Spatial Distribution of *Synchytrium endobioticum*, the Cause of Potato Wart Disease, in Field Soil

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ABSTRACT

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The spatial distribution of resting spores of *Synchytrium endobioticum* was studied in three extents in two fields. The extents were sampled with a device that removed blocks of soil. Two extents were divided into three sections, 6 m apart, and one extent retained as a single block. The extents were divided into grains that measured either 20 cm^2 × 5 cm or 5 cm^2 × 5 cm, and the spore population of each grain estimated. The data were mapped, and subjected to analysis using Lloyd's Index of Patchiness. There were differing levels of spore aggregation across the grains. No particular pattern of aggregation emerged; the aggregates assumed random patterns of distribution. Biological, edaphic, and cultural dispersal mechanisms were considered, and it was concluded that the pattern of distribution likely followed gall production (biological dispersal).

Additional keywords: contour, sampling, soilborne

Wart disease of potato (*Solanum tuberosum* L.) is incited by the Chytridiomycete *Synchytrium endobioticum* (Schilb.) Perc. Its primary symptom is a gall (13). The polycyclic character of the pathogen results in countless dormant (diameter approximately 30 to 100 µm) resting spores in the galls (10). At harvest time, the galls rot to black, smelly, decomposing masses—hence Black Wart (24)—and release resting spores into the soil (12). These form the inoculum carryover from season to season. Laidlaw (20) estimates resting spore longevity at ≥40 years.

Little attention has been given to the spatial distribution of resting spores of *S. endobioticum* in soil, and hence its role in the incidence of wart disease. In the literature, field work appears to have been carried out on the assumption that *S. endobioticum* is spatially homogenous in soil. It is a matter of common observation, however, that the disease is often discontinuous in infested soils. In two fields in the St. John's environs, the susceptible (to pathotype 2) potato cv. Arran Victory scored 25, 70, and 100% infection at Avondale, and 100, 90, and 7% at Goulds in 1990, 1991, and 1992, respectively. Unmapped spore data in these fields ranged from 8 to 123 spores per g of field soil (7,9).

Failure or success in potato wart disease incidence has been attributed to weather conditions (6,25), clone susceptibility (19), resting spore germination (11), and soil temperatures (16). It is proposed that disease incidence discontinuity in potato wart disease is related primarily to the spatial distribution of resting spores.

On the basis of these observations, a study was undertaken to characterize resting spore distribution in naturally infested field soil. Recourse was made to Campbell and Benson's (2) recent review and discussion of the suite of characters involved in spatial analysis of soil propagule distribution. To challenge the assumption of spatial homogeneity of *S. endobioticum* in field soil, the random variables (propagule counts) were assembled from three extents (sensu Campbell and Benson) and 160 grains (sensu Campbell and Benson), and analyzed in the manner of Campbell and Benson's (2) two-element program: (i) mapping and (ii) index of dispersion. The ecological terms extent and grain are defined as the total area studied and the size of an individual sampling unit, respectively (2). In this study each grain is a quadrat.

MATERIALS AND METHODS

Field sites. Sites were selected at the Canadian Government Potato Wart Disease Testing Station in Avondale and at a private farm in Goulds. These fields represent typical "wart"-infested land in Newfoundland monocultured to potato. The soil in the Avondale field has been kept well-infested since 1954 by allowing galls to rot in situ; soil at the private site has been in potato cultivation for an undetermined number of (≥25) years and reinfested through poor sanitation methods. Each field displays a southern slope (Avondale, 1:12; Goulds, 1:45). The northerly end of each site is at the top of the slope. To indicate the position of each sampling area in this study in relation to its topography, the designations top (T), middle (M) and bottom (B) are used.

