Host Range and Cultural Characteristics of *Cercospora zebrina* from White Clover in North Carolina

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ABSTRACT

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An isolate of *Cercospora zebrina* from white clover (*Trifolium repens*) was pathogenic to 24 species in 12 genera of legumes in greenhouse tests. This report is apparently the first evidence of pathogenicity of a white clover isolate of *Cercospora* to species of *Apios, Arachis, Glycine, Lespedeza, Lotus, Phaseolus*, and *Vigna*. The isolate was not always more virulent on white clover; pea cultivars (*Pisum sativum*) were more severely diseased. Five isolates of *C. zebrina* from white clover in four counties in North Carolina manifested significant ($P < 0.05$) isolate × host interactions on six leguminous hosts. The Vance Co. isolate was the only one that was more virulent on white clover than on other hosts and caused more disease on white clover, subclover (*T. subterraneum*), and alfalfa (*Medicago sativa*) than the other isolates. The Franklin Co. isolate was more virulent on arrowleaf clover (*T. vesiculosum*) than on other hosts. Optimum radial growth on V-8 juice agar occurred at 24 C for all five isolates. Growth differed ($P = 0.05$) among isolates at 16, 20, 24, and 28 C, which indicated that some isolates were more tolerant of extremes in temperature in vitro. One isolate produced significantly more conidia in vitro than the other four. Mean length of conidia produced in vitro did not differ among isolates.

Leaf and stem diseases caused by *Cercospora* spp. on white clover (*Trifolium repens* L.) and other small-seeded forage legumes are often referred to as "summer black stem" and are characterized by leaf spotting, defoliation, and petiole and stem discoloration. Symptoms develop most commonly on mature host tissue during periods of relatively warm temperatures (24–28 C) and high humidity in late spring and summer (2,4,11).

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Few morphological differences are manifest among isolates of *Cercospora* spp. from different forage legumes (5,7), and the taxonomy of the pathogen(s) involved is not clearly defined. At least six species of *Cercospora* have been described on species of *Trifolium, Medicago*, and *Melilotus* (7), and evidence for host species-level and/or genus-level specificity has been presented (2,8,15,16). Thus, there exists some question as to whether these organisms should be ascribed to species based upon fungal morphology or host range and symptoms. On the basis of a morphological study of *C. medicaginis* Ellis & Everh. on *Medicago* spp., *C. davisii* Ellis & Everh. on *Melilotus* spp., and *C. zebrina* Pass. on *Trifolium* spp., Horsfall (7) reduced the first two species to synonomy with *C. zebrina*. Conversely, Chupp (6) and Lieneman (12) treated *C. medicaginis, C. davisii*, and *C. zebrina* as distinct species.

Cross-infection sometimes occurs following inoculations with isolates of *Cercospora* spp. from different host genera and species, but isolates are usually most virulent on the host from which they are isolated. Results of cross-inoculation experiments (8,15) indicated that *C. medicaginis, C. davisii*, and *C. zebrina* are specialized at the host genus level. Pathogenicity of *C. medicaginis* from alfalfa (*Medicago sativa* L.) was restricted to *Medicago* spp. in a previous study (2). A "host-specific" form of *C. zebrina* has been isolated from subterranean clover (*T. subterraneum* L.) in Mississippi (16), although rose clover (*T. hirtum* Alioni) also developed severe and consistent disease symptoms. Conversely, an Australian isolate of *C. zebrina* from subterranean clover readily infected many species of *Trifolium* and *Medicago* (1). In addition, isolates of *Cercospora* from red clover (*T. pratense* L.) and alfalfa have been established as efficient pathogens of several legume genera (3,5,9).

Comparatively little is known of the pathogenicity and host range of *C. zebrina* from white clover, despite the economic importance of this legume and the severe disease problems associated with it (13). White clover is widely used in grass-legume mixtures in most temperate, humid regions and is the primary grazing forage legume in North Carolina. Cercospora leaf spot and a combination of other diseases, insect damage, adverse environmental conditions, and management practices have been associated with the rapid decline of stands of white clover 2–3 years after seeding (10). The fundamental objective of our investigation was to evaluate the pathogenicity, host ranges, morphology, and cultural characteristics of isolates of *C. zebrina* collected from

white clover growing in the piedmont region of North Carolina.

