Isolate Characteristics and Epidemic Components of Leptosphaerulina Leaf Spots on Alfalfa and White Clover

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ABSTRACT

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Isolates of *Leptosphaerulina* spp. from alfalfa (*Medicago sativa*) and white clover (*Trifolium repens*) were compared for growth rate on V-8 juice medium at 12–28 C, colony characteristics, production of pseudothecia, ascospore morphology, and pathogenicity on alfalfa and white clover. Optimum growth rate occurred at 20–24 C. Isolates from white clover generally had slower, restricted growth with irregular colony margins on agar medium, whereas isolates from alfalfa generally grew rapidly with regular colony margins. Production of pseudothecia, ascospore characteristics, and pathogenicity on detached leaves of alfalfa and white clover were similar among isolates. The conspecificity of *L. briosiana* and *L. trifolii* was supported. Under controlled-environment conditions, disease severity was lower at 30 C than at 22 or 15 C. At all temperatures, mean disease severity was similar for white clover and alfalfa when inoculated with isolates of *L. trifolii* from white clover, but was greater on alfalfa than on white clover with isolates from alfalfa. Disease severity was similar among isolates tested from each host. Mean incubation period (3–4 days), latent period (14–15 days), and infectious period (21 days) were similar, but sporulation varied among isolate-host combinations.

Leptosphaerulina leaf spots of alfalfa (*Medicago sativa* L.) and white clover (*Trifolium repens* L.) caused by *Leptosphaerulina briosiana* (Poll.) Graham & Luttrell and *L. trifolii* (Rost.) Petrak, respectively, are important foliar diseases in the southeastern United States (4). These diseases also occur in other temperate regions of the world. Both *L. briosiana* and *L. trifolii* cause similar, pepper spot symptoms, primarily on young leaves and petioles. Expression of symptoms depends on leaf age, cultivar, and environmental conditions (7).

Confusion exists regarding variation among isolates and species designation within the genus *Leptosphaerulina*, particularly with regard to *L. briosiana* and *L. trifolii*. Graham and Luttrell (5) revised the taxonomy of the genus and recognized six species: *L. australis* McAlp., *L. arachidicola* Yen, Chen & Huang, *L. trifolii, L. briosiana, L. americana* (Ell. & Ev.) Graham & Luttrell, and *L. argentinensis* (Speg.) Graham & Luttrell. They separated the species on the basis of size and septation of ascospores, colony characteristics on media, and the host on which the pathogen occurs. Booth and Pirozynski (1) disagreed with this revision, postulating that the large number of species resulted from overemphasizing morphological characteristics and that undue variability could be reduced by growing pure cultures under standardized conditions. Irwin and Davis (6) argued, in a similar fashion to Booth and Pirozynski, and recognized only four species: *L. argentinensis, L. arachidicola, L. americana,* and *L. trifolii*. They regarded *L. australis* and *L. briosiana* as synonyms for *L. trifolii*.

Although selected aspects of the epidemiology of Leptosphaerulina leaf spots in alfalfa have been studied (8,14) and the possible pathogenicity of *Leptosphaerulina* spp. on a range of leguminous plants has been reported (6), no attempts have been made to characterize cross-infection and disease components with isolates from alfalfa and white clover on these two hosts. Graham and Luttrell (5) reported that *L. briosiana* and *L. trifolii* primarily are pathogenic on alfalfa and clover, respectively. When infected leaves of southern bur clover (*Medicago maculata* L. (= *M. minima* (L.) Desr.)) were used as an inoculum source (10), typical symptoms occurred on *M. maculata* and *M. hispida*; few lesions developed on *M. sativa*, *T. repens*, and *T. pratense*; and no lesions developed on *T. hybridum*, *T. reflexum*, *T. incarnatum*, or *T. procumbens*. Irwin and Davis (6) observed, however, that effective cross-infection among isolates of *Leptosphaerulina* spp. can occur on some leguminous plants.

