Comparative Spatial Analysis of Foliar Epidemics on White Clover Caused by Viruses, Fungi, and a Bacterium

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ABSTRACT

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Incidence and spatial patterns of eight foliar pathogens and diseases were monitored during two 6-wk growth periods per year from June to September in 1990 and 1991 on regularly spaced plants of white clover (*Trifolium repens*) planted in a tall fescue (*Festuca arundinacea*) sward in a 10-ha pasture in Wake County, North Carolina. Disease ratings were made in four plots on a total of 512 plants in eight square lattices of 64 plants each. Each plot comprised two proximal lattices of either the virus-susceptible white clover cultivar, Regal, or the Southern Regional Virus Resistant white clover germ plasm. Incidence of three viruses (alfalfa mosaic virus, clover yellow vein virus, and peanut stunt virus) was assessed by indirect enzyme-linked immunosorbent assay in June or July, August, and September. Per-plant incidence of the following leaf spot diseases was assessed on 16 dates during 1990–1991: black spot (*Pseudomonas andropogonis*), Cercospora leaf spot (*Cercospora zebrina*), Curvularia leaf spot (*Curvularia trifolii*), summer blight (*Rhizoctonia solani*), and Stagonospora leaf spot (*Stagonospora meliloti*). Two-dimensional distance class analysis was used to quantify spatial attributes of viral and leaf spot epidemics on the two white clover host populations. Virus-resistance altered the spatial pattern of the virus disease complex. Viral epidemics in populations of Regal were characterized by higher disease incidence, stronger aggregation of diseased individuals, more numerous and larger clusters of virus-infected plants, and stronger edge effects than for those in populations of the Southern Regional Virus Resistant germ plasm. Spatial patterns of the leaf spot diseases were similar in the virus-resistant and virus-susceptible host populations. In general, spatial patterns of the fungal and bacterial leaf spot diseases reflected the gradient and mode of pathogen dispersal. Significant edge effects and large, well-defined, expanding clusters were characteristic of the splash-dispersed pathogens, *P. andropogonis* and *S. meliloti*. Smaller clusters (in plot interiors rather than plot edges) were found for the aerially dispersed pathogens *C. zebrina* and *Curvularia trifolii*, which have shallower dispersal gradients. Defoliation throughout the epidemics was related to continual changes in disease incidence, strength of aggregation and cluster location, and morphology. Variability in spatial attributes between and within years, among plots, and between growth periods suggest the importance of environment (e.g., rainfall, temperature) and cultural practices (e.g., harvest) as determinants of spatial patterns in this pathosystem.

Additional keywords: AMV, CYVV, PSV.

Spatial attributes of diseases and pathogen populations are of central importance to the analysis, understanding, and management of plant disease epidemics. Spatial processes are linked with biotic and abiotic forces and reflect the interaction between organisms and their spatially and temporally dynamic environment (4,21). The ecology of plant pathogens is, in reality, a spatiotemporal succession of events with spatial pattern as one of the most characteristic properties of species (46).

An increase in intensity of foliar diseases through time implies a concomitant change in spatial pattern, i.e., the arrangement of disease entities relative to each other and to the architecture of the host crop (13). However, most disease progress models that are applied traditionally to the analysis of epiphytotics contain the implicit assumption of spatial regularity or randomness of host and pathogen populations and, thereby, of disease (27,50). Whereas spatial regularity is characteristic of many row crops, spatial randomness is not a characteristic of most plant diseases. A wide range of soilborne and aerial pathogens and their resulting diseases are characterized by spatial aggregation and anisotropy (4,5).

Several techniques are available for analyzing spatial patterns of plant parasitic organisms and diseases for those pathosystems in which discrete or continuous measures and estimates of disease (e.g., estimates of severity or continuous count and proportion data), are biologically relevant (39,48). In contrast, disease intensity with regard to systemic diseases such as those caused by viruses is often expressed as incidence of diseased plants. Thus, spatial pattern analysis with systemic diseases often relies upon binomial data (i.e., presence/absence of disease). All three data types—severity, continuous count, and binomial—are amenable to spatial characterization; however, the appropriate analytical techniques (and what the data reflect) differ for each type. For example, disease severity data often reflect the importance of environmental conditions for pathogen reproduction and spread and/or disease increase upon the host (autoinfection). Incidence and binomial data are more representative of plant-to-plant dispersal gradients (alloinfection).

Currently, there are relatively few techniques available in the phytopathological literature for the descriptive and quantitative analysis of spatial pattern based upon binomial data. Distance-based analyses have been used successfully to describe spatial patterns in ecological studies (7,26,38). These analyses consider distances between individuals of a population within a continuous area. An underlying assumption is that members of the sampled

population can occupy any location within the continuum. Although this assumption of occupancy is not met for most agronomic or horticultural row crops, distance-based analyses have been applied successfully for bacterial blight of soybean (40) and citrus tristeza on orange (29).

Spatial analyses based upon the position of healthy or diseased plants within a row or series of rows such as doublet (49) and runs analyses (28) and two-term local quadrat variance analysis (25) are appropriate for transect data but were not designed for and are less effective in quantifying spatial attributes of two-dimensional (row and column) grids of plants. A distance-class method proposed for describing two-dimensional spatial patterns of diseased/healthy plants arranged or demarcated on a lattice was developed by Gray et al (17,18) for characterizing spatial relationships among virus-infected plants within row crops or plant lattices. Recently, we (37) introduced microcomputer software capable of performing Gray's two-dimensional distance class analysis, which facilitates rapid and concise description and quantification of spatial patterns based upon binomial data.

We have described a multiple-pathogen, "leaf spot" complex on white clover (*Trifolium repens* L.) in clover-grass pastures in the Piedmont region of North Carolina (36). The most important bacterial and fungal pathogens in the complex were *Pseudomonas andropogonis* (black spot), *Cercospora zebrina* Pass. (Cercospora leaf spot), *Colletotrichum* spp. (anthracnose), *Curvularia trifolii* (Kauffm.) Boedjin (Curvularia leaf spot), *Leptosphaerulina trifolii* (Rostr.) Petr. (pepper spot), *Rhizoctonia solani* Kühn (summer blight), and *Stagonospora meliloti* (Lasch) Petr. (Stagonospora leaf spot). These pathogens vary greatly with regard to modes of reproduction and dispersal, and little quantitative information is available on the ecology of these pathogens individually or as a group.

In addition to the bacterial and fungal leaf spot complex, diseases caused by viruses are endemic on white clover throughout the southeastern United States (3). In our research pasture in March 1990, we detected alfalfa mosaic virus (AMV), clover yellow vein virus (CYVV), peanut stunt virus (PSV), and red clover mosaic virus (RCMV). There is little information available on spatial or temporal attributes of virus epidemics on white clover in pastures. However, the recent availability of white clover germ plasm with resistance to the viral disease complex in the southeastern United States (12) provides an opportunity to quantify and compare the epidemiological effects of resistance versus susceptibility to viruses in white clover.

The purpose of this paper is to present a comparative analysis of the spatial patterns of the most important components of a foliar disease complex on white clover in North Carolina. A comparative epidemiological approach, as suggested by Kranz (22), was used as a vehicle to test and generate hypotheses with regard to pathogens and their ecology during epidemics. Specific objectives of the study were 1) to characterize the spatial patterns of virus-infected plants on virus-resistant and virus-susceptible white clover, 2) to quantify and compare spatial attributes of five leaf spot diseases, and 3) to examine the effects of introducing virus-resistant white clover germ plasm into a pasture upon spatial patterns of the five leaf spot diseases.

MATERIALS AND METHODS

Experimental plots. Experiments were conducted during 1990 and 1991 in a 10-ha pasture of white clover and tall fescue (*Festuca arundinacea* Schreber), which was grazed by dairy cattle, in Wake County at the Unit 2 Forage Research Facility of North Carolina State University. Pasture history and cultural practices during the experiment were described in detail in a previous paper (36).

Four plots were established in arbitrary locations that represented dissimilar microenvironments within the pasture. Plots consisted of two, proximal, eight row by eight column lattices of 64, 10-wk old transplants each of either Southern Regional Virus Resistant germ plasm (SRVR) (12) or of the virus-susceptible white clover cultivar Regal. Transplanting occurred on 4 May in 1990 and on 15 April in 1991. Clover plant lattices were placed within the existing sward of tall fescue. Lattice dimension was 10 m × 10 m, with plants on 1.25-m centers. Host genotype was assigned randomly to one of two lattice positions within each plot. Plots were enclosed by an electric fence (Gallagher Mini Strip Grazer, Gallagher Electronics, Hamilton, New Zealand) to prevent bovine interference. Plot orientation (long axis) was either north-south or east-west (two plots for each orientation).

In 1990 and 1991, disease assessments were made during two 6-wk growth periods. The first of the two consecutive 6-wk growth periods began on 25 June 1990 and on 10 June 1991. Plots were harvested to a height of approximately 6–10 cm with a flail-chop harvester (Carter Manufacturing, Brookston, IN) at the termination of growth period 1 (harvest dates: 4 August 1990 and 24 July 1991). Harvested material was removed from plot areas and discarded. Monitoring of growth period 2 began 10–14 days after harvest of growth period 1.

Beginning in late July 1990, incidence of foliar diseases caused by several fungi and one bacterium was assessed every 2 wk on each of the 512 plants (five assessments). Virtually each leaf on every plant was examined in situ for pathogen incidence. Pathogen presence/absence was confirmed via recognition of diagnostic symptoms or periodic, destructive sampling prior to microscopic inspection (×40) of leaves for pathogen reproductive structures. Pathogens were (disease in parentheses) *C. zebrina* (Cercospora leaf spot), *Colletotrichum trifolii* (anthracnose), *R. solani* (summer blight), *S. meliloti* (Stagonospora leaf spot), and *P. andropogonis* (black spot). Late in the 1990 growing season and during the 1991 season, additional pathogen species were observed, identified, and monitored: *Curvularia trifolii* (Curvularia leaf spot), *L. trifolii* (pepper spot), *Polythrincium trifolii* (sooty blotch), and *Uromyces* sp. (rust). During 1991, incidence of leaf-spotting organisms was assessed for each plant once per week; thus, 16 disease assessments were made (five in 1990 and 11 in 1991).

Virus sampling. In February 1990, the presence of several viral diseases in the 10-ha pasture was confirmed (M. R. McLaughlin, USDA-ARS, Mississippi State, *personal communication*) via enzyme-linked immunosorbent assay (ELISA). These were identified as RCMV, AMV, CYVV, and the eastern strain (20) of PSV. Because PSV, AMV, and CYVV are considered to be the most important viruses in North Carolina and the southeastern United States (3,31), incidence of PSV, AMV, and/or CYVV was determined for each of the 512 plants three times each year. Sampling dates were 9–16 July, 20 August, and 25 September (1990) and 4–7 June, 6 August, and 16 September (1991). Three to five leaves were selected arbitrarily from each plant. Plant sap was extracted, and the antigen (1:10, w/v) was stored in carbonate buffer.

