Disease Progress, Defoliation, and Spatial Pattern in a Multiple-Pathogen Disease Complex on White Clover

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The use of trade names in this publication does not imply endorsement by the North Carolina Research Service of the products named nor criticism of similar ones not mentioned. This work was supported by funds provided by the North Carolina Agricultural Research Service and a grant-in-aid to the first author by Sigma Xi, the Scientific Research Society. We thank Charles Harper and Mike Munster, NCSU Department of Plant Pathology, and Ken Snyder, NCSU Unit 2 Forage Research Facility, for technical assistance. We sincerely appreciate the advice and virus antisera provided by O. W. Barnett, Clemson University, antisera and indirect-ELISA protocol provided by S. A. Ghabrial, University of Kentucky, and seeds of the Southern Regional Virus Resistant white clover germ plasm provided by G. A. Pederson and M. R. McLaughlin, USDA-ARS Mississippi State. We thank Dan Phillips, University of Georgia, for advice on automated data collection.

Accepted for publication 6 January 1993.

ABSTRACT

Nelson, S. C., and Campbell, C. L. 1993. Disease progress, defoliation, and spatial pattern in a multiple-pathogen disease complex on white clover. Phytopathology 83:419-429.

Disease progress, host growth and defoliation, and spatial patterns for a multiple pathogen, leaf spot disease complex on white clover (*Trifolium repens*) were monitored in four 6-wk growth periods during 1990–1991 in a natural, 10-ha pasture of white clover and tall fescue (*Festuca arundinacea*) in North Carolina. The disease complex comprised summer blight (*Rhizoctonia solani*), black spot (*Pseudomonas andropogonis*), Stagonospora leaf spot (*Stagonospora meliloti*), Cercospora leaf spot (*Cercospora zebrina*), Curvularia leaf spot (*Curvularia trifolii*), anthracnose (*Colletotrichum trifolii*), sooty blotch (*Polythrincium trifolii*), and rust (*Uromyces* sp.). Estimates of disease severity (percent leaf area diseased) for the disease mixture and leaf area (cm^2) were obtained twice per week for 20 leaves on each of 512 plants in four plots. Each plot consisted of two proximal, square 8×8 lattices of 64 plants of the virus-susceptible cultivar Regal and the Southern Regional Virus Resistant (SRVR) white clover germ plasm in an existing sward of tall fescue. SRVR populations are resistant to alfalfa mosaic virus, clover yellow vein virus, and peanut stunt virus. A disease severity/leaf area rating scale with 45 categories (nine for disease severity, five for leaf area) was used to estimate percent leaf area diseased and total leaf area (cm^2) for each leaf sampled. Also in 1991, disease severity and leaf area were estimated once per week for all individual leaves on 20 plants each of Regal and SRVR during each growth period. Most progress curves for disease incidence (percent of plants diseased) and severity were nonmonotonic, and poor fits were obtained to linearized, classic growth-curve models (logistic, Gompertz, monomolecular). Incidence of virus-infected plants was significantly greater for most plots of Regal than for the SRVR germ plasm; however, both host populations were similar with regard to shape of progress curves and overall disease values (disease maxima and AUDPC) for the leaf spot disease complex. No differences were detected between virus-free and virus-infected plants in total AUDPC for leaf spot severity per 6-wk epidemic. Interactions between host growth (leaf expansion or leaf addition) and defoliation accounted for observed declines in leaf spot severity between consecutive disease assessments. Loss of diseased tissue (defoliation) occurred most often as disease severity increased in the latter part of each growth period, and in patches or near plot edges. Spatially, values for disease severity indicated strongly nonrandom patterns for diseased plants at most assessment dates. Temporal shifts in cluster size and shape were associated with increasing disease severity, or with defoliation. A blocked-quadrat variance procedure detected aggregation for leaf spot severity at several, concurrent spatial scales.

The union of classic growth curve or population dynamics models with plant disease data was founded upon a series of implicit assumptions that relate to uniformity and constancy of environment, host, and pathogen. The assumptions are that in a uniform environment, a single and genetically uniform pathogen species on a uniformly susceptible host population (in which there is a constant amount of host area to be infected), results in a continuous change in disease that is immediately visible and spatially random or uniform (5). Deterministic models, such as the logistic model, continue to be valuable simplifications of epidemics in which extreme or chaotic violations of the implicit assumptions do not occur or do not impair a model's descriptive or predictive utility. The models have been adaptable enough to describe more complex epidemics, wherein biologically realistic parameters for host growth and/or defoliation (5,15,33,34) and spatial aggregation (35) have been proposed.

In reality, the assumptions implicit in the use of growth curve models to describe plant disease epidemics often are not satisfied. The environment changes substantially throughout most epidemics (28). The amount of available host tissue is temporally and spatially variable in certain pathosystems (31). Plant pathogens and diseased plants are often aggregated in fields (5). Thus, experiments or hypotheses designed to identify the relative fit of the implicit assumptions to complex systems will identify the areas of model extension necessary for the theoretical improvement and practical management and prediction of epidemics.

Several attributes of leaf spot epidemics on white clover (*Trifolium repens* L.) within pastures suggest a theoretically poor fit between this complex of multiple pathosystems and the implicit assumptions of deterministic growth curve models. Genetically, the synthetically derived, cross-pollinated populations of white clover are extremely diverse. (3,4). Spatial occurrence of clover plants in pastures is often patchy and temporally variable, and the nonrandom occurrence of pathogens and diseases is superimposed on clover plants within pastures (25). The sporadic loss and gain (8) of large numbers of leaves from this perennial plant throughout the year indicate foliation and pathogen-induced defoliation during epidemics. Finally, foliar epidemics of white clover comprise many concurrent diseases, i.e., at least three viruses, eight fungi, and a bacterium comprise this 'leaf spot' disease complex (24).

One approach to modeling multiple-pathogen systems is to describe disease dynamics independently, e. g., with separate or linked equations for the respective diseases (18). However, with

a system as complex as the white clover leaf spot pathosystem, more than 10 equations would be needed to describe the system and analytical solutions would be difficult to achieve and interpret (5). A much simpler approach would be to treat a commonly occurring disease mixture as one entity and to analyze spatial and temporal attributes of disease progress for the group of diseases as a whole. Then, in conjunction with spatial and temporal data on individual pathogens and diseases, the impact of potential pathogen interactions (e.g., competition, predisposition, etc.) on overall disease progress could be assessed.

The availability of virus-resistant and -susceptible populations of white clover germ plasm (9) allows a test of the hypothesis that resistance or susceptibility to viruses at a plant population level has a significant effect on spatiotemporal aspects of leaf spot epidemics on white clover. Recently, however, we reported that spatial patterns of the individual leaf spot diseases were similar in virus-resistant and -susceptible host populations (25). Significant nonrandomness was detected for all diseases, although strength of aggregation and attributes of cluster morphology varied among diseases with different modes of pathogen dispersal. However, given the existence of specific, fungus/virus interactions that may alter leaf spot intensity (23), it would be of interest to assess the impact of virus-resistant populations of white clover on the spatial and temporal severity of the leaf spot disease complex as a whole.

Previous findings have suggested that defoliation may be a driving force with regard to the incidence and spatial patterns of individual diseases in the leaf spot complex on white clover (22). Through their interaction, two 'competing' forces—host growth and defoliation—lead to observed changes in disease incidence (nonmonotonic increase) through sporadic removal of diseased tissue and addition of healthy tissue during epidemics. Nonmonotonic progress curves for the clover leaf spot complex suggest violations of the 'constant host area' assumption that is implicit in the use of the deterministic models (5). For example, a significant decline in estimated disease severity between disease ratings may result from leaf addition or expansion ('diluting' estimates of disease severity), from removal of diseased tissue (defoliation), or from a combination of host growth and defoliation.

Thus, we designed experiments and a disease rating system to assess the relative importance of leaf addition versus leaf expansion, and of host growth versus defoliation as determinants of nonmonotonic disease progress in this pathosystem. Specifically, we wished to test two hypotheses: 1) that virus/leaf spot interactions have significant effects (at a population level) upon spatial and temporal attributes of leaf spot epidemics, and 2) that defoliation and host growth during epidemics are determinants of the nonmonotonic progress curves that have been observed in this and other forage systems (6,31).

MATERIALS AND METHODS

Experimental plots. Experiments were conducted in a 10-ha, white clover/tall fescue grass pasture grazed by dairy cattle in Wake County at the Unit 2 Forage Research Facility of North Carolina State University during 1990–1991. Pasture history and cultural practices during the experiment were described in detail in a previous paper (24). A brief description of the plot design follows.