The objectives of this study were: to examine and compare the morphological and cultural variability of *Leptosphaerulina* spp. isolated from alfalfa and white clover in North Carolina; to examine the pathogenicity of isolates of *L. briosiana* from alfalfa and *L. trifolii* from clover on the host of origin and the reciprocal host at several temperatures; and to quantify components of development of Leptosphaerulina leaf spot diseases such as incubation, latent, and infectious periods. A preliminary report has been published (12).

MATERIALS AND METHODS

Acquisition and maintenance of isolates. Single ascospore isolates of *Leptosphaerulina* spp. were obtained from actively sporulating pseudothecia on leaflets of alfalfa and white clover. Leaflets of each host were collected from the three physiographic regions of North Carolina: the Mountains, the Piedmont, and the Coastal Plain. Leaflets were surface-disinfested in 0.26% NaOCl for 20–30 sec, rinsed in sterile distilled water, and incubated in petri dishes with acidified water agar in the lid. Ascospores ejected from pseudothecia onto the water agar were transferred after germination to V-8 juice agar (11). From 34 counties in the Mountain, Piedmont, and Coastal Plain regions, six and two, 11 and 15, and five and eight isolates were obtained from alfalfa and white clover, respectively.

Effects of temperature on growth rates on V-8 juice agar. Mycelial plugs (5 mm diameter), cut from the margins of 20-day-old cultures, were placed in the centers of petri dishes (100

mm diameter) containing approximately 20 ml of V-8 juice agar. Initially, a series of nine isolates from Wake County, four from alfalfa (A82Wa1, A86Wa1, A86Wa4, A85Wa1) and five from white clover (C85Wa1, C86Wa1, C86Wa2, C86Wa3, C86Wa4), were used. One plate per isolate was incubated in the dark at 12, 16, 20, 24, 28, and 32 C. Mean radial growth rate was determined from measurements of colony diameter in two perpendicular directions after 5 days of incubation. The experiment was repeated twice. A similar experiment was conducted for each of the 22 isolates from alfalfa and 25 isolates from white clover from the different physiographic regions. Analysis of variance (13) was used to determine if mean growth of isolates differed at $P = 0.05$ with respect to temperatures, host of origin, or physiographic region.

Ascospore characteristics. Ascospores were measured from cultures grown on V-8 juice agar. Isolates were incubated in the dark at 20 C for 3 days and then exposed to fluorescent light (GTE F96T12/CW 75 W, 65 μmol $m^{-2}s^{-1}$) with an alternating 16/8 hr light-dark period at room temperature (23–24 C) to induce ascospore formation and ejection. For ascospores impacted on the coverslip in the lid of the petri dish, spore size (length and width) and number of transverse and longitudinal septations were determined at 400×. In a preliminary experiment (C. L. Campbell, *unpublished data*), length, width, and number of transverse and longitudinal septa were recorded for 100 arbitrarily selected, ejected ascospores for each of four arbitrarily chosen isolates of *L. trifolii* from white clover. Values for the coefficient of variation were 7, 7, 8, and 8% for width measurements and 10, 10, 12, and 14% for length measurements. Number of transverse septa ranged from two to four and from zero to three for longitudinal septa. Because of the relatively low degree of variability in ascospore measurements, only 20 ascospores/isolate were measured for the remainder of the isolates.

Pathogenicity studies on detached leaves. For inoculation, sporulating cultures of each isolate on V-8 juice agar were inverted for 2 days over detached leaves from greenhouse-grown plants of alfalfa cultivar Arc and white clover cultivar Regal on moistened filter paper placed in petri dishes. During and after inoculation, leaves were incubated at room temperature (23–24 C) with supplemental fluorescent light (65 μmol $m^{-2}s^{-1}$) and a 16/8 hr light-dark period. An isolate was considered pathogenic if typical pepper spot lesions developed within 96 hr after inoculation.