Samples were tested for the presence/absence of virus via indirect ELISA (1). Positive and negative virus controls were maintained on *T. repens* in screen (32 × 32 mesh) cages (0.96 × 0.087 × 1.22 m) in a greenhouse. Antisera were obtained from O. W. Barnett, Department of Plant Pathology, Clemson University (AMV, CYVV) and from S. A. Ghabrial, Department of Plant Pathology, University of Kentucky (PSV). Microtiter ELISA plates were evaluated visually in 1990. In 1991, colorimetric responses were recorded as the absorbance at 480 nm read on a microtiter plate reader (Molecular Devices, Palo Alto, CA) after 1 h of incubation at 25–30 C. Samples were interpreted as virus-positive when absorbance values exceeded the negative controls by at least two standard deviations of the mean absorbance for the negative controls that were included on each plate.

Data analysis. Two-dimensional distance class analysis was developed originally to characterize spatial relationships of virus-infected plants within row crops (17) but is equally applicable to any disease or pest occurrence where plants are regularly spaced and presence/absence data are biologically meaningful. Specific guidelines for the use and interpretation of two-dimensional distance class analysis were presented in another paper (37). For the current study, we applied the following analytical and interpretive criteria to data sets for diseases caused by viruses, fungi,

and a bacterium. Data sets with fewer than nine diseased plants (out of a total of 64 plants), more than 54 diseased plants, or more than 16 dead plants were not analyzed. Data sets were interpreted as having significantly nonrandom (i.e., aggregated) spatial patterns if the total number of significant standardized count frequencies (SCFs) exceeded two, otherwise the pattern was interpreted as random. Data sets with more than 54 diseased plants were interpreted as having a uniform pattern of diseased plants. Strength of aggregation (nonrandomness) was directly proportional to the total number of significant SCFs. Data sets were interpreted as having strongly nonrandom spatial patterns if the total number of significant SCFs exceeded five. The "minimum core cluster size" was defined as the number of significant and adjacent $[X, Y]$ distance classes (including the $[X, Y]$ distance class, [0,0]) that formed a discrete, contiguous group in the upper left-hand corner of the distance class analysis matrix. Minimum "reflected core cluster size" was defined as the number of significant and adjacent $[X, Y]$ distance classes that form discrete, contiguous groups elsewhere in the two-dimensional distance class analysis matrix. Cluster shape was defined as the shape of the contiguous groups comprising the minimum core and minimum reflected core clusters. Minimum cluster number was defined as the number of contiguous groups of significant $[X, Y]$ distance classes within the distance class analysis matrix. If only n reflected cluster(s) are evident in the two-dimensional distance class analysis matrix (and no core cluster in the upper left-hand corner of the two-dimensional distance class analysis matrix), then the minimum number of clusters is $n + 1$, because groups of significant SCFs within the two-dimensional distance class analysis matrix are "reflections" of at least one additional cluster in the data set. "Edge effects" were interpreted as significant if more than two SCFs were significantly greater than expected in the $[X, Y]$ distance classes [0–7,7], [7,0–7] (the outermost row and column of the distance class analysis matrix). Conversely, "anti-edge" effects were interpreted as significant if more than two SCFs were significantly less than expected in the $[X, Y]$ distance classes [0–7,7], [7,0–7]. Row, column, and diagonal effects represented the maximum number of significant and adjacent SCFs per data set within the two-dimensional distance class analysis matrix (in the X, Y, and diagonal directions, respectively).

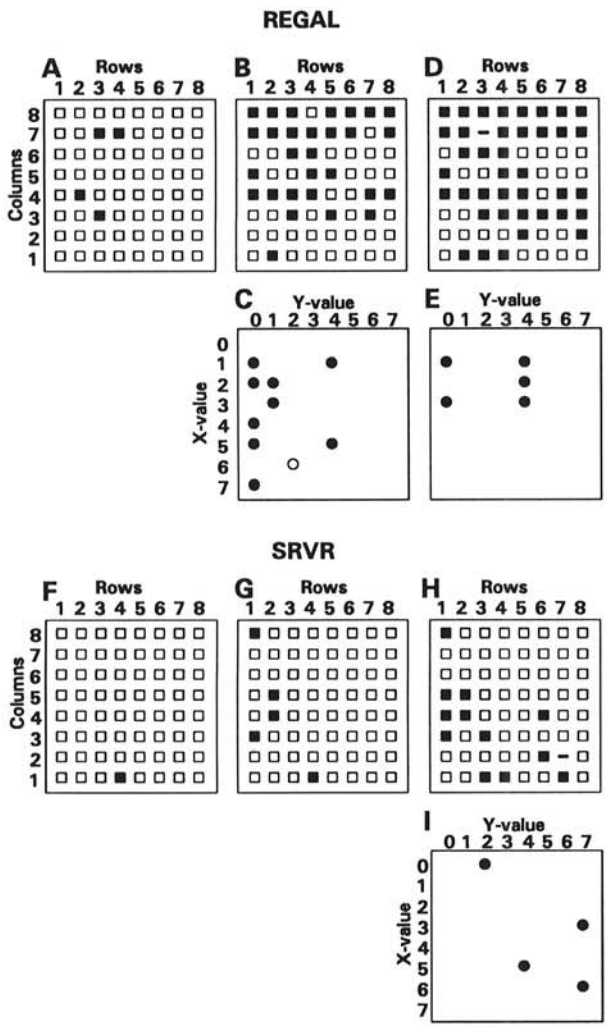

Fig. 1. Spatial patterns and two-dimensional distance class analyses of white clover plants infected or not infected with alfalfa mosaic virus, clover yellow vein virus, and/or peanut stunt virus in eight-column by eight-row lattices of white clover plants of cv. Regal and the Southern Regional Virus Resistant (SRVR) germ plasm in plot 4 in a 10-ha pasture of white clover and tall fescue grass in 1990. **A** and **F,** Maps of plants on 13 July. Symbols: □ = healthy plant, ■ = diseased plant, – = dead plant. Distance class analysis not conducted due to insufficient number of diseased plants. **B** and **G,** Maps of plants on 20 August; **D** and **H,** on 25 September. **C, E,** and **I** show two-dimensional distance class analyses of the data in the plots above them. Symbols: ● = $[X, Y]$ class with a standardized count frequency greater than expected ($P \leq 0.05$), ○ = $[X, Y]$ class with a standardized count frequency lower than expected ($P \geq 0.95$). Data sets without corresponding distance class analyses did not meet the minimum criteria for application of two-dimensional distance class analysis.

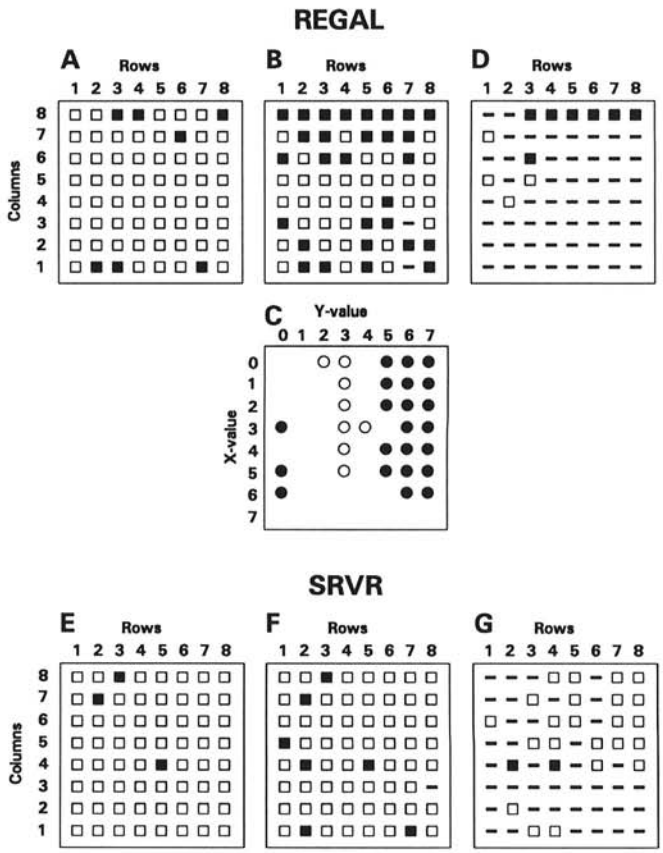

Fig. 2. Spatial patterns and two-dimensional distance class analyses of white clover plants infected or not infected with alfalfa mosaic virus, clover yellow vein virus, and/or peanut stunt virus in eight-column by eight-row lattices of white clover plants of cv. Regal and the Southern Regional Virus Resistant (SRVR) germ plasm in plot 1 in a 10-ha pasture of white clover and tall fescue grass in 1991. **A** and **E,** Maps of plants on 7 July. Symbols: □ = healthy plant, ■ = diseased plant, – = dead plant. **B** and **F,** Maps of plants on 6 August and **C,** two-dimensional distance class analysis of the data in **B**. Symbols: ● = $[X, Y]$ class with a standardized count frequency greater than expected ($P \leq 0.05$), ○ = $[X, Y]$ class with a standardized count frequency lower than expected ($P \geq 0.95$). **D** and **G,** Maps of plants on 16 September. Data sets without corresponding distance class analyses did not meet the minimum criteria for application of two-dimensional distance class analysis (insufficient numbers of diseased plants in **A** and **E,** insufficient numbers of living plants in **D** and **G**).

RESULTS

Incidence of viral diseases. PSV was detected in 91% of virus-infected plants in 1990 (99 of 109 plants by September) and in more than 99% of virus-infected plants in 1991. In 1990, CYVV and AMV were detected in only 14 and six plants, respectively, out of 512 plants. In 1991, only one plant was infected with CYVV and none were infected with AMV. Multiple infections (plants infected by more than one virus) were not common; five plants in 1990 and one plant in 1991 were infected with two viruses. Thus, the subsequent data and spatial analyses treat the virus mixture as a single disease comprising a disease complex that was dominated by PSV.

Regal, the virus-susceptible cultivar, had a significantly ($P = 0.05$) greater proportion of virus-infected plants than did SRVR, the virus-resistant germ plasm, at all sampling dates in both years. In 1990, the proportion of virus-infected plants for cv. Regal was 0.09 in July, 0.27 in August, and and 0.35 in September; the proportion of virus-infected plants for SRVR germ plasm was 0.03, 0.06, and 0.12. Similar trends and disease values were observed in 1991 (*data not shown*), although the overall incidence of virus-infected plants was greater in 1991 than in 1990.