Four plots were established in arbitrary locations representing 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 (9), or of the virus-susceptible white clover cultivar, Regal. SRVR populations are resistant to alfalfa mosaic virus, clover yellow vein virus, and peanut stunt virus. Plants were transplanted on 4 May in 1990 and on 15 April in 1991. Clover plant lattices were placed within the existing sward of tall fescue (*Festuca arundinacaea* Schreb.). Lattice dimension was 10- × 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 Ltd., 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, two 6-wk growth periods were monitored. The first of the two consecutive 6-wk epidemics began on 25 June 1990 and 10 June 1991, respectively. Plots were harvested to a height of approximately 6–10 cm with a flail-chop harvester (Carter Manufacturing Co., Inc., Brookston, IN) at the termination of period 1 (harvest dates were 6 August 1990 and 25 July 1991). Harvested material was removed from plot areas and discarded. Monitoring of period 2 began 10–14 days after harvest of period 1.

Assessment of disease severity. Disease severity was defined as the percentage of leaf area diseased. The 'leaf spot' disease was caused by a mixture of many pathogens: *Cercospora zebrina* Pass. (Cercospora leaf spot), *Colletotrichum trifolii* Bain (anthracnose), *Curvularia trifolii* (Kauffm.) Boedijn (Curvularia leaf spot), *Polythrincium trifolii* Kunze in J. C. Schmidt & Kunze (sooty blotch), *Pseudomonas andropogonis* (Smith) Stapp., *Rhizoctonia solani* Kühn (summer blight), *Stagonospora meliloti* (Lasch) Petr. (Stagonospora leaf spot), and a *Uromyces* sp. (rust) (24).

Nondestructive estimates of disease severity were made twice per week on 20 leaves on each of the 512 white clover plants for 12 wk (June-September) during 1990–1991. Estimates of disease severity per plant were expressed as the mean disease severity for 20 leaves per plant. For plants with fewer than 20 leaves, every leaf was sampled. The samples were obtained from a rectangular swath (10–15 cm wide) from the perimeter of each plant towards its center. There were eight possible positions for the rectangular sampling area. The positions were aligned approximately with the compass points: N, NE, E, SE, S, SW, W, and NW. For example, an NE sample began at plant perimeter from the northeast and towards plant center. Leaves were sampled equally from lower to upper canopy until 20 leaves were sampled. Swath position was rotated one compass point (counterclockwise) if more leaves were needed. The starting position was selected arbitrarily before entering the pasture each day.

The disease rating scale. Both disease severity and leaf area (cm^2) were estimated visually with the aid of rating diagrams. Disease severity classes 0–8 were based on percentage of leaf area diseased: 0, 0.1–2.5, 2.6–5.0, 5.1–10.0, 10.1–15.0, 15.1–25.1, 25.1–35.0, 35.1–50.0, and 50.1–65.0. Y_{max}, or maximum percentage of leaf necrosis, was assigned a value of 65% because of the tendency of clover leaves to defoliate at or below this level of disease (23). Leaves with collapsed petioles or complete necrosis were considered to be removed from the system, and, thus, were not rated for disease severity and leaf area.

A complementary leaf area rating scale was developed to account for leaf expansion and the variability in leaf size within and among clover plants. Based on a preliminary sample of typical clover leaves, leaf area categories 1–5 (based on total square centimeters for the three trifoliolates per leaf) were established (values are midpoint of range): 5, 10, 15, 20, and 25 cm^2. Thus, with nine severity classes and five leaf area classes, a total of 45 disease severity/leaf area categories were used. By multiplying estimates of total leaf area by estimates of percent disease, accurate and precise estimates of the actual amount of diseased tissue (cm^2) in each sample were possible. Graphs of total square centimeters of leaf area and total square centimeters of diseased tissue versus time were compared with progress curves for disease severity (percent leaf area diseased) to identify the occurrence and effects of host growth (leaf expansion, leaf addition) and defoliation (loss of diseased tissue from plants) during the leaf spot epidemics.

Virus sampling. In February 1990, the presence of several white clover viruses in the experimental pasture was confirmed by M. R. McLaughlin, USDA-ARS, Mississippi State, via enzyme-linked immunosorbent assay (ELISA): red clover mosaic virus (RCMV), alfalfa mosaic virus (AMV), clover yellow vein virus (CYVV), and the Eastern strain (14) of peanut stunt virus (PSV). Because PSV, AMV, and CYVV are considered to be the most

important viruses in North Carolina and the southeastern United States (2,19,27), the incidence of PSV, AMV, and/or CYVV was determined for each of the 512 plants three times each year for 1990 and 1991. Sampling dates were 9–16 July, 20 August, and 25 September 1990 and 4–7 June, 6 August, and 16 September 1991. On each sampling date, three to five leaves were selected arbitrarily from the circumference of each plant, plant sap was extracted, and the antigen (1:10, w/v) 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 (AMV, CYVV), Department of Plant Pathology, Clemson University, and S. A. Ghabrial (PSV), Department of Plant Pathology, University of Kentucky. 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 Corporation, 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.

Host growth versus defoliation. The relative effects of host growth and defoliation upon progress curves for disease severity were assessed by two methods during 1990–1991. One approach used the leaf area/disease severity rating scale to estimate the actual amount (cm^2) of symptomatic versus healthy tissue for each sample of 20 leaves. Because temporal changes in disease severity reflect an interaction between the relative proportions of healthy tissue (including leaf addition and expansion) versus diseased tissue (including defoliation) that are observed in a

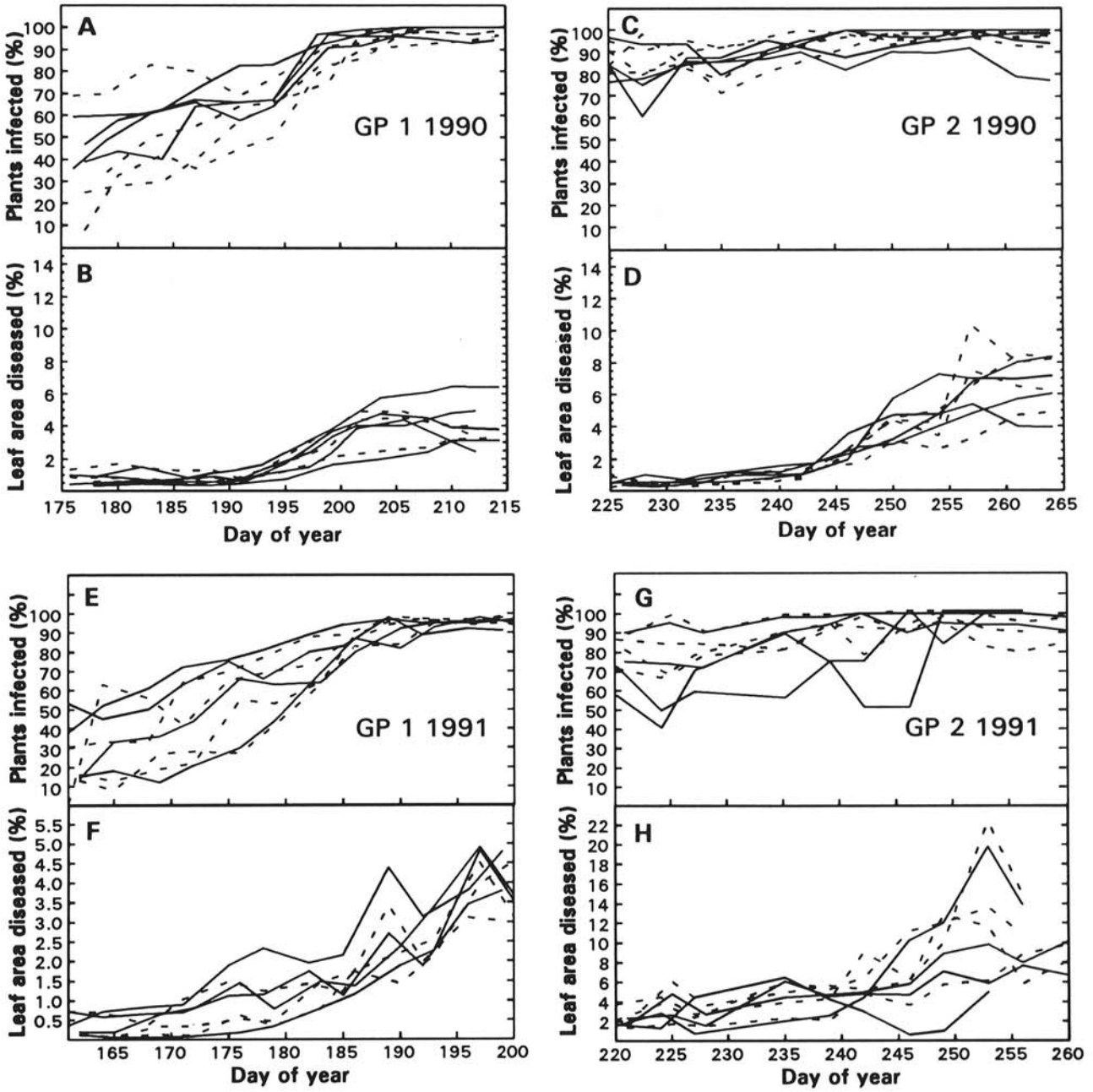

Fig. 1. Disease progress curves for leaf spot epidemics in four plots of Regal (virus-susceptible) and the Southern Regional Virus Resistant (SRVR) white clover germ plasm in a 10-ha pasture of *Trifolium repens* and *Festuca arundinacea* (tall fescue) in Wake County, North Carolina, in 1990 and 1991: disease severity (percent leaf area diseased) and disease incidence (percentage of plants infected with at least one leaf spot pathogen) versus day of year. **A,B,** Disease incidence and severity, respectively for growth period 1 (GP 1) 1990. **C,D,** Disease incidence and severity for GP 2 1990. **E,F,** Disease incidence and severity growth period 1 (GP 1) 1991. **G,H,** Disease incidence and severity for GP 2 1991. Solid lines represent data for plants of cultivar Regal in each plot; dashed lines represent data for plants of the SRVR germplasm in each plot.