Extents and grains. The experimental layouts at each site are presented in Figures 1 through 3. At each site, represented in Figures 1 and 2, a 1.92-m^2 extent (i.e., sum of area of all sampling units) was selected along a north-south transect and divided into three equal-sized sections (0.8 × 0.8 m): T, M, and B, respectively, each separated by a 6-m gap. The gaps (T to M and M to B) are not shown on Figures 1 and 2 for reasons of space. Each section was divided into a 4 × 4 grid of sampling units (0.04 m^2), i.e., grains. At Goulds, a further sampling (Goulds^2), illustrated in Figure 3, involved dividing a 0.16-m^2 extent (0.4 × 0.4 m) into an 8 × 8 grid of 0.0025 m^2 grains. The grains (quadrats) in sections Avondale-T through to Goulds^1-B and the single Goulds^2 extent were 5 cm deep.

Sampling device. Soil core quadrats were obtained with a device constructed from 20 × 5 cm aluminum strips spot-welded to form a 4 × 4 comb of 16 cells, each measuring 5 × 5 × 5 cm. An aluminum plate (20 × 20 cm) was held on the top side by wing-nuts. The cell edges on the lower side were sharpened to facilitate soil-coring. Total soil volume was 2 liters.

Sampling procedure. The device was worked into the soil so that each cell filled with soil. The top plate was attached and the device removed by sliding a flat spade beneath it, lifting, and turning it over. Excess soil was removed to give each cell an equal volume of soil (5 cm^3).

In sampling the 4 × 4 extents at Avondale and Goulds^1, the total soil component of the device (i.e., all 16 cells) was emptied after each application into individual "5-pound" double paper bags; by applying the device in a 4 × 4 square, 16 grains were sampled.

In sampling the 2 × 2 extent at Goulds^2, the soil was removed carefully from each cell and stored in individual double paper bags; by applying the device in a 2 × 2 square, 64 grains were sampled.

The soils were stored at 3±1°C.

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Spore enumeration. A protocol was developed to retrieve and enumerate spores. Wet sieving was carried out in a Fritsch "Analysette 3," with 106-µm (ASTM 140) and 36-µm (ASTM 400) mesh; an IEC Centra-8 was used for centrifugation; resting spores were collected on 47-mm gridded (100 grids) acetate membrane filter circles (pore size 1.2 µm), and were counted at magnification ×50.

Retrieval

Sieve bagged, infested soil through 9-mesh to make 3 × 100-g subsamples

↓

to each subsample add 120 ml of 0.1% Tween 80 solution

↓

sonicate for 0.5 h

↓

hold under vacuum for 24 h

↓

wet-sieve for 1 h

↓

dry subsample over night at 60°C

↓

weigh (x grams) material collected on 36-µm sieve.

(A)

↓

Enumeration

To a 1-g subsample from a sievate

↓

add approximately 15 ml of reagent grade $CHCl_3$

↓

centrifuge suspension for 10 min at 250 × g

↓

decant, dilute ×10

↓

collect on membrane filter circle

↓

count spores on central 25 grids of circle (17)

(B)

Numbers of spores per g of infested soil = (A × 40B)/100, where A = x grams, B = spore number. The spore numbers were averaged (n = 3) for each bag of soil.

Spatial analysis: mapping. Box and contour plots were generated (Spyglass Transform package; Spyglass, Inc., Savoy, IL) with estimated counts of propagules per g of soil per quadrat for the extents to yield descriptive data. Box plots are illustrated in Figures 1A, 2A, and 3A. Contour plots are shown in the companion diagrams (Figures 1B, 2B, and 3B), in which similar spores per g of soil values are joined and reflect the levels of aggregation of the distribution of resting spores.

For the box plots, each grid yielded 16 values; the grids were mounted in Figures 1 and 2 in the descending order T, M, and B. At Avondale, 37/48 sampling units recorded spores per g of soil at <200 and 2/48 at ≥600; to avoid a map with low contrast between the low values, the numbers of spores per g of soil were ranked and each assigned a number (1 to 48) consonant with its rank to give relative densities (represented by intensities of gray value). This display mode was used for both sites. For the single extent at Goulds[2] (Fig. 3), the spores per g of soil values were likewise ranked and each assigned a number from 1 to 64.