MATERIALS AND METHODS

Isolates of *Cercospora zebrina*. Five monoconidial isolates of *C. zebrina* were obtained from leaves of diseased white clover collected from plants growing in four counties in the piedmont region of North Carolina. Isolates were designated Cabarrus, Franklin, Vance, Wake1, and Wake2. Leaves were air-dried before incubation in moisture chambers for 12 hr at 24 C to induce sporulation. Single conidia were removed from leaf surfaces and transferred to V-8 juice agar (14). After growing for 10–15 days, cultures were maintained at 4 C until 5 days before inoculation, when inoculum was produced by the method of Latch and Hanson (9).

Host range test 1. Nineteen plant species or cultivars (Table 1) were tested for susceptibility under greenhouse conditions to isolate Wake1 of *C. zebrina*. White clover cv. Regal was included in the test as the susceptible control. Seeds of each species or cultivar were direct-seeded into a steam-pasteurized mixture of washed river sand and sandy loam soil (1:3, v:v) in clay pots (500 cm^3). Ten pots of each host were established with five plants per pot. Seeds were inoculated with compatible strains of *Rhizobium* at planting. Plants were grown for 8 wk before inoculation with *C. zebrina*. Pots were arranged randomly on a greenhouse bench.

Fungal inoculum was obtained (9), calibrated to 6×10^4 spores per milliliter, and sprayed on leaves until runoff. Tween 20 (Fisher Scientific, Orangeburg, NY 10962) was added (80 μl/L) to facilitate inoculum dispersal and adhesion of conidia to leaf surfaces. Plants were enclosed in clear plastic bags for 96 hr after inoculation to maintain high humidity, which is necessary for infection (5). Greenhouse temperatures ranged from 22 to 28 C. A disease rating scale was used to assess disease severity at 10 days after inoculation, with the disease 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. In order to confirm the presence of infection by *C. zebrina*, symptomatic leaves were detached and placed in petri dishes containing moistened filter paper to induce sporulation. The experiment was conducted twice.

Host range test 2. Six cultivars of bean (*Phaseolus vulgaris* L.), one of soybean (*Glycine max* (L.) Merr.), one of cowpea (*Vigna sinensis* (Torner) Savi), one of lima bean (*Phaseolus limensis* Macf.), and two of pea (*Pisum sativum* L.) were tested for susceptibility to isolate Wake1 of *C. zebrina* under greenhouse conditions. White clover cv. Regal was included as the susceptible control. Seeds of each cultivar were direct-seeded into pots as in test 1. Ten pots of each host were established with three plants per pot. Pots were arranged randomly on a greenhouse bench. Seeds were inoculated with compatible strains of *Rhizobium* at time of planting.

Fungal inoculum was prepared and applied as in test 1 (6×10^5 spores per ml) at 4 wk after planting. The entire bench was enclosed in clear plastic (to maintain humidity) and covered with brown paper (to minimize heat accumulation within the enclosure) for 96 hr after inoculation. High humidity was provided by two cool-mist vaporizers that operated for 30 sec every 3 min for 96 hr after inoculation. Temperatures within the plastic enclosure ranged from 22 to 30 C and from 22 to 28 C within the greenhouse after the plastic was removed. Disease severity was assessed as in test 1 at 10 days after inoculation. Symptomatic leaves were detached and placed into humidity chambers to induce sporulation, thereby confirming infection by *C. zebrina*. The experiment was conducted twice.

Host range test 3. The five isolates of *C. zebrina* were compared for virulence on six leguminous hosts that represented a range of susceptibility to isolate Wake1 of *C. zebrina*. Hosts were white clover, alfalfa, arrowleaf clover (*T. vesiculosum* Savi.), red clover, subterranean clover, and bigflower vetch (*Vicia grandiflora* Scop.). Plants were seeded, inoculated, and assessed for disease severity as described in test 1, with five pots of each treatment and three plants per pot. Pots were arranged in a randomized complete block design on the greenhouse bench. The experiment was conducted twice.

