Distribution of Hypocotyl Rot Caused in Snapbean by *Rhizoctonia solani*

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ABSTRACT

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Hypocotyl rot caused by *Rhizoctonia solani* in snapbean (*Phaseolus vulgaris*) is a potentially destructive disease wherever snapbeans are grown. In order to investigate this disease system quantitatively, six snapbean fields in central Pennsylvania were selected and a 0.4-ha section of each field was divided into 100 contiguous 6 × 6 m quadrats. Plants were removed from each quadrat and hypocotyls were evaluated to determine the number of infected plants per quadrat and number of lesions induced by *R. solani* per quadrat. The presence of *R. solani* in lesions was verified by standard isolation and identification techniques. Variance-to-mean ratios of infected plants per quadrat were not significantly greater than unity for all samples and data for each sample were adequately fit by the Poisson distribution function which indicated a random dispersion of infected plants. Fungal lesions were clustered as indicated by variance-to-mean ratios greater than unity for all samples and the goodness of fit of all data sets by the negative binomial distribution function.

Additional key words: soilborne pathogens, epidemiology.

Quantitative epidemiology, which integrates the five dimensions of plant disease —host, pathogen, environment, time, and space, has been limited largely to diseases induced by airborne pathogens (16,26). The dispersal and subsequent distribution of airborne pathogens has been extensively studied (1,13).

In plant pathological literature there is a paucity of experimental evidence concerning the planar and spatial distribution of soilborne pathogens. Soilborne pathogens have been suggested or demonstrated to have the following spatial distributions: tetrahedral or regular (for modeling purposes) (2,17), uniform (18), random (4–6,13,23), uneven (25), and nonuniform (clustered) (11,22). Many organisms are not distributed randomly according to a Poisson distribution in nature (8,9,19,24). The negative binomial distribution has been particularly applicable in describing data involving aggregation or clustering of organisms (9,19).

Rhizoctonia solani Kühn is a ubiquitous pathogen that is perhaps the most studied of all soilborne pathogens (21). The number of *R. solani* lesions induced on a host is a reflection of the amount of inoculum present (3,6,7).

In this study, the distribution of Rhizoctonia-infected bean plants as well as the distribution of lesions was examined to gain an indication of the distribution of *R. solani* in field soil. The implications of the distribution of *R. solani* with respect to inoculum density determination and disease dynamics studies are discussed.

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MATERIALS AND METHODS

In 1977, a 0.4-ha field (soil type: Hagerstown silt loam) at the Plant Pathology Research Farm, Rock Springs, Centre County, Pennsylvania was planted to the snapbean cultivar Tendercrop and was divided into 100 contiguous quadrats. Each 6 × 6 m quadrat had a row spacing of 0.92m and a plant density of approximately 20 plants per meter of row. The field had not been previously planted to beans, but was planted to spring barley in 1976, to wheat in 1975, and to potatoes in 1973 and 1974. A cultural history for the Rock Springs field is presented in Table 1.

Twelve plants were removed arbitrarily from each quadrat at 10, 20, 30, 40, and 50 days after planting. Plants obviously infected by *Pythium* sp., as indicated by wilted foliage and gray, water-soaked stem tissue (27), were not included in any sample. On each of the five sample dates, 1,200 plant specimens were brought into the laboratory, washed for 8–10 min under running tap water, and inspected to determine number of plants infected. Samples taken 10 and 20 days after planting were evaluated for number of lesions. Samples taken at 30, 40, and 50 days after planting were not evaluated for number of lesions because of lesion coalescence.

Lesions present on plants in samples taken 10 and 20 days after planting were characteristic of those induced by *R. solani* (27). Lesions were considered to be induced by *R. solani* if they were brick red in color, sunken, had irregular margins, and generally measured greater in vertical length than in width. The presence of *R. solani* in lesions of this type was verified by culture testing at least 50 plants, recorded as having *R. solani* lesions, from each sample. Hypocotyl sections containing characteristic lesions were cut and surface sterilized in 0.5% sodium hypochlorite solution for 2–3 min. A small tissue sample was then aseptically removed from the margin of each lesion and placed on acidified 1.5% water agar in a 9-cm-diameter petri dish. Cultures were incubated for 7–10 days at room temperature. Hyphae growing from each tissue segment were examined microscopically at ×100 to ascertain if mycelium with the morphological characteristics of *R. solani* was present. Characteristics used included: the presence of constrictions of branch hyphae at the point of origin, hyphal branching at nearly right angles, and presence of a septum in the hyphal branch near the point of origin (21). Brown hyphal pigmentation also was used as a determining characteristic.