Actual spore load values (n spores per g of soil) for each grain were used to create contour line plots. Min-max values for Figures 1B, 2B, and 3B were set at 52-750, 28-580, and 22-111, respectively. Contour line differentials in Figures 1B and 2B were set at 15 spores per

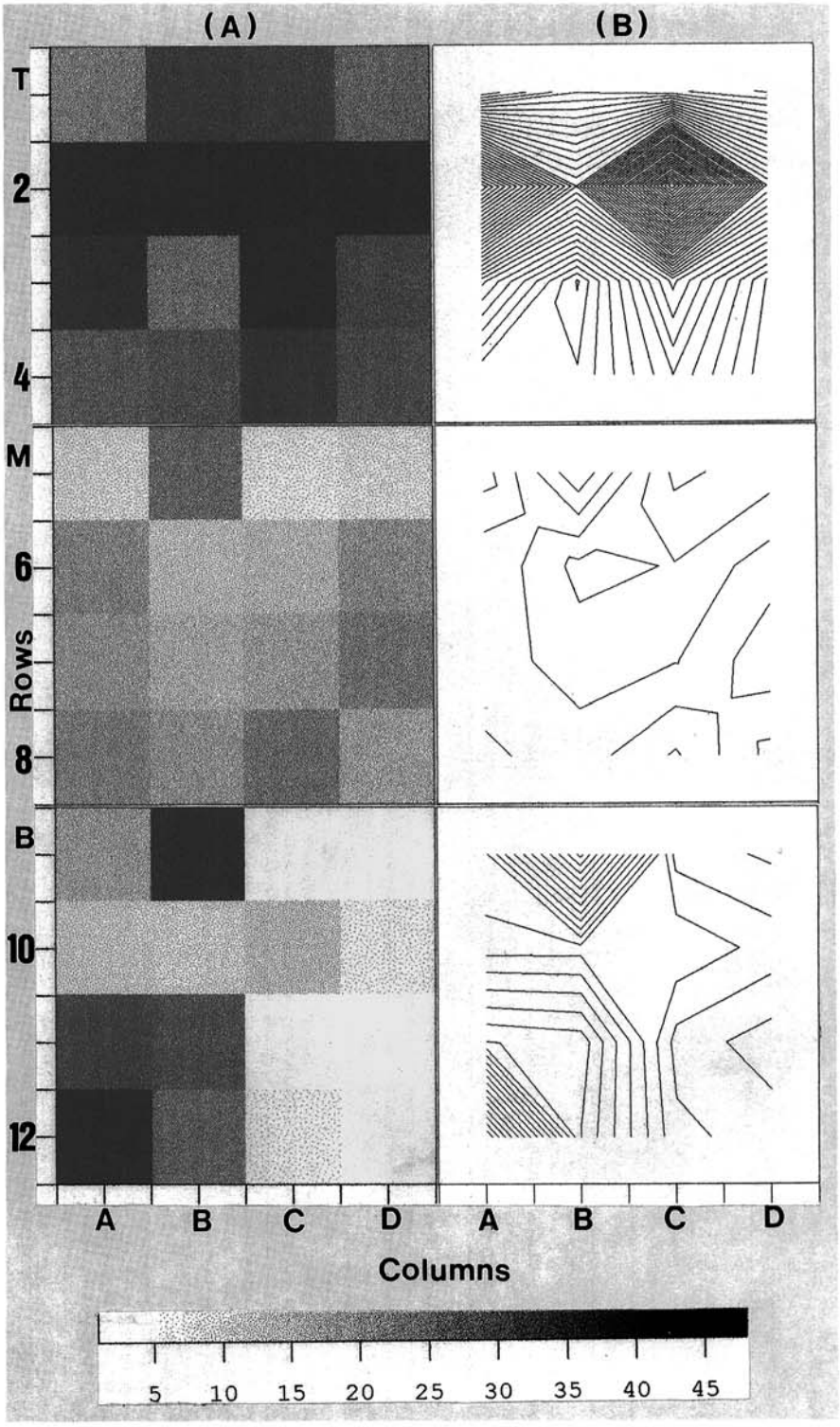

Fig. 1. Box (**A**) and contour (**B**) plots of distributions of numbers of *Synchytrium endobioticum* resting spores at Avondale site. Extent comprises three sections (top, T; middle, M; bottom, B), each 0.8 × 0.8 m, aligned on a north-south transect and subdivided into 16 grains. **(A)** Grains are ranked according to spore density; each assigned a value of 1 (low) to 48 (high), as represented by gray scale. **(B)** Contour plots are based on actual spore range of 52 to 750 spores per g of soil, with line differential set at 15 spores.

line, and in Figure 3B at 3 spores per line.

Spatial analysis: index of dispersion. Lloyd's Index of Patchiness (γ) (21) was used as the index of dispersion. To indicate the degrees of randomness or aggregation in blocks of grains (5), $\gamma = \{m + [(\sigma^2/m) - 1]\}/m$, where m = mean resting spore density, σ^2 = variance, and values of $\gamma < 1$, $= 1$, or > 1 indicate spatial uniformity (regularity), randomness, or aggregation, respectively.

For sites Avondale and Goulds[1], γ was computed for each composite section, for each column of each section, and the 12 rows in each extent. For site Goulds[2], γ was calculated for the whole extent, eight rows and eight columns.

