# Seasonal Occurrence of Leaf Spot Pathogens of Alfalfa in North Carolina

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**ABSTRACT**

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In order to monitor leaf spot pathogens, 25 alfalfa stems were collected at 2-wk intervals from March to October in 1985 and 1986 at two sites in Wake County and 50 stems at monthly intervals from May to October in 1985 in Rowan and Washington counties of North Carolina. Disease severity estimates were made on each leaf. Fungi were identified after incubation of leaflets in petri dish moist chambers or on V-8 juice agar. Correlations between pathogen occurrence and weather data (maximum and minimum temperature, amount and frequency of rain) from the NOAA weather stations nearest to each alfalfa plot were examined. *Leptosphaerulina briosiana* was the most frequently occurring pathogen during both 1985 and 1986, and occurrence was not limited to cool and wet weather. *Stemphylium botryosum* was present early in the 1985 season but occurred primarily in the spring and fall in 1986; occurrence decreased as mean temperature and rainfall increased. *Phoma medicaginis* var. *medicaginis* was more frequent in the spring than in the fall, and occurrence seemed to be related to frequency of rain rather than to mean rainfall. *Cercospora medicaginis* was present from mid-June until October. The seasonal distribution of each of the four leaf spot pathogens is not mutually exclusive.

Leaf spot diseases are important economically wherever alfalfa (*Medicago sativa* L.) is grown. These diseases cause premature leaf senescence and defoliation, which reduce dry-matter yields (6,7,9, 10,22–24) and forage quality (5,9,12,22). Leaf-spotting fungi can also stimulate production of toxic compounds in alfalfa (4,11,12,17,18).

Leaf spot diseases of alfalfa are caused by several fungal pathogens, including *Leptosphaerulina briosiana* (Poll.) Graham & Luttrell, *Pseudopeziza medicaginis* (Lib.) Sacc., *Phoma medicaginis* Malbr. & Roum. var. *medicaginis* Boerema, *Pleospora herbarum* (Pers.:Fr.) Rab. (anamorph *Stemphylium botryosum* Waller), *Cercospora medicaginis* Ell. & Ev., and *Colletotrichum destructivum* O'Gara (6,14,23). More than one of these fungal pathogens can be present simultaneously in diseased leaves of alfalfa (7,14,20,21). Species in several other genera of fungi, i.e., *Alternaria, Epicoccum, Pithomyces*, and *Helminthosporium*, have been isolated from symptomatic alfalfa leaves, but their role in disease etiology has not been established.

The spectrum of alfalfa leaf spot pathogens varies with geographic location (6,14,21,22,24). Limited information is available, however, concerning the time and frequency of occurrence of specific pathogens during the growing season in North Carolina. The relationship between weather parameters and seasonal distribution of the predominant pathogens also has not been studied. This study was conducted to determine the frequency and time of occurrence of four leaf spot pathogens—*L. briosiana, P. herbarum (S. botryosum), P. m.* var. *medicaginis*, and *C. medicaginis*—and to examine the possibility of accounting for variations in pathogen occurrence with changes in weather.

## MATERIALS AND METHODS

The alfalfa cultivar Arc, selected because it is widely grown in North Carolina and is relatively susceptible to alfalfa leaf spot diseases (21), was monitored at four locations. In Wake County, the two broadcast-planted fields were located at University Research Unit 1 and Unit 9, had an area of 0.13 and 0.4 ha, respectively, and were separated by a

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distance of 8 km. In Rowan and Washington counties, the fields had an area of 0.05 ha and were located at the Piedmont Research Station, Salisbury, and Tidewater Research Station, Plymouth, respectively. The Salisbury and Plymouth fields were part of the 24-cultivar, North Carolina official variety test; only the cultivar Arc was sampled, however.

Since only gross variations in environmental conditions were of interest, weather data were acquired from climatological reports from Raleigh-Durham Airport WSFO AP, Raleigh 4 S.W., Salisbury, and Plymouth 5E weather stations (1,2). Weather stations were located approximately 6.4 and 1.5 km from each plot in Wake County, 0.5 km in Rowan County, and 1.6 km in Washington County. Variables examined were daily maximum and minimum temperatures and daily rainfall.