sample, five categories ('interaction types') were used to classify the types of interactions between increasing (↑), decreasing (↓), or unchanging (→) leaf area (LA) and diseased leaf area (DA) between consecutive ratings that result in an observed decline in leaf spot severity. The interaction types reflected temporal changes in amounts of healthy and diseased tissue and were used to categorize any observed decrease in leaf spot severity (>2% disease) between assessments. Interaction types were: I (↑LA,↑DA), II (↑LA,→DA), III (↑LA,↓DA), IV (→LA,↓DA), and V (↓LA,↓DA). Other possible interactions (e.g., →LA,→DA) do not result in observed declines in leaf spot severity between consecutive ratings.

In 1991, an additional method for assessing host growth and defoliation during epidemics was used. Estimates of disease severity and leaf area were made for every leaf on each of 40 plants during the two growth periods. Five plants were chosen randomly from lattices of Regal and five from the SRVR germ plasm in each plot for period 1. The plants were rated once per week for 6 wk. For period 2, indirect-ELISA was used to identify all virus-infected plants. Thereafter, 20 virus-infected plants were chosen randomly from plots of Regal (five plants each) and 20 virus-free plants from plots of the SRVR germ plasm, and the same plants were rated for leaf spot severity once per week during the second growth period. The following variables were plotted against time to examine interactions during periods of increasing and decreasing disease severity: leaf number, disease incidence (percentage of leaves infected by a least one foliar pathogen), disease severity (mean percentage of leaf area diseased, total leaf area (cm^2), and total leaf area diseased (cm^2).

Analysis of disease progress. Epidemic models (exponential, monomolecular, logistic, and Gompertz) (5) were fitted to data on disease severity for each host/plot combination to obtain descriptions of the progression of leaf spot epidemics and to assess the appropriateness of implicit assumptions in fitting simple, deterministic models. Models were linearized (16) and then fit to the data using ordinary least squares regression. After fitting each model to the data, the goodness of fit was determined using standardized residual plots, coefficients of determination (R^2), and additional statistics as needed (17). Disease progress curves also were analyzed and compared by calculating the area under the curve (AUDPC) (29) for each lattice and plant of Regal and the SRVR germ plasm.

Analysis of spatial pattern. Spatial patterns of disease severity for plants of Regal and the SRVR germ plasm in each plot were analyzed using a blocked-quadrat variance procedure (the Greig-Smith procedure) (12,13,32) and spatial autocorrelation analysis (10,20). The Greig-Smith procedure was used to identify the existence of nonrandom spatial patterns at several spatial scales, to identify the sizes and shapes of clusters of diseased plants, and to assess temporal changes in clusters of diseased plants. The Greig-Smith procedure utilizes variances calculated for successively larger blocks of sample quadrat counts to estimate 'cluster size'. A peak or local maximum in the plot of variance versus block size was taken to represent the primary cluster size. Secondary clusters (clustering at different spatial scales) were indicated by smaller peaks in the plots of variance versus block size. Absence of a peak in the plot of variance versus a given block size was taken to represent a random or uniform spatial pattern for disease severity. Shape, or two-dimensional orientation of the clusters, was assessed by performing the Greig-Smith analyses in relation to the north-south and east-west ("horizontal" versus "vertical") plot axes (30). Plots of block size (approximate cluster size) versus time were examined for each plot to identify temporal changes and trends with regard to cluster size and morphology. Two-dimensional distance class analysis (11,25,26) was used to analyze the spatial patterns of disease severity, with decline in disease severity as a binomial variable.

Spatial correlation analysis. Spatial autocorrelation analysis (10,20) was applied to data for AUDPC for plants of Regal and the SRVR germ plasm in each plot. To obtain a simple, single measure on which to base the spatial autocorrelation analysis for each plot that reflected progress of the epidemics in entirety, a value for AUDPC (29) was calculated for each quadrat (i.e., each plant) and utilized in a computer program (10) that calculated correlations among values for AUDPC in various directions and at various distances within the grids of plants.

RESULTS

Disease progress for the leaf spot complex. Progress curves for plants of Regal were similar to those for the SRVR germ plasm with regard to overall shape and levels of disease severity within growth periods. Higher levels of disease severity were observed during period 2 than during period 1 in both 1990 and 1991 for both host populations. (Fig. 1B,D,F,H). Least squares means for AUDPC (percent-days) for Regal and SRVR, respectively, were: 82.6 and 109.4 (period 1, 1990); 119.9 and 107.8 (period 2, 1990); 63.5 and 61.2 (period 1, 1991); and 135.6 and 165.2 (period 2, 1991). Progress curves for disease severity (especially period 2 in 1990 and period 1 and period 2 in 1991) generally were nonmonotonic; multiple peaks in disease severity were observed over time. For example, during period 1 in 1991, three distinct and concurrent peaks in disease severity occurred for most plots at approximately 1-wk intervals—at day of year 182–183, 189–190, and 196–197 (Fig. 1F). Distinct peaks in disease severity often occurred in the final week of the epidemics.

Several simple models (linearized versions of the monomolecular, logistic, and Gompertz) commonly used in phytopathology and a simple linear model were examined for goodness-of-fit to the disease severity data (via simple linear regression). No data sets were described adequately by these models, and fits were equally poor for data sets of Regal and the SRVR germ plasm. For the models, the coefficient of determination (R^2) ranged from 0.05 to 0.27 in 1990 and from 0.005 to 0.13 in 1991. Nonrandom and systematic patterns were found in the graphs of residuals (versus the independent variable and/or the predicted values), which indicated violations of the regression assumption that the error term of the model had constant variance at all levels of time. Coefficients of determination for the simple linear regressions ranged from 0.09 to 0.17.

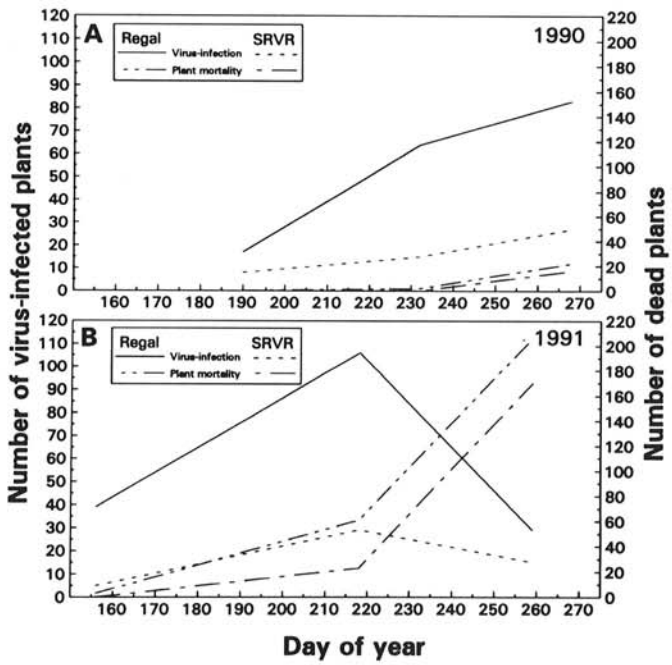

Fig. 2. Total numbers of virus-infected plants and dead plants at three sampling dates each in 1990 and 1991 for viral/leaf spot epidemics in four plots of Regal (virus-susceptible) and the Southern Regional Virus Resistant (SRVR) white clover germ plasm in a 10-ha pasture of *Trifolium repens* and *Festuca arundinacea* (tall fescue) in Wake County, North Carolina, in 1990 and 1991. **A,** 1990. **B,** 1991. Two hundred and fifty-six plants each (64 per plot) of Regal and the Southern Regional Virus Resistant (SRVR) germ plasm were assayed via indirect-ELISA for alfalfa mosaic virus, clover yellow vein virus, and peanut stunt virus.