Data analysis. Before analysis of variance (17), disease scores for all host range tests were converted to percentages using the midpoint of each rating class. In the analysis of variance for test 3, isolates and hosts were considered as sources of variation in the fixed effects model. Significance of the isolate and host main effects were determined by using the runs × isolate and runs × host interactions as the error terms, respectively. Significance of the isolate × host interaction was determined by using the runs × isolate × host interaction as the error term.

Effect of temperature on fungal growth. A sterile needle tip was touched to an actively sporulating culture of *C. zebrina* on V-8 juice agar and spores transferred to three sites in a triangular pattern on the surface of V-8 juice agar (25 ml per dish). Petri dishes were sealed with parafilm, randomized, enclosed in plastic bags, and incubated in darkness at 12, 16, 20, 24, 28, 32, and 36 C. The five isolates and five replications per treatment were used for each run of the experiment. Daily radial growth at each temperature was calculated as an overall mean (of the three colonies per petri dish) from two perpendicular measurements of colony diameter made after 12 days of growth. The experiment was conducted twice.

Spore size, spore production, and spore germinability. Actively growing,

Table 1. Disease severity induced by an isolate of *Cercospora zebrina* from white clover in nineteen legume species 10 days after inoculation

Plant	Percentage of leaf area diseased[z]	
	Test 1	Test 2
Apios americana Medik. groundnut	2.0 f	1.7 f
Arachis hypogaea L. 'Florigiant' peanut	2.8 ef	2.0 f
Lespedeza cuneata (Dum. Cours.) G. Don sericea lespedeza	1.5 f	1.5 f
L. stipulacea Maxim. Korean lespedeza	3.1 def	1.1 f
Lotus tenuis Waldst. & Kit. ex Willd. 'Viking' birds'-foot trefoil	1.7 f	2.6 def
Medicago sativa L. 'Raidor' alfalfa	3.2 def	4.1 cdef
Melilotus alba Desr. 'Floanna' white sweetclover	3.4 def	3.0 def
Trifolium alexandrinum L. berseem clover	6.8 b	10.8 a
T. dubium Sibth. small hop clover	1.6 f	2.0 def
T. hirtum Allioni rose clover	5.0 bcde	4.7 cd
T. hybridum L. alsike clover	5.8 bc	3.2 def
T. incarnatum L. crimson clover	3.9 cdef	4.6 cde
T. pratense L. 'Redman' red clover	5.1 bcde	7.9 b
T. repens L. 'Regal' white clover	9.9 a	8.1 b
T. resupinatum L. 'Abon' persian clover	6.6 b	6.3 bc
T. subterraneum L. 'Mt. Barker' subterranean clover	5.3 bcd	4.9 cd
T. vesiculosum Savi. 'Yuchi' arrowleaf clover	3.5 def	2.6 def
Vicia grandiflora Scop. bigflower vetch	3.3 def	3.4 def
V. villosa Roth. hairy vetch	2.2 f	3.5 def

[z] Disease severity based upon a visual estimate. Run × host interaction significant ($P < 0.01$). Means in a column not followed by the same letter are significantly different (Student-Newman-Keuls mean separation test, $P < 0.05$).

sporulating isolates of *C. zebrina* in petri dishes were flooded with 10 ml sterile distilled water to dislodge the spores. V-8 juice agar (25 ml per dish) in four petri dishes for each isolate was flooded with 5 ml of the resulting spore suspensions. Cultures were incubated at 24 C for 7 days with a 12-hr light/dark photoperiod. Mean light intensity at the upper, exterior surface of the petri dish was 22 $\mu E\ m^{-2}\ s^{-1}$, provided by two cool white fluorescent lights.