Effects of temperature and isolate/host combination on disease severity. Nine isolates of *Leptosphaerulina* spp. (four from alfalfa [A85Wa1, A86Wa1, A86Wa2, A82Wa1] and five from white clover [C86Wa1, C86Wa2, C86Wa3, C86Wa4, and C85Wa1]) obtained from Wake County, NC, and one isolate (A82R1) obtained from alfalfa in Rowan County, NC, were used for the study. Isolates were maintained on V-8 juice agar at 20–23 C in the laboratory (11). Isolates stored this way maintained pathogenicity for at least 5 mo.

Seedlings of alfalfa cultivar Arc and white clover cultivar Regal were grown in 11.4-cm-diameter (600 cm^3) pots containing a mixture of vermiculite and gravel (1:1, v/v) with inoculant of *Rhizobium* (Southern States Cooperative, Inc., Richmond, VA) in the greenhouse. These cultivars were selected because of their extensive use in North Carolina and because Arc alfalfa is highly susceptible to Leptosphaerulina leaf spot (15). No information was available concerning the relative susceptibility of white clover cultivar Regal to this disease. Plants were grown for 6–8 wk before inoculation. Plants with apparently equal vigor, as judged by leaf size, plant height, and color, were used.

Inoculum for each trial of the experiment was increased by transferring mycelial plugs from stored cultures onto V-8 juice agar. Cultures were grown in the dark at 20 C for 3 days before transferring to the lab bench at 23–25 C with 8–14 hr/day of fluorescent light (GTE F96T12/CW, 75 W; 65 μmol $m^{-2}s^{-1}$). Sporulation occurred approximately 5 days after incubation under light and continued for 1 wk. Ascospore suspensions were prepared by scraping sporulating cultures gently, blending mycelial fragments and pseudothecia with 30 ml of sterile distilled water in a commercial blender (Waring Products Division, Dynamics Corp. of America, New Hartford, CT) on high speed for 1 min and on low speed for 45 sec, and filtering them through cheesecloth to remove mycelial debris. Ascospore concentration was measured with the aid of a hemacytometer and adjusted to 10^4 ascospores/ml by dilution with distilled water. Tween 20 (2–5 drops/100 ml suspension; polyoxyethylene sorbitan monolaurate, Sigma Chemical Co., St. Louis, MO) was added to the spore suspension to increase the wetting of leaves.

Inoculations were conducted by spraying an ascospore suspension of a specific isolate onto seedlings of alfalfa and white clover until runoff occurred (9). After inoculation, plants were moved into a dew chamber (Percival, Boone, IA) at 20 C, with relative humidity $\geq$ 90% for 72 hr of incubation in the dark. Inoculated plants then were transferred from the dew chambers to controlled-environment chambers. Temperature was maintained at 15, 22, or 30 C with a relative humidity of 60–70%. A 12-hr light/dark photoperiod was utilized, with a mean photosynthetic photon flux density (400–700 nm) of 411 μmol $m^{-2}s^{-1}$. Illumination was provided by cool-white fluoresecent and incandescent lamps.

Disease severity for entire plants was determined as percent of total leaf area with pepper spots with the aid of a rating diagram (15). The experiment was arranged as a split-split-plot in a completely randomized design, with temperature as the main plots, pathogen (alfalfa or clover as host of origin) as subplots (isolates nested within pathogens), and hosts (alfalfa and clover) as subsubplots. There were three samples (pots) per isolate per host in one trial for a total of 60 pots per chamber and two samples per isolate per host for a total of 40 samples per chamber in two additional trials. Analysis of variance for disease severity was conducted with the PROC ANOVA of the Statistical Analysis System (13), and differences were considered significant if the probability of obtaining a greater F-value was ≤ 0.05.

Effects of temperature and isolate/host combination on components of disease. Incubation period was determined by noting the day after inoculation on which symptoms were evident. Latent period (time from inoculation to sporulation), infectious periods (duration of sporulation), and sporulation patterns were determined initially on leaflets of alfalfa or clover with 10% disease severity that were detached from symptomatic plants in the 15 C treatment. Four and five detached leaflets from white clover and alfalfa, respectively, were placed on moistened filter paper in a petri dish. A single petri dish was used as the experimental unit, and the experiment was replicated twice. Solidified water agar was inverted above the petri dishes to trap ascospores ejected from the leaflets. Water agar was replaced daily, and ascospores with one or more germ tubes after a subsequent 24-hr incubation period were counted.