During the 1978 growing season, five commercial bean fields in Clinton County, Pennsylvania were identified for sampling purposes. Cultural histories for these fields are presented in Table 1. All fields were planted to beans for at least one growing season during the past five. In each field a level 0.4-ha section was selected and was divided into 100 contiguous 6 × 6-m quadrats. Plant density was ~36/m of row and row spacing was 0.92 m. The selected 0.4-ha section was inspected to confirm that a similar soil type occurred throughout the entire sample area.

On one sampling date in each of the five selected fields, ten plants were randomly selected and removed from each quadrat. Sample intervals after planting ranged from 12 to 23 days and, as in 1977 plants obviously infected by *Pythium* sp. were excluded from all samples. The 1,000 plant specimens were brought into the laboratory, washed for 8–10 min under running tap water, and visually inspected to determine the number of plants infected and the number of hypocotyl lesions induced by *R. solani*. Verification of the presence of *R. solani* in characteristic lesions was performed in the same manner as in 1977.

The mean number of lesions, the disease incidence, and the resultant unbiased variance were calculated for the sample of plants taken from each field. A variance-to-mean ratio was calculated for each sample for infected plants per quadrat and number of lesions per quadrat. The statistical equality of calculated variance-to-mean ratios to unity was tested by means of a chi-square statistic (9) of the form:

$$\chi^2 = ns^2 / \bar{x} \qquad (1)$$

in which, s^2 = unbiased estimate of variance, n = degrees of freedom (sample size less one), and $\bar{x}$ = sample mean.

A variance-to-mean ratio not significantly different from unity indicates a distribution of infected plants or lesions conforming to a Poisson series and, therefore, represents an even or purely random dispersion of sampled individuals. A variance-to-mean ratio significantly greater than unity could indicate a clustering or aggregation of infected plants or lesions.

Frequency data were further analyzed for goodness of fit to frequency distributions with a FORTRAN IV program for fitting discrete count data to frequency distributions (12). Distributions examined were the Poisson and negative binomial distributions. For the Poisson distribution (9) the probability, P_x, such that a frequency class x will contain 0, 1, 2, . . . individuals is

$$P_x = e^{-m}(m^x / x!) \qquad (2)$$

in which, m = the arithmetic mean of the population, e = base of the natural logarithm, and x = frequency class. For the negative binomial distribution (9) the probability, P_x, that a frequency class x will contain 0, 1, 2, . . . individuals is

$$P_x = \{(k+x-1)! / [x!(k-1)!]\} p^k q^x, \qquad (3)$$

in which, p = probability of lesion occurrence, q = 1−p, k = relative index of aggregation, and x = frequency class. In all cases, the probability of a given x was multiplied by N, the total number of units; eg, 100 quadrats, counted to obtain the expected frequency of classes with x individuals. Goodness of fit of expected frequency values to observed values for each distribution function was evaluated by chi-square analysis.

RESULTS

Verification of fungi present in bean hypocotyl lesions. In 1977, *R. solani* was isolated from all selected lesions from plants sampled 10 and 20 days after planting. *Fusarium solani* f.sp.*phaseoli* was not isolated from any hypocotyl lesion during 1977. For bean plants removed 30, 40, and 50 days after planting, *R. solani* was isolated from 96, 94, and 90% of selected characteristic lesions. At 30 days after planting, lesion coalescence was occurring and secondary invading fungi, such as *Fusarium oxysporum* and *F. roseum* frequently were isolated from lesions in addition to *R. solani*. In 4, 6, and 10% of lesions examined for the 30-, 40-, and 50-day samples, respectively, only secondary fungal invaders were isolated. The secondary invaders may have contributed to lesion expansion and coalescence.

Pythium sp. were isolated only three times from the plants sampled from the Rock Springs field in 1977. The relatively low incidence of isolation of *Pythium* sp. was probably due in part to the exclusion of plants obviously infected by *Pythium* at the time of sampling and to the use of an acidified medium for isolation of fungi from hypocotyl lesions which was not conducive for growth of *Pythium* sp. (15).

