Infection by Clover Yellow Vein Virus Alters Epidemic Components of Cercospora Leaf Spot on White Clover

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ABSTRACT

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Epidemic components of leaf spot caused by *Cercospora zebrina* were evaluated in repeated experiments on two clones of *Trifolium repens* 'Tillman', which vary in response to infection by clover yellow vein virus (CYVV). CYVV-free and CYVV-infected plants of clones T7 (low virus titer, virtually asymptomatic) and T17 (high virus titer, symptomatic) were grown at 28/23 or 22/17 C and monitored for 17 days after inoculation with *C. zebrina*. Altered epidemic components for CYVV-infected plants of T17 compared with those of CYVV-free plants of T17 included diminished lesion density, shortened latent period, larger lesions, greater proportion of leaves with sporulating lesions, reduced defoliation, and reduced disease incidence and severity. For CYVV-infected plants of T7, incubation period (at 28/23 C) and latent period were shortened compared with CYVV-free plants. A clone × virus status × temperature interaction was found for incubation period and lesion diameter. Incidence of infected leaves was greater at 22/17 C than at 28/23 C, regardless of clone/virus status combination. In a separate study, CYVV-infected plants of each clone had a reduced incidence of petioles infected by *C. zebrina* at 9 days after inoculation and more conidia per square millimeter of lesion at 10, 12, and 14 days after inoculation at 24 C than CYVV-free plants.

White clover (*Trifolium repens* L.) is used widely in a legume-grass mixture for pastures in most humid, temperate regions. The ladino (large) type of white clover is the principal grazing/forage legume in the United States. White clover provides nitrogen to the sward, thereby diminishing the need for chemical fertilizer. It reduces soil erosion and is a high-protein component of animal diets. However, a combination of stresses (diseases, insect damage, adverse environmental and management practices) has been associated with the premature decline of stands of white clover in pastures 2–3 yr after seeding (10,13).

The destructive and debilitating effects of plant pathogenic viruses and fungi are an important constituent of the clover decline syndrome. Infection by viruses that are transmitted nonpersistently by aphids occurs commonly in white clover (1), reduces plant vigor, and decreases forage and seed yields (2,4,14,15,21). These viruses have wide host ranges and, after overwintering in white clover and/or other perennial legumes, may pose a severe threat to proximal commercial legumes such as soybean or peanut. The viruses most prevalent in white clover in the southeastern United States are alfalfa mosaic virus, peanut stunt virus, and clover yellow vein virus (CYVV) (1). Virus incidence increases within a stand of clover from year to year, but may reach 80% within the first year after planting (7).

Leaf spotting and blighting fungi and bacteria also commonly infect white clover. Species of *Cercospora, Colletotrichum, Leptosphaerulina, Phoma, Pseudomonas, Rhizoctonia, Stagonospora,* and *Stemphylium* can be isolated readily from lesions on leaves and/or petioles throughout the life span of a clover plant (17, S. C. Nelson and C. L. Campbell, *unpublished data*). When conditions are conducive for dissemination of and infection by these organisms, losses in clover yield may be high (3).

Interrelationships among viruses and foliar, fungal pathogens are poorly understood in forage systems. Little is known of components of resistance and susceptibility to leaf-infecting fungi (alone or in combination with viral pathogens). Such information would be of value from both a theoretical and an applied perspective. From a theoretical standpoint, disease progress models that accommodate specific epidemic components contain parameters that may have a more apparent biological interpretation than simpler, less refined models (5,11). Also, the practical understanding and management of natural epidemics may be enhanced through an examination of the correlations between controlled-environment assessments of components of disease resistance and quantitative descriptions of leaf spot epidemics in the field (12). The objective of this research was to investigate the effects of viral infection of white clover upon components of a fungal leaf spot epidemic. The pathogens chosen for this investigation, CYVV and *Cercospora zebrina* Pass., often infect ladino clover in North Carolina. Experiments were conducted in controlled environments to remove the influences of unmanageable stresses imposed under field conditions.