Spatial patterns of virus-infected plants. With the exception of one plot on one date, spatial patterns of virus-infected plants were significantly nonrandom for populations of both Regal and the SRVR germ plasm in all plots and for all months for which two-dimensional distance class analysis was appropriate (e.g., Figs. 1A–I, 2A–G). Spatial attributes of the virus-infected populations (e.g., number and size of clusters of diseased plants, and the existence and magnitude of edge, row, and column effects) differed between plants of Regal and the SRVR germ plasm. For instance, the relative degree or strength of nonrandomness (indicated by the relative number of distance classes in which observed SCFs were significantly greater or fewer than expected under a random spatial pattern of diseased plants) generally was greater for cv. Regal than for the SRVR germ plasm. In 1990 and 1991, evidence for stronger overall aggregation and larger, more numerous and more distinctive clusters of virus-infected plants was detected in plots of Regal compared with SRVR germ plasm (Table 1). For example, in plot 4 in July 1990 (Fig. 1C) and in September 1990 (Fig. 1E) the significant $[X, Y]$ distance classes for plants of Regal generally were associated in tightly organized groups or adjacent pairs, indicating that diseased plants were more often closer together and in larger groups than would be expected with a random or nearly random spatial pattern. An examination of the mapped data for this plot (Fig. 1B and D) reveals two elongated, relatively discrete clusters of diseased plants, each approximately two rows wide. For the SRVR germ plasm in September 1990 in plot 4 (Fig. 1I), however, the significant distance classes are not close, which indicates much more loosely defined and less distinct patterns of aggregation. Similar patterns of discrete, compact, relatively larger clusters for Regal (Fig. 2B and C) and relatively fewer, indistinct, loose, and relatively smaller clusters of diseased plants for the SRVR germ plasm were observed in several other plots in 1991.

Populations of Regal and the SRVR germ plasm differed with regard to frequency and magnitude of edge effect. A significant and relatively strong edge effect or near-edge effect was found more often for Regal (plots 2 and 3 in 1990, *data not shown*) and plots 1–4 in 1991 than for the SRVR germ plasm. For example, a very strong edge effect was detected in plot 1, cv. Regal in August 1991 (Fig. 2B and C) as indicated by the significant $[X, Y]$ distance classes [0–6,7] in column 7 of the two-dimensional distance class analysis matrix (Fig. 2C) and in plot 4, cv. Regal in June 1991 (data not shown). Edge effects in these plots were found to be perpendicular to the east-west axis.

Leaf spot pathogens. Incidence and associations among leaf spot pathogens during this experiment were addressed previously (35). Briefly, black spot (*P. andropogonis*), summer blight (*R. solani*), and Stagonospora leaf spot (*S. meliloti*) were the most prevalent diseases in the plots and surrounding pasture in 1990 and 1991. Cercospora leaf spot (*C. zebrina*) and Curvularia leaf spot (*Curvularia trifolii*) were of lesser incidence, which restricted the application of two-dimensional distance class analysis for these diseases.

Black spot (*P. andropogonis*). Disease incidence data and spatial statistics generated by two-dimensional distance class analysis of 82 data sets are presented for black spot of white clover during 1990 and 1991 (Table 2). Similar, detailed analyses for the other leaf spot diseases are available (34). Only nine of the 60 data sets that met the minimum criteria for application of two-dimensional distance class analysis were characterized by

TABLE 1. Spatial statistics generated by two-dimensional distance class analysis of foliar diseases of white clover in experimental plots of cv. Regal (virus-susceptible) and Southern Regional Virus Resistant germ plasm in a 10-ha pasture of white clover and tall fescue during 1990 and 1991 in Wake County, North Carolina

	Data sets		Spatial pattern				1990			1991		
Pathogen	Possible no.	No. meeting criteria[a]	Random[b] (%)	Nonrandom[c] (%)	Strongly nonrandom[d] (% of nonrandom)	Uniform[e] (%)	Cluser size[f]	Cluster no.[g]	Edge effect[h]	Cluster size	Cluster no.	Edge effect
Virus complex[i]												
SRVR	24	5	0	100	40	0	2.7 (1–4)	1.7 (1–2)	1	1.0 (1)	1.0 (1)	1
Regal	24	10	10	90	89	0	3.0 (1–7)	2.8 (1–5)	2	5.6 (1–19)	2.4 (1–3)	3
Pseudomonas andropogonis	128	60	15	65	56	20	6.7 (1–19)	1.9 (1–3)	4	3.3 (1–8)	1.7 (1–3)	3
Rhizoctonia solani	128	76	12	88	58	0	3.4 (1–11)	2.0 (1–3)	1	3.4 (1–19)	2.0 (1–3)	6
Stagonospora meliloti	128	51	6	94	49	0	3.3 (1–10)	2.3 (1–4)	4	3.1 (1–11)	2.0 (1–3)	2
Curvularia trifolii	128	29	0	100	41	0	...	...	...	2.5 (1–5)	1.9 (1–3)	2
Cercospora zebrina	128	14	14	86	75	0	2.6 (1–3)	2.8 (2–3)	0	2.2 (1–3)	1.5 (1–2)	1

[a] Criteria for analysis by two-dimensional distance class method: more than eight and fewer than 55 diseased plants out of 64 plants per lattice (two lattices per plot) and fewer than 17 dead plants (missing values).
[b] Data sets in which the total number of significant SCF values (standardized count frequencies significantly greater or fewer than expected) were less than three.
[c] Data sets in which the total number of significant SCF values exceeded two.
[d] Expressed as a percentage of nonrandom sets. More than five significant SCF values.
[e] More than 55 diseased plants out of 64.
[f] Mean minimum core cluster size (range in parentheses) determined in one of two ways: 1) core cluster size was the number of adjacent, significant distance classes forming a contiguous group in the upper left-hand corner of the two-dimensional distance class analysis matrix (usually when only one discrete cluster of diseased plants is in the data set); or 2) the reflected core cluster size, defined as the number of significant and adjacent distance classes that form discrete, contiguous groups within the body of the two-dimensional distance class analysis matrix (an approximation or one of at least two clusters of diseased plants in the data set).
[g] Mean minimum number of clusters of diseased plants (range in parentheses).
[h] The total number of SCFs that were significantly greater than expected in the $[X, Y]$ distance classes [7,0–7] and [0–7,7] in the 8×8 two-dimensional distance class analysis matrix. Edge effects were considered to be significant if more than two SCFs were significantly greater than expected in the [X,Y] distance classes [7,0–7], [0–7,7]. Values represent number of host-plot combinations in which at least one significant edge effect was detected during 1990 and 1991.
[i] The viral disease complex comprised peanut stunt virus, clover yellow vein virus, and alfalfa mosaic virus.

a random spatial pattern (Table 1). Thirty-nine data sets were interpreted as having a significantly nonrandom pattern of diseased plants; 22 of these 39 data sets were characterized as strongly nonrandom. Twelve data sets had a uniform spatial pattern.

Temporal changes within plots with regard to shifts from overall randomness to nonrandomness (or vice versa) were observed in one plot in 1990 (plot 1 SRVR) and in most plots during 1991. Shifts from significant nonrandom spatial patterns to random spatial patterns occurred at various times throughout the growth periods and followed no consistent trend. In 1991, changes in disease incidence (e.g., plot 1 SRVR, plot 2 Regal, plot 4 SRVR), or midseason harvests (e.g., plot 2 SRVR) were followed by a shift from aggregation to randomness in some plots (Table 2).

Plants with black spot tended to occur in closer proximity than would be expected with a random spatial pattern. Cluster size (the minimum core and/or reflected) was highly variable between years and among plots and sampling dates. For example, core and/or reflected cluster size ranged from one (plot 4 SRVR on 21 July) to 19 (plot 4 SRVR on 23 August) in 1990 and from one to eight in 1991. Examination of Table 2 (e.g., plots 1 and 4, SRVR and Regal in 1990) confirms that, generally, larger clusters of *Pseudomonas*-infected plants were found in 1990. These larger clusters were associated with plots that had a higher disease incidence. Cluster size tended to increase over time within or between growth periods in some plots, especially after the midseason harvest (e.g., after harvest in plot 1 SRVR and plot 4 SRVR in 1990, plot 1 Regal in 1991). In other plots, cluster size decreased with time after the midseason harvest (e.g., plot 4 SRVR and Regal in 1990) (Table 2).

Cluster shape in 1990 and 1991 for black spot was also variable.

TABLE 2. Incidence of black spot of white clover (*Trifolium repens*) caused by *Pseudomonas andropogonis* in field plots in Wake County, North Carolina in 1990 and 1991, and spatial statistics generated by two-dimensional distance class analysis

Plot[a]		No. of plants[d]		Significance[e]						Effect[j]		
Host[b]	Date[c]	Infected	Dead	SCF+	SCF−	Pattern[f]	Core[g]	Shape[h]	Cluster[i]	Edge	Row	Col
1990												
Plot 1												
SRVR	21 Jul	43	1	1	0	R	...[k]	...	...	...	...	...
	2 Aug	49	1	3	0	A	2	/	2	0	1	1
	*23 Aug	42	1	1	1	R	...	...	...	...	...	...
	7 Sept	45	5	18	7	A	16	□	2	11	2	8
	22 Sept	35	7	8	1	A	7	L	2	0	4	2
Regal	21 Jul	54	0	12	3	A	12	□	2	0	7	2
	2 Aug	59	0	...	...	U	...	...	...	...	...	...
	*23 Aug	43	0	3	0	A	3*	−	1	0	3	1
	7 Sept	60	0	...	...	U	...	...	...	...	...	...
	22 Sept	50	1	4	2	A	2*	−	1	1	2	1
Plot 2												
SRVR	21 July	43	0	9	2	A	5	1	2	6	1	5
	2 Aug	59	0	...	...	U	...	...	...	...	...	...
	*23 Aug	41	0	2	3	A	2	/	2	0	1	1
	7 Sept	56	0	...	...	U	...	...	...	...	...	...
	22 Sept	57	0	...	...	U	...	...	...	...	...	...
Regal	21 Jul	57	0	...	...	U	...	...	...	...	...	...
	2 Aug	64	0	...	...	U	...	...	...	...	...	...
	*23 Aug	49	0	4	1	A	2*	/,1	3	0	1	2
	7 Sept	60	0	...	...	U	...	...	...	...	...	...
	22 Sept	61	1	...	...	U	...	...	...	...	...	...
Plot 4												
SRVR	21 Jul	49	0	3	0	A	1	~	1	1	1	1
	2 Aug	61	0	...	...	U	...	...	...	...	...	...
	*23 Aug	54	0	23	6	A	19	□	2	3	8	4
	7 Sept	52	1	12	2	A	10	□	2	6	5	3
	22 Sept	53	1	13	3	A	11	□	2	3	7	3
Regal	21 Jul	57	0	...	...	U	...	...	...	...	...	...
	2 Aug	59	0	...	...	U	...	...	...	...	...	...
	*23 Aug	51	0	19	7	A	2,16	−,□	3	12	2	8
	7 Sept	47	0	4	1	A	2*	1	1	0	1	3
	22 Sept	45	1	3	1	A	2	/	2	0	1	1

(*continued on next page*)

[a] Data from plot 3 in 1990 are omitted, due to poor plant establishment. Data not shown for plots 3 and 4 after midseason harvest, due to extensive plant death.

[b] Southern Regional Virus Resistant white clover germ plasm and white clover cv. Regal.

[c] Plots were harvested 4 Aug 1990 and on 24 July 1991 and plants were allowed to re-grow. Data for some dates omitted, due to absence of symptoms of black spot. Asterisk signifies the first disease assessment after harvest of growth period 1.

[d] Number of white clover plants with symptoms of black spot and number of dead plants out of a total of 64 white clover plants in eight-column by eight-row plant matrices in a clover–tall fescue pasture.

[e] Number of $[X, Y]$ distance classes in which the observed standardized count frequency (SCF) was significantly greater (+) or less (−) than expected under a random spatial pattern. Plots of 64 plants in which fewer than nine or more than 55 were diseased were not analyzed, nor were data sets with >16 dead plants.