Samples of 25 alfalfa stems per field, collected at 2-wk intervals from March to October in 1985 and 1986 in Wake County, were obtained following a "W"-shaped path that allowed the field to be sampled with six or seven stems per sampling arm. At 4-wk intervals from May to October 1985 at Salisbury and Plymouth, 50 stems were collected following a zigzag path within each of the five row-planted replications. Only the leaf phase of the diseases caused by the various pathogens was considered in this study. Overall disease severity (for all pathogens) was estimated on each leaf present using a pictorial assessment key based on a modification of a portion of the Horsfall-Barratt rating system (20). Missing leaves were not accounted for in the disease assessment.

Fungi were isolated by surface-disinfesting one leaflet selected arbitrarily on each stem in an aqueous 0.525% sodium hypochlorite solution. Half of the leaflet was placed on V-8 juice agar and the other half was placed in a moist chamber. Petri plates were incubated for 10 days at room temperature (23–25 C) under alternate periods of 12 hr dark and 12 hr light (cool-white fluorescent). Compound and dissecting microscopes were used to identify the fungi present.

Correlations among number of leaflets with each fungus (*L. briosiana, S. botryosum, P. m.* var. *medicaginis*, and *C. medicaginis*), percent disease severity, and frequency of rain days, mean rainfall per rain day (cm), and mean maximum and mean minimum temperatures (C) for 1, 2, and 3 wk prior to the sampling date were examined (16). Although it was probably present in 1985, occurrence of *C. medicaginis* was monitored only in 1986.

## RESULTS

Severity of alfalfa leaf spot varied throughout both growing seasons and among locations. In 1985, disease

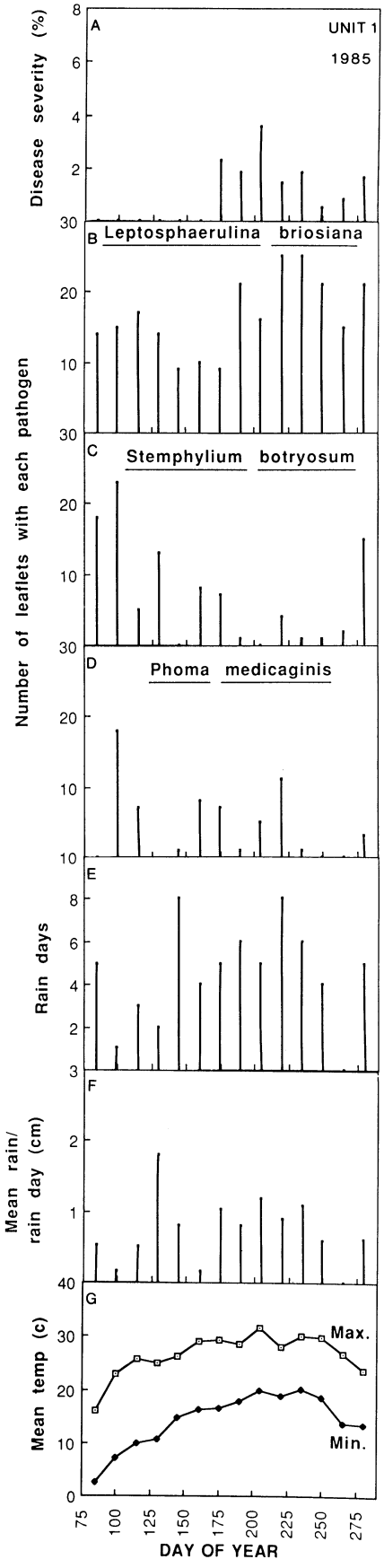


**Fig. 1.** Disease severity, number of leaflets of alfalfa ($n = 25$) infected with *Leptosphaerulina briosiana, Stemphylium botryosum*, and *Phoma medicaginis*, frequency of rain, mean rainfall, and mean temperature from 26 March to 7 October 1985 at University Research Unit 1, Wake County.