Graphs of disease incidence (percentage of plants infected with at least one leaf spot pathogen) over time also were similar between years and generally were nonmonotonic (Fig. 1A,C,E,G). Values for disease incidence at the beginning of period 1 in 1990 and period 1 in 1991 were highly variable among plots and ranged from approximately 5 to 70% and from 5 to 55% for 1990 and 1991, respectively. By the end of period 1 in both 1990 and 1991, the percentage of plants infected ranged from approximately 90 to 100% for all plots of both Regal and the SRVR germ plasm.

After harvest, disease incidence was reduced ranging from 76 to 96% (Fig. 1A,C) and 56 to 90% (Fig. 1E,G) in 1990 and 1991, respectively. Values for disease incidence generally remained at high levels during period 2 in both years. Values for disease severity had greater, relative reductions after harvest in 1990 and 1991 than did values for disease incidence (Fig. 1C or D and G or H).

Values for overall mean leaf spot AUDPC for virus-infected plants were not significantly different (based on Student's t tests, $P \leq 0.05$) from values for leaf spot AUDPC for virus-free plants in 1991. Data from 1990 were not analyzed due to low incidence of virus-infected plants. In 1991, mean AUDPCs for virus-infected versus virus-free plants (Regal and SRVR combined) were 69.9 and 61.7%-days, and 153.6 and 151.7%-days, respectively, for period 1 and period 2.

Disease progress for virus epidemics. The incidence and spatial patterns of virus-infected plants in this experiment were described previously (25). PSV was the predominant virus within the experimental plots although AMV-infected and CYVV-infected plants were present in the pasture and in the plots (at low frequencies) (25). Thus, the disease progress curves (Fig. 2A,B) reflect the incidence of PSV infection. Incidence of virus-infection increased more rapidly in populations of Regal than in the SRVR germ plasm in 1990 and 1991 (Fig. 2A,B), and number of virus-infected plants of Regal was greater ($P = 0.05$) than for the SRVR germ plasm at most sampling dates. More plants died in 1991 in plots of Regal and the SRVR germ plasm than in 1990 (Fig. 2A,B). More plants died after midseason harvest in 1991 than in 1990. Harvest in 1991 was conducted on the hottest day of the year (maximum temperature approximately 37 C). Many virus-infected plants died in 1991, i.e., as numbers of dead plants increased, numbers of virus-infected plants decreased (Fig. 2B). Slopes for plant mortality (Regal versus SRVR) from day of year 220–260 (in 1991) were parallel.

Host growth versus defoliation: total leaf area versus diseased leaf area (1990–1991). Declines in leaf spot severity (greater than 2%) between consecutive ratings were observed for most plants in 1990 and 1991 (Table 1). Data from period 1 (1990) are not shown because data on leaf area and diseased tissue were not collected for the entire growth period. Maximum declines in disease severity on individual plants ranged from approximately 32 to 42% during 1990–1991. Repeated declines in disease severity were observed for many plants during each growth period.

Interactions between total leaf area and total diseased leaf area in which leaf area increased between ratings (interaction types I, II, and III) accounted for 30, 23, and 29% of the total number of severity-reducing interactions during period 2 1990, period 1 1991, and period 2 1991, respectively (Table 1). Interactions involving a loss of diseased tissue (interaction types III, IV, and V) during period 2 1990, period 1 1991, and period 2 1991 accounted for 97, 99.6, and 98.5% of the total number of interactions, respectively (Table 1). Data for plants of the SRVR germ plasm and Regal were not different ($P < 0.05$) with regard to total number of plants for which declines in disease severity were observed, the total number of severity-reducing interactions, and the total number of interactions occurring for each interaction type (data not shown).

Loss of diseased tissue from samples (interaction types III, IV, and V) was associated with increasing incidence and severity for the leaf spot complex. For example, during period 2 in 1990, most diseased tissue was lost from plants in the latter part of the epidemic (day of year 250–257) (Fig. 3A). This loss of diseased tissue was associated with an approximate doubling of overall disease severity from day of year 250–257 (Fig. 1D). During period

Table 1. Summary of interactions between total leaf area (cm^2) and total diseased tissue (cm^2) resulting in >2% decline in leaf spot disease severity between consecutive ratings of 512 white clover plants during 1990–1991 in experimental plots in a 10-ha pasture of white clover and tall fescue at the NCSU Forage Research facility in Wake County, North Carolina

			Interaction types[d]					
Growth period[a]	Plants[b]	Interactions[c]	I	II	III	IV	V	Max. decline[e]
GP 2 1990	336	399	7	4	108	142	138	40.1%
GP 1 1991	355	550	0	2	125	267	156	32.2%
GP 2 1991	300	620	2	7	169	202	240	42.4%
Totals	991	1569	9	13	402	611	534	

[a]Two growth periods (GP) per year were 6 wk in duration and separated by harvest; data not shown for GP 1 1990. Dates for GPs were: Day of year 175–215 (GP 1 1990), 223–265 (GP 2 1990), 160–202 (GP 1 1991), and 218–260 (GP 2 1991).

[b]Total number of plants (maximum value = 512) for which at least one decline in disease severity (>2%) occurred between consecutive rating dates.

[c]Total number of interactions between total leaf area (cm^2) and total diseased leaf area (cm^2) that resulted in a decline in disease severity (>2%) between consecutive ratings.

[d]Five types of interactions are possible between the total amount of healthy leaf tissue (cm^2) versus total amount of diseased leaf tissue (cm^2) that may result in an observed decline in disease severity between consecutive disease ratings. The interaction types reflect increasing (↑) and decreasing (↓) (increasing or decreasing by >10% of the previous day's value), or unchanging (→) total leaf area (LA) and diseased leaf area (DA) between consecutive ratings that result in a decline in estimated leaf spot severity (>2% disease) between assessment dates. Interaction types were: I (↑LA,↑DA), II (↑LA,→DA), III (↑LA,↓DA), IV (→LA,↓DA), and V (↓LA,↓DA), respectively.

[e]Maximum observed decline in disease severity between consecutive ratings that was observed for an individual plant.

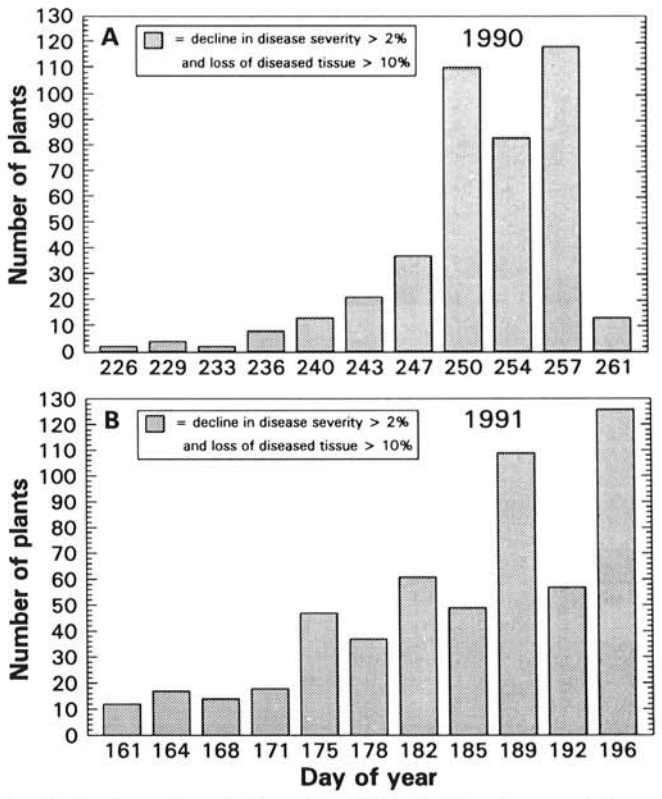

Fig. 3. Total number of plants (per 512) of white clover and day of year for which disease severity began to decrease (>2%) between two of 12 consecutive assessment dates and for which a loss in total diseased tissue (greater than 10% of the value at the preceding assessment date) was initiated for leaf spot epidemics on white clover in four plots in a 10-ha pasture of *Trifolium repens* and *Festuca arundinacea* (tall fescue) in Wake County, North Carolina. **A,B,** Growth period (GP) 2 in 1990 and GP 1 in 1991, respectively.

1 in 1991, major peaks in loss of diseased tissue over time (Fig. 3B, days 189 and 196) corresponded precisely with two major peaks in disease severity that were observed during this epidemic (Fig. 1F).

Significant aggregation of plants with relatively large declines in disease severity (≥5%) was detected via two-dimensional distance class analysis for most plots in all growth periods in both years. For example, during period 2 in 1990 (for plants in which a decline in disease severity ≥ 5% was observed between consecutive ratings), significantly nonrandom patterns (25) were observed in seven of eight host/plot combinations (Fig. 4A–P). Two-dimensional distance class analysis revealed significant clusters of such plants existed in most plots (e.g., Fig. 4M–P). Cluster size ranged from approximately 2–8 (25). Significant edge effects were detected in some plots (e.g., plot 4 Regal, Fig. 4).