[f] R = Random (total number of + or − significant SCFs <3), A = aggregated (total number of + or − significant SCFs >2), and U = uniform (total number of diseased plants >55).

[g] Minimum core cluster size, determined in two ways: 1) core cluster size (followed by * when >1), the number of significant and adjacent distance classes forming a contiguous group in the upper left hand corner of the two-dimensional distance class analysis matrix (usually when there is only one discrete cluster of diseased plants in the plot); and 2) the reflected core cluster size, defined as the number of significant and adjacent distance classes that form discrete groups within the $[X, Y]$ distance class analysis matrix (a reflection, or approximation of one of at least two clusters of diseased plants in the plot).

[h] Shape symbols represent clusters of significant SCFs in the distance class analysis matrix. Symbol L = cluster resembling the letter. Symbols □ = roughly rectangular, ○ = roughly isodiametric, / = cluster along diagonals of the plot, − = horizontal, 1 = vertical, and ~ = irregular, amorphous shape, which may be a combination of others. Rows are parallel with east-west axis in plots 1 and 4, columns parallel with the east-west axis in plots 2 and 3.

[i] Minimum number of clusters of diseased plants in the plot.

[j] Edge effect is the total number of SCFs that were significantly greater than expected in the $[X, Y]$ distance classes [7,0–7], [0–7,7] in the two-dimensional distance class analysis matrix. Edge effect was significant if more than two SCFs were significantly greater than expected. Negative numbers indicate number of SCFs that were significantly less than expected in the $[X, Y]$ distance classes [7,0–7], [0–7,7]. Row and column (Col) effects are the number of significant, adjacent SCFs detected in the distance class analysis matrix. For row and column effects that were adjacent to the $[X, Y]$ distance class [0,0], a value of one was added to the total number of adjacent, significant SCFs.

[k] Indicates that either minimum criteria for two-dimensional distance class analysis were not satisfied or cluster attributes were not listed for random or uniform data sets.

Larger clusters were rectangular (□) or nearly rectangular (L), or roughly isodiametric (○) (Table 2). Smaller clusters reflected small, within-row (−) or within-column (I) aggregates of diseased plants. Approximate cluster number ranged from one to three in both years. Mean cluster number (per 64-plant lattice) was 1.9 in 1990 and 1.7 in 1991 (Table 1).

Significant edge effects were detected in four of six host-plot combinations in 1990 and in three of eight host-plot combinations during 1991, but in only 10 out of the total number of data sets. Two plots were characterized by a significant, strong, early-season edge effect: plot 2 SRVR in 1990 and 1991. Other strong edge effects were detected during growth period 2 (after the midseason harvest) (e.g., plot 4 cv. Regal, 23 August 1990) (Table 2).

Significant row and/or column effects were detected in most data sets, indicating that diseased plants tended to occur in closer proximity within rows or columns of the plots. Significant diagonal effects were detected with less frequency than were significant row and column effects. Variability and temporal shifts among and within host-plot combinations within growth periods and between years was observed for row and column effects. Row and/or column effects were not aligned consistently with either the east-west or north-south axes.

Spatial attributes of black spot epidemics were similar for populations of Regal and the SRVR germ plasm with regard to overall disease incidence, existence and strength of nonrandomness, core and reflected cluster sizes, cluster shapes, and the number and magnitude of edge, row, and column effects. Some commonly observed spatial attributes that are characteristic of black spot are presented (Fig. 3A–T). For example, several groups of significant $[X, Y]$ distance classes in the two-dimensional distance class analysis matrices indicate that diseased plants were often closer than expected with a random spatial pattern, e.g., distance classes [3,7], [4,6–7], [5,5–7] and [6,5–7] (Fig. 3H); and [1,0], [0–1,1], [2–5,6], [4–7,7] (Fig. 3J). Significant edge, row, column and/or diagonal effects were detected, and the minimum number of

TABLE 2. (*continued from preceding page*)

Plot[a] Host[b]	Date[c]	No. of plants[d] Infected	Dead	Significance[e] SCF+	SCF−	Pattern[f]	Core[g]	Shape[h]	Cluster[i]	Effect[j] Edge	Row	Col
1991												
Plot 1												
SRVR	24 Jun	1	0	...	...	...	...	...	...	...	...	...
	1 Jul	20	0	5	2	A	3	L	2	0	2	2
	8 Jul	31	0	6	2	A	7*	□	1	0	3	3
	15 Jul	34	0	3	2	A	2	I	2	0	1	2
	*12 Aug	24	5	6	5	A	7*	○	1	0	3	3
	26 Aug	39	6	1	0	R	...	...	...	...	...	...
	2 Sept	40	8	7	1	A	3*	○	3	2	3	1
	9 Sept	36	14	1	0	R	...	...	...	...	...	...
	16 Sept	24	19	...	...	...	...	...	...	...	...	...
Regal	24 Jun	2	0	...	...	...	...	...	...	...	...	...
	1 Jul	15	0	2	0	R	...	...	...	...	...	...
	8 Jul	30	0	2	2	A	1	~	1	1	1	1
	15 Jul	25	0	5	0	A	1	~	1	0	1	1
	*12 Aug	14	10	11	1	A	3*,9	○	2	4	3	4
	26 Aug	27	14	11	2	A	4*,8	○,I	2	4	2	4
	2 Sept	31	20	...	...	...	...	...	...	...	...	...
	9 Sept	24	26	...	...	...	...	...	...	...	...	...
	16 Sept	18	32	...	...	...	...	...	...	...	...	...
Plot 2												
SRVR	24 Jun	2	0	...	...	...	...	...	...	...	...	...
	1 Jul	17	0	7	0	A	2	I	2	4	1	2
	8 Jul	33	0	5	0	A	2	/	2	2	1	1
	15 Jul	46	0	4	1	A	2	−	2	0	2	1
	*12 Aug	48	0	1	1	R	...	...	...	...	...	...
	26 Aug	55	0	3	1	A	2*	−	1	0	2	1
	2 Sept	58	0	...	...	...	...	...	...	...	...	...
	9 Sept	56	2	...	...	...	...	...	...	...	...	...
	16 Sept	46	11	8	5	A	8	□	1	−2	2	3
Regal	24 Jun	2	0	...	...	...	...	...	...	...	...	...
	1 Jul	21	0	5	1	A	2	/	2	0	1	1
	8 Jul	31	0	6	2	A	1	~	1	1	1	1
	15 Jul	39	0	0	1	R	...	...	...	...	...	...
	*12 Aug	43	5	9	4	A	6	□	2	1	3	2
	26 Aug	52	5	2	0	R	...	...	...	...	...	...
	2 Sept	55	5	...	...	...	...	...	...	...	...	...
	9 Sept	51	9	...	...	...	...	...	...	...	...	...
	16 Sept	45	17	...	...	...	...	...	...	...	...	...
Plot 3												
SRVR	24 Jun	1	0	...	...	...	...	...	...	...	...	...
	1 Jul	8	0	...	...	...	...	...	...	...	...	...
	8 Jul	25	0	4	1	A	2	−	2	1	2	1
	15 Jul	34	0	2	1	A	2	I	2	0	1	2
Regal	24 Jun	0	3	...	...	...	...	...	...	...	...	...
	1 Jul	12	3	3	0	A	1	~	1	2	1	1
	8 Jul	14	3	4	0	A	2	/	2	1	1	1
	15 Jul	22	6	3	0	A	2	I	2	0	1	2
Plot 4												
SRVR	24 Jun	1	0	...	...	...	...	...	...	...	...	...
	1 Jul	16	0	5	0	A	2*	I	1	0	1	2
	8 Jul	34	0	1	0	R	...	...	...	...	...	...
	15 Jul	37	1	7	4	A	5	I	2	3	2	3
Regal	24 Jun	0	0	...	...	...	...	...	...	...	...	...
	1 Jul	1	0	...	...	...	...	...	...	...	...	...
	8 Jul	3	0	...	...	...	...	...	...	...	...	...
	15 Jul	6	2	...	...	...	...	...	...	...	...	...

clusters detected by two-dimensional distance class analysis ranged from roughly one to two in this plot. An increase in cluster size over time is evident in an examination of the mapped data and two-dimensional distance class analysis of plot 1 cv. Regal (Fig. 3A–J), wherein an increase in reflected minimum cluster size was detected from 15 July to 12 August (Fig. 3F and H). A commonly observed effect of plot harvest (e.g., harvest on 24 July 1991) upon increased departure from nonrandomness and disruption or change in cluster morphologies was detected for both cv. Regal and the SRVR germ plasm in this plot (Fig. 3H versus 3F and 3R versus 3P).

Summer blight (*R. solani*). Seventy-six data sets for summer blight met the minimum criteria for two-dimensional distance class analysis in 1990 and 1991 (Table 1). Nine data sets were classified as having a random spatial pattern (one in 1990 and eight in 1991). The remaining 67 data sets were characterized as having a significantly nonrandom (aggregated) spatial pattern. Thirty-nine of these data sets were classified as strongly nonrandom. None of the plots at any date was classified as having a uniform spatial pattern.

Diseased pairs of plants tended to be closer than would be expected with random, or nearly random, spatial patterns. Long runs of *Rhizoctonia*-infected plants were observed in almost every plot for Regal and the SRVR germ plasm during 1990 and 1991 (e.g., Fig. 4); significant and strong row and column effects were detected in most data sets. The significant row and column effects were found to be variable through time (e.g., significance and magnitude of the effects were not always consistent between successive rating days). Row and column effects for summer blight were not found to be aligned consistently with either the east-west or north-south axes.