For the 1978 samples, *R. solani* was isolated from 94, 92, 98, 92,

TABLE 1. Cultural history and cultivars grown for production of snapbeans (*Phaseolus vulgaris*) in a research plot at the Plant Pathology Research Farm, Rock Springs (RS), Centre County, Pennsylvania (1977) and for commercial production of snapbeans in fields in Clinton County, Pennsylvania (1978)

Field	Cultivar	Field size (ha)	Soil type[y]	Crop History[z]
RS	Tendercrop	0.4	SiL	wheat–barley
AA	G.V. 50	3.9	SL	beans–field corn
BA_2	G.V. 50	3.9	SL	wheat–potatoes
CF_2	Yellow Wax	4.2	SCL	beans–beans
DB_1	Blue Lake	3.2	SL	wheat–potatoes
EE	Ramano	1.0	SL	wheat–sweet corn

[y]SiL = silt loam, SL = sandy loam, SCL = sandy clay loam.
[z]Crop history for field RS for 1975 and 1976; crop history for all other fields for 1976 and 1977.

TABLE 2. Variance-to-mean ratio analysis for number of *Rhizoctonia solani* lesions per quadrat on hypocotyls of snapbean (*Phaseolus vulgaris*) in Pennsylvania

Field[x]	Ratio (variance:mean)	Chi-square[y]	Probability of exceeding chi-square value
RS77-10[z]	2.97	294.03	<0.005
RS77-20	5.84	578.16	<0.005
AA78-19	1.97	195.03	<0.005
$BA_2$78-16	2.12	209.88	<0.005
$CF_2$78-12	2.04	201.96	<0.005
$DB_1$78-12	1.57	155.43	<0.005
EE78-23	1.56	154.44	<0.005

[x]Twelve plants sampled per 6 × 6 m quadrat in Rock Springs research field RS77; ten plants per quadrat were sampled in Clinton County fields during 1978.
[y]99 degrees of freedom; Chi-square analysis of Bliss and Fisher (9), H_0: variance:mean = 1, H_a: variance:mean > 1.
[z]Numbers indicate the year and time of sampling in days after planting.

and 98% of the tissue samples of lesions considered to be characteristic of *R. solani* from field AA, BA_2, CF_2, DB_1, and EE, respectively. In tissue not yielding *R. solani*, other fungi were found. A *Pythium* sp. was again isolated several times.

Isolates of *R. solani* obtained during both growing seasons varied in color from light tan to dark brown when grown on potato dextrose agar medium and exposed to diffuse light from laboratory windows. Most isolates formed sclerotia abundantly in culture. Thirty randomly selected isolates from each growing season were all assignable to anastomosis group two (20). This particular group includes many isolates of *R. solani* which induce root rot diseases.

Pattern of lesion distribution. The mean numbers of lesions observed per quadrat and variance about the mean were calculated for each field and the null hypothesis that the variance-to-mean ratio was equal to one was tested by using a chi-square statistic (9). For each field sampled, the ratio differed significantly from one (Table 2).

The seven sets of lesion count data (Table 2) were analyzed for goodness of fit to the Poisson and negative binomial distributions, which could represent random and clustered dispersal patterns of individuals, respectively. A representative frequency-class table utilizing data obtained for field AA78 is presented in Table 3. In that table, goodness of fit of the expected frequencies for the negative binomial to the observed frequencies and the lack of fit of the Poisson expected frequencies to the observed frequencies are identified by the relative magnitude of the respective chi-square values and the associated probabilities of a greater chi-square value.

The lesion count data for all seven fields adequately fit the negative binomial distribution as determined by chi-square analysis (Table 4). The Poisson distribution did not give a good fit for any of the lesion count data sets. The 'k' parameter of the negative binomial distribution, which gives an indication of the degree of aggregation or clustering of lesions, is presented for each field in Table 4. Calculated values for the 'k' parameter for the lesion count data ranged from 0.37 to 5.22.

Pattern of distribution of infected plants. The mean number of infected plants and variance about the mean were determined for each field. Chi-square analysis (9) indicated the variance-to-mean ratio of the number of infected plants per quadrat for the five sample dates in 1977 and the five commercial fields in 1978 were not significantly greater than one (Table 5).

Since the variance and mean were nearly equal for all samples, no departure from a purely random distribution of infected plants was indicated. This was confirmed by the fitting of all count data for infected plants per quadrat by the Poisson distribution.