For quantification of spore size, production, and germinability, 10 circular plugs (5 mm in diameter) were cut in a systematic pattern from each petri dish containing the 7-day-old, sporulating isolates of *C. zebrina*. Plugs from each petri dish were placed into 15-ml conical, graduated, screw-cap, centrifuge tubes with 10 ml of sterile distilled water. Spores were dislodged from plugs into suspension by placing tubes onto a Vortex-genie mixer (Scientific Industries, Inc., Bohemia, NY) for 30 sec. For quantification of spore size, one 30-μl drop of the resulting spore suspension from each culture was placed upon a glass slide and covered with a cover slip, and the first 50 spores observed were measured (length, width, number of septations) at 400×. Spore size was expressed as a mean of the four replicate cultures per isolate.

Table 2. Disease severity induced by an isolate of *Cercospora zebrina* from white clover in six legume species 10 days after inoculation

Plant	Percentage of leaf area diseased[z]
Glycine max (L.) Merr. 'Centennial' soybean	6.0 cd
Phaseolus limensis Macf. 'Nemagreen' baby lima bean	1.7 d
P. vulgaris L. 'Topcrop' snap bean	8.2 c
P. vulgaris 'Stinger' navy bean	5.9 cd
P. vulgaris 'Stringless Blue Lake' pole bean	2.1 d
P. vulgaris 'Stretch' garden bean	3.8 cd
P. vulgaris 'Redkloud' kidney bean	0.7 d
P. vulgaris 'Ouray' pinto bean	5.0 cd
Pisum sativum L. 'Spring' freezer pea	56.6 a
P. sativum 'Dawn' canner pea	56.8 a
Trifolium repens L. 'Regal' white clover	38.4 b
Vigna unguiculata (L.) Walp. 'Ramshorn' cowpea	1.1 d

[z] Disease severity 10 days after inoculation, based upon a visual estimate. Data are means of two tests. Means not followed by the same letter are significantly different (Student-Newman-Keuls mean separation test, $P = 0.05$).

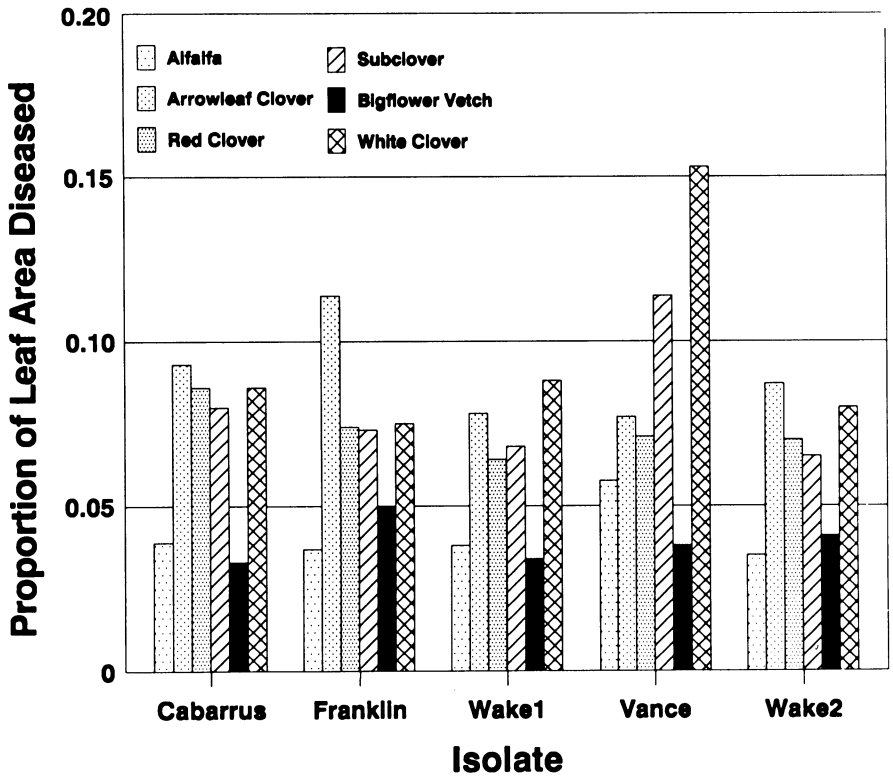

Fig. 1. Virulence of five isolates of *Cercospora zebrina* on six leguminous hosts as measured by percentage of leaf area diseased 10 days after inoculation. Data are means of two tests. Host × isolate interaction significant ($P < 0.05$).