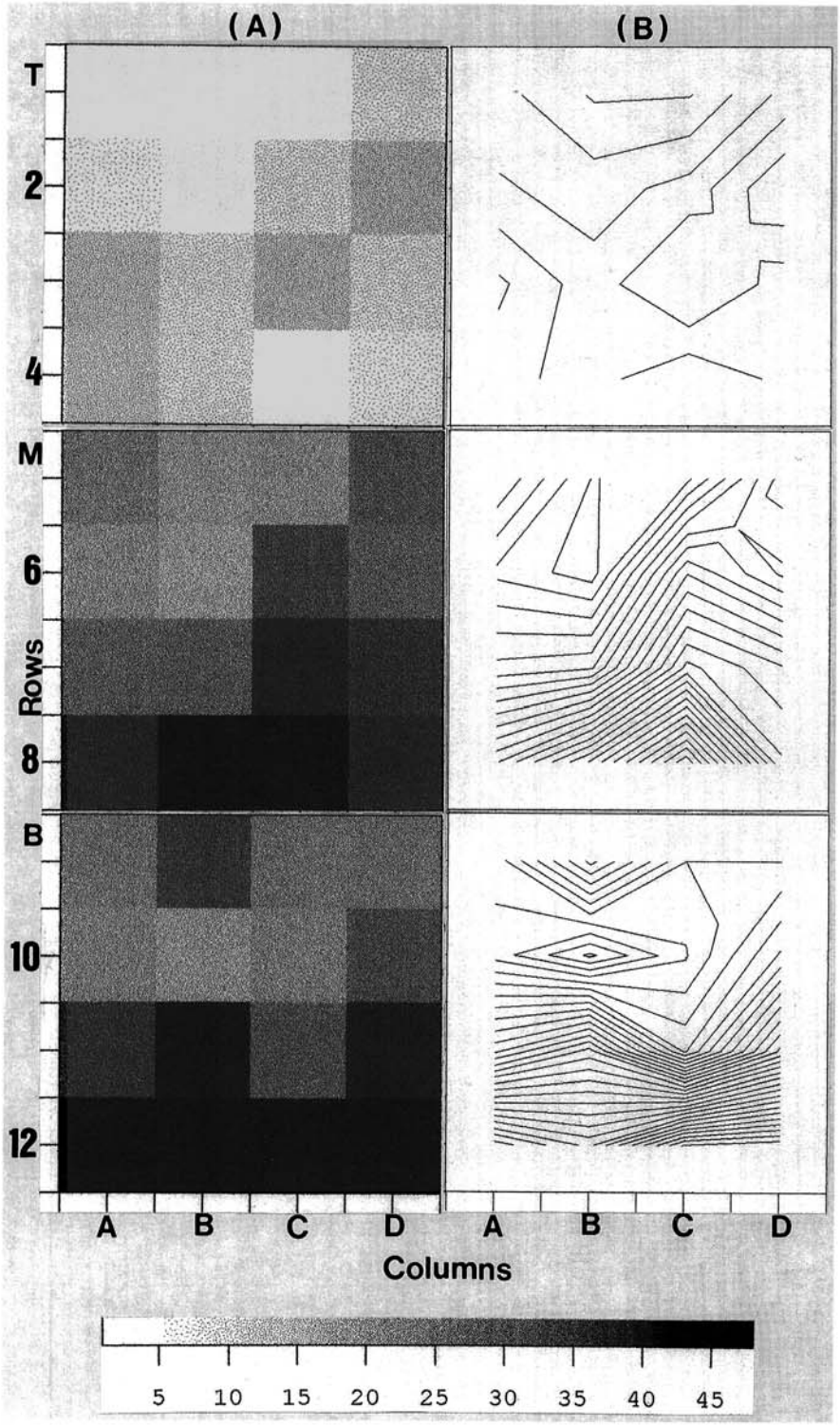

Fig. 2. Box (**A**) and contour (**B**) plots of distributions of numbers of *Synchytrium endobioticum* resting spores at Goulds[1] site. Extent comprises three sections (top, T; middle, M; bottom, B), each 8 × 0.8 m, aligned on a north-south transect and subdivided into 16 grains. (**A**) Grains are ranked according to spore density; each assigned a value of 1 (low) to 48 (high), as represented by gray scale. (**B**) Contour plots are based on actual spore range of 28 to 582 spores per g of soil, with line differential set at 15 spores.

RESULTS

Box and contour plots of the extent in Avondale are shown in Figure 1A and B, respectively. Areas of aggregation occur in the T and B sections of the extent. From Table 1, it can be seen that, taking the rows as blocks, γ varies from random (1.00) to aggregation (1.72). No particular pattern is evident in the increasing order of γ (1.00 to 1.72) row-by-row: 7 = 10, 6 = 8, 1, 4, 3 = 5, 2, 11, 9, 12. Similarly, from Table 2 there is no recognizable pattern to the distribution of γ. The T and B sections of the Avondale extent display aggregation to a high ($\gamma \geq 1.40$) degree compared with the M section ($\gamma = 1.02$). The only γ value (0.99) tending to regularity (uniform distribution) (i.e., <1.00) was in column B of the M section.

Box and contour plots of the extent at the site Goulds[1] are shown in Figure 2A and B, respectively, and display the wide range of γ values shown in Table 2. Within the M and B sections, aggregation predominates. Clumping is more evident in the B section; the overall γ (Table 2) increases from 1.10 through 1.19 to 1.27. Column γ (Table 2) ranges between 1.05 and 1.48. Within sections, however, no particular pattern is evident (Table 1) in the increasing γ order 1.01 to 1.17: 12, 3, 4 = 9 = 11, 8, 5 = 7 = 10, 2, 1, 6.