Cluster characteristics and morphology for summer blight were similar for populations of Regal and the SRVR germ plasm and between growth periods and years. For example, the minimum core and/or minimum reflected core cluster size ranged from one to 11 (mean 3.4) in 1990 and from one to 19 (mean 3.4) in 1991. Minimum cluster number ranged from one to three (mean 2.0) in 1990 and from one to four (mean 2.0) in 1991. Cluster shape was highly variable. Shapes for smaller clusters (I, −, /) represented significant within row or column or diagonal effects. Larger

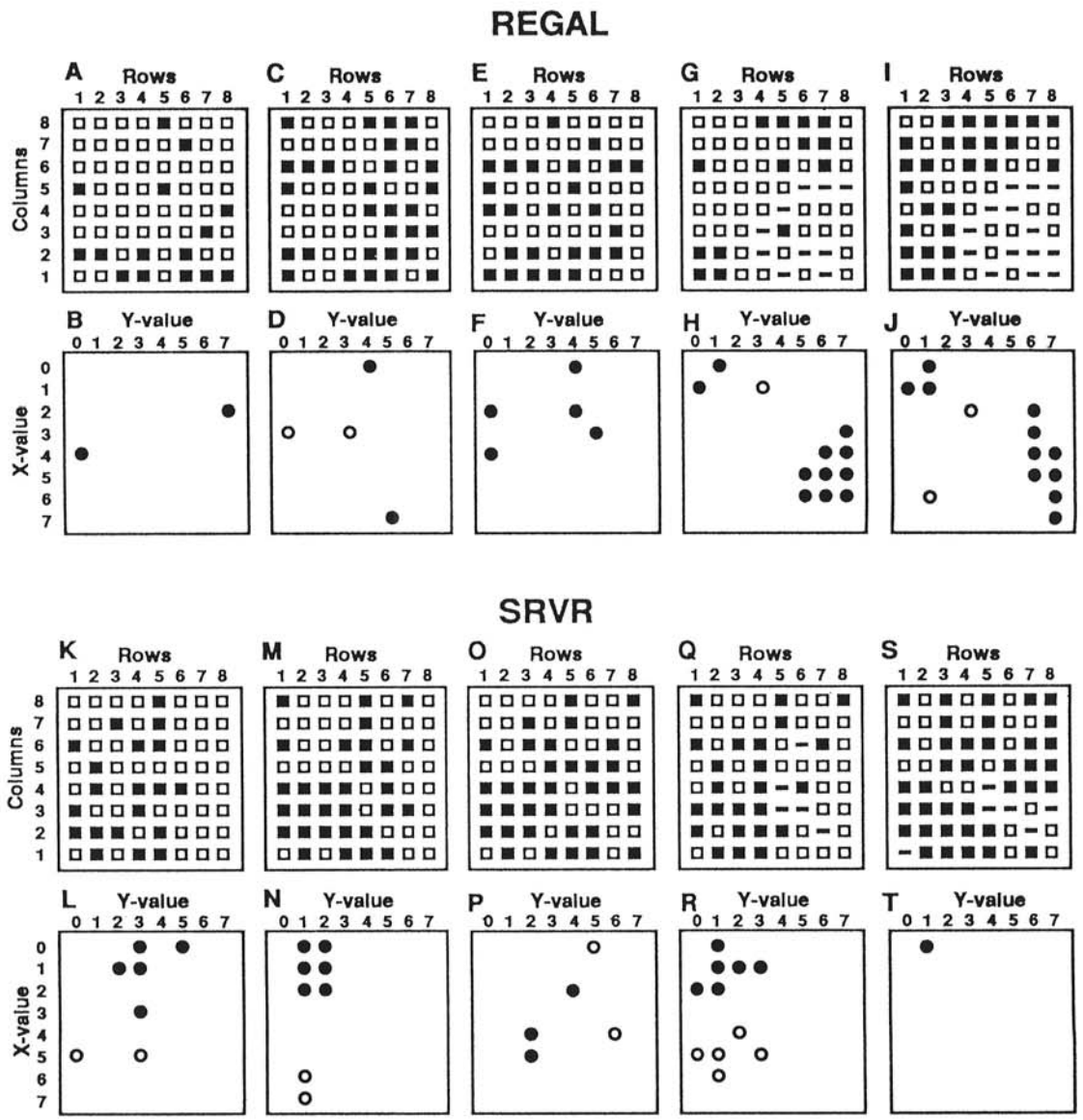

Fig. 3. Spatial patterns and two-dimensional distance class analyses of white clover plants infected or not infected with *Pseudomonas andropogonis* in eight-column by eight-row lattices of white clover plants of cv. Regal and the Southern Regional Virus Resistant (SRVR) germ plasm in plot 1 in a 10-ha pasture of white clover and tall fescue grass in 1991. **A** and **K,** Maps of plants on 1 July. Symbols: □ = healthy plant, ■ = diseased plant, − = dead plant. **C** and **M,** Maps of plants on 8 July; **E** and **O,** on 15 July (harvest date was 4 August); **G** and **Q,** on 12 August; and **I** and **S,** on 26 August. **B, D, F, H, J, L, N, P, R,** and **T** show two-dimensional distance class analyses of the data in the plots above them. Symbols: ● = $[X, Y]$ class with a standardized count frequency greater than expected ($P \leq 0.05$), ○ = $[X, Y]$ class with a standardized count frequency lower than expected ($P \geq 0.95$).

clusters were found to have roughly rectangular or isodiametric shapes. However, some large clusters arose from lengthy, within or across row aggregation (e.g., plot 4 SRVR on 22 September 1990) (34).

Significant edge effects were found for one of six host-plot combinations in 1990, for all host-plot combinations in 1991, and for a total of 20 data sets during 1990 and 1991. Significant edge effects were relatively stronger and more frequently detected in 1991. No significant anti-edge effects were detected for summer blight during 1990 and 1991.

Within plots, plants of Regal and SRVR responded similarly to harvest with regard to disease incidence. Incidence of summer blight generally decreased (range 14–42%) after harvest in 1990 and 1991, although an increase in disease incidence was observed in plot 4 in 1990. Intrinsic spatial patterns (e.g., intrinsically random or nonrandom) were not altered significantly by harvest. Plots characterized as having intrinsically nonrandom or aggregated spatial patterns before harvest had the same spatial patterns after harvest. However, the effects of harvest upon degree of departure from nonrandomness and spatial attributes of clusters of plants infected by *R. solani* on white clover followed no consistent patterns. For example, in some plots during 1990 and 1991, cluster size and number or degree of departure from nonrandomness increased after harvest (e.g., plot 1 cv. Regal in 1991). The opposite effect was observed for other plots.

Important characteristics of summer blight observed frequently during 1990 and 1991 were great temporal variability with regard to location of diseased individuals, size and shape of clusters, and degree of departure from nonrandomness; cluster size and morphology; and significant row, column, and edge effects (Fig. 4A–S). Symptoms of summer blight on specific plants often were not constant or consistent between sampling dates. For example, in plot 2 (SRVR) during growth period 1 in 1991, 17 plants exhibited symptoms of summer blight (Fig. 4J). Two-dimensional distance class analysis indicated a strong and significant departure from randomness (Fig. 4K). Three groups of three significant

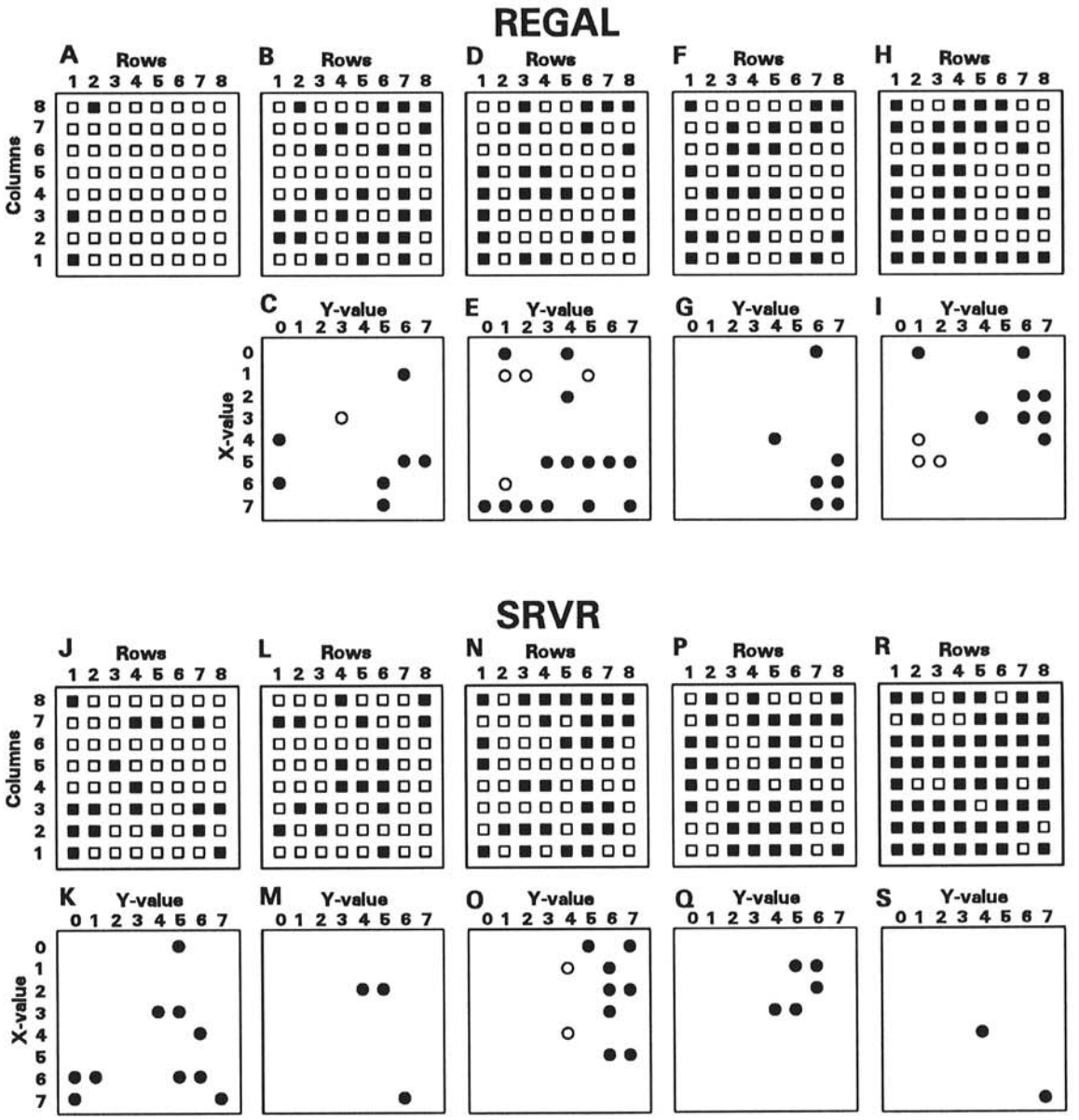

Fig. 4. Spatial patterns and two-dimensional distance class analyses of white clover plants infected or not infected with *Rhizoctonia solani* in eight-column by eight-row lattices of white clover plants of cv. Regal and the Southern Regional Virus Resistant (SRVR) germ plasm in plot 2 in a 10-ha pasture of white clover and tall fescue grass in 1991. **A** and **J,** Maps of plants on 10 June. Symbols: □ = healthy plant, ■ = diseased plant, − = dead plant. **B** and **L,** Maps of plants on 17 June; **D** and **N,** on 24 June; **F** and **P,** on 1 July; and **H** and **R,** on 8 July. **C, E, G, I, K, M, O, Q,** and **S** show two-dimensional distance class analyses of the data in plots above them. Symbols: ● = $[X, Y]$ class with a standardized count frequency greater than expected ($P \leq 0.05$), ○ = $[X, Y]$ class with a standardized count frequency lower than expected ($P \geq 0.95$). Data sets without corresponding distance class analyses did not meet the minimum criteria for application of two-dimensional distance class analysis.

[X, Y] distance classes indicate the presence of at least four relatively small aggregates of diseased plants in the plot. One week later, 18 plants were symptomatic, but 12 of the plants that were diseased in the previous week had no detectable symptoms of summer blight the following week. The resulting spatial pattern was nearly random (three significant SCFs), with seven fewer significant SCFs than the previous week. In the third disease assessment in the following week, the spatial pattern was characterized as significantly and strongly nonrandom. Significant edge, row and column effects and a general increase in disease incidence during this growth period are evident in an examination of the mapped data and two-dimensional distance class analyses for this plot.

