0.01	0.78	0.10	0.91	0.97	0.79	0.10	0.01	1.65
	0.9 × 3.0 m	0.01	0.19	0.69	0.35	0.48	0.56	0.69	0.01	1.29
	1.2 × 3.0 m	0.01	0.40	0.01	0.49	0.55	0.17	0.01	0.01	1.62
	0.9 × 0.9 m	0.88	0.46	0.43	0.46	0.37	0.46	0.43	0.14	1.26
	2.1 × 1.8 m	0.01	0.18	0.27	0.27	0.35	0.34	0.27	0.01	1.10
2	0.3 × 3.0 m	0.01	0.83	0.05	0.88	0.67	0.43	0.05	0.05	1.73
	0.6 × 3.0 m	0.01	0.49	0.04	0.51	0.83	0.66	0.04	0.01	1.62
	0.9 × 3.0 m	0.01	0.02	0.01	0.75	0.10	0.06	0.01	0.01	1.55
	1.2 × 3.0 m	0.01	0.06	0.01	0.07	0.12	0.01	0.01	0.01	1.55
	1.2 × 1.2 m	0.01	0.19	0.55	0.05	0.83	0.77	0.55	0.01	1.76
	2.1 × 2.4 m	0.01	0.03	0.01	0.69	0.05	0.01	0.01	0.01	1.59
3	0.3 × 3.0 m	0.01	0.06	0.01	0.01	0.01	0.01	0.01	0.01	3.62
	0.6 × 3.0 m	0.01	0.76	0.01	0.01	0.11	0.01	0.01	0.98	3.14
	0.9 × 3.0 m	0.01	0.06	0.01	0.01	0.01	0.01	0.01	0.41	3.19
	1.2 × 3.0 m	0.01	0.33	0.01	0.01	0.16	0.01	0.01	0.65	2.29
	1.5 × 1.5 m	0.01	0.91	0.03	0.14	0.25	0.06	0.04	0.96	2.41
	0.3 × 3.3 m	0.01	0.46	0.01	0.18	0.40	0.09	0.01	0.22	1.53
4	0.3 × 3.0 m	0.01	0.71	0.01	0.57	0.65	0.53	0.01	0.01	1.35
	0.6 × 3.0 m	0.01	0.85	0.01	0.50	0.76	0.06	0.04	0.96	1.39
	0.9 × 3.0 m	0.01	0.93	0.01	0.01	0.07	0.09	0.01	0.22	1.46
	1.2 × 3.0 m	0.01	0.43	0.01	0.25	0.45	0.29	0.01	0.01	1.37
	0.6 × 0.6 m	0.01	0.53	0.01	0.01	0.10	0.08	0.01	0.01	1.38
	1.2 × 1.2 m	0.01	0.47	0.01	0.21	0.34	0.21	0.01	0.11	1.42
5	0.3 × 3.0 m	0.01	0.99	0.01	0.01	0.14	0.01	0.01	0.01	2.14
	0.6 × 3.0 m	0.01	0.68	0.01	0.01	0.04	0.01	0.01	0.01	2.06
	0.9 × 3.0 m	0.01	0.73	0.01	0.01	0.02	0.01	0.01	0.21	1.91
	1.2 × 3.0 m	0.01	0.68	0.01	0.01	0.01	0.01	0.01	0.01	1.96
	0.9 × 0.9 m	0.01	0.45	0.05	0.65	0.85	0.47	0.05	0.01	1.97
	1.8 × 1.8 m	0.01	0.72	0.01	0.01	0.01	0.01	0.01	0.13	1.84
6	0.3 × 3.0 m	0.01	0.19	0.08	0.17	0.18	0.17	0.08	0.08	1.45
	0.6 × 3.0 m	0.01	0.49	0.01	0.05	0.16	0.03	0.01	0.21	1.58
	0.9 × 3.0 m	0.01	0.91	0.01	0.40	0.57	0.31	0.01	0.01	1.42
	1.2 × 3.0 m	0.01	0.19	0.01	0.09	0.11	0.06	0.01	0.01	1.44
	0.9 × 1.2 m	0.01	0.58	0.01	0.24	0.33	0.20	0.01	0.13	1.60
	2.1 × 2.7 m	0.01	0.92	0.01	0.77	0.86	0.68	0.01	0.01	1.34

^a NB = negative binomial; PB = positive binomial; TDP = Thomas' double Poisson; NTA = Neyman type A; PoB = Poisson binomial; PwZ = Poisson with zeros; LwZ = logarithmic with zeros; P = Lloyd's index of mean patchiness.

quadrat sizes, whereas the negative binomial, Neyman Type A, and Poisson binomial distributions resulted in probability values greater than 0.05.

In Field 5, 5.91% of the 15,612 lettuce plants were affected by lettuce drop (Fig 1). The Poisson, positive binomial, and Poisson-with-zeros distributions did not have a χ^2 probability value greater than 0.05 with any of the quadrat sizes. The negative binomial did not have a probability value less than 0.05. The other distributions varied by quadrat size.