MATERIALS AND METHODS

Plant material. Clonal host material was used to remove variation due to genetic variability. The two clones, T7 and T17, selected originally from a population of plants of ladino white clover cultivar Tillman, were chosen for study based on their disparate reaction to infection by CYVV with respect to yield, symptom expression, and virus titer and distribution within plants (21). Plants of T7 have a lower CYVV titer and are virtually asymptomatic, whereas plants of T17 support a higher CYVV titer and display classic and severe symptoms of CYVV infection (veinal yellowing and necrotic flecks). T7 and T17 were maintained in a greenhouse as CYVV free and CYVV infected (infected naturally) in screen (32 × 32 mesh) cages (0.96 × 0.87 × 1.22 m) (6). Plants for all experiments were derived originally from a single plant of each clone. Clonal populations were increased by allowing 2.5- to 4.5-cm-long stolon cuttings to root in

pasteurized sand for 2 wk. Rooted stolons were inoculated with a compatible *Rhizobium* spp. Virus status of stock plants was confirmed by an enzyme-linked immunosorbent assay (ELISA) (8) by Mike McLaughlin (USDA-ARS, Mississippi State University).

The 2-wk-old plants were transferred to the Southeastern Plant Environment Laboratory at the North Carolina State University (NCSU) Phytotron. Plants were sprayed with malathion for insect control and transplanted into 15.2-cm-diameter (1,650 ml) plastic pots containing a steam-pasteurized mixture of pea gravel and peat (1:1, v/v). Plants were sorted by virus status into two groups (CYVV infected and CYVV free) and placed into separate greenhouses (23/18 C). After 4 wk, plants were moved to two walk-in, controlled-environment chambers (8 m^2 floor area with a vertical clearance of 2.13 m) where plants grew for 3 days before inoculation with *C. zebrina*. Chamber temperature regime, 22/17 or 28/23 C (12/12 h day/night), represented early- or late-season growing conditions in the Piedmont region of North Carolina. A 12-h light/dark photoperiod with an illuminance at pot level of 250 μmol m^{-2} s^{-1} was provided by incandescent and cool-white fluorescent lamps. Each plant was watered automatically with 100 ml of deionized water twice daily.

Preparation and application of fungal inoculum. *C. zebrina* was isolated from infected ladino white clover at the NCSU Unit 9, Forage Research Facility in Wake County, NC, in October 1988 (20). The monoconidial isolate was maintained in a greenhouse on white clover cultivar Regal. Dried, infected leaves stored at room temperature were used to initiate cultures on V8 juice medium (19). Cultures were prepared and conidia obtained for inoculum by the method of Latch and Hanson (16). Inoculum concentration in sterile, distilled water was adjusted to 0, 1×10^5, or 2×10^5 conidia per milliliter. Tween 20 (Fisher Scientific, Orangeburg, NY) was added to spore suspensions (80 μl/L) to facilitate dispersal of conidia and their adhesion to leaf surfaces. Plants were inoculated within the controlled-environment chambers by spraying spore suspensions onto clover leaves with an atomizer until runoff. Uninoculated controls were included, in part, to detect the presence of any unwanted pathogens. Plants were enclosed in clear plastic bags for 84 h after inoculation to maintain leaf wetness. After removal from plastic bags, plants were maintained at 98–100% RH (night) and 85–90% RH (day) via automatic misting with deionized water (5 sec every 5 min and 5 sec every 15 min, respectively).

Experimental design. The experiment was designed and analyzed as a split-plot, with temperature as the whole plot and a factorial arrangement of the subplot. Twelve treatments were assigned randomly to six blocks within each chamber. One plant was assigned randomly to each treatment-block combination, for a total of 72 plants in each of the two temperature regimes. Treatments were combinations of two clones (T7, T17), virus infection status (CYVV free and CYVV infected), and two levels of fungal inoculum (1×10^5 and 2×10^5 conidia per milliliter of sterile distilled water). No disease developed on the uninoculated controls, and these plants were not included in the analysis. Chamber temperature was assigned randomly for each of the two runs (replicates in time) of the experiment. The following notation is used to identify clone/virus status combinations: T17 = clone T17, CYVV free; T17-CYVV = clone T17, CYVV infected; T7 = clone T7, CYVV free; and T7-CYVV = clone T7, CYVV infected. The experiment was repeated once.