TABLE 3. Expected and observed frequencies of number of *Rhizoctonia solani* lesions on 10 snapbean (*Phaseolus vulgaris*) plants per quadrat for the Poisson and negative binomial distributions of data for field AA78

Class[y]	Observed[z] frequency	Expected frequency: Poisson	Expected frequency: Negative binomial
0	21	9.4	19.8
1	20	22.3	22.9
2	22	26.3	19.1
3	12	20.7	13.9
4	9	12.2	9.3
5	7	5.8	6.0
6	3	2.3	3.7
7	3	0.8	2.2
8	2	0.2	1.3
9	0	0.6	0.8
10	1	0.2	1.0
Calculated chi-square		42.91	2.86
Probability of exceeding chi-square value		0.00	0.94
Variance-to-mean ratio = 1.97		k value = 2.27	

[y]Number of *R. solani* lesions on 10 plants per 6 × 6 m quadrat.
[z]Number of quadrats having specified number of *R. solani* lesions on 10 plants; 100 quadrats sampled.

DISCUSSION

Soilborne fungi have been described as being tetrahedrally (regularly), uniformly, randomly, unevenly, or nonuniformly distributed in soil. Different distribution patterns may be expected with different cultural and experimental practices; however, few field studies have been conducted to demonstrate the proposed distribution patterns for soilborne fungi.

Trujillo and Snyder (25) reported an "uneven" distribution of *F. oxysporum* f.sp. *cubense*, which attacks banana, in soils in Honduras. The authors related this to the survival of the fungal propagules in host tissue combined with a lack of cultivation and plowing of banana stubble in Honduras.

In contrast to the banana-Fusarium system, practices for bean culture in Salinas, California, involve cultivation and turning under of bean plants after harvest. On this basis, Trujillo and Snyder (25) concluded that these practices would lead to an "even"

TABLE 4. Fit of distribution functions to observed frequency data for number of *Rhizoctonia solani* lesions per 6 × 6 m quadrat on snapbean (*Phaseolus vulgaris*) plants

Field[x]	Distribution function	Probability of exceeding chi-square value	'k'[y]
RS77-10[z]	Poisson	0.000	
	Negative binomial	0.630	0.37
RS77-20	Poisson	0.000	
	Negative binomial	0.450	0.42
AA78-19	Poisson	0.000	
	Negative binomial	0.943	2.27
$BA_2$78-16	Poisson	0.000	
	Negative binomial	0.996	2.73
$CF_2$78-12	Poisson	0.000	
	Negative binomial	0.348	3.10
$DB_2$78-12	Poisson	0.000	
	Negative binomial	0.336	5.22
EE78-23	Poisson	0.000	
	Negative binomial	0.456	4.91

[x]Twelve plants sampled per quadrat in Rock Springs research field RS77; 10 plants sampled per quadrat in Clinton County fields during 1978.
[y]Relative measure of aggregation—calculated for negative binomial distribution only.
[z]Numbers in field designations indicate the year and time of sampling in days after planting.

TABLE 5. Variance-to-mean ratio analysis for number of *Rhizoctonia solani*-infected snapbean (*Phaseolus vulgaris*) plants per 6 × 6 m quadrat

Field[x]	Variance:mean	Chi-square[y]	Probability of exceeding chi-square value
RS77-10[z]	1.03	101.97	>0.25
RS77-20	1.07	105.93	>0.25
RS77-30	1.12	110.88	>0.10
RS77-40	0.99	98.70	>0.50
RS77-50	0.84	83.95	>0.75
AA78-19	0.92	91.08	>0.50
$BA_2$78-16	0.79	78.21	>0.90
$CF_2$78-12	0.78	77.22	>0.95
$DB_1$78-12	0.82	81.18	>0.90
EE78-12	0.81	80.19	>0.90

[x]Twelve plants sampled per quadrat in Rock Springs field RS77; 10 plants sampled per quadrat in Clinton County fields during 1978.
[y]99 degrees of freedom; chi-square analysis of Bliss and Fisher (9), H_0: variance:mean = 1, H_a: variance:mean = 1.
[z]Numbers in the field designations indicate the year and time of sampling in days after planting.

distribution of *F. solani* f.sp. *phaseoli* in such soils and cited the work of Nash and Snyder (18) as evidence of even distribution of the pathogen in bean fields.