The influence of temperature (15, 22, or 30 C) on the latent period during disease development was tested with detached leaves exhibiting 10% disease severity from each controlled environment chamber and the procedure described above for monitoring latent period. The experiment consisted of a 4×2 factorial arrangement (four isolates, two from alfalfa and two from clover, and two hosts). The experimental unit consisted of a single petri dish moist chamber with four or five symptomatic leaves obtained from white clover or alfalfa, respectively, which were inoculated with either an alfalfa or clover isolate of *Leptosphaerulina* spp. The experiment consisted of two replicates in time. Duration of sporulation was observed only for a single trial of the experiment.

Sporulation and isolate characteristics from naturally infected leaves. Lesions were monitored on detached, symptomatic leaves obtained from the field to determine to what degree chlorotic and necrotic detached leaves contribute to epidemics by way of ascospore ejection, and to examine if sporulation curves on naturally infected leaves differ from those obtained from infected leaves from the controlled-environment studies. Green leaves refer to samples where >50% of leaf area is green with few lesions, and chlorotic leaves are those with >50% of leaf area have chlorosis and numerous lesions.

Leaves of alfalfa and clover with symptoms of Leptosphaerulina leaf spot were obtained from established stands at two sites in

a field in Wake County, NC. Samples of leaves obtained from an area where diseased alfalfa and white clover plants were growing adjacent to each other were sample 1. Leaves obtained from the middle of plots of established, pure stands of alfalfa and white clover were designated sample 2. The treatment combination consisted of two sites × two hosts (alfalfa, clover) × two levels of infection (green or chlorotic). The proportion of leaves with sporulating lesions was monitored by the water agar inversion technique, as described previously, for a period of 5–6 days.

Colony morphology of isolates of *Leptosphaerulina* spp. obtained from leaves of adjacent or nonadjacent alfalfa and white clover plants was compared on V-8 juice agar. Cultures were incubated at room temperature (23–24 C), with supplemental fluorescent light (65 μmol $m^{-2}s^{-1}$) and a 16/8 hr light/dark period.

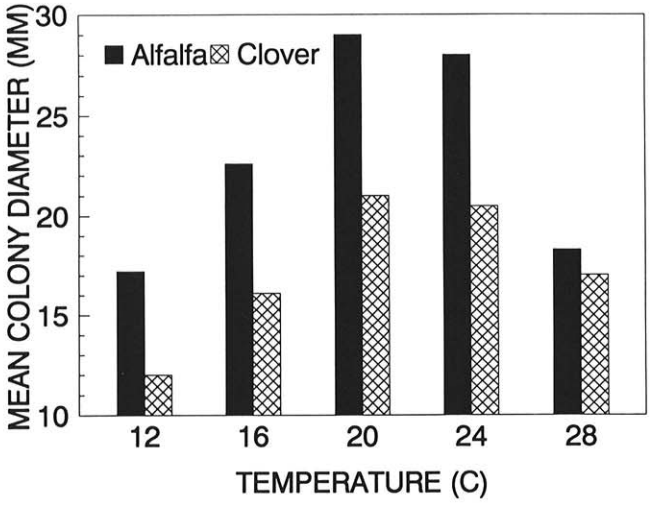

Fig. 1. Mean colony diameters of 22 and 25 isolates of *Leptosphaerulina trifolii* from alfalfa and white clover, respectively, after 5 days of incubation at five temperatures on V-8 juice agar.

Colonies were classified after 7–10 days of growth as expanded, with radial, concentric growth and smooth colony margins, or as restricted, and with nonconcentric, scattered growth and irregular margins.