The extent Goulds[2] represents a quarter of a Goulds[1] section (Fig. 3A, B). Within this small area, aggregation can be seen to occur. No particular pattern of aggregation can be discerned. Ascending γ values for

Table 1. Lloyd's Index of Patchiness applied to rows of quadrats in two fields infested with resting spores of *Synchytrium endobioticum*[a]

	Lloyd's Index of Patchiness, γ		
	Threefold extent		Single extent
Row[b]	Avondale	Goulds[1]	Goulds[2]
Top			
1	1.02	1.15	1.08
2	1.13	1.09	1.05
3	1.09	1.03	1.11
4	1.03	1.04	1.05
Middle			
5	1.09	1.07	1.04
6	1.01	1.17	1.11
7	1.00	1.07	1.05
8	1.01	1.05	1.15
Bottom			
9	1.60	1.04	NV[c]
10	1.00	1.07	NV
11	1.32	1.04	NV
12	1.72	1.01	NV

[a] Sampling units measured: Avondale and Goulds[1], 20 cm² × 5 cm; Goulds[2], 5 cm² × 5 cm. Designations Top, Middle and Bottom do not apply to the single extent.
[b] Values in Avondale and Goulds[1] are of 4 grains each; in Goulds[2] of 8 grains each.
[c] No value.

rows and columns are in the orders: 5, 2 = 4 = 7, 1, 3 = 6, 8, and A, H, C, D, G, F, B = E, respectively. Although the overall γ for the extent = 1.10, the 64 individual grains analyzed by row (×8) and column (×8) displayed a range of γ, viz.: 1.02, 1.04 (×2), 1.05 (×4), 1.07, 1.08. 1.09, 1.10, 1.11 (×2), 1.15, and 1.18 (×2).

DISCUSSION

The results of this study show that spore distribution displays spatial inhomogeneity in "wart"-infested fields, and that changes in sampling distances of 6 m or less influence spore load evaluation and, it is suggested concomitantly, disease incidence. Analysis of spore loads in the single extent in Goulds[2] echoes the observation by Campbell and Benson (2) that scale profoundly affects the detection of spatial patterns. This extent was sampled adjacent to the Goulds[1] B section (spore range: 28 to 105), and its spore range was 22 to 111. By reducing the size of grain from 20 cm^2 × 5 cm to 5 cm^2 × 5 cm, a seemingly homogenous grain is seen to be composed of a heterogeneity of grains.

Campbell and Benson (2) observed that the spatial framework for soilborne pathogens varies along a continuum from a high degree of aggregation to randomness, thus imposing an observable pattern. Plainly, this pattern is not immediately apparent in any of the three extents examined in this study. Without further testing, it is unwise to infer that all *S. endobioticum*—infested soils are likewise, but the evidence weighs strongly in favor of seemingly unordered patterns of aggregation. It is obvious that, with time, inocula will disperse through gradients from high to low levels, Data to support this contention may be allied entirely to scale (5).

Mechanisms invoked to account for spore dispersion include biological, edaphic and cultural ones. The maps of the spatial pattern for *S. endobioticum* in the Avondale and Goulds extents are strikingly different, but both fields containing these extents appear to be subject to similar environmental factors q.v. dispersal mechanisms.

Under natural conditions, galls rot and release resting spores in situ. This is an obvious biological mechanism for both dispersal over an infested area and a means of aggregation. *S. endobioticum* is a zoosporic pathogen. Dispersal by zoospores has been well studied (2) but this seems to be circumscribed by emphases on *Phytophthora* spp., and the scant work on zoospore activity in *S. endobioticum* falls outside the general scientific interest in dispersal by zoospores. Weiss (25) and Curtis (3) report a life span of about 1 h for zoospores of *S. endobioticum*, and Hampson and Coombes (14) estimate zoospore competency (spatial ability to induce infection) to be approximately 30 mm. By comparison with other zoosporic pathogens, its