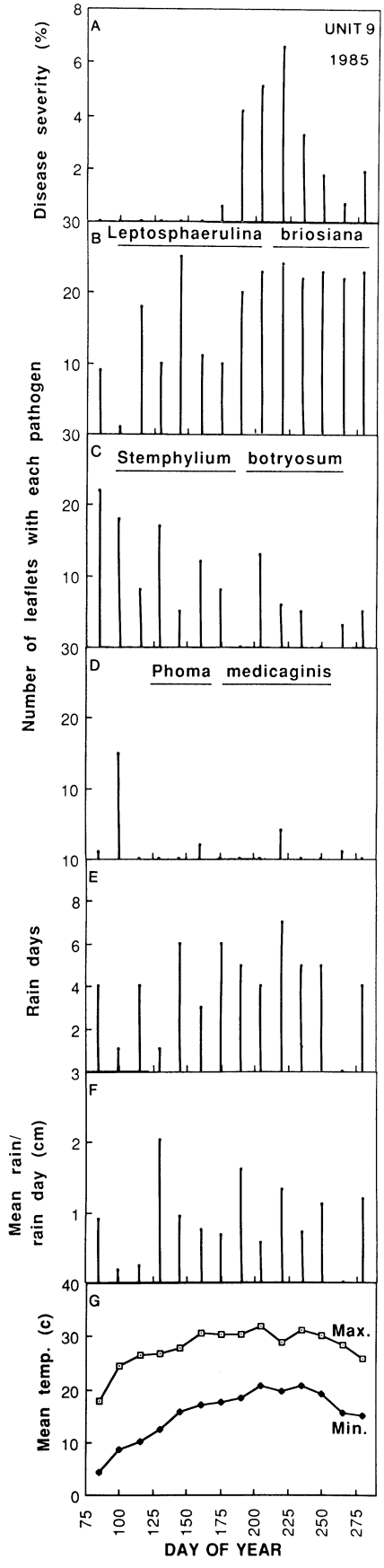


**Fig. 2.** Disease severity, number of leaflets of alfalfa ($n = 25$) infected with *Leptosphaerulina briosiana, Stemphylium botryosum*, and *Phoma medicaginis*, frequency of rain, mean rainfall, and mean temperature from 26 March to 7 October 1985 at University Research Unit 9, Wake County.

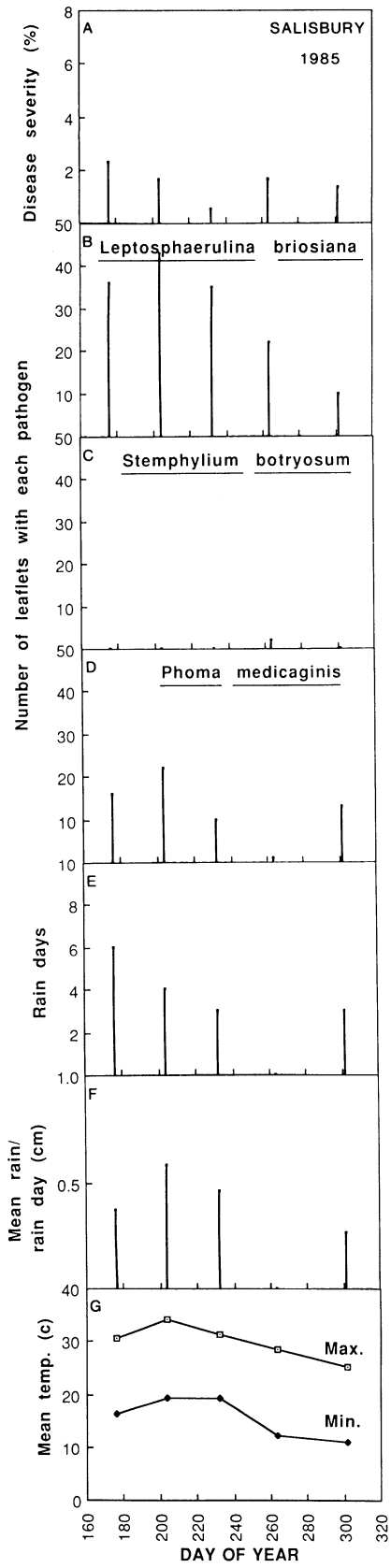


**Fig. 3.** Disease severity, number of leaflets of alfalfa ($n = 50$) infected with *Leptosphaerulina briosiana, Stemphylium botryosum*, and *Phoma medicaginis*, frequency of rain, mean rainfall, and mean temperature from 25 June to 29 October 1985 at Piedmont Research Station, Salisbury, Rowan County.