Runs, temperatures, clones, virus status, and fungal inoculum levels were considered as sources of variation in the fixed-effects model. Based on a previous experiment, blocking within chambers was done to account for variation in relative humidity in the controlled-environment chambers. In the analysis of variance (23), significance of the temperature effect was determined by using the runs × temperature interaction as the error term; significance of the main effects of clone, virus status, fungal inoculum level (and their interactions), and effects of temperature × clone/virus status/fungal inoculum level interactions were determined by using the runs × clone × virus status × fungal inoculum level interaction term.

Epidemic components. Each run of the experiment was terminated 17 days after inoculation to ensure that disease components were evaluated before a second incubation period (second generation of lesions) could occur (S. C. Nelson and C. L. Campbell, *unpublished data*). From 7 to 17 days after inoculation, the following components of Cercospora leaf spot epidemics were assessed at 2-day intervals: disease incidence (percentage of total number of leaves infected per plant), disease severity (percentage of leaf area diseased), defoliation (percentage of total number of leaves defoliated per plant), and sporulation (percentage of total number of leaves with at least one sporulating lesion per plant). Disease severity was estimated visually with the aid of a rating scale with severity classes 0–8 based on percentage of leaf area diseased: 0, 0.1–2.5, 2.6–5.0, 5.1–10.0, 10.1–15.0, 15.1–25.0, 25.1–35.0, 35.1–50.0, 50.1–65.0. Y_{max} (maximum proportion of leaf area diseased) was assigned a value of 65% due to the tendency of clover leaves to defoliate at $\leq 65\%$ disease severity (S. C. Nelson and C. L. Campbell, *unpublished data*). Leaves with collapsed petioles or complete necrosis due to Cercospora leaf spot were classified as defoliated and were assigned a value of Y_{max} for the purpose of calculating disease severity. Estimates of disease incidence and disease severity were obtained for each leaf on each rating day and a composite value of disease was calculated for each plant. Sporulation was assessed visually.

The following variables were calculated from data on disease incidence, disease severity, defoliation and sporulation:incubation period, latent period, area under the curve (AUC) for disease severity, disease incidence, and defoliation, and rate of disease progress (slope parameter from simple linear regression). Slope values were subjected to analysis of variance to identify significant main effects or interactions. Incubation period was defined as the mean days after inoculation when 50% of the total infected leaves (as tabulated at 17 days after inoculation) were symptomatic. Latent period was defined as the mean day after inoculation when sporulation first was observed.

At 11 days after inoculation, lesion density and lesion size were evaluated. Lesion density was determined from a nondestructive sample of three leaves per plant. Leaves were selected on the basis of position on stolons to remove variability associated with leaf age. Leaf positions were numbered in ascending order from the plant center to the distal leaflet on each of three arbitrarily selected stolons per plant. Leaves at position three (the third oldest leaf on the stolon) were assessed for lesion density. Leaflets were assumed to approximate rectangles, and leaflet area was calculated from measurements of length and width of leaflets. Total lesion number was determined from visual counts, and lesion density expressed as lesions per square millimeter of leaf. Lesion size was estimated from a nondestructive census of each infected leaf on every plant; a visual approximation of mean size of discrete lesions on each leaf was obtained by using a rating scale consisting of lesion size classes 1–7 based on lesion diameter in millimeters (values are midpoint of range): 1, 2, 3, 4, 5, 6, and 7.

Petiole infection and spore production. In a separate study, plants of clones of T7 and T17 (CYVV free and CYVV infected) were propagated and maintained as described in the controlled-environment experiment. Twelve cuttings each from plants of clones T7 and T17 (CYVV free and CYVV infected) were allowed to root for 3 wk in pasteurized sand on a greenhouse bench before transplanting plants singly into 10-cm-diameter clay pots (500 ml) containing Terra-Lite Metro Mix 220 (W. R. Grace & Co., Cambridge, MA). Plants then were grown for 3–4 wk before inoculation with *C. zebrina*.