For quantification of spore number, two 10-μl drops of the spore suspension from each culture were placed onto glass slides, where spores were counted at 100×. Total number of spores per isolate was expressed as a mean (number of spores per square millimeter of V-8 juice agar) of the four replicate cultures per isolate. For quantification of spore germinability, one 50-μl drop of the suspension from each isolate was placed on a glass slide and incubated in darkness in a petri dish containing moistened filter paper at 24 C for 12 hr. Conidia and germ tubes were stained with 1% acid fuchsin in lactophenol, and the first 100 conidia from each replication were examined at 100× for the presence or absence of at least one germ tube. Percent germination was expressed as a mean of the four replicate cultures per isolate. Data for all experiments for spore size, number, and germinability were subjected to an analysis of variance (17).

RESULTS AND DISCUSSION

Host range test 1. Authorities and scientific names for host plants are in Table 1. Ranking of hosts by disease severity varied between runs, as indicated by the significant ($P < 0.05$) runs × host interaction (Table 1). Plants were never killed by the pathogen. However, infected leaves died on the most severely diseased plants. *Cercospora zebrina* was recovered from lesions on every host species, although symptoms were variable. Symptoms on most of the forage legumes, e.g., clovers, alfalfa, and white sweet clover (*Melilotus alba* Desr.) were typical of Cercospora leaf spot on white clover: relatively large, gray to black, circular to rectangular, often interveinal, lesions on leaves that caused defoliation, and girdling lesions on petioles with concomitant petiole collapse. Symptoms on less susceptible hosts varied with respect to lesion shape, size, and color. Peanut (*Arachis hypogaea* L.) and birds'-foot trefoil (*Lotus tenuis* Waldst. & Kit. ex Willd.) had small (1–2 mm in diameter), brown leaf spots. Bigflower vetch, hairy vetch (*V. villosa* Roth), and Korean lespedeza (*Lespedeza stipulacea* Maxim.) had small (2–3 mm in diameter), oval to circular lesions with tan centers and dark red margins. White clover and berseem clover were significantly more susceptible ($P = 0.05$) than most other hosts in both tests (Table 1). However, Persian clover (*T. resupinatum* L.), red clover, subclover, and rose clover were relatively good hosts in both tests.

Our data are in partial agreement with the findings of Berger and Hanson (5). They were able to infect red clover and alsike clover with an isolate of *C. zebrina* from white clover in Wisconsin and showed that this isolate was more pathogenic on white clover than on red clover. However, they were unable to infect alfalfa or white sweet clover with the

Wisconsin isolate. In contrast, we were able to infect alfalfa and white sweet clover with a North Carolina isolate of *C. zebrina* (Table 1). These differences may be construed as evidence for the existence of host specialization among geographically diverse isolates of *C. zebrina.*

Host range test 2. *Cercospora zebrina* was recovered from leaves of each host cultivar. With the exception of the two cultivars of *Pisum sativum*, plants were never killed by the pathogen. The runs × host interaction was not significant ($P < 0.05$). This indicates the disease severity ranking of hosts was consistent between runs. Mean values for disease severity were similar between runs. The Wake1 isolate of *C. zebrina* caused significantly ($P = 0.05$) more disease on peas than on white clover and other hosts (Table 2). Symptoms on *P. sativum* were similar to those caused by Cercospora leaf spot on white clover, for example, numerous gray, circular to rectangular, coalescing lesions leading to rapid defoliation and collapse of petioles and stems. A significant proportion of freezer pea and canner pea plants died within 7 days after inoculation from the effects of stem-girdling lesions. White clover also sustained severe defoliation in this test. Symptoms on *Phaseolus* spp. varied in shape and size of lesions, and generally consisted of oval to circular lesions with tan centers and brown to dark red peripheries. Disease severity was significantly ($P = 0.05$) less on cultivars of *Phaseolus* spp. and cowpea (*Vigna unguiculata* (L.) Walp.) than on peas and white clover. Kidney bean (*Phaseolus vulgaris* L.) and cowpea were exceptionally poor hosts. The higher inoculum concentration in this test is thought to be largely responsible for the greater severity of disease in comparison with test 1 (Tables 1 and 2).