Host growth versus defoliation: leaf addition versus leaf removal (1991). Declines in disease severity (greater than 2%) between consecutive ratings were observed on approximately one-half of the 80 plants for which disease severity was estimated on each leaf during 1991 (10 of 40 plants in period 1 and 28 of 40 plants in period 2). A total of 12 severity-reducing interactions between total leaf area and total diseased tissue were observed during period 1, and all interactions involved a loss of diseased tissue from plants (interaction types III [3], IV [2], and V [7]). A total of 30 severity-reducing interactions between total leaf area and total diseased tissue were observed during period 2, and all interactions involved a loss of diseased tissue from plants (interaction types III [2], IV [0], and V [28]).

Peaks occurred in the progress curves of disease severity for individual plants over time. For example, disease progress for a plant of Regal in plot 1 (period 1 in 1991) (Fig. 5B) was characterized by two significant peaks in disease severity and in the incidence of diseased leaves. Two different interaction types resulted from the relationship between total leaf and diseased leaf areas (type II, day 170–177; type V, day 191–198) (Fig. 5B). Most plants had more than one decline in disease severity during epidemics. For example, one such plant in 1990 (Fig. 5A) had several peaks in disease severity, each peak with a corresponding peak in total amount (cm^2) of disease tissue in the sample.

An overall decline in disease severity for the 40 plants in this study was not observed during period 1 (Fig. 6B, day of year 160–200). Progress curves for disease severity during period 1 were similar for plants of Regal and plants of the SRVR germ plasm with regard to maximum percent disease and shape (both curves were roughly sigmoidal). Curves of leaf number were

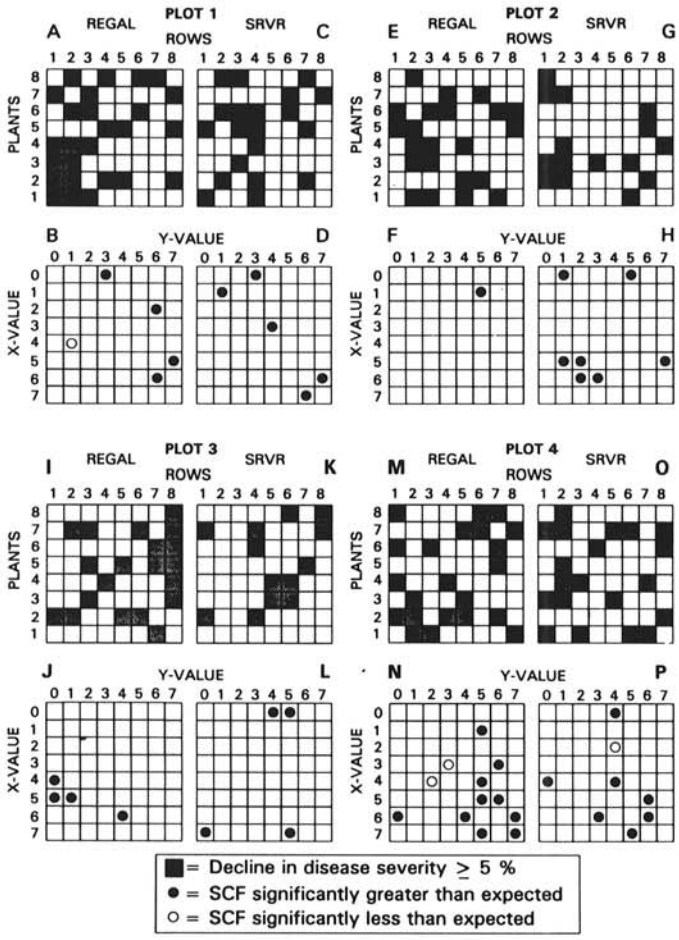

Fig. 4. Spatial patterns and two-dimensional distance class analyses of white clover plants for which a decline in disease severity ≥5% was observed between two consecutive rating days in eight-column by eight-row lattices of white clover plants of cv. Regal and the Southern Regional Virus Resistant (SRVR) germ plasm in four plots in a 10-ha pasture of white clover and tall fescue grass at the Unit 2 Forage Research Facility in Wake County, North Carolina, during growth period 2 in 1990. **A, C,** Spatial patterns for Regal and SRVR in plot 1. **B, D,** Two-dimensional distance class analyses for data in A and C, respectively. **E, G,** Spatial patterns for Regal and SRVR in plot 2. **F, H,** Two-dimensional distance class analyses for data in E and G, respectively. **I, K,** Spatial patterns for Regal and SRVR in plot 3. **J, L,** Two-dimensional distance class analyses for data in I and K, respectively. **M, O,** Spatial patterns for Regal and SRVR in plot 4. **N, P,** Two-dimensional distance class analyses for data in M and O, respectively. Significance of the SCF (standardized count frequency) for each distance class was determined at $P \leq 0.05$ and $P \geq 0.95$ for SCF values that were significantly greater and less than expected, respectively, than with a random spatial pattern.

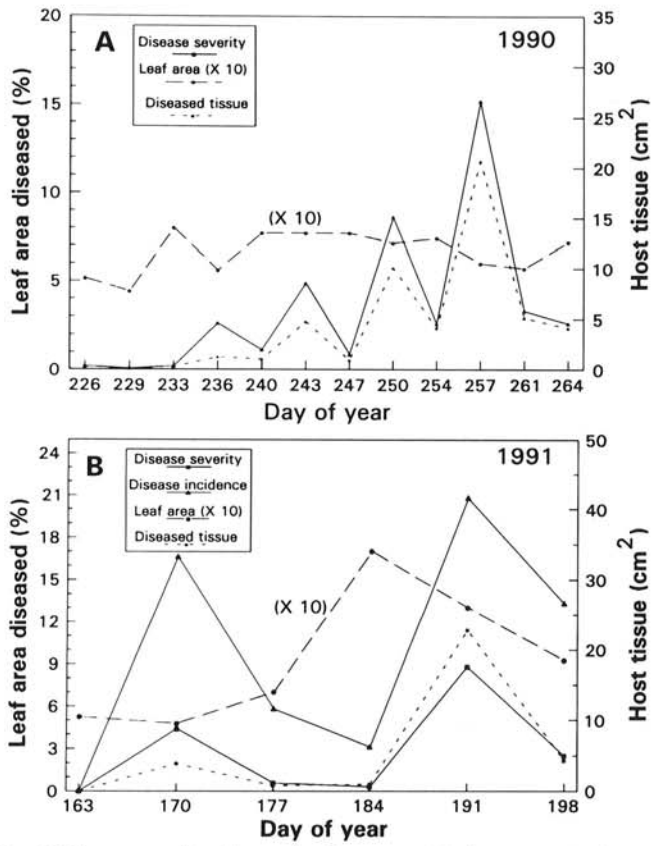

Fig. 5. Disease severity, disease incidence, total leaf area, total diseased leaf area, and leaf number versus time for a leaf spot epidemic on a single plant of white clover during a 6-wk growth period in a 10-ha pasture of *Trifolium repens* and *Festuca arundinacea* (tall fescue) in Wake County, North Carolina, in 1990 and 1991. **A,** Mean disease severity (percent leaf area diseased), disease incidence (percent leaves infected), total diseased leaf area (cm^2), and total leaf area (cm^2) versus day of year. Data are from one plant (Regal, plot 1, growth period 1 in 1991); disease severity estimates were made on each leaf of the plant at each of five assessment dates. **B,** Mean disease severity (percent leaf area diseased), total leaf area (cm^2), and total diseased leaf area (cm^2) versus day of year from a sample of 20 leaves per day from one plant (Regal, plot 4, growth period 2 in 1990).

similar for both hosts, and nearly linear over time (excluding the last sampling date) (Fig. 6B). Reduced rates of increase in disease severity were observed in the last week of period 1, and were correlated with a concurrent decline in total leaf number for plants of Regal and the SRVR germ plasm.

The effects of harvest on foliation and disease severity differed between the 20 virus-infected plants of Regal and the 20 virus-free plants of the SRVR germ plasm. Mean leaf number (175) for plants of the SRVR germ plasm was much greater than for plants of Regal (leaf number = 58) (Fig. 6B). During period 2, leaf addition kept pace with defoliation until day of year 236, then leaf number declined rapidly in a linear fashion for both host populations to the lowest number of the entire season (day of year 261) (Fig. 6B). Level of disease severity was reduced after harvest for plants of both Regal and the SRVR germ plasm (Fig. 6B). Progress curves were similar during period 2 (Regal versus SRVR); significant declines in disease severity occurred for both host populations in the latter part of the epidemic.

Progress curves for mean incidence of diseased leaves per plant (Fig. 6A) were similar in shape to progress curves for disease severity (Fig. 6B). Values for disease incidence at the beginning and end of each growth period were virtually the same for plants of both Regal and the SRVR germ plasm (Fig. 6A). Incidence of diseased leaves began to increase rapidly after day of year 177. Harvest reduced disease incidence by 8–10%. Incidence of diseased leaves was significantly greater for plants of the SRVR germ plasm during some weeks of period 2, but the number of infected leaves declined rapidly for both host populations in latter weeks of the epidemic.