Field 6 had 11,608 plants, of which 4.69% showed symptoms of lettuce drop. As in Field 5, the Poisson, positive binomial, and Poisson-with-zeros distributions had probability values less than 0.05, regardless of the quadrat size. The negative binomial and Neyman Type A distributions had probability values greater than 0.05 in all of the quadrat sizes.

Overall, the negative binomial distribution was fit (χ^2 probability greater than 0.05) more often than any other, in 34 of 36 cases, followed by the Neyman Type A, in 30 of 36 cases (Table 1). The Poisson distribution was fit only one time, and the Poisson-with-zeros distribution only seven times.

Lloyd's index of mean patchiness is not affected by the mean (14), and a nonsignificant linear correlation occurred between it and the mean, $r = -0.43$, $P > 0.10$. When a mixed model analysis of variance was calculated from 75 quadrats of each of five sizes randomly taken 10 times in each field, there was insufficient evidence to conclude that quadrat size had an effect on Lloyd's index of mean patchiness ($P > 0.05$); however, the field effect on the index was significant ($P < 0.01$).

An ordinary-runs test (3) was conducted over each of the entire fields, sampling with the rows. The value Z of the ordinary-runs test

is a large negative number (less than -1.64) if there is clustering of diseased plants in the field ($P \leq 0.05$) (10). All of the fields had clustered populations as determined by the ordinary-runs test. The value of Z was -5.49 , -5.56 , -5.15 , -10.21 , -13.12 , and -3.27 for Fields 1, 2, 3, 4, 5, and 6, respectively.

DISCUSSION

Quadrat size affected the type of distribution best fit and the goodness of fit of the frequency distributions. This has been reported previously, from a nonhelpful approach by Shimwell (18) who merely stated, "quadrat size is one obvious factor affecting frequency figures and is one which needs no further elaboration except to emphasize that quadrat size used should be stated," to a mathematical approach in which Peilou (13) developed a procedure from artificial data by using a series of quadrat sizes to calculate the regression of log of percentage absence on density. Unfortunately, both approaches are not very applicable to the problem of determining the degree of aggregation or the level of disease severity in an agricultural field. The importance of considering frequency data is obvious, for we found that only data for one quadrat size in only one field fit the Poisson distribution.

The runs test was used by Madden et al (10) to determine the possibility of plant-to-plant spread. They stated that if the runs test value Z was less than -1.64 , clustering of diseased plants would be indicated. In all of the fields in this study, Z was less than -1.64 . The runs test indicated that diseased plants were adjacent to each other more often than can be explained by randomness. Madden et al (10) concluded that this would indicate plant-to-plant spread in an

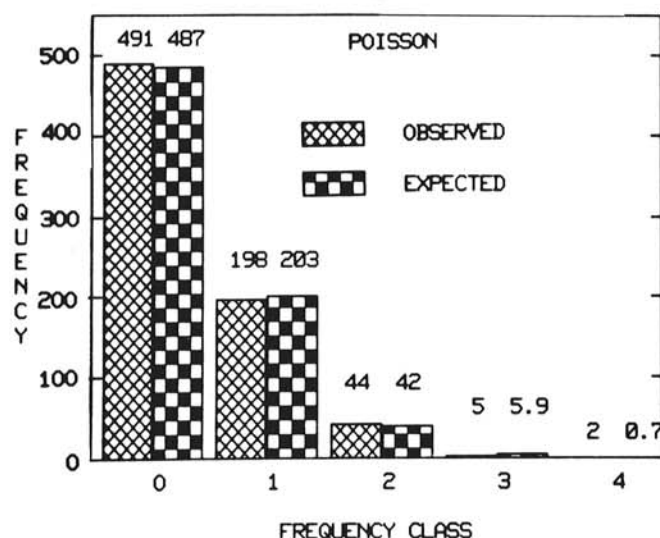


Fig. 2. Frequency data from Field 1 when sampled with a quadrat size of 0.9×0.9 m. The data were fit by the Poisson distribution (χ^2 probability 0.88).

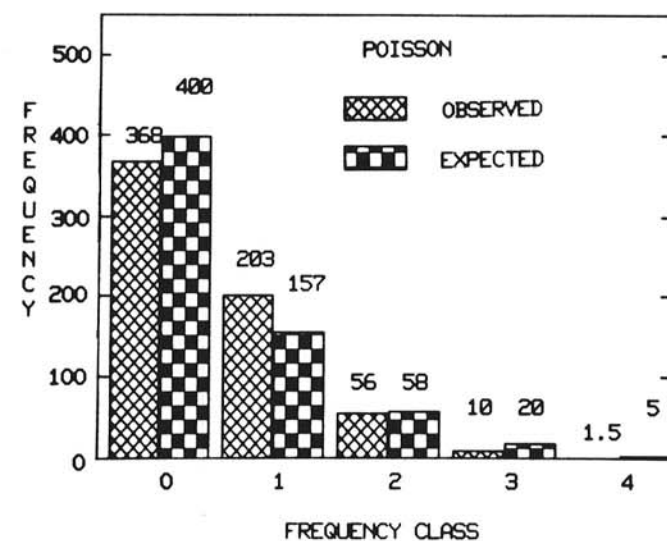


Fig. 3. Frequency data from Field 1 when sampled with a quadrat size of 0.3×3.0 m. The data were not fit by the Poisson distribution (χ^2 probability 0.01).