Stagonospora leaf spot (*S. meliloti*). A total of 51 data sets for Stagonospora leaf spot met the minimum criteria for two-dimensional distance class analysis (Table 1). Spatial patterns for 48 of the 51 data sets were characterized as significantly nonrandom; only three data sets had a random or nearly random spatial pattern (one in 1990 and two in 1991). The spatial patterns for 25 data sets were classified as strongly nonrandom (highly aggregated). Uniform spatial patterns were not observed for Stagonospora leaf spot.

Diseased pairs of plants tended to occur in closer proximity than would be expected with random spatial patterns. Lengthy runs and/or tight clusters of *Stagonospora*-infected plants were observed often during 1990 and 1991. Significant and relatively strong row and column effects, or significant associations in clusters along diagonals were detected in every plot on every sampling date for Stagonospora leaf spot. In 1990, significant row and column effects ranged from one to eight and from one to six, respectively. In 1991, significant row and column effects ranged from one to four and from one to six, respectively. Significance of row and column effects was temporally variable, and no specific and consistent orientation with either the east-west

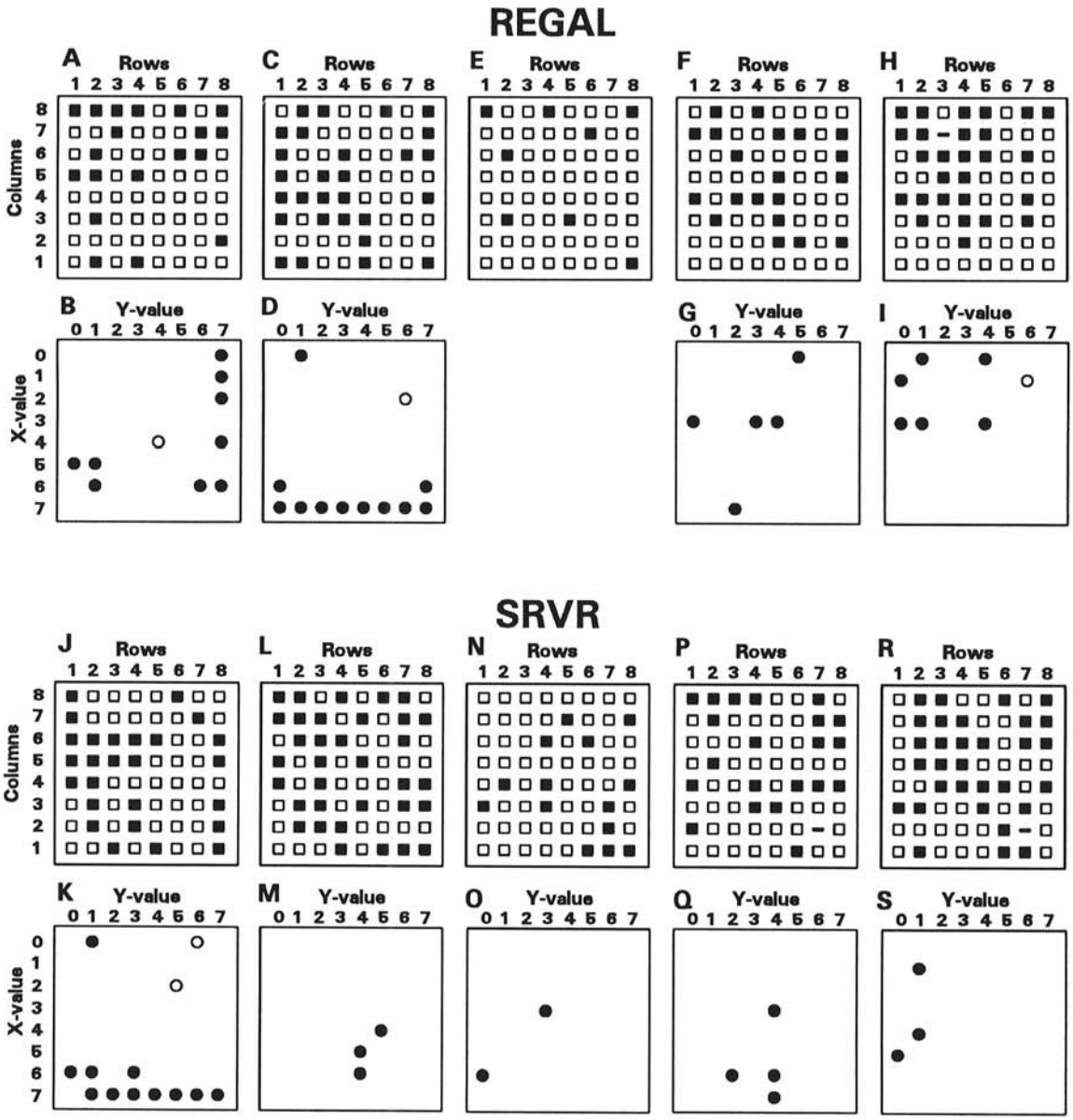

Fig. 5. Spatial patterns and two-dimensional distance class analyses of white clover plants infected or not infected with *Stagonospora meliloti* in eight-column by eight-row lattices of white clover plants of cv. Regal and the Southern Regional Virus Resistant (SRVR) germ plasm in plot 4 in a 10-ha pasture of white clover and tall fescue grass in 1990. **A** and **J**, Maps of plants on 21 July. Symbols: □ = healthy plant, ■ = diseased plant, − = dead plant. **C** and **L**, Maps of plants on 2 August; **E** and **N**, on 23 August; **F** and **P**, on 7 September; and **H** and **R**, on 22 September. **B, D, G, I, K, M, O, Q,** and **S** show two-dimensional distance class analyses of the data in plots above them. Symbols: ■ = [X, Y] class with a standardized count frequency greater than expected ($P \leq 0.05$), ○ = [X, Y] class with a standardized count frequency lower than expected ($P \geq 0.95$). Data sets without corresponding distance class analyses did not meet the minimum criteria for application of two-dimensional distance class analysis.

or north-south axes was observed. However, the relative magnitude (number of plants in a run) was greater for most plots during the earliest disease ratings in 1990 and 1991 (e.g., plot 1 SRVR, plot 4 SRVR, plot 4 Regal during 1991, *data not shown*) (34).

Minimum core cluster and/or reflected core cluster size was similar between clover germ plasm and years and ranged from one to 10 (mean 3.3) in 1990 and from one to 11 (mean 3.1) in 1991 (Table 1). Minimum number of clusters per grid of 64 plants for each host (means in parentheses) ranged from one to four (mean 2.3) in 1990 and from one to three (mean 2.0) in 1991. Cluster shape was highly variable and followed no discernible or consistent pattern with respect to plot axes. Roughly rectangular, isodiametric, and within row or column aggregates were detected most frequently.

Significant edge effects were detected in four of six host-plot combinations in 1990 and in two of six in 1991. In 1990 and 1991, several of the significant edge effects were detected in early to midseason disease ratings as Stagonospora leaf spot became established in plots and disease incidence increased (e.g., plot 4 in 1990) (34). Significant edge effects were detected in a total of seven of the 51 data sets analyzed during 1990 and 1991.

Plot harvest had significant effects upon incidence and spatial patterns of Stagonospora leaf spot. In 1990, incidence of Stagonospora leaf spot declined by an average of 63.3% from 2 August (the last disease assessment date in growth period one) to 23 August (the first disease assessment date in growth period two). In 1991, the decline in incidence of Stagonospora leaf spot followed a similar trend. Harvest also affected strength of aggregation and cluster size and morphology. In general, the strength of aggregation decreased (fewer significant $[X, Y]$ distance classes were detected after harvest) and minimum core cluster size and minimum reflected cluster size was diminished. For example, in plot 2 (Regal) in 1991, incidence of Stagonospora leaf spot declined by 53.8% after harvest of growth period one, the number of significant $[X, Y]$ distance classes declined by eight (six positive and two negative), and the cluster size decreased from 11 to two

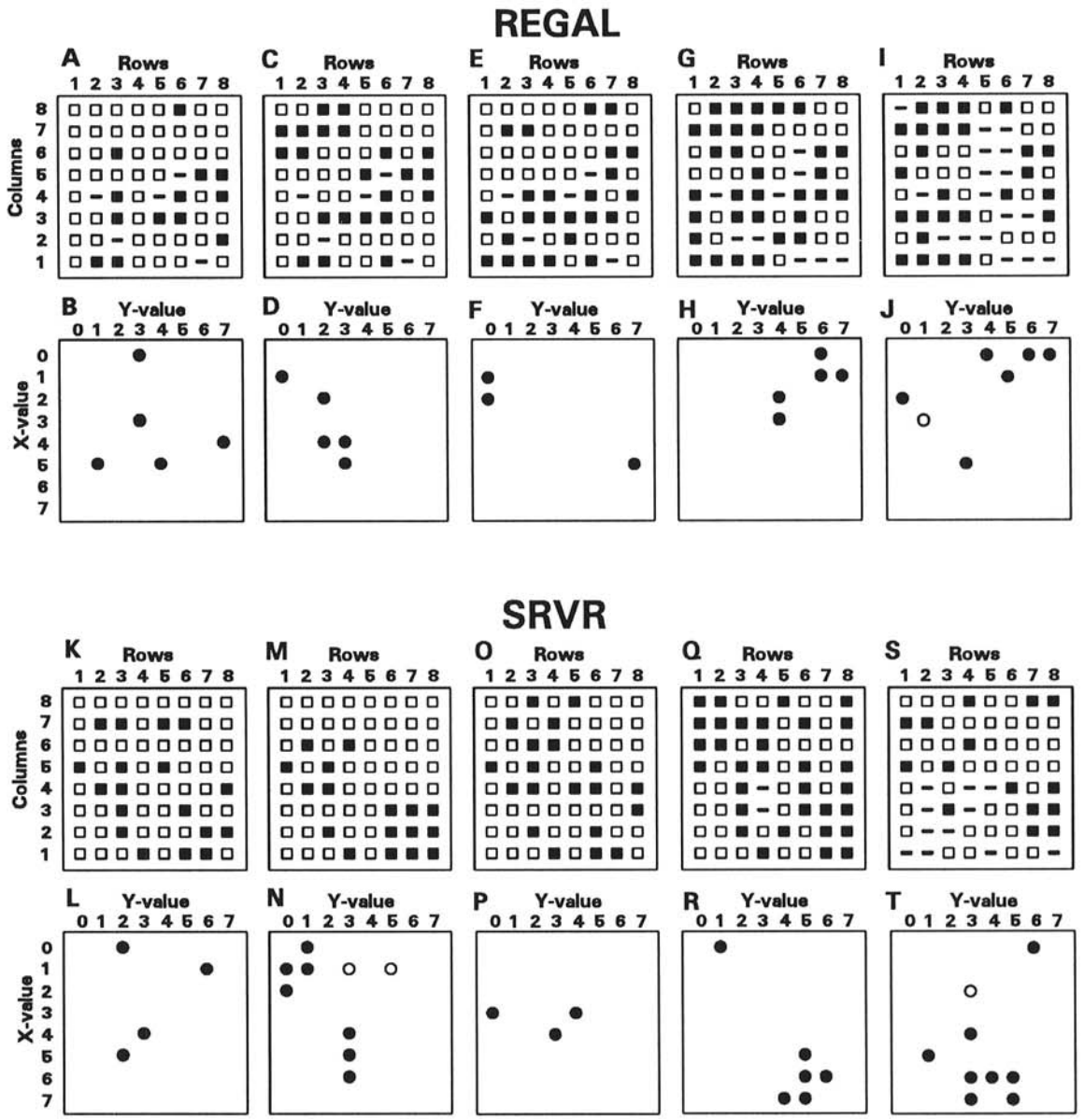

Fig. 6. Spatial patterns and two-dimensional distance class analyses of white clover plants infected or not infected with *Curvularia trifolii* in eight-column by eight-row lattices of white clover plants of cv. Regal and the Southern Regional Virus Resistant (SRVR) germ plasm in plot 2 in a 10-ha pasture of white clover and tall fescue grass in 1991. **A** and **K,** Maps of plants on 12 August. Symbols: □ = healthy plant, ■ = diseased plant, − = dead plant. **C** and **M,** Maps of plants on 26 August; **E** and **O,** on 2 September; **G** and **Q,** on 9 September (harvest date was 4 August); and **I** and **S,** on 16 September. **B, D, F, H, J, L, N, P, R,** and **T** show two-dimensional distance class analyses of the data in plots above them. Symbols: ● = $[X, Y]$ class with a standardized count frequency greater than expected ($P \leq 0.05$), ○ = $[X, Y]$ class with a standardized count frequency lower than expected ($P \geq 0.95$).

and three. In some plots (e.g., plot 2 Regal 1990) cluster size began to increase in the weeks subsequent to the first week after harvest.