Plants (four of each clone/virus status combination) were arranged randomly on a greenhouse bench for each of three runs of the experiment. Fungal inoculum (6×10^5 conidia per milliliter) was prepared and applied as in the controlled-environment experiment. The greenhouse bench was enclosed in clear plastic supported by a wooden frame (to maintain high RH) and covered with brown paper (to minimize heat accumulation within the enclosure) for 96 h after inoculation. Leaf wetness and high RH were provided by two cool mist vaporizers that operated for 30

sec every 3 min. After incubation, plants were removed from the plastic enclosure and placed on a greenhouse bench until 10 days after inoculation. At 9 days after inoculation, incidence of petioles with at least one lesion was determined. At 10 days after inoculation, the plants were arranged randomly within a growth chamber at a constant temperature of 24 C. Cool-white fluorescent lights (illuminance at pot level of 64 μmol m^{-2} s^{-1}) provided a 12 h light/dark photoperiod.

In vivo production of conidia by *C. zebrina* was measured at 10, 12, and 14 days after inoculation. A single groove (3 × 8 mm) was cut into the lateral face of both lid and base of plastic petri dishes (100 × 15 mm), so that upon joining the lid and base and aligning the grooves, a 3- × 6-mm opening was created. The base of the petri dish was affixed with adhesive tape to a 2-mm-diameter steel rod (10 to 20 cm long). Filter paper (9 cm diameter) was placed within the petri dish and moistened with sterile distilled water. The steel rod with attached petri dish was inserted into the soil near the base of each plant at approximately a 45-degree angle. The petri dish was opened, and a clover leaf with lesions characteristic of Cercopsora leaf spot was placed inside with petiole extending through the 3- × 6-mm opening. After 24 h at 24 C, the petiole was cut before assessment of sporulation.

Leaves were examined at 40× to identify the three lesions per leaf with the most abundant production of conidia. One 4- × 4-mm square was cut with dissecting scissors from the center of each of the three lesions and suspended in 400 μl of distilled water with Tween 20 (400 μl/L) in a 1.5-ml microcentrifuge tube. Conidia were dislodged into suspension by placing the microcentrifuge tubes on a Vortex-Genie mixer (Scientific Industries, Inc., Bohemia, NY) for 30 sec. Based on estimates of variance and mean from a preliminary sample (coefficients of variation ranged from 0.03 to 0.23), sample size was three 20-μl aliquot samples of the resulting spore suspension. Conidia were counted directly at 40× and data expressed as conidia per mm^2 lesion (the mean of the three aliquot samples per leaf over the three sample dates). Virus status of each plant was confirmed by ELISA (8) for CYVV at the conclusion of each of the three runs.

The experiment was designed and analyzed as a randomized complete block, with runs as blocks in time. In the analysis of variance (23), significance of the main effects of clone, virus status, and days (and their interactions) with respect to sporulation was determined by using the runs × clone × virus × day interaction as the error term. Regarding petiole infection, significance of the main effects of clone and virus status (and their interaction) was determined by using the runs × clone × virus interaction term. For significant ($P \leq 0.05$) interactions, Student's t tests were used to identify significantly different pairs of means.

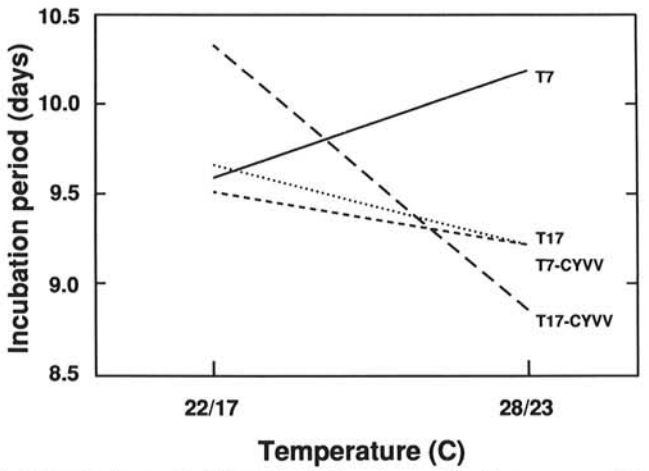

Fig 1. Incubation period (days) for leaf spot caused by *Cercospora zebrina* on plants of two clones (T7 and T17) of ladino white clover cv. Tillman, infected with clover yellow vein virus (CYVV) or CYVV free, grown for 17 days after inoculation at two temperature regimes. Incubation period was defined as the mean days after inoculation when 50% of the total infected leaves (as tabulated at 17 days after inoculation) were symptomatic. Temperature × clone × virus interaction significant ($P < 0.01$). Means are data averaged over two levels of fungal inoculum.