Nash and Snyder (18) found no difference at the 95% confidence level among three sample types within each specific area sampled for *F. solani* f.sp. *phaseoli* in a field. The authors stated: "This indicated even distribution of the fungus population within each area to the plow depth (15–20 cm)." Their study did not involve the planar or spatial distribution of the fungus over a large expanse or over the entire field, but rather the sampling of several selected areas within a field. Although the conclusion of an "even" distribution of the fungus population within the sampled area may be valid, this does not imply that the fungus is necessarily randomly distributed according to a Poisson distribution in the horizontal dimension of the field.

In this study, the distribution of infected plants and lesions was studied rather than the distribution of the pathogen itself. As Strandberg (24) indicated, however, most epidemiological studies are based on the assumption that lesions, disease damage, or diseased plants all give adequate estimates of localized pathogen population densities. Thus, infection sites of *R. solani*, as indicated by the number of infected plants per quadrat, were randomly distributed according to the Poisson distribution in all fields sampled. The number of lesions induced at infection sites, as given by lesions per quadrat, however, indicated a clustering of lesions.

The pathogen, *R. solani*, proliferates and forms survival structures in bean tissue and is often present in the soil in decaying plant tissue (5,10). It is this decaying plant tissue which is dispersed throughout the soil by the customary cultural practices such as cultivation, turning under of bean plants, and subsequent spring plowing. It is hypothesized that these practices result in a random dispersal of decaying plant tissue throughout a field which would account for the observed randomness of infection sites according to the Poisson distribution. Further, this decaying host tissue may not be broken up or dispersed in a way which would randomly release fungal propagules. This could then account for at least some of the observed clustering of lesions.

An additional factor contributing to the observed clustering of lesions could be the induction of more than one hypocotyl lesion by a single fungal propagule. If the propagules of *R. solani* are, in fact, present in plant debris, the energy for colonization of the host available to the propagules could vary with the size, age, and the type of debris particles. For example, larger debris particles could have more nutrient substrate providing energy, and the propagules with more available energy might induce more lesions than those particles with less available energy (14). As the number of lesions induced by a single propagule increased, the amount of observed clustering also would increase.

With regard to the clustering of lesions, the value of the 'k' parameter of the negative binomial distribution gives an indication of the degree of aggregation present. This parameter has been referred to as the "dispersion parameter" for several sets of insect count data (8,9). Small values of 'k', ie, values approaching zero, indicate extreme aggregation; as 'k' approaches infinity, clustering decreases and a random distribution of units according to the Poisson distribution is defined (8,9,24).

For the Rock Springs research field (Table 4), which was not previously planted to beans, values of the 'k' parameter were 0.37 and 0.42 for samples taken 10 and 20 days after planting. This indicated a relatively high degree of aggregation. For the commercial fields, previously planted to beans, 'k' parameter values were 2.27 to 5.22 indicating less aggregation of lesions than that found in the Rock Springs research field. This lesser aggregation of lesions in field used for commercial production of snapbeans may possibly be due to continuous or intermittent bean culture over many years and the associated cultivation and plowing which would continually break up and disperse decaying tissue pieces. The greater aggregation for the Rock Springs field may be due to less cultivation of crops previously planted.

The clustering or "nonrandom" distribution of lesions induced by *R. solani* has several important implications for studies on the ecology and epidemiology of this pathogen. One consequence of a clustered versus a random distribution of lesions induced by soilborne pathogens is the possible reduction of accuracy of inoculum density determinations when lesion clustering is present. If number of lesions induced is taken as a biological index of pathogen inoculum density, and if sampling is done randomly according to a poisson distribution in a location or field where a high degree of clustering is present, a mean value with a relatively high variance would be obtained for the number of lesions and thus the inoculum density in the soil. If lesions are randomly dispersed according to the Poisson distribution, a lower variance about a sample mean would be obtained than with similar sample size and a clustering of lesions.

Where lesions are clustered, it may be necessary to increase sample size to obtain a variance similar to that obtained with a smaller sample size where lesions are randomly dispersed according to the Poisson distribution. In a case of clustered lesions in which a random distribution of lesions is assumed, a high variance about a sample mean could erroneously be interpreted as the actual variance of disease in the host population.

In studying the epidemiology of disease induced by *R. solani*, the relative distributions of the resultant disease should be a factor considered in field sampling procedures. If lesions are clustered and infection sites are purely randomly dispersed, a larger sample size would be required to accurately determine disease severity than for accurate disease incidence determinations. The sample size required would be dependent on the degree of clustering present.

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