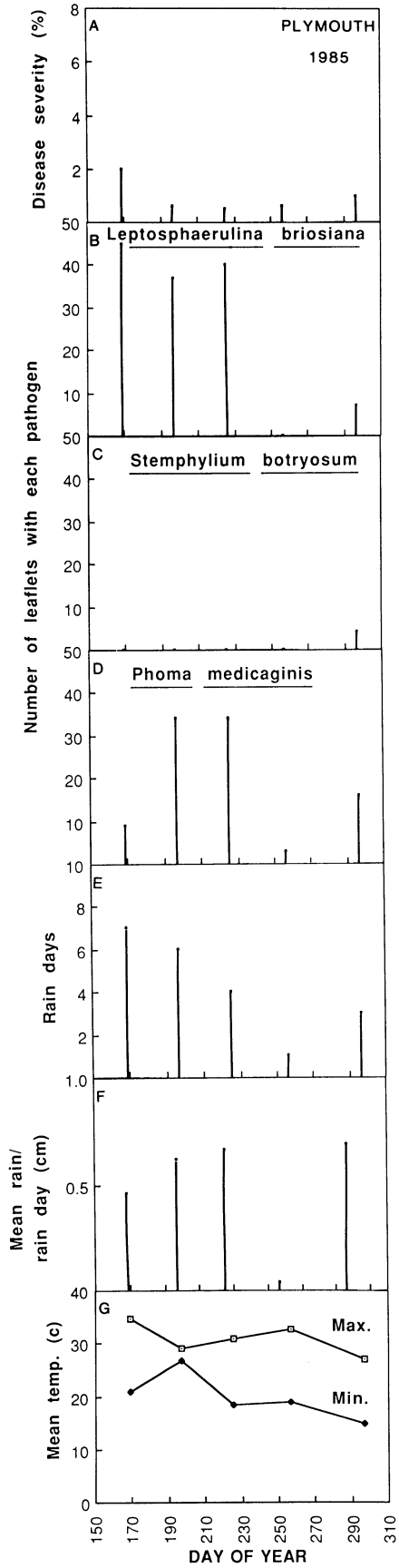


**Fig. 4.** Disease severity, number of leaflets of alfalfa ($n = 50$) infected with *Leptosphaerulina briosiana, Stemphylium botryosum*, and *Phoma medicaginis*, frequency of rain, mean rainfall, and mean temperature from 18 June to 24 October 1985 at Tidewater Research Station, Plymouth, Washington County.

severity ranged from 0.53 to 3.58, 0.57 to 6.58, 0.51 to 2.28, and 0.52 to 1.98% at the Wake County Units 1 and 9, Salisbury, and Plymouth locations, respectively (Figs. 1–4). In 1986, only the locations in Wake County were sampled; disease severity ranged from 0.13 to 2.55 and 0.20 to 2.37% at Units 1 and 9, respectively (Figs. 5 and 6). *L. briosiana* was detected in all samples in both years. In 1985, it increased in frequency during the second half of the season in Wake County; no such pattern occurred at Salisbury or Plymouth. In 1986, frequency of occurrence decreased in early August and increased again in early September. *S. botryosum* was detected infrequently at Salisbury and Plymouth. At Units 1 and 9 in 1985, it was present early in the season and decreased as the season continued; a slight end-of-season increase occurred at Unit 1. In 1986, *S. botryosum* was detected infrequently but occurred primarily in the spring and fall at both Wake County locations. *P. m.* var. *medicaginis* was detected irregularly but was more frequent in the spring than in the fall at all locations. *C. medicaginis* was detected irregularly from the second half of June 1986 until the end of the season at Units 1 and 9 in Wake County (Figs. 5 and 6).

In 1985, the seasonal occurrence of *L. briosiana* at Unit 9 and Salisbury increased with an increase in temperature (Table 1), whereas at Unit 9 in 1986, the occurrence of this pathogen was related negatively to temperature. There was some indication in 1985 that an increase in mean rainfall positively influenced occurrence of *L. briosiana*; this was most apparent at Plymouth.

Frequency of detection of *S. botryosum* decreased as mean maximum and mean minimum temperatures increased at all locations in both years (Table 1). Also, as mean rainfall and frequency of days with rain increased, incidence of *S. botryosum* in alfalfa leaflets decreased.

Frequency of occurrence of *P. m.* var. *medicaginis* at Unit 9 decreased during 1985 and 1986 as mean maximum and minimum temperatures increased (Table 1). In 1985, sampling commenced later at Salisbury than at the other three locations, and incidence of *P. m.* var. *medicaginis* was positively related to mean minimum temperatures, which were highest for the 4-wk period preceding days 204 and 232 (Fig. 3). Rainfall measurement and detection of *P. m.* var. *medicaginis* were also positively related at the Plymouth and Salisbury locations.

Occurrence of *C. medicaginis* increased in 1986 at Unit 1 as temperatures the week before the sample date increased (Table 1). At Unit 9, as mean rainfall increased so did frequency of detection of *C. medicaginis*.