RESULTS

Effects of temperature on growth of isolates. Colony morphology was similar among temperatures but differed among isolates from alfalfa and white clover on V-8 juice agar. Isolates from alfalfa had a regular, smooth colony margin and produced concentric growth rings corresponding to zones of daily increase in growth. Isolates from clover had an irregular colony margin and often did not develop concentric growth rings. For isolates from both hosts, growth and production of pseudothecia were greatly suppressed at the lower temperatures.

Growth rate of the nine isolates and the isolates from the three physiographic regions of North Carolina differed ($P = 0.005$) among temperatures tested, but not among isolates from a specific host. Optimal growth rate of isolates from both hosts occurred at 20 or 24 C; reduced growth rate occurred at 12 and 28 C (Fig. 1). No growth occurred at 32 C. Mean growth rate for the 22 isolates from alfalfa was greater than for the 25 isolates from clover at all temperatures tested (Fig. 1). Alfalfa isolates of *Leptosphaerulina* spp. from the Mountain region had faster ($P = 0.05$) growth rates at all the temperatures than isolates obtained from the other regions (data not shown). Growth rate of clover isolates of *Leptosphaerulina* spp. did not differ ($P = 0.05$) among regions.

Ascospore characteristics. Isolates from alfalfa and white clover generally were similar with regard to ascospore dimensions and septations (Table 1). Coefficients of variation ranged from 9.1 to 13.0% for mean length and from 11.9 to 15.9% for mean width among isolates of the two hosts. Thus, isolates within Wake County and from North Carolina had similar characteristics, i.e., length, width, length/width ratios, and numbers of transverse and longitudinal septations when grown on V-8 juice agar (Table 1).

Pathogenicity of isolates on detached leaves of alfalfa and white

TABLE 1. Mean ascospore dimensions and number of septations for isolates of *Leptosphaerulina* spp. from *Medicago sativa* and *Trifolium repens* in North Carolina and as reported by Graham and Luttrell (5) and Irwin and Davis (6)

Species	Source	Length × width		Septa	
		Range (μm)	Mean (S.D.) (μm)	Number[a]	Mean (S.D.)
Wake County[b]					
L. trifolii	Culture (from *M. sativa*)	24–41 × 9–15	30.8 (4.0) × 11.3 (1.5)	2–5 × 0–2	3.2 (0.5) × 0.6 (0.7)
	Culture (from *T. repens*)	25–39 × 10–17	31.7 (13.2) × 13.2 (2.1)	2–3 × 0–2	2.8 (0.4) × 0.3 (0.5)
North Carolina[c]					
L. trifolii	Culture (from *M. sativa*)	25–41 × 13–20	34.2 (3.1) × 15.1 (1.8)	3–4 × 0–2	3.1 (0.2) × 0.4 (0.6)
	Culture (from *T. repens*)	28–51 × 10–20	37.4 (4.3) × 15.3 (2.2)	2–5 × 0–3	3.6 (0.6) × 0.8 (0.8)
Graham and Luttrell[d]					
L. briosiana	*M. sativa*	26–46 × 11–18	32.2–39.7 × 14.3–16.2	3–5 × 0–2	3.1–3.8 × 1.0–1.7
	Culture	26–50 × 11–20	38.1–42.4 × 16.1–16.8	3–5 × 0–4	3.6–4.5 × 1.3–1.8
L. trifolii	*T. repens*	25–49 × 11–21	29.2–43.3 × 13.4–18.5	3–4 × 0–2	3.0–3.1 × 0.0–1.0
	Culture	38–62 × 17–26	46.5–54.2 × 18.2–22.3	3–5 × 0–4	3.1–4.5 × 0.4–2.0
Irwin and Davis[e]					
L. trifolii	*M. sativa*	28–35 × 11–15	31 (1.3) × 13 (1.0)	3–4 × 0–2	3.1 (0.3) × 1.4 (0.6)
	Culture	26–35 × 12–14	33 (2.1) × 13 (0.6)	3–4 × 0–3	3.8 (0.9) × 1.7 (0.7)
	Culture	30–39 × 13–17	34 (2.1) × 13 (1.1)	3–4 × 0–3	3.5 (0.6) × 0.9 (0.7)