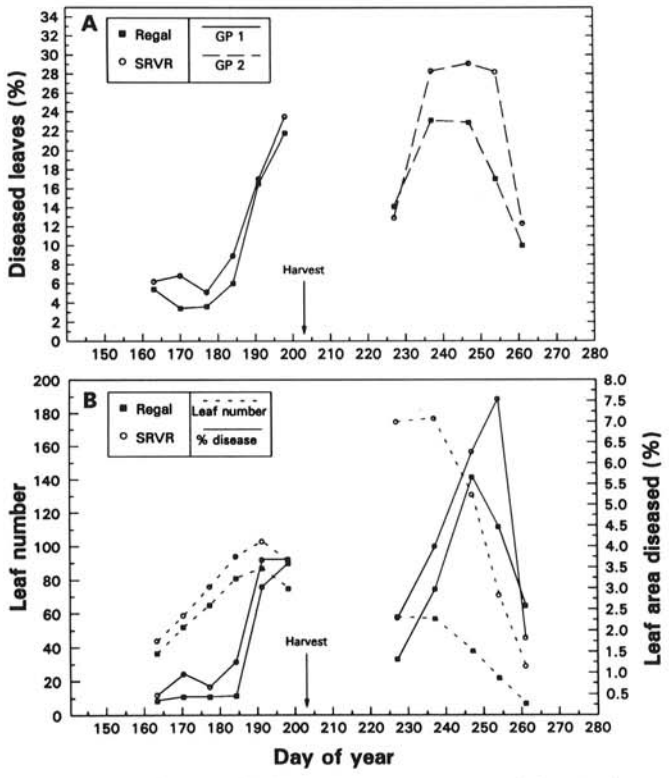

Fig. 6. Mean incidence of diseased leaves per plant (infected with at least one leaf spot pathogen), disease severity (percent leaf area diseased), and leaf number for a total 40 plants in four plots of either Regal or the Southern Regional Virus Resistant germ plasm (SRVR) (20 plants each) during two 6-wk growth periods (GP) on white clover in four plots in a 10-ha pasture of *Trifolium repens* and *Festuca arundinacea* (tall fescue) in Wake County, North Carolina, in 1991. A. Mean percentage of leaves infected per plant during GP 1 and GP 2 1991. **B,** Leaf number and percent leaf area diseased during GP 1 (day of year 161–199) and GP 2 (day of year 225–264). Plants of Regal were infected with peanut stunt virus during GP 2, whereas plants of the SRVR germ plasm were virus-free.

Spatial pattern analysis. Analysis of data for leaf spot disease severity via the method of Greig-Smith indicated significant aggregation (i.e., the presence of peaks in plots of scaled variance versus block size) for disease severity in more than 90% of the 288 data sets analyzed (three growth periods × eight host-plot combinations × 12 assessment dates per growth period). Data from period 2 in 1991 were not analyzed because many data points were lost with extensive plant mortality. Plots of scaled variance (equivalent to mean squares in nested analyses of variance) versus block size had multiple peaks in almost all cases, which may indicate more than one level of aggregation at levels of spatial resolution greater than the single quadrat (plant) (Fig. 7). No consistent differences between plots of both Regal and the SRVR germ plasm were detected concerning the existence of aggregation for disease severity or the presence of multiple peaks in graphs of scaled variance versus block size.

Primary cluster size for all plots was never constant for entire growth periods; temporal shifts in cluster size were observed in all plots (Figs. 7 and 8C,F). Drastic shifts in primary cluster size often were associated with significantly increasing or de-

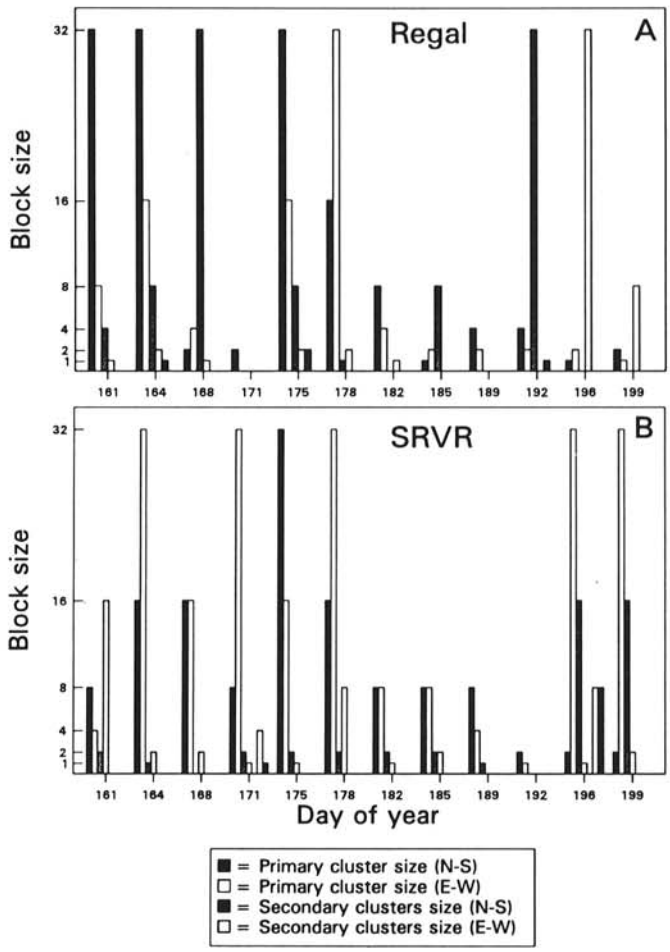

Fig. 7. Block size versus day of year as determined from analysis of leaf spot disease severity data via the Greig-Smith procedure for white clover plants of Regal and the Southern Regional Virus Resistant germ plasm (SRVR) in two, 64-plant, square lattices in plot 2 during growth period 1, 1991, in a 10-ha pasture of *Trifolium repens* and *Festuca arundinacea* in Wake County, North Carolina. Two orientations (parallel to the north-south or east-west plot axes), or methods of combining individual quadrats were used to obtain block sizes of 1, 2, 4, 8, 16, and 32. Blocks of size 1, 4, and 16 were square and occupied the same position for each orientation. Block sizes 2, 8, and 32 were rectangular, with the long axis of the rectangle parallel with either the north-south or east-west axes. Peaks were assessed subjectively. Peaks in scaled variance were taken to approximate cluster size. The block size at maximum variance was considered to represent the primary cluster size. Other peaks in variance represented secondary cluster sizes, or aggregation at different spatial scales.

creasing levels of disease severity (Figs. 8B,C,E,F and 9B,C,E,F). Shape of primary clusters also was temporally variable, shifting from elongate to roughly isodiametric or vice versa for most data sets. Consistent orientations of elongate, primary clusters with either the north-south or east-west axes were not detected, i.e., orientations were temporally variable within and among data sets (Figs. 7, 8C,F, and 9C,F). Average, primary cluster size and shape for specific plots (estimated visually from an examination of the graphs of block size versus day of year [Figs. 8C,F and 9C,F]) often was found to correspond with visual estimates of overall cluster size from scrutiny of spatial pattern maps of AUDPC for individual plants in each plot (Figs. 8A,D and 9A,D).

Overall, scaled variances for disease severity increased over time during epidemics. Progress curves for maximum scaled variance were very similar to progress curves for disease severity (Figs. 8B,E and 9B,E) in most plots. In some plots (e.g., plot 4 SRVR in period 2 1990; Fig 9B), declines in disease severity associated with defoliation were associated with a reduction in scaled variance and primary cluster size. In general, differences in magnitude of scaled variance between north-south and east-west orientations during epidemics reflected primary cluster shapes (elongate rectangles versus roughly isodiametric clusters) evident in graphs of block size versus day of year.

Spatial correlation analysis was not performed on data for AUDPC from period 2 in 1991 due to extensive number of missing values (dead plants). Analysis of the other 24 data sets for AUDPC (eight host/plot combinations × three growth periods) revealed that only 1% of the total number of possible mean autocorrelations (RHO-BAR, see Gottwald et al; 10) among quadrats were significant, positive spatial correlations. No negative correlations were detected. Analysis revealed well-defined and elongated (approximately two × four to six plants) groups of plants with similar values for AUDPC for only two data sets.