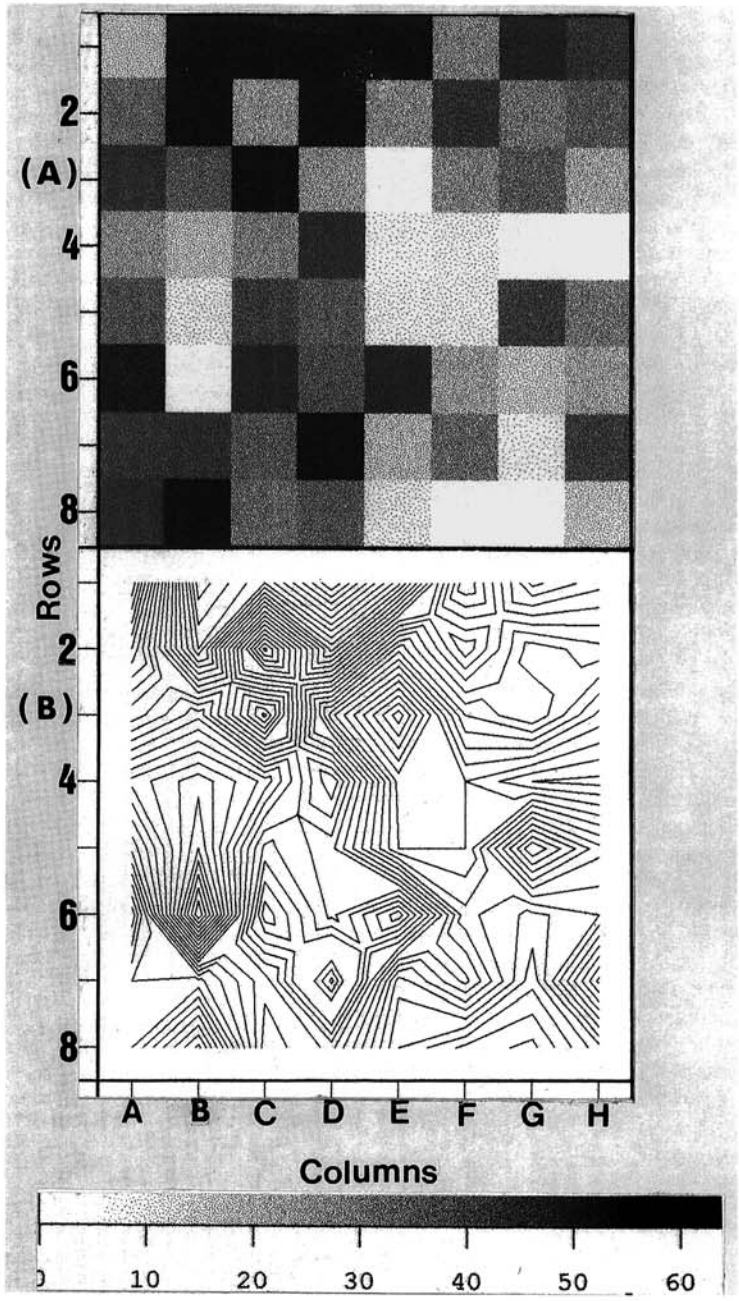

Fig. 3. Box **(A)** and contour **(B)** plots of distributions of numbers of *Synchytrium endobioticum* resting spores at Goulds[2] site. Extent measures 0.4 × 0.4 m and is subdivided into 64 grains. **(A)** Grains are ranked according to spore density; each assigned a value of 1 (low) to 64 (high), as represented by gray scale. **(B)** Contour plot spore range is 22 to 111, with line differential set at 3 spores.

Table 2. Lloyd's Index of Patchiness applied to columns of quadrats, four at a time in two fields infested with resting spores of *Synchytrium endobioticum*[a]

	Lloyd's Index of Patchiness, γ								
	Column[b]								
Site	A	B	C	D	E	F	G	H	Overall[c]
Avondale-Top	1.50	1.20	1.50	1.21	NV[d]	NV	NV	NV	1.40
Avondale-Middle	1.01	0.99	1.05	1.02	NV	NV	NV	NV	1.02
Avondale-Bottom	1.40	1.16	1.03	1.03	NV	NV	NV	NV	1.48
Goulds[1]-Top	1.06	1.10	1.23	1.06	NV	NV	NV	NV	1.10
Goulds[1]-Middle	1.12	1.38	1.26	1.05	NV	NV	NV	NV	1.19
Goulds[1]-Bottom	1.33	1.24	1.48	1.29	NV	NV	NV	NV	1.27
Goulds[2]	1.02	1.18	1.05	1.07	1.18	1.10	1.09	1.04	1.10