This report is apparently the first evidence of pathogenicity of a clover isolate of *Cercospora* to species of *Apios, Arachis, Glycine, Lespedeza, Lotus, Phaseolus*, and *Vigna* and could have important ramifications for our understanding of the disease epidemiology and fungal taxonomy of *Cercospora* diseases on these crops. The results of this study confirm a prior report (5) of the susceptibility of *P. sativum* to a red clover isolate of *C. zebrina.*

Host range test 3. There was a significant ($P < 0.05$) host × isolate interaction with respect to disease severity in this experiment, indicating that the five isolates differed in degree of virulence among the six host species (Fig. 1). Mean values for disease severity for host-isolate combinations were similar between runs. All interactions involving the runs source of variation were not significant ($P < 0.05$). This indicates the disease severity ranking of host-isolate combinations was consistent between runs. The Vance isolate was significantly ($P = 0.05$) more virulent on white clover, subclover, and alfalfa than the remaining four isolates (Fig. 1). Only the Vance isolate induced significantly more severe disease on its host of origin, white clover, than on the other hosts. In addition, the Franklin isolate caused significantly ($P = 0.05$) more disease on arrowleaf clover than on white clover. With the exception of the Vance isolate, alfalfa and bigflower vetch were less susceptible ($P = 0.05$) to all isolates of *C. zebrina* than were the other host species. These data contradict previous evidence (2,5,7) that isolates of *Cercospora* are more virulent on the hosts from which they were isolated and suggest the existence of host specialization among isolates of *C. zebrina.* Also from these data we conclude that species-level and/or genus-level host specificity among isolates is not attributable to host of origin (e.g., white clover may not necessarily be the primary host for the Franklin isolate).

Effects of temperature on fungal growth. A significant ($P < 0.001$) isolate × temperature interaction was found for rate of radial growth on V-8 juice agar. No significant interactions involving runs were detected. Mean values and ranking of isolates were consistent between runs. In general, radial growth rate of each of the five isolates was greatest at 24 C (Fig. 2), but comparatively good growth occurred from 20 to 28 C. These optimum temperatures for mycelial growth agree with previous data (4). Mycelial growth for all isolates decreased as temperature increased or decreased (relative to 24 C), and little and no measurable growth occurred at 32 and 36 C, respectively.

Growth rates among the five isolates at 16, 20, 24, and 28 C differed ($P = 0.05$). No differences were found at 12, 32, and 36 C. The Cabarrus and Vance isolates had faster ($P = 0.05$) growth than all other isolates at 16 and 28 C. The Cabarrus isolate had greater ($P = 0.05$) radial growth than all other isolates at 20 and 24 C. The two Wake isolates had the slowest ($P = 0.05$) mycelial growth among isolates from 16 to 24 C, whereas the Franklin isolate had the slowest growth at 28 C. The Cabarrus and Vance isolates appear to be more tolerant of extremes in temperature in comparison with the two Wake isolates: the maximum growth rate (24 C) for the latter two isolates was equalled or exceeded by the former's growth from 20 to 28 C. Further studies may be necessary to determine whether the variability among isolates with respect to mycelial growth at specific temperatures is due to temperature alone or to nutritional requirements.