[a] Number of transverse × number of longitudinal septa.
[b] Ascospore characteristics of five isolates from each host. Dimensions represent the mean ($n = 5$) of the mean characteristics of 20 ascospores per isolate.
[c] Ascospore characteristics of each of eight isolates from alfalfa and eight isolates from clover chosen randomly from isolates collected in the Piedmont, Coastal Plain, and Mountain regions of North Carolina. Dimensions represent the mean ($n = 8$) of the mean characteristics of 20 ascospores per isolate.
[d] Ascospore characteristics as measured by Graham and Luttrell; data presented are mean minimum and maximum length and width, respectively.
[e] Ascospore characteristics as measured by Irwin and Davis.

clover. Pepper spot symptoms were produced on detached leaves of both alfalfa and white clover. Among the 18 isolates from alfalfa tested, 14 were pathogenic on both alfalfa and clover, two were pathogenic only on alfalfa, and two were nonpathogenic. Among the 13 isolates from white clover tested, six were pathogenic on both alfalfa and clover, three were pathogenic only on white clover, and four were nonpathogenic.

Effect of temperature and isolate/host combination on disease severity. All 10 isolates of *Leptosphaerulina* spp. used were pathogenic on both alfalfa and white clover. There was a significant temperature × trial interaction, indicating that the level of disease at the various temperatures varied and was not consistent among the three trials of the experiment. For example, disease severity (averaged across isolates and hosts) was greatest at 15 C in the first trial, but was similar at 15 and 22 C in the second trial and slightly lower at 15 C than at 22 C in the third trial (data not shown). In general, however, disease was more severe at 15 and 22 C than at 30 C (Fig. 2).

Mean disease severity was affected by the pathogen-host combination, i.e., there was a significant pathogen × host interaction. Mean disease severity ranged from 0.5 to 2.8% on alfalfa and from 1.0 to 1.7% on white clover. Isolates from white clover caused a similar level of disease on both hosts at 15 C and only slightly less disease on alfalfa than on clover at 22 and 30 C (Fig. 2). The mean disease severity caused by the isolates from alfalfa on alfalfa was greater than that on white clover (Fig. 2). Isolates from an individual host did not differ significantly in the amount of disease caused on both hosts.

Effect of temperature and isolate/host combination on disease components. Most lesions on a specific leaf appeared on the same day, and the incubation period was 3–4 days. Isolates sporulated only on detached leaflets of both hosts. The latent period on plants incubated at 15 C during disease development ranged from 14 to 15 days, whereas the mean period from leaf detachment to ejection of ascospores from infected leaves ranged from 6.9 to 9.3 days. Latent period for each isolate/host combination was similar. Because tissue of detached leaves began to decay in the moist chamber, infectious period was difficult to estimate accurately. Ascospores were ejected from pseudothecia on leaves for 18–21 days with the different isolate/host combinations. The latent period, averaged over the two hosts, ranged from 14 to 15 days, 15 to 18 days, and 14 to 18 days when symptomatic leaves were obtained from plants at 15, 22, and 30 C, respectively, during disease development.

Sporulation of *Leptosphaerulina* spp. occurred only on detached, senescent leaves from inoculated plants in the controlled-environment studies. Mean sporulation, which was quantified on a per leaf basis, varied among the pathogen/host combinations (Fig. 3). Isolates did not consistently sporulate at a greater rate on the host of origin or the other host. Four of five isolates from white clover had a numerically lower sporulation rate than the isolates from alfalfa. Sporulation rate for isolates C86WA1 was greater than for the other clover isolates.