RESULTS

Epidemic components. Results of the two runs of the experiment were similar; rankings of means between runs were consistent for all components evaluated. No runs × treatment or runs × temperature interaction terms were significant in the analysis of variance. Because repeatability of the results was confirmed, the data presented are combined means.

Lesion density. When plants of clone T17 were infected with CYVV, lesion density was reduced significantly from that on CYVV-free plants of T17 (Table 1). Conversely, the number of lesions per square millimeter leaf was virtually identical on CYVV-infected and CYVV-free plants of clone T7. Neither temperature nor concentration of fungal inoculum had an effect ($P = 0.05$) on lesion density.

Incubation period. A complex, three-way interaction (temperature × clone × virus status) was found for incubation period (Fig. 1). Incubation period on plants of T17-CYVV was longer than on plants of T17 at 22/17 C, but was shorter on plants of T17-CYVV than on plants of T17 at 28/23 C. On plants of T7 and T7-CYVV, incubation period did not differ at 22/17 C. However, incubation period on T7-CYVV was significantly shorter than on plants of T7 at 28/23 C. At 22/17 C, the incubation period was longer than at 28/23 C for plants of all clone/virus status combinations except for plants of T7. Plants of T17-CYVV had the greatest disparity in incubation period at the two temperature regimes.

Latent period. Virus infection of plants of both clones resulted

TABLE 1. Effects of clover yellow vein virus (CYVV) infection of plants of clones T7 and T17 of white clover, cv. Tillman, on epidemic components[a] of leaf spot caused by *Cercospora zebrina* at 28/23 and 22/17 C

Component	C × V[b] interaction	Clone-virus status combination[c] T7	T7-CYVV	T17	T17-CYVV
Lesion density[d]	***	0.30	0.30	0.30	0.06
Latent period[e]	+	14.8	13.9	12.5	10.3
AUC[f]					
Disease incidence	*	520	570	609	474
Disease severity	*	189	186	187	118
Defoliation	*	241	228	196	122
Rate of disease progress[g]					
Disease incidence	−	0.05	0.05	0.05	0.04
Disease severity	*	0.04	0.03	0.04	0.02
Defoliation	−	0.05	0.04	0.05	0.03
Disease at 17 days after inoculation					
Disease incidence	*	0.68	0.70	0.76	0.62
Disease severity	*	0.33	0.32	0.35	0.23
Defoliation	−	0.47	0.43	0.47	0.28

[a] Values are "marginal" means: the mean of one treatment factor averaged over two levels of other treatment factors (e.g., clone-virus status combination averaged over two temperature regimes and two levels of fungal inoculum concentration) on plants monitored for 17 days after inoculation.

[b] Symbols denote level of significance for clone × virus interaction. − = $P > 0.05$ (not significant); + = $P \leq 0.10$; * = $P \leq 0.05$; *** = $P \leq 0.001$.

[c] Infection by CYVV denoted by -CYVV appendage.

[d] Lesion density expressed as number of lesions per square millimeter leaf at 11 days after inoculation.

[e] Latent period defined as mean days after inoculation when sporulation was first observed.

[f] Area under the disease progress curve (percent-days).

[g] Rate expressed in percent disease per day, estimated from linear regression analysis. Epidemic component and range in coefficient of determination (R^2) in parentheses: disease incidence (0.48–0.54), disease severity (0.64–0.70), and defoliation (0.41–0.55).

in a significantly reduced latent period (Table 1). Conidia formed from 1 to 2 days earlier on CYVV-infected plants. Latent period was shorter on plants of clone T17 than on clone T7 for both CYVV-free and CYVV-infected plants.