DISCUSSION

Defoliation and host growth during leaf spot epidemics on white clover are keys to understanding the apparently chaotic and ever-changing temporal and spatial progression of disease in this complex of multiple pathosystems. The nonmonotonic disease progress curves that we observed are indications of the violation of one of the implicit assumptions, e.g., 'constant host area', upon

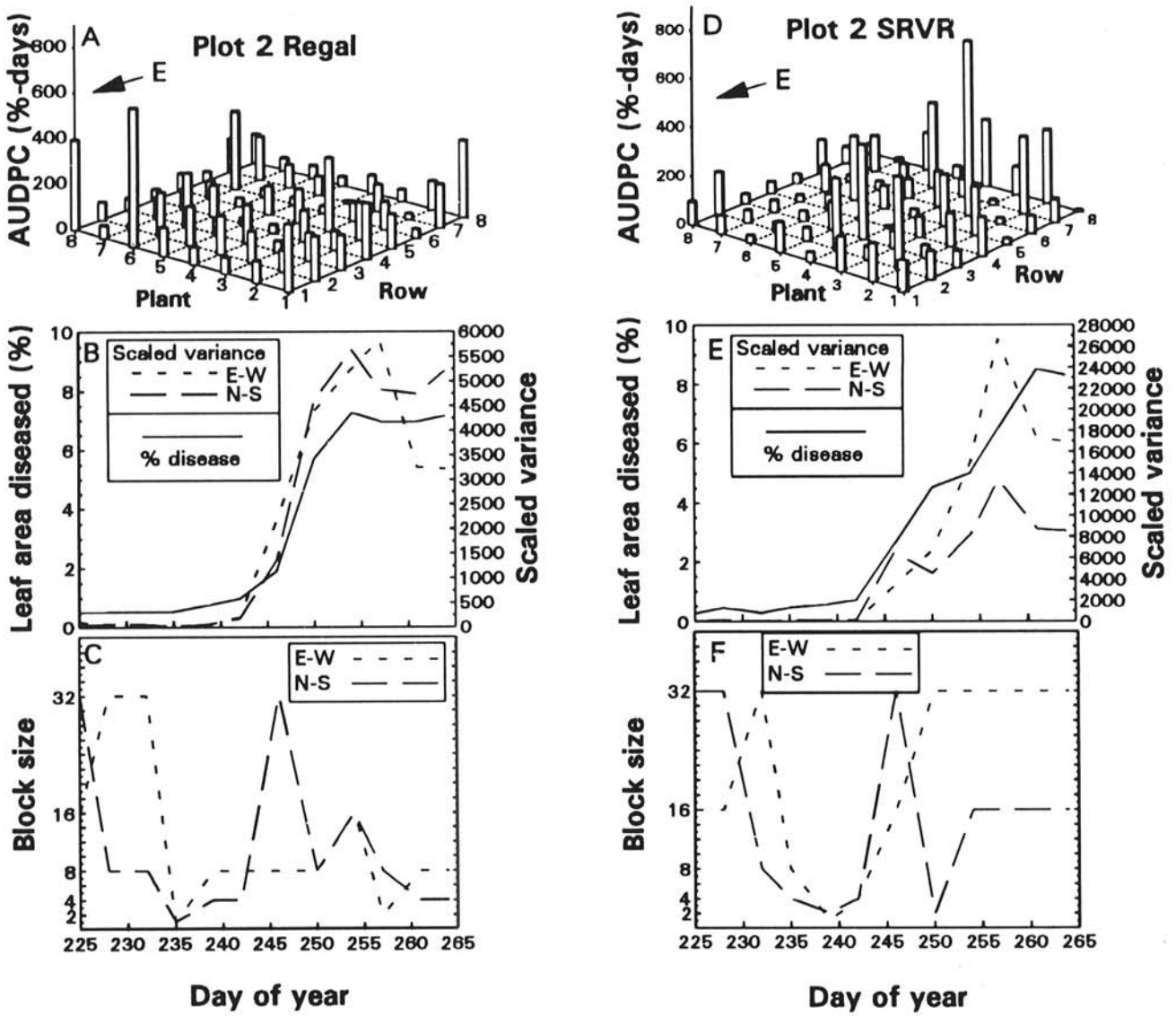

Fig. 8. Spatial pattern analysis and disease progress for disease severity data (percent leaf area diseased) for 64 white clover plants of both Regal and the Southern Regional Virus Resistant germ plasm (SRVR) in plot 2 for the second of two, 6-wk growth periods in 1990 during leaf spot epidemics on white clover in four plots in a 10-ha pasture of *Trifolium repens* and *Festuca arundinacea* (tall fescue) in Wake County, North Carolina. **A,** Map of total area under the disease progress curve (AUDPC) for plants of Regal. **B,C,** Scaled variance (with progress of disease severity) and block size, respectively (north-south and east-west orientations) versus day of year for plants of Regal. Scaled variance and block size determined by the Greig-Smith procedure. **D,** Map of area under the disease progress curve (AUDPC) for plants of SRVR. **E,F,** Scaled variance (with disease progress) and block size, respectively (north-south and east-west orientations), versus day of year for SRVR.

which growth curve models have been applied to the description of plant diseases. We believe that the intermittent addition and removal of large numbers of leaves (through host growth and defoliation) during epidemics are responsible for significant declines in observed disease severity in this pasture.

These two 'competing' forces—defoliation of diseased leaves and host re-growth—are mechanisms that result in the maintenance of relatively low levels for disease severity and disease incidence during leaf spot epidemics on white clover. The 'sawtooth' appearance of disease progress curves for individual plants and plant populations in this pathosystem indicates that values of Y_{max} for disease severity never approach 100%, due either to 'dilution' of disease severity (foliation in excess of disease increase) or loss of diseased tissue (pathogen-induced defoliation). Our disease rating scale was based on the assumption that Y_{max} for individual leaves does not exceed 65% diseased leaf area for most leaf spot pathogens in this system, because defoliation occurs at or below this level of disease. However, at the plant or population level, host foliation and defoliation during leaf spot epidemics created an asymptotic maximum disease severity of approximately 8–25%. Host growth and defoliation also resulted in the maintenance of incidence of infected leaves per plant at $\leq 30\%$. Estimates of disease severity or counts of infected leaves at a single point in time lend the appearance that the epidemics are not severe (e.g., 5% leaf area diseased). However, the cumulative effects of continual, pathogen-induced defoliation during periods of weather that were unfavorable for host growth (very hot and dry) contributed to reduced yield and plant debility and mortality.

Defoliation, disease incidence, and patterns of diseases (and pathogens) during epidemics are natural components influencing the growth and ecology of white clover plants. Garren (8) outlined a damage/growth cycle for white clover plants during multiple-pathogen epidemics of concurrent leaf spot and petiole diseases for conditions in Alabama. From January to mid-February was the 'period of debility', in which defoliation was due largely to cold-induced debility. Mid-February through April was the 'period of maximum vegetative growth' (minor pathogen activity and considerably more growth of foliage than defoliation). May