Some of the important spatial characteristics of Stagonospora leaf spot observed during 1990 and 1991 were as follows (Fig. 5A–S): diseased pairs in close proximity (i.e., significant and adjacent SCFs in the two-dimensional distance class analysis matrices); strong, early to midseason edge effects; significant row and column effects; and the effect of harvest (on 2 August 1990) upon disease incidence and the spatial structure of the epidemic. For instance, strong edge effects are evidenced by those significant $[X, Y]$ distance classes in the row and/or column 7 of the two-dimensional distance class analysis matrices (Fig. 5B, D, and K). The disruptive effect of harvest upon spatial patterns is evident through an examination of maps of the observed data (before and after harvest) and the corresponding two-dimensional distance class analyses (Fig. 5C, D, E, L, M, N, and O). For example, harvest disrupted the significant edge effect that was detected for cv. Regal (Fig. 5C and D versus Fig. 5E). Harvest also disrupted cluster size and morphology, or the tendency for diseased pairs to be in very close proximity for the SRVR germ plasm (as evidenced by the clustering of significant SCFs in Fig. 5M and lack of such clustering in Fig. 5O).

Curvularia leaf spot (*Curvularia trifolii*). Curvularia leaf spot was not detected in the experimental plots until late August 1990, when occurrence of the disease was limited to only a few plants in plot 2 (36). In 1991, incidence of Curvularia leaf spot began to increase, and was greatest in plots 2 and 1, wherein disease incidence values were similar for plants of Regal and the SRVR germ plasm. Low incidence of Curvularia leaf spot in plots 3 and 4 in 1991 prevented spatial pattern analysis for data from these plots.

Twenty-nine data sets for Curvularia leaf spot in 1991 met the minimum criteria for application of two-dimensional distance class analysis (Table 1). All 29 data sets were characterized as having significantly nonrandom, or aggregated spatial patterns. Twelve of the 29 data sets were classified as strongly aggregated. None of the plots at any date had a uniform spatial pattern of *Curvularia*-infected plants.

Pairs of *Curvularia*-infected plants tended to occur in closer proximity within rows and columns of plants than would be expected with random spatial arrangement of diseased plants. Significant row, column, and diagonal effects were detected for each host-plot combination and in 22 of the 29 data sets. In general, clusters of plants with Curvularia leaf spot were smaller and fewer than clusters of plants infected by *P. andropogonis, R. solani,* and *S. meliloti*. Minimum core cluster size and/or minimum reflected core cluster size ranged from one to five (mean 2.5). Minimum cluster number ranged from one to three (mean 1.9). Significant edge effects were detected in two of four host-plot combinations but in only three of the 29 data sets. The significant edge effects were relatively weaker than those for *P. andropogonis, R. solani,* and *S. meliloti*. Cluster shape was not often rectangular or isodiametric but usually was represented by shorter, discrete runs of diseased plants within and across rows.

The effect of midseason harvest upon incidence and patterns of Curvularia leaf spot was variable. For example, incidence of Curvularia leaf spot declined after harvest for plot 1 (for Regal and the SRVR germ plasm), whereas disease incidence increased or remained constant in plot 2. Influence of harvest upon relative strength of aggregation and cluster size, number, and morphology also varied and no consistent trends were observed.

Several characteristics of Curvularia leaf spot were observed during 1991 (Fig. 6A–T): diseased pairs of plants in close proximity, a general lack of significant edge effects, relatively small and tight and/or small and loosely defined aggregates of diseased plants, and a higher disease incidence in the second growth period of 1991. The lack of strong edge effects is evidenced by the overall absence of significant $[X, Y]$ distance classes in row 7 and column 7 of the distance class analysis matrices (Fig. 6B, D, F, H, J, L, N, P, R, and T). Plants infected with *Curvularia trifolii* tended to occur in the central regions of the plots (Fig. 6A, C, E, G, I, L, N, P, R, and T). For most plots at the beginning of growth periods, strength of aggregation (number and organization of significant SCFs) was relatively weaker than in most other subsequent weeks (e.g., the SRVR germ plasm, Fig. 6K–T). Evident in the first week of the growth period (Fig. 6A, B, K, and L) was the tendency for clusters to be less well-defined (note the relative 'scatter' of significant SCFs in Fig. 6B and L) than in subsequent weeks. As Curvularia leaf spot spread

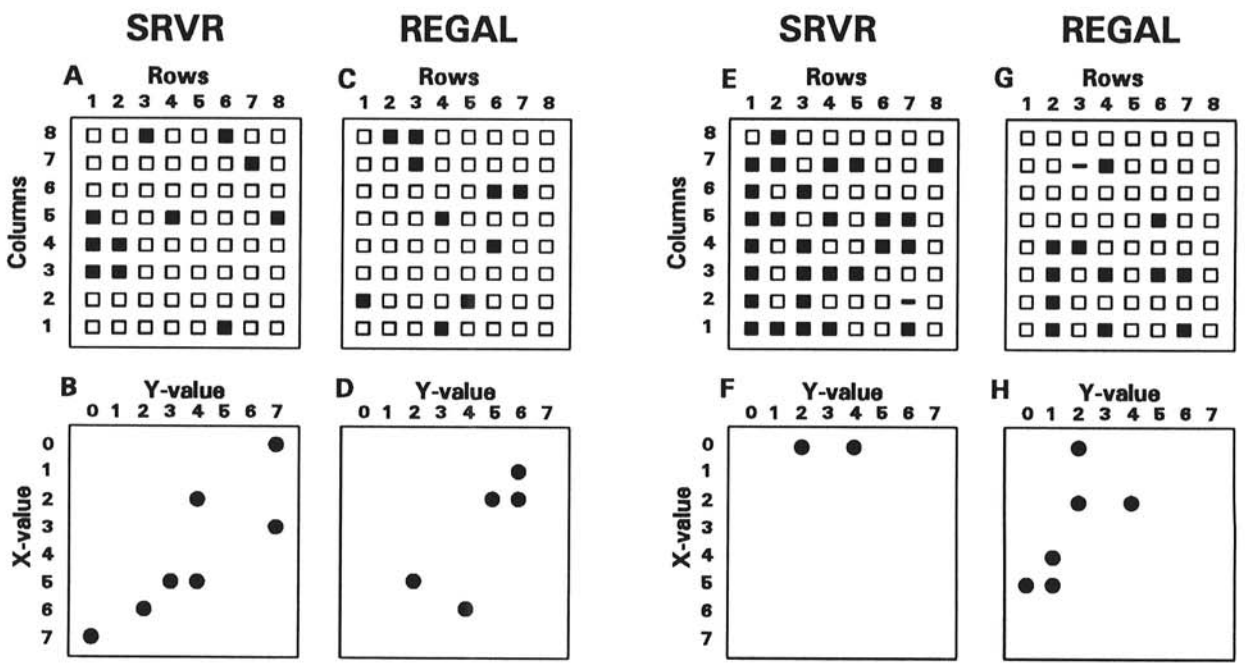

Fig. 7. Spatial patterns and two-dimensional distance class analyses of white clover plants infected or not infected with *Cercospora zebrina* in eight-column by eight-row lattices of white clover plants of cv. Regal and the Southern Regional Virus Resistant (SRVR) germ plasm in plot 4 in a 10-ha pasture of white clover and tall fescue grass in 1990. **A** and **C**, Maps of plants on 21 July. Symbols: □ = healthy plant, ■ = diseased plant, − = dead plant. **E** and **G**, Maps of plants on 22 September. **B, D, F,** and **H** show two-dimensional distance class analyses of the data in plots above them. Symbols: ● = $[X, Y]$ class with a standardized count frequency greater than expected ($P \leq 0.05$), ○ = $[X, Y]$ class with a standardized count frequency lower than expected ($P \geq 0.95$).

throughout the host populations, more discrete and tightly defined clusters of *Curvularia*-infected plants developed (Fig. 6D, H, J, N, R, and T).

Cercospora leaf spot (*C. zebrina*). Incidence of Cercospora leaf spot was much lower during 1990 and 1991 in the experimental plots than was incidence of the other leaf spot diseases. In most plots, low values for disease incidence prevented analysis of spatial pattern. The disease was more severe in 1990 than in 1991 and reached the highest incidence in plot 4 (Fig. 7A, C, E, and G).

For those data sets analyzed by two-dimensional distance class analysis, spatial patterns were most often characterized as significantly and strongly nonrandom (Fig. 7A–H). Cluster size, shape, and number were similar to clusters of Curvularia leaf spot. Diseased plants tended to occur in relatively small, tight groups (Fig. 7A–D) or in more loosely defined aggregates (Fig. 7E–H). Only one significant edge effect was detected for Cercospora leaf spot (in 1990). Significant row and column effects exhibited no consistent or specific alignment with either plot axes.

DISCUSSION

Spatial knowledge of disease is essential to an understanding of epidemic ontogeny and disease management because of the close relationship between spatial pattern and disease increase and spread within plant populations. Patterns in space suggest the concept of distance. Accordingly, analyses that are based upon distances have great power as descriptive, analytical, and comparative tools. The utility of a distance-based approach with continuous count (6,15,33,43,44) and binomial data (17,18,37,42) has been demonstrated in several pathosystems for pattern description and for the generation and testing of hypotheses about spatial patterns. Although data requirements vary, all of these distance-based techniques permit interpretations of spatial patterns and cluster attributes that allow an analytical approach to pattern analysis, rather than simple descriptions. Thus, the use of these procedures in synecological and comparative studies in an integrated spatial and temporal framework will permit analytical studies of epidemic processes in multiple-pathogen disease systems such as the virus–leaf spot disease complex on white clover, in which a complex network of biological and physical forces impel disease increase and spread.