Lesion diameter. Plants of T17-CYVV had larger lesions ($P = 0.01$) at both temperature regimes than did the other three clone/virus status combinations (Fig. 2). Lesion diameter for plants of T7 and T7-CYVV did not differ within or between temperatures. Lesion diameter on plants of T17-CYVV was greater ($P = 0.05$) at 28/23 C than at 22/17 C. Lesions were significantly larger at both temperature regimes on CYVV-infected plants of T17 (the CYVV-sensitive clone) than on CYVV-free plants of clone T17.

Leaves with sporulating lesions. Plants of T17-CYVV had a significantly greater percentage of leaves with at least one sporulating lesion at each day after inoculation than did other clone/virus status combinations. A clone × virus status interaction was found at most days after inoculation for percentage of leaves with sporulating lesions (Fig. 3). Plants of T7-CYVV had a numerically greater proportion of leaves with sporulating lesions than did plants of T7 at 11, 13, and 15 days after inoculation. Plants of T17 had a greater proportion of leaves with sporulating lesions than did plants of T7 at 11 and 15 days after inoculation.

Disease incidence, severity, and defoliation. For disease incidence, plants of T17-CYVV had a significantly smaller AUC and final percent disease than did plants of T17 and the other clone/virus status combinations (Table 1). Plants of T17, T7, and T7-CYVV did not differ with respect to AUC and final percent disease. Rate of disease progress was not affected by CYVV infection of either clone. Temperature affected ($P = 0.05$) disease incidence at 17 days after inoculation; over all levels of the other sources of variation, percent disease was 64 at 28/23 C and 74 at 22/17 C. With regard to disease severity, CYVV-infected plants of clone T17 had a significantly smaller AUC, rate of disease progress, and final percent disease (17 days after inoculation) than did CYVV-free plants of clone T17 (Table 1). Conversely, virus infection of clone T7 had no apparent effect on disease severity. CYVV-free plants of both clones did not differ with respect to the measures of disease severity. For the epidemic component defoliation, plants of T17-CYVV had a significantly smaller AUC and final percent defoliation (17 days after inoculation) than did plants of T17 and T7 (CYVV infected or CYVV free) (Table 1). Plants of T17, T7, and T7-CYVV did not differ with respect to AUC and final percent defoliation. The rate of defoliation was reduced numerically on CYVV-infected plants of both clones.

Petiole infection and spore production. Results of the three runs of the experiment were similar; rankings of means among runs were consistent for the components evaluated. No runs × treatment interaction terms were significant in the analysis of variance. Because repeatability was confirmed, the data presented are combined means. With respect to sporulation capacity, only the main effects of clone and virus status were significant. Production of conidia was more profuse ($P = 0.05$) on CYVV-infected plants of both clones. Number of conidia per square millimeter of lesions averaged over the three assessment dates was 145.5 for T7, 221.7 for T7-CYVV, 178.7 for T17, and 276.5 for T17-CYVV. With respect to petiole infection at 9 days after inoculation, only the main effect of virus status was significant in the analysis of variance. CYVV-infected plants of both T7 and T17 had a reduced ($P = 0.05$) incidence of infected petioles at 9 days after inoculation as compared with their CYVV-free counterparts. Proportion of infected petioles was 0.52 for T7, 0.39 for T7-CYVV, 0.48 for T17, and 0.19 for T17-CYVV.

DISCUSSION

Viral infection (CYVV) of ladino white clover altered components of a fungal leaf spot epidemic (*C. zebrina*). The number and magnitude of affected components were largely a function of clonal identity and the disparate reaction of T7 and T17 to infection by CYVV. Clone T17 exhibits severe and classic symptoms when infected with CYVV (stunting, veinal yellowing, mottling, necrotic flecks, and veinal necrosis), whereas CYVV-infected plants of T7 are virtually asymptomatic (mild stunting only). Furthermore, Ragland et al (21) found that CYVV-infected plants of clone T17 had higher virus titer in most leaves and roots and greater yield reduction than did CYVV-infected plants of clone T7. They suggested that plants of clones T7 and T17 differ in the ability to support and maintain multiplication of CYVV and in distribution of CYVV within and among plant organs. Our analysis was congruent with the earlier differences found between the clones, i.e., virus-induced effects on host reaction to *C. zebrina* varied between clones. Thus, CYVV-symptom expression, virus titer, and/or virus distribution among plant organs and tissues may have an explicit influence on the epidemic components of Cercospora leaf spot on white clover.