Spore size, spore production, and

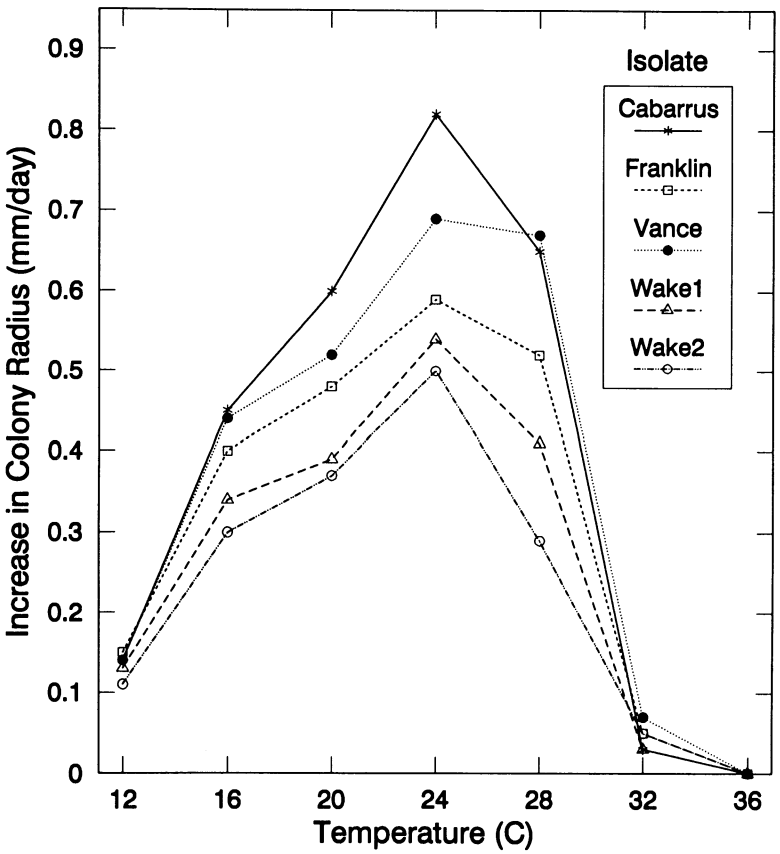

Fig. 2. Growth rates of five isolates of *Cercospora zebrina* on V-8 juice agar at seven temperatures in the dark. Data are the average of five replications (with three colonies per replication) for two tests. Temperatures (C), with least significant difference ($P = 0.05$) in parentheses: 12 (0.022), 16 (0.035), 20 (0.031), 24 (0.032), 28 (0.096), 32 (0.032), 36 (0.0).

Table 3. Spore characteristics and spore production for five white clover isolates of *Cercospora zebrina* grown on V-8 juice agar[v]

Isolate	Length[w]			Width[w]			Septa[x]	Spore production[y]
	Min	Max	Mean	Min	Max	Mean		
Cabarrus	31	229	78	3	5.5	4.9	2–14	1011 b
Franklin	38	145	73	3	5.5	4.8	2–11	2029 a
Vance	33	176	80	3	5.5	4.8	2–15	1187 b
Wake1	36	193	83	3	5.5	4.9	2–18	987 b
Wake2	33	171	73	3	5.5	4.9	2–18	841 b
			NS[z]			NS		

[v] Data are means of four replicates of each isolate grown for 7 days at 24 C.
[w] Length or width (μm), on the basis of a total of 200 spores per isolate.
[x] Range in the number of septations for 200 spores per isolate.
[y] Spore production expressed as number of spores per square millimeter of V-8 juice agar in petri dishes incubated at 24 C for 7 days. Means not followed by the same letter are significantly different (Student-Newman-Keuls mean separation test, $P = 0.05$).
[z] NS = no significant difference.

spore germinability. Mean conidial dimensions (length and width) among isolates did not differ ($P = 0.05$), but measures of maximum spore length were numerically different and indicate potential variability among these isolates of *C. zebrina* (Table 3). The primary purpose of these measurements was for comparisons among isolates. No taxonomic inferences are to be drawn from these data, because previous studies (4) have demonstrated that conidial dimensions of isolates of *Cercospora* spp. from forage legumes are highly dependent upon incubation conditions (humidity and temperature). With respect to spore production per square millimeter of V-8 juice agar, the Franklin isolate was more prolific ($P = 0.05$) than the other isolates, once again demonstrating variability among isolates (Table 3).