The white clover leaf spot system allowed a comparative study of the incidence and spatial patterns of diverse foliar pathogens in virus-resistant and virus-susceptible plant populations. The white clover pathosystem studied was not precisely a typical clover and fescue pasture, because clover plants occurred with spacings in the grass sward. The experimental system did, however, contain many of the ecologically important attributes of a pasture ecosystem. Our analyses indicated that four factors—host resistance to viruses, pathogen ecology, defoliation, and environment—were the important forces that drove spatial pattern in this complex ecosystem.

Host resistance to viruses altered the spatial pattern of the viral disease complex normally associated with virus-susceptible white clover populations. In the SRVR germ plasm, resistance is expressed in the parental clones by resistance to mechanical inoculation and by remaining virus-free in the presence of natural inoculum (12). The exact mechanism(s) of resistance are unknown. Due to genetic diversity in cross-pollinated white clover, not all progeny in the SRVR germ plasm (which are all derived from polycrosses of the parental clones) are virus-resistant. However, virus resistance in the SRVR germ plasm did disrupt epidemic patterns that we observed in the virus-susceptible host populations of Regal (Figs. 1–2, Table 1). For example, significant edge effects were common for plots of Regal and probably were the result of primary infestation and movement of viruliferous aphids into plots (24). Diseased plants often were close, which suggests secondary spread of disease or inoculum from plant to plant in a uniformly susceptible host population (11). The more numerous large, rectangular, and elongate clusters of diseased plants characteristic of populations of Regal also were indicative of patterns of secondary spread and cluster expansion. For the SRVR germ plasm, edge effects were uncommon, overall strength of aggregation was less pronounced, and clusters of diseased plants were smaller, less numerous, and more loosely defined than clusters of virus-infected plants of Regal (Figs. 1–2, Table 1).

These patterns for the SRVR germ plasm reflected spatial effects due to a comparatively greater proportion of virus-resistant plants (i.e., fewer successful infections per aphid) and their presumably random placement within plots. Thus, host resistance to viruses probably altered patterns of primary viral infection and subsequent patterns of virus spread within plots. Similar phenomena were observed by Gray et al (18) in a study of the effects of resistance to aphids in *Cucumis melo* upon spatial and temporal spread of watermelon mosaic virus 2 in North Carolina. In that pathosystem, resistance to viruliferous aphids affected size and morphology of virus-infected clusters of plants. Although clusters of virus-infected plants appeared in the resistant host population, the clusters were smaller, did not increase in size, and were more loosely arranged than were the rather large, well-defined, expanding clusters of virus-infected plants in the aphid-susceptible host populations.

Resistance to viruses at the population level had no apparent or consistent effect upon the occurrence and spatial pattern of diseases caused by *C. zebrina, Curvularia trifolii, P. andropogonis, S. meliloti,* and *R. solani.* It is likely that specific virus-fungus interactions affecting disease intensity operate in forage pathosystems (35), as they do in other pathosystems (8,11,32). However, we found little evidence to suggest that leaf spot epidemics differ spatially in virus-susceptible versus virus-resistant white clover at the population level. These data suggest that while the effects of specific interactions between a host plant and pathogen genotype may be significant with regard to changes in disease components (i.e., pathogen reproduction rate, latent period, etc.), these effects may not be important as determinants of spatial characteristics of epidemics in genetically diverse populations of *T. repens.*

In general, spatial characteristics of the various diseases reflected host and pathogen ecology and modes of dispersal. For example, significant edge effects were characteristic of black spot and Stagonospora leaf spot, both caused by organisms that are dispersed primarily by splashing and wind-driven rain. Because of steeper dispersal gradients for splash-dispersed organisms than for aerially (dry) dispersed organisms (10,19), the inoculum that was splashed/blown from the source (natural pasture) to the research plots had a greater chance of infecting plants in the outermost rows and columns than interior plants. Edge effects were not common for Cercospora and Curvularia leaf spots. Dispersal gradients for aerially (dry) dispersed pathogens (e.g., *C. zebrina, Curvularia trifolii*) are shallower than the steeper gradients that are characteristic of splash-dissemination (10). Accordingly, plants closer to the source of inoculum (the edge plants) were no more prone to infection than were plants within the plot.

Cluster size also was probably a function of pathogen dispersal gradient. Steep dispersal gradients imply the potential occurrence of diseased plants to be in close proximity. In contrast, clusters of *Cercospora*- or *Curvularia*-infected plants (relatively shallow dispersal gradients) were smaller and more loosely arranged than the large, distinct clusters of *Pseudomonas*- or *Rhizoctonia*-infected plants (steeper dispersal gradients). Thus, large and distinct clusters of diseased individuals imply a comparatively rapid and efficient mode of plant-to-plant dissemination for the pathogens that cause black spot, summer blight, and Stagonospora leaf spot.

Summer blight of white clover in pastures is an unusual disease, because its occurrence and spatial pattern depend upon the underlying occurrence and pattern of another disease (same pathogen) and host, brown patch and tall fescue. Under conducive conditions (warm temperature, heavy dew) the incidence of brown patch increased in the population of tall fescue plants (45). Concomitantly, large clusters of blighted clover plants developed in areas where there was the greatest chance for contact between clover foliage and infected or infested tall fescue. Tan, circular

to elliptical patches (0.25–0.5 m diameter) of completely blighted fescue were areas in which white clover foliage rarely survived for more than a few days. Thus, the underlying spatial influence of the primary inoculum of *R. solani*, which characteristically has an aggregated pattern in tall fescue turf (30), may have been related to the nonrandom spatial patterns that we observed in *Rhizoctonia*-infected white clover.

Patterns of black spot reflected the unusual ecology of the disease-causing bacterium. *P. andropogonis* requires foliar wounds for infection, and thus actually thrives in situations where activity of humans (9) or grazing animals provides dispersal of bacteria, wounding, and inoculation of the host. Foliar pathogens of forage crops generally are suppressed by grazing activity (2,41), which destroys dense canopies that are favorable to infection and pathogen reproduction. During May 1990, rainy and warm weather conditions favored production and dispersal of inoculum, and black spot spread quickly throughout the grazed, 10-ha pasture. When disease assessment began, black spot was already spreading from the surrounding pasture into some plots. The large clusters and uniform disease patterns that we observed for *P. andropogonis* reflect the prevalence and uniform occurrence of black spot in this pasture and suggest a pathogen with effective modes of primary and secondary spread of inoculum. Thus, the rapid coalescence of primary and secondary foci of diseased plants that was observed for black spot was a function of efficiency of dispersal and copious production of inoculum.

The leaf spot epidemics were not spatially static. As would be expected in most pathosystems, as disease(s) spread through the host population, cluster size and strength of aggregation changed. Superimposed upon this natural spatial change were the forces of sporadic defoliation and host growth. Defoliation, either disease-induced or such as occurs with harvest of forage, can remove from a plant all symptomatic foliage for a given disease. Thus, defoliation can and did alter intrinsic spatial pattern (i.e., from random to aggregated), strength of aggregation, cluster size and morphology, and disease incidence between assessment dates. Significant change in spatial parameters between weeks was observed for all diseases (e.g., summer blight 1991).

Cycles of defoliation occurred at different rates for the various, leaf spot pathogens. *C. zebrina* and *Curvularia trifolii* caused rapid (7–10 days) defoliation of clover foliage during 1990 and 1991. Due to slower lesion expansion, *Stagonospora*- or *Pseudomonas*-infected leaves usually persisted at least 2 wk before defoliation. However, *R. solani* caused the most rapid (2–3 days) defoliation of white clover foliage in our pasture. The probability of observing summer blight on a given plant is a function of chance, physical contact between white clover foliage and infected or infested tall fescue, environmental conditions, and the amount of time that elapses between infection and disease assessment. On clover plants with a low incidence of *Rhizoctonia*-infected leaves, and under conducive conditions, rapid defoliation created a quickly changing, dynamic pathosystem with regard to disease incidence between assessment dates. Similar, defoliation-driven spatial flux has been observed in other pathosystems. For example, slopes of disease gradients associated with citrus canker (caused by *Xanthomonas campestris* pv. *citri*) fluctuated over time because of disease-induced defoliation on severely diseased citrus nursery plants (14,16). Because spatial and temporal phenomena are linked, the significant effect of defoliation upon temporal disease progress that has been observed in this and other pathosystems (23,36,47) must also be expressed spatially.

Environment (e.g., year, growth period, weather, plot location, microclimate) affected spatial patterns and contributed to spatial flux of white clover leaf spots. Between-year, weather-driven variability with regard to disease incidence and spatial attributes was observed for most diseases. For example, the nearly uniform spatial pattern and large clusters for black spot that we observed in most plots during 1990 (disease incidence near 100%) was contrasted by lower disease incidence and stronger aggregation during 1991 for this disease. As mentioned earlier, the spring of 1990 (above average temperature and 17 cm of rain) was more conducive than the spring of 1991 for the establishment and rapid spread of black spot through the grazed pasture and the experimental plots. Seasonal and weather-driven changes in pathogen abundance and associations are common in this pathosystem (36), and such changes coincide with the spatial differences among epidemics that we observed.

Between-year variability in disease onset, disease incidence, and size of clusters of virus-infected plants of Regal may have been influenced by weather effects upon overwintering populations of aphid vectors and virus populations. The number of aphids surviving the winter and entering crops in the spring depends on weather conditions in the winter (11). For example, in mild winters large numbers of adult aphids and aphid eggs may survive on weeds and the continually growing clover within the pasture. After mild winters, alate adult aphids are produced earlier and in larger numbers than after severe winters (11). Beginning in February 1990 and continuing for 22 consecutive months, mean monthly temperatures were above the 30-yr average for the Raleigh, NC, area. Thus, the earlier disease onset, increased incidence of virus diseases, and larger clusters of virus-infected plants in plots of Regal in 1991 may represent the cumulative effects of a conducive environment upon expanding populations of the aphid vectors.

As the environment became more or less conducive for reproduction and dispersal of specific pathogens within a year or between growth periods, changes in incidence of diseased plants occurred, cluster number and size changed, clusters coalesced or disintegrated, defoliation occurred, and plants died. The observed effects of changing temperatures upon spatial patterns within a year (e.g., for summer blight and Curvularia leaf spot) and of changing microclimates (e.g., reduced canopies after harvest and defoliation), indicated that environmental data are critical to explaining spatial variability in this pathosystem.

Binomial data are biologically relevant simplifications of many pathosystems. Binomial data reflect disease gradients and modes of plant-to-plant dispersal, forces that drive foliar epidemics. This research demonstrates an array of quantitative applications for two-dimensional distance class analysis that extend the previous applications of the technique. Other binomial-based analytical methods (e.g., runs analysis, mapping, two-term local quadrat variance) do not have the power to quantify in two dimensions spatial attributes such as cluster size, shape, number, and edge effects. Two-dimensional distance class analysis is a powerful tool that allows the quantification of many spatial attributes of epidemics and thereby the testing of important spatial and ecological hypotheses of disease occurrence and spread.

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