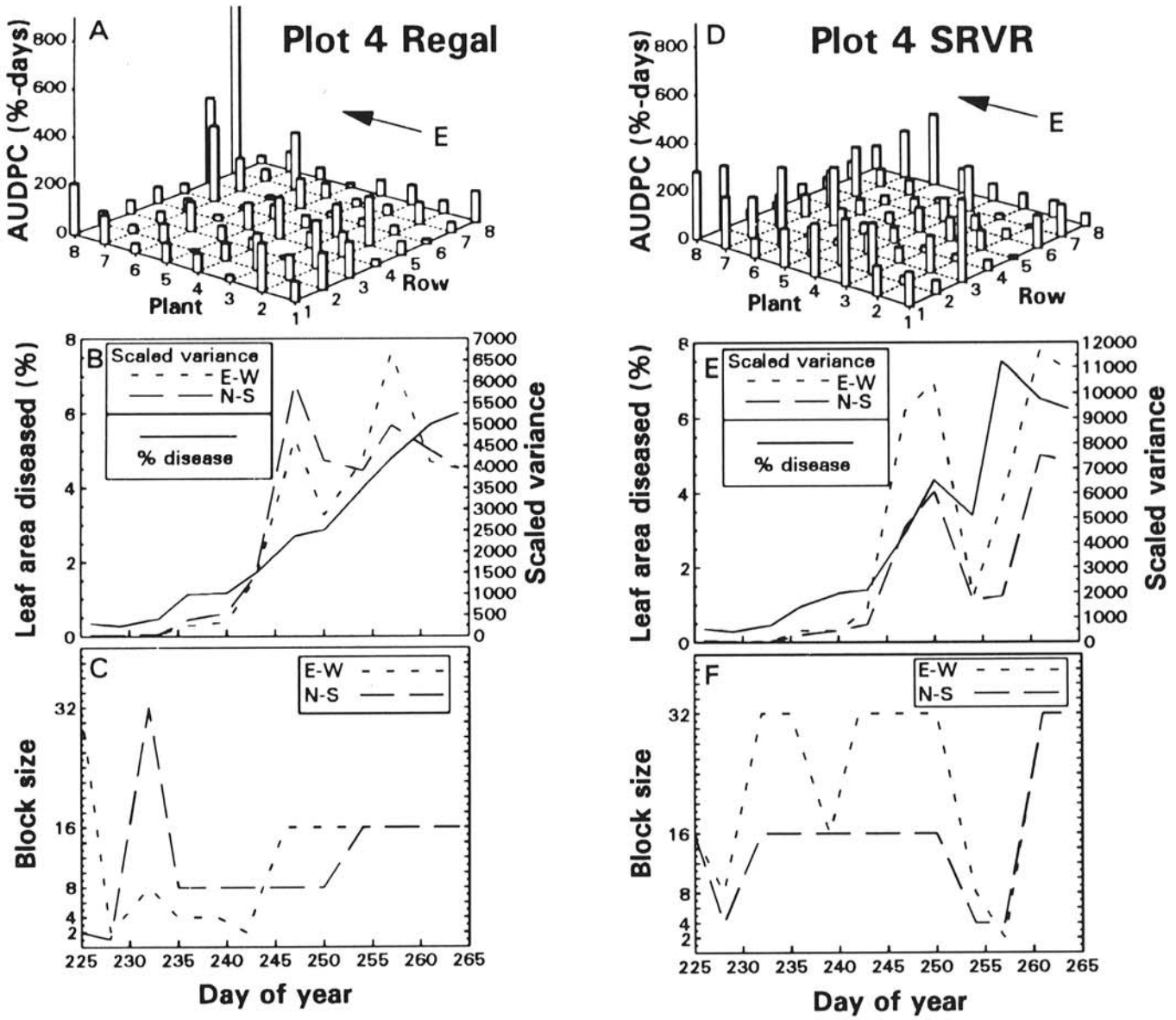

Fig. 9. Spatial pattern analysis and disease progress for disease severity data (percent leaf area diseased) for 64 white clover plants of both Regal and the Southern Regional Virus Resistant germ plasm (SRVR) in plot 4 for the second of two, 6-wk growth periods in 1990 during leaf spot epidemics on white clover in four plots in a 10-ha pasture of *Trifolium repens* and *Festuca arundinacea* (tall fescue) in Wake County, North Carolina. **A,** Map of area under the disease progress curve (AUDPC) for plants of Regal. **B,C,** Scaled variance (with disease progress) and block size, respectively (north-south and east-west orientations) versus day of year for plants of Regal. Scaled variance and block size determined by the Greig-Smith procedure. **D,** Map of area under the disease progress curve (AUDPC) for plants of SRVR. **E,F,** Scaled variance (with disease progress) and block size, respectively (north-south and east-west orientations), versus day of year for plants of SRVR.

and June was a 'static period' in which vegetative growth was still good, but slowed by flowering (and in which the 'high temperature society' of pathogens, e.g., *Rhizoctonia* spp. and *Curvularia* spp., began activity). July-October was the 'period of maximum defoliation', during which high temperatures, erratic precipitation, and extreme activity of the 'high temperature society' (7) of pathogens overwhelmed host growth (which continue through this period), resulting in twice as much defoliation as growth. November-December was a 'transition period'.

The maxima for leaf damage to white clover in the Alabama leaf spot system occurred just before and just after the period of maximum vegetative growth. Period 1 and period 2 in our study correspond roughly with Garren's static period and period of maximum defoliation, respectively. Disease severity and defoliation were greater during period 2 than for period 1 in both 1990 and 1991, perhaps because of the activity of a summer society of leaf spot pathogens (e.g., *R. solani, P. andropogonis,* and *C. trifolii*). In period 1, for some plants leaf addition outpaced pathogen spread and disease development, which resulted in reduced estimates of disease severity between assessments. Increasing incidence of *R. solani* and *Curvularia trifolii* was associated with much defoliation during period 2. Thus, our leaf spot epidemics were similar to those in Alabama with regard to the composition of the disease complex (24) and the temporal occurrence and relative, temporal importance of host growth versus defoliation.

Defoliation and host growth also may be keys to understanding changing spatial patterns in this complex of multiple pathosystems. The spatial attributes of the leaf spot complex as a whole were similar to the spatial patterns of the component pathogen organisms (25): aggregated, and with continual shifts in strength of aggregation or cluster size, number, and morphology. Clusters of similar levels of disease severity for the disease complex underwent rapid shifts in size and number during the leaf spot epidemics in 1990 and 1991. As in the alfalfa leaf spot system (30), these shifts often were associated with increasing levels of disease severity or with periods of defoliation and declining disease severity, which suggests that as defoliation occurs in some areas and disease increases in other areas, new foci of disease occur.

The existence of aggregation at several spatial scales that we observed is similar to that found in an alfalfa leaf spot pathosystem (30). The idea that clustering occurs at various levels of spatial resolution suggests that over large areas of relatively homogeneous disease, there are areas, and areas within areas, in which disease is progressing either more rapidly or more slowly than expected, perhaps due to environment. In general, primary cluster (e.g., peaks in scaled variance) size was much larger than the individual plant, indicating a disease complex that can spread rapidly through a population, yet cause locally severe damage.

It is not clear how aggregation at several spatial scales may be related to differences in spatial occurrence and cluster size that we observed for the individual pathogens (25). For example, summer blight and Curvularia leaf spot were both severe diseases in our pasture, but clusters of *R. solani*-infected plants generally were larger than those for *C. trifolii*-infected plants (25). Thus, aggregation at different spatial scales for the leaf spot complex in this pathosystem may imply more than just the existence of conducive microclimates within plots; the overall patterns may reflect the predominance, or spatial occurrence, of individual pathogen species. However, the net effect (in terms of AUDPC) of shifting size, number, and location of clusters during epidemics was revealed in the autocorrelation analysis of AUDPC values. Significant, positive autocorrelations among plants for AUDPC were observed rarely, suggesting an equilibrium of disease within plots in relation to larger time frames.

We used a spaced-plant system to study the leaf spot and virus epidemics both on individual plants and in populations of plants. This would have been exceedingly difficult (if not impossible) in a natural setting wherein plant stolons are intertwined. However, we feel that there were enough similarities between our plots and a natural pasture (e.g., genetic variability, plants within a grass sward) to warrant some initial conclusions about processes that may occur in natural pasture pathosystems. We also feel justified in analyzing spatial and temporal aspects of the disease complex as a whole and in genetically diverse populations, because these are the very attributes of leaf spot epidemics as they occur in natural populations of white clover. Nonetheless, it must be noted that the spatial (e.g., blocked-quadrat techniques) and temporal analyses for genetically diverse populations for multiple-pathogen systems should be interpreted with caution.

The implicit assumption of 'one disease' may have some relevance to the white clover leaf spot pathosystem, but only in reference to 'leaf spot' versus 'viral' diseases. For example, we found little evidence to suggest that leaf spot disease incidence, severity or spatial patterns were different in a virus-resistant population versus a virus-susceptible population of white clover plants. However, differences and variability between genotypes could mask any effect of virus on leaf spot diseases in this and other environments. Thus, more study is needed to determine whether future modeling of this complex of pathosystems may ignore the potential interactions among the viruses and leaf spot organisms. Populations of the SRVR germ plasm contained numerous virus-susceptible individuals; hypothetically, future improvement for virus-resistance in this germ plasm, or efforts to enhance genetic uniformity, may have a significant impact upon progress of leaf spot epidemics.

The 'one disease' assumption may not hold universally for the leaf spot complex on white clover. Our observations indicate that a large proportion of the leaf spot disease on white clover was attributable to *R. solani, P. andropogonis,* and *S. meliloti.* However, all of the other leaf spot pathogens caused severe disease at certain times, or in certain areas, or on certain plants (24,25), perhaps due to genetic differences among plants with regard to disease susceptibility. Previous evidence for strong, negative associations among leaf spot pathogens of white clover (24) suggests that individual pathogens usually occur alone on leaves. Thus, estimates of disease severity on leaves may reflect the effects of individual pathogens. Accordingly, the relative incidence of the various leaf spot pathogens may influence specific temporal and spatial aspects of disease progress (e.g., greater defoliation that is associated with summer blight than for black spot may give progress curves a more 'sawtooth' appearance). We believe that the basic spatial attributes (strong aggregation, relatively large clusters of plants with similar disease levels, temporal shifts in spatial parameters) of the leaf spot complex on white clover reflect the predominance of the defoliating summer blight (*R. solani*) and the rapidly spreading black spot (*P. andropogonis*) pathogen. The fact that some species are predominantly cool-weather pathogens (i.e., *L. trifolii*) suggests that disease progress curves during periods, when pepper spot dominates (spring, fall), would reflect primarily the attributes of vigorous host growth and the interaction of *T. repens* and *L. trifolii.*

The classic growth curve or population dynamics models did not fit the disease progress curves for the spatially and temporally variable, white clover leaf spot pathosystem. They failed to describe the epidemics because some of the implicit assumptions were inadequate approximations of reality. To correct for these inadequacies, areas of future research and model expansion for prediction and management of this pathosystem should focus upon host growth/defoliation and multiple pathogens as top priorities. Also, the question of Y_{max} (21), or maximum level of disease possible—at a leaf level, plant level, population level—should be addressed to optimize disease assessment. Once these issues are addressed, efficient prediction, modeling, and management of this complex pathosystem may be possible.

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