Spore germination was assessed to determine its possible role as a determinant in differences in disease severity among isolates. No differences ($P = 0.05$) among isolates for conidial germinability were found. Isolates and percent germination were Wake1, 99.8; Vance, 99.8; Cabarrus, 98.5; Wake2, 89.5; and Franklin, 88.0. Correlations (Pearson's correlation coefficient) between conidial germinability at 24 C and disease severity on white clover and between viability and disease severity averaged over all six hosts (host range test 3) were not significant. Thus, differences in severity of disease caused by these isolates are not attributed to germinability of conidia.

In summary, these studies establish a host range for *C. zebrina* of heretofore unreported extent, and demonstrate that North Carolina isolates may induce severe disease on species other than their hosts of origin. These studies contribute to a growing body of evidence for host specialization of this organism. That an isolate of *Cercospora* sp. from white clover may attack species of *Phaseolus, Arachis*, and *Glycine* suggests a need for a reevaluation of the current understanding of the disease epidemiology and taxonomy of *Cercospora* spp. causing leaf spots on these and other legumes. It is not known whether the disease caused by *C. zebrina* on species of *Arachis, Phaseolus*, and *Glycine* is serious enough to be of concern. However, the potential for economic loss to pea growers from epidemics caused by *C. zebrina* from white clover deserves serious consideration. Further studies of fungal morphology and host-parasite relationships with respect to *C. zebrina* are needed to resolve the taxonomic and epidemiological issues raised by these and other investigations.

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LITERATURE CITED

1. Barbetti, M. J. 1985. Infection studies with *Cercospora zebrina* on pasture legumes in Western Australia. Aust. J. Exp. Agric. 25:850-855.
2. Baxter, J. W. 1956. Cercospora black stem of alfalfa. Phytopathology 46:398-400.
3. Berger, R. D., and Hanson, E. W. 1962. Host ranges of three isolates of *Cercospora* and effects of environment on pathogen and disease development. (Abstr.) Phytopathology 52:3-4.
4. Berger, R. D., and Hanson, E. W. 1963. Relation of environmental factors to growth and sporulation of *Cercospora zebrina*. Phytopathology 53:286-294.
5. Berger, R. D., and Hanson, E. W. 1963. Pathogenicity, host-parasite relationships, and morphology of some forage legume *Cercosporae*, and factors related to disease development. Phytopathology 53:500-508.
6. Chupp, C. 1953. A monograph of the fungus genus *Cercospora*. Published by the author, Ithaca, New York. 667 pp.
7. Horsfall, J. G. 1929. Species of *Cercospora* on *Trifolium, Medicago*, and *Melilotus*. Mycologia 21:304-312.
8. Jones, F. R. 1944. Life history of *Cercospora* on sweetclover. Mycologia 36:518-525.
9. Latch, G. C. M., and Hanson, E. W. 1962. Comparison of three stem diseases of *Melilotus* and their causal agents. Phytopathology 52:300-315.
10. Leath, K. T. 1985. General diseases. Pages 206-227 in: Clover Science and Technology. N. L. Taylor, ed. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America. Madison, WI.
11. Leath, K. T., Erwin, D. C., and Griffin, G. D. 1988. Diseases and nematodes. Pages 206-227 in: Alfalfa and Alfalfa Improvement. A. A. Hanson, D. K. Barnes, and R. R. Hill, Jr., eds. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America. Madison, WI.
12. Lieneman, C. 1929. A host index to the North American species of the genus *Cercospora*. Ann. Mo. Bot. Gard. 16:1-52.
13. Main, C. E., and Gurtz, S. K., eds. 1988. 1987 Estimates of Crop Losses in North Carolina due to Plant Diseases and Nematodes. Department of Plant Pathology Special Publication 7, North Carolina State University, Raleigh. 209 pp.
14. Miller, P. M. 1955. V-8 juice agar as a general-purpose medium for fungi and bacteria. Phytopathology 45:461-462.
15. Nagel, C. M. 1932. The sporulation and host range of six species of *Cercospora*. Ph.D. thesis. Iowa State University, Ames.
16. Pratt, R. G. 1984. A new Cercopsora leaf and stem disease of subterranean clover. Phytopathology 74:1152-1156.
17. SAS Institute, Inc. 1985. SAS user's guide: Statistics, version 5 ed. SAS Institute, Cary, NC.