The day of peak sporulation for each isolate/host combination was similar, occurring 15–20 days after leaf inoculation. Sporulation occurred in waves or cycles, indicating a possible occurrence of different ages or differential maturation of pseudothecia on senescent leaves. Nearly 80% of the ascospores were ejected within 1 wk after leaf detachment and incubation. The cumulative number of ascospores ejected was slightly higher on alfalfa than white clover leaves when inoculated with isolates from alfalfa.

Sporulation and isolate characteristics from naturally infected leaves. Ascospores were ejected only from senescent parts of the detached leaves obtained from the field. The proportion of lesions on which *Leptosphaerulina* spp. sporulated consistently was higher on chlorotic than on green leaves. All isolates from alfalfa leaflets ($n = 25$), regardless of whether leaves were obtained from the middle of pure alfalfa stands or from alfalfa adjacent to clover, had radial concentric growth with smooth colony margins. From white clover, 14 of 22 isolates had nonconcentric, restricted growth with irregular colony margins, whereas colonies of eight of the 22 isolates were similar to those obtained from alfalfa leaves.

DISCUSSION

Ascospore characteristics that have been used routinely as a taxonomic feature (1,5,6) in classifying *Leptosphaerulina* spp. were not useful in distinguishing among isolates or between isolates from the two hosts in our study. The description by Booth and Pirozynski (1) of *L. trifolii* included those isolates with ascospores 25–50 × 10–20 μm, having three to four transverse septa and zero to two vertical septa. Very little deviation of the ascospore characteristics from their description was observed among the limited sample of isolates we studied from North Carolina. Graham and Luttrell (5) and Irwin and Davis (6) also noted extensive overlap of ascospore characteristics. Graham (3), in a comparative study of ascospores from alfalfa and ladino, alsike, and red clovers grown on V-8 juice agar, reported that, in general, ascospores from alfalfa isolates had more transverse septa than those from the clovers. Among our isolates, the number of transverse septa was not different regardless of whether the original host was alfalfa or white clover. The most notable difference between our isolates of *Leptosphaerulina* spp. from

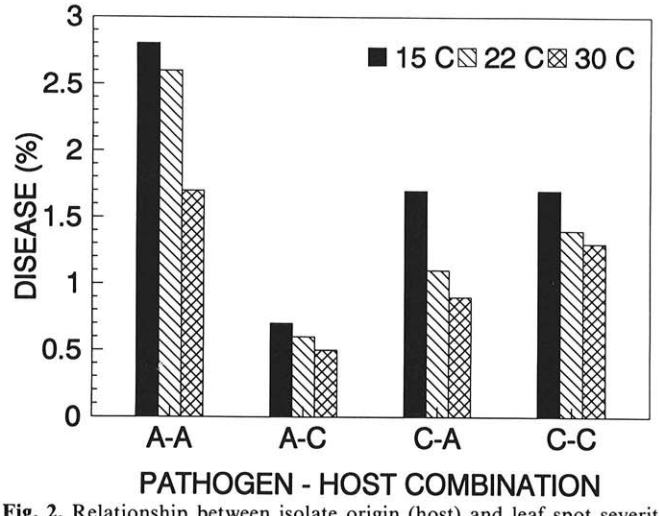

Fig. 2. Relationship between isolate origin (host) and leaf spot severity at 15, 22, and 30 C. A, Alfalfa and C, white clover; the first letter is the host of isolate origin; second letter is the host inoculated. Data represent means of three trials and two plant samples per isolate per host combination, with five isolates from each host.