Fewer lesions formed on CYVV-infected plants of clone T17 than on similarly infected plants of clone T7. One explanatory hypothesis is that expression of virus symptoms somehow restricts

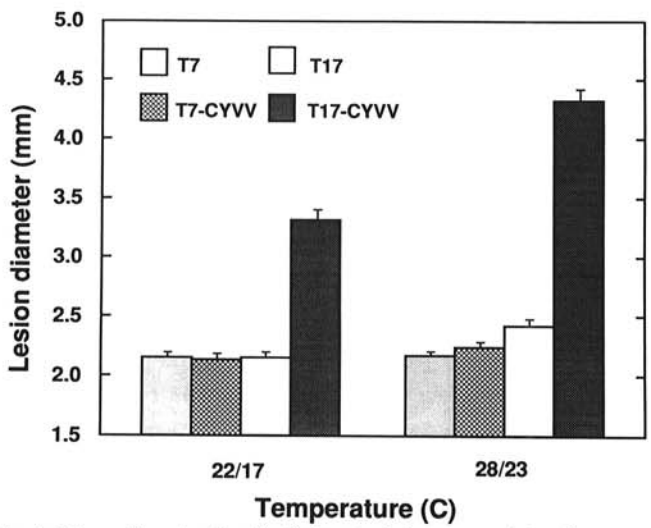

Fig 2. Mean diameter (mm) of discrete lesions caused by *Cercospora zebrina* on plants of two clones (T7 and T17) of ladino white clover cv. Tillman, infected with clover yellow vein virus (CYVV) or CYVV free, grown for 11 days after inoculation at two temperature regimes. Lesion diameter was obtained via a visual estimate on each diseased leaf, for a composite value of mean lesion diameter for each plant. Temperature × clone × virus interaction significant ($P < 0.01$). Means are data averaged over two levels of fungal inoculum. Error bars represent the standard errors of individual means.

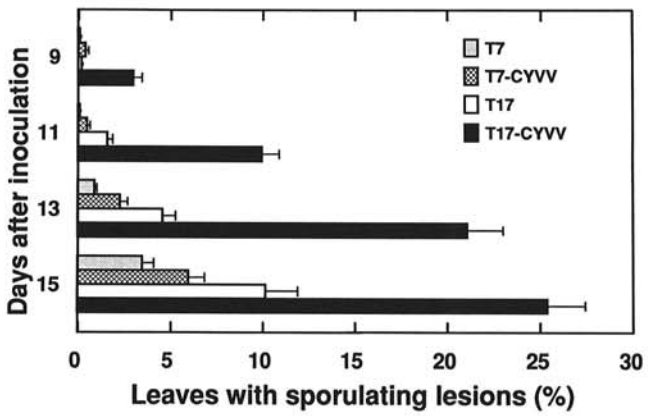

Fig 3. Proportion of leaves with at least one sporulating lesion caused by *Cercospora zebrina* on plants of two clones (T7 and T17) of ladino white clover cv. Tillman, infected with clover yellow vein virus (CYVV) or CYVV free, at 9, 11, 13, and 15 days after inoculation. Clone × virus interaction significant ($P < 0.01$). Means are data averaged over two levels of fungal inoculum and two temperature regimes. Error bars represent the standard errors of individual means.

the number of successful infections by *C. zebrina,* i.e., numerous, necrotic flecks and veinal necrosis associated with CYVV infection of T17 effectively reduce the number of potential infection courts (viable stomata) available to spores of *C. zebrina.* CYVV-induced, necrotic flecking was absent from T7-CYVV, and lesion density was virtually the same for both CYVV-free and CYVV-infected plants of clone T7.