[a] Sampling units measured: Avondale and Goulds[1], 20 cm^2 × 5 cm; Goulds[2], 5 cm^2 × 5 cm.
[b] Values in Avondale and Goulds[1] are of 4 grains each; in Goulds[2] of 8 grains.
[c] Values in Avondale and Goulds[1] are of 16 grains each; in Goulds[2] of 64 grains.
[d] No value.

ability to spread in soil by zoospores appears limited.

Soils in the field sites are typical of soils on the Avalon Peninsula: subject to precipitation (average annual rainfall at St. John's Research Centre for 1951 to 1980 = 1,606.5 mm), but stony and drain well. Although Hampson (8) found that resting spores will move down a percolation column of sand of particle size 600 to 1,000 µm, nevertheless the slopes of the soils did not appear to contribute to spore dispersal. The slope of the field at Goulds was slight but the concentration of aggregates was at the lower part of the extent; the slope at Avondale was quite pronounced but the aggregates were concentrated in the upper part of the extent. Other edaphic dispersal mechanisms at the sites encompass Hampson and Coombes's (14) finding that earthworms facilitate dissemination of resting spores, inducing infection at 250 mm away from inoculum source. In a well-fertilized soil, it can be expected that some destruction of inoculum-aggregates by microfauna will occur over time.

Dispersal by cultural means centers on plowing and disking the soils in May prior to planting, and hand-harvesting in September/October. At the Avondale site, plowing is carried out across the extent, whereas in Goulds it is carried out along the length of the extent. Planting potatoes involves the extents and their contiguous areas; thus, at Avondale the field width is approximately 30 m, and at Goulds the length is approximately 35 m. Each 0.8 × 0.8 m section would receive at least two furrows each season in addition to other cultural perturbations. These cultural mechanisms do not appear to be powerful enough to create the displayed distributions.

None of the previous studies on potato wart disease or hypotheses for disease incidence discontinuity in the field address spatial distribution as an underlying cause. The extensive experiments in the U.K., U.S., and Germany in the 1920s, and former Soviet Union in the 1950s and 1960s, lack information even on spore loads. For example, Glynne (6) comments that a certain soil is ". . . heavily infected [sic] with wart disease"; Potocek (23) states, "Field experiments were carried out in severely and *uniformly* infected [sic] plots . . ." (our italics). Malec (22), in Poland, samples a field for 8 years to assay changes in viability of spores with time but does not report on any changes in spore distribution or loads. Discrete knowledge of spore loads and their distribution must be paramount in explaining variations in disease incidence in wart disease (15).

A search of the extensive literature on this disease (12,18) did not reveal correlations between field experiments (disease incidence and intensity) and spore loads, let alone distribution of the spores. Gilligan (4) draws attention to the scarcity of studies on spatial patterns of soilborne pathogens. That scarcity, coupled with the scarcity of information on the distribution of *S. endobioticum* in field soil, emphasizes the contribution of this study to understanding spatial aspects of soilborne propagules in general and potato wart disease in particular.

The "good year/bad year" syndrome of disease incidence suggests that the spatially discontinuous production of gall material in soil may not assume a similar pattern from season to season. By default, however, the primary cause of the spatial inhomogeneity illustrated in this study is probably the scattered infection in the early period of crop production that would "set up" local centers of inoculum. With the passing of years, notwithstanding edaphic and cultural perturbations, and yearly climatological fluctuations, these local centers could constitute foci. It is possible that gall tissue, although mainly of thin-walled parenchyma (1), in rotting, does not release all its spores, which can amount to >1 million per gall. In a laboratory bench experiment (M. C. Hampson, unpublished), intact gall material was still evident after 6 mo incubation in soil at room temperature.

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