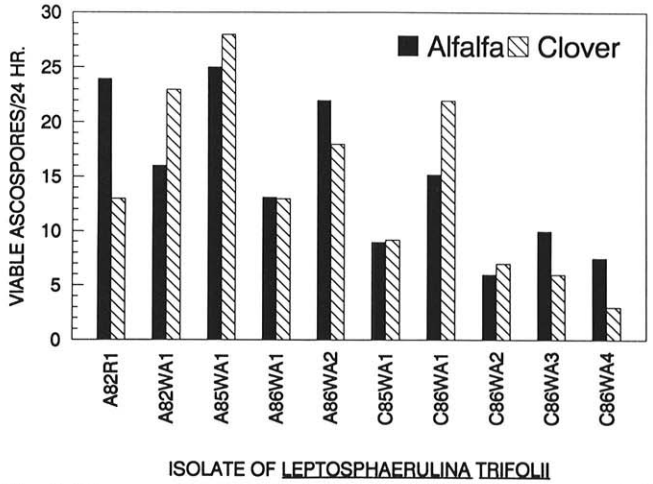

Fig. 3. Mean number of viable ascospores ejected per day per leaflet of isolates of *Leptosphaerulina trifolii* on detached symptomatic leaves of alfalfa and white clover. An ascospore was considered to be viable if one or more germ tubes were observed after 24 hr of incubation on water agar. Isolate designations are: host, A, alfalfa and C, white clover; year of isolation; county of isolation; and number of isolates within the county.

alfalfa and white clover was colony morphology on V-8 juice agar; the importance of this difference is unknown. The similarity among isolates of *Leptosphaerulina* spp. from North Carolina also was reflected in the pepper spot symptom obtained in pathogenicity studies on detached leaves and on intact plants. These results are consistent with those of Miles (10) and Irwin and Davis (6), thus, supporting the view that the two taxa, *L. briosiana* and *L. trifolii* are conspecific.

Mean disease severity was affected by the pathogen/host combination. *L. trifolii* from alfalfa caused more disease on alfalfa than on white clover plants (Fig. 2), but isolates from white clover caused nearly equal amounts of disease on both hosts at each of the three temperature regimes. Although isolates from alfalfa can infect white clover and isolates from white clover can infect alfalfa, some degree of host preference apparently exists, especially with regard to isolates from alfalfa. Consistent, distinct morphological types of fungal colonies have been obtained with isolates of *L. trifolii* from alfalfa; several colony types were observed, however, for isolates obtained from white clover that had been infected naturally in the field. Conceivably, isolates from alfalfa are biotypes or strains of *L. trifolii*.

The incubation period of all isolates examined was 3–4 days; no difference in incubation period was observed among the isolates or hosts. This can be attributed to the rapid spore germination and infectious process under conditions of high relative humidity in the controlled environment. After infection, symptom expression readily occurs under moderate light intensity (7).

Although an effect of host on mean disease severity was observed, such an effect was not observed for latent period. Thus, for the susceptible white clover and alfalfa cultivars used in our study, relative disease severity does not appear to be a determinant of latent period. Latent period (2), with respect to fungal pathogens, usually refers to the time elapsed from infection to sporulation. In many cases, e.g., late blight of potato and stem rust of wheat, sporulation occurs in lesions while uninfected host tissue remains green. In our study, ascospore ejection was detected only on detached, senescent leaves. Thus, events that lead to leaf senescence are important in determining time to first ascospore ejection and subsequent rate of sporulation. These events may not depend specifically on the particular host, i.e., alfalfa or white clover, because host did not affect the mean and peak sporulation for each isolate/host combination. Because detachment of leaves probably accelerated the rate of leaf senescence relating to the rate of leaf death under field conditions, actual latent period may be longer than what we estimated.

We have confirmed that cross-infectivity of *L. trifolii* from alfalfa and white clover occurs on these hosts under controlled conditions. In our study, isolates of *L. trifolii* from a specific host, either alfalfa or white clover from North Carolina, apparently did not differ in their virulence on the cultivar of alfalfa and white clover tested. Differences in disease severity were related to temperature, i.e., increasing temperature within the range used in our study resulted in decreased disease severity (Fig. 2). Although disease did develop at the higher temperature of 30 C, disease development was limited and epidemics of pepper spot should not be expected to occur during July and August in North Carolina. This observation agrees with previous field studies (16) in which inoculum of *L. trifolii* was not observed and incidence of disease caused by this pathogen was reduced on alfalfa under summer conditions when the mean maximum weekly temperatures exceeded 30 C.

LITERATURE CITED

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