TABLE 2. Standard error (SE) of distance estimate (D) to standardize chi square values of Field 1

Quadrat size	Model ^a															
	Poisson		NB		PB		TDP		NTA		PoB		PwZ		LwZ	
	D ^b	SE ^c	D	SE	D	SE	D	SE	D	SE	D	SE	D	SE	D	SE
0.3 × 3.0 m	847	380	-5	3	-2	2	-5	29	-9	40	-26	67	425	85	91	120
0.6 × 3.0 m	522	421	-81	166	3,758	1,131	575	442	553	434	291	314	120	202	410	373
0.9 × 3.0 m	1,147	790	107	241	-51	167	21	107	-26	119	-115	250	-88	219	1,549	918
1.2 × 3.0 m	5,765	1,980	28	139	218	385	-37	160	-73	223	881	774	1,320	947	2,073	1,188
0.9 × 0.9 m	-23	59	-6	31	-6	30	-62	30	-12	42	3	20	-13	44	26	62
2.1 × 1.8 m	1,060	79	124	270	30	132	63	192	25	122	81	218	64	194	1,618	975

^a NB = Negative binomial; PB = positive binomial; TDP = Thomas' double poisson; NTA = Neyman type A; PoB = Poisson binomial; PwZ = Poisson with zeros; and LwZ = logarithmic with zeros.

^b $D = (\chi^2/n - df/n)1,000$.

^c $SE = 2\sqrt{(D/n)1,000}$.

environmentally homogeneous field. However, with lettuce drop a large number of sclerotia are produced on the diseased plant which, after disturbance by field preparation for the next season, may infect a number of plants. Further investigations of the biology of the disease will be required to determine if increase is due to plant-to-plant spread within a season or redistribution of sclerotia between seasons.

For effective integrated pest management systems, the level of disease severity must be known. This may be the weakest link in such a program (20). Goodell and Ferris (4,5) developed a sampling procedure for nematodes which minimized the deviation of the samples within practical limits. They used the negative binomial distribution after fitting it to data derived from a single quadrat size, a 2.54×45 cm core. The results from our research and that of Pielou (13) indicate that a particular distribution cannot be chosen in nondiscrete populations due to the effect of sample size. The choice of the type of distribution used may not be critical, as concluded by Griffin and Tomimatsu (6). In contrast, Lin et al (8) and Onsager (12) concluded that clustered disease patterns must be considered when determining sampling designs and making pest management decisions.

The development of a method to determine biologically defined discrete quadrats for soil systems would be needed to study distributions of soilborne plant pathogens. Grogan et al (7) have proposed such a method where the volume of a soil sample is equal to the competence volume (that volume of soil in which a pathogen propagule has the potential to cause disease to an individual plant). A limitation of this method is that if the competence volume of a pathosystem is also equal to the volume of clusters of the pathogen, sampling error will result due to the problem of quadrat size and cluster size being equal. Equal quadrat and cluster size increases the chance of erroneous data due to quadrats crossing over the boundaries of the clusters (12).

If a biologically defined discrete quadrat is obtainable, one still has the problem of comparing different distributions which may fit different formulas to different degrees. Even if the same formula does describe different populations, the comparison of the parameters of the formulas is difficult because of different χ^2 goodness-of-fit values. A method that eliminates the χ^2 test and one that is not affected by the mean or quadrat size would be very useful (14). It appears that Lloyd's index of mean patchiness is such a method.

Lloyd's index of mean patchiness was not affected by the mean and was significantly different in the fields analyzed in this study. It was possible to use only one quadrat size for each field and still have an index of aggregation which could be compared to other fields, even if disease severity varies from field to field. Taylor et al (21) also found that Lloyd's index of mean patchiness was applicable to the study of the aggregation of soilborne plant pathogens.

In conclusion, quadrat size as a function of frequency distribution analyses must be considered in developing sampling procedures, for quadrat size and shape affect the quality and quantity of goodness-of-fit analyses. Whenever possible, biological rather than logistic factors should determine quadrat size. Other

methods to indicate clustering should be considered, especially Lloyd's index of mean patchiness since it is independent of the mean, is not affected by quadrat size, and is applicable to all frequency distribution data sets.

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