Lesion density (or infection efficiency) may be of central importance as a determinant of observed changes in lesion size on CYVV-infected plants of clone T17. Lesion size may depend directly on lesion number. The phenomenon of larger lesion size at lower inoculum densities has been documented in the *C. arachidicola* Hori/*Arachis hypogaea* L. system (22). Observations in greenhouse experiments (S. C. Nelson and C. L. Campbell, *unpublished data*) indicate that when inoculum density is low, lesion size on CYVV-free plants is equal to those on CYVV-infected plants. The relationship between lesion density and lesion size (fewer, larger lesions on plants of T17-CYVV) may indicate a potentially "antagonistic" relationship among lesions, such that lesion expansion or size is inhibited by the presence of many proximal lesions. In studies on V8 juice agar, colony expansion of *C. zebrina* may be inhibited by proximal colonies (S. C. Nelson and C. L. Campbell, *unpublished data*). Studies with wider ranges of inoculum concentration are needed to clarify the relationship between lesion number and lesion size.

Altered epidemic components of leaf spot in other Cercospora-legume-virus systems have been reported. Crane and Calpouzos (9) detected a synergism of *C. beticola* Sacc. and beet yellows virus in enhancing disease severity and subsequent defoliation of sugar beet (*Beta vulgaris* L.) leaves. Melouk and Sherwood (18) found fewer conidia of *C. arachidicola* produced per peanut leaflet, larger lesion area, and fewer lesions per leaflet on plants infected with peanut mottle virus (PMV). In our study of the white clover/*C. zebrina*/CYVV system, disease severity and defoliation were not enhanced; however, lesion density and lesion size were affected in a manner similar to that in the peanut/*C. arachidicola*/PMV system. However, we failed to observe an adverse effect of CYVV infection of white clover on capacity for lesions to sporulate. On the contrary, CYVV-infected plants of both clones tended to have more profusely sporulating lesions.

The influence of temperature on epidemic components was significant only for incubation period, lesion size, and final disease incidence, implying that the isolate of *C. zebrina* selected for this study may cause epidemics over a relatively wide temperature range. A previous report (3) established that symptoms of leaf spot on virus-free red clover (*Trifolium pratense*), caused by *C. zebrina,* developed most rapidly at 28 C and progressed slowest and were least severe at 16 C. In our study, differences in clones were found for incubation period, in that development of leaf spot symptoms on CYVV-free plants of T7 was favored by a cooler regime (22/17 C), whereas symptoms developed more rapidly on T17 at 28/23 C. Thus, the effect of temperature on disease development may vary among clones within a population of *T. repens* and among isolates of *C. zebrina,* which confounds our understanding of host resistance and epidemic processes. The optimum temperature for radial growth of the isolate of *C. zebrina* used in this study is 24 C on V8 juice medium, with relatively good growth occurring from 20 to 28 C (20). Measures of disease severity and defoliation (AUC, rate of progress, disease at 17 days after inoculation) were unaffected by temperature, and disease incidence at 17 days after inoculation was greater at 22/17 C, suggesting that optimums for radial growth in vitro and for infection and disease development may not be correlated.

The reduction in intensity of certain epidemic components (e.g., lesion density, disease severity, defoliation, disease incidence) and an increase in other components (e.g., lesion diameter, sporulation, latent period, incubation period at 28/23 C) for plants of clone T17 underscores the complexity of virus-fungus-host interactions in this pathosystem. Measures of disease intensity (e.g., severity and defoliation) indicate potential resistance to *C. zebrina* for individual CYVV-infected plants when one cycle of disease is assessed. Paradoxically, the value of such resistance becomes questionable when the contribution by these same "resistant" individuals to the host population in a polycyclic epidemic (in terms of latent period, spore production, and infectious period) is considered. For example, plants of T17-CYVV generally had fewer but larger lesions that tended to produce conidia earlier and more often than did plants of T17. Plants of T17-CYVV were less prone to defoliation than plants of T17 and effectively extended the infectious period of Cercospora leaf spots on CYVV-infected leaves. The implications for field epidemics become obvious in the light of the reduced latent period, enhanced sporulation, and lengthened infectious period inherent in certain CYVV-infected plants, and they emphasize the need for understanding epidemics at a population or pathosystem level.

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