The occurrence of *L. briosiana* was not correlated significantly with that of any

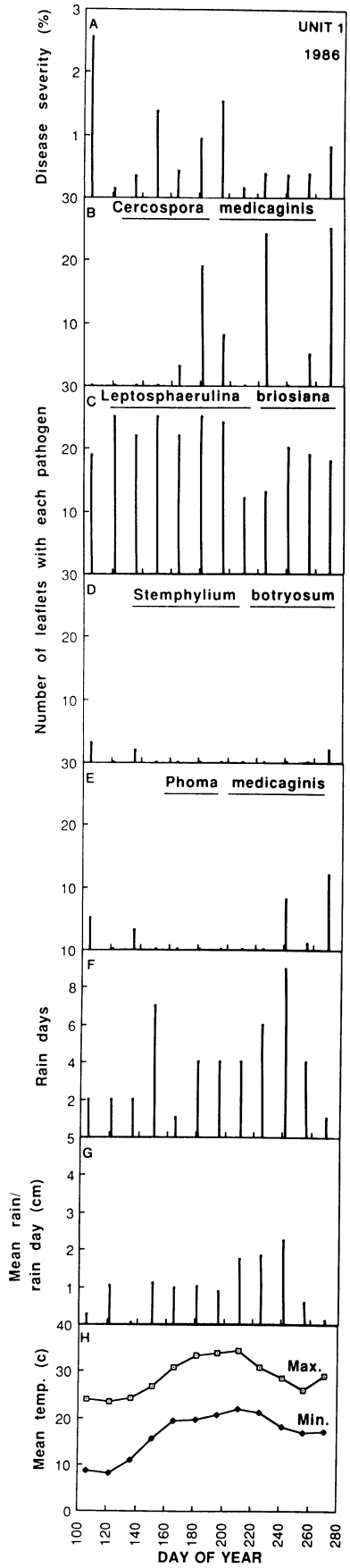


**Fig. 5.** Disease severity, number of leaflets of alfalfa ($n$ = 25) infected with *Cercospora medicaginis, Leptosphaerulina briosiana, Stemphylium botryosum*, and *Phoma medicaginis*, frequency of rain, mean rainfall, and mean temperature from 16 April to 28 September 1986 at University Research Unit 1, Wake County.

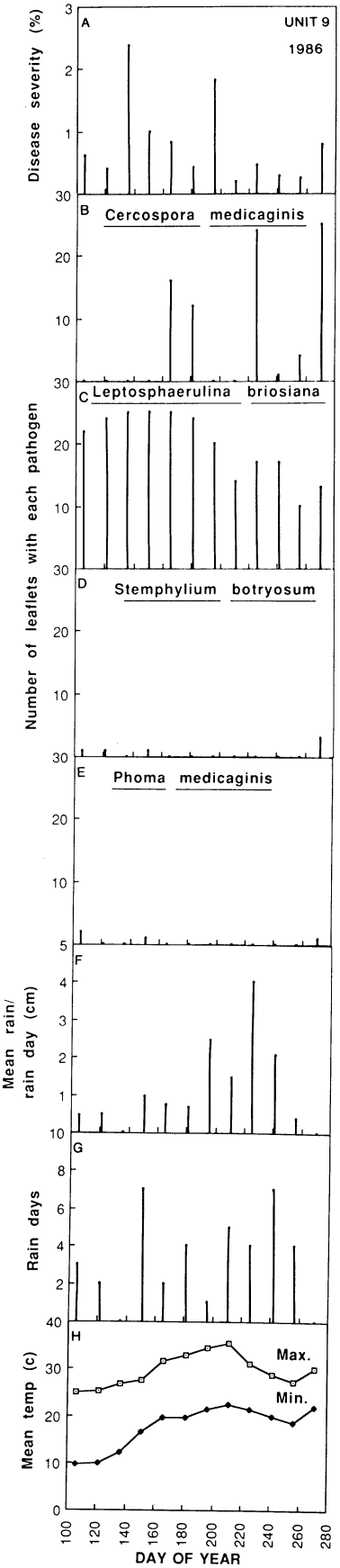


**Fig. 6.** Disease severity, number of leaflets of alfalfa ($n$ = 25) infected with *Cercospora medicaginis, Leptosphaerulina briosiana, Stemphylium botryosum*, and *Phoma medicaginis*, frequency of rain, mean rainfall, and mean temperature from 16 April to 28 September 1986 at University Research Unit 9, Wake County.

of the other pathogens except at Unit 9 in 1985, where its presence was correlated negatively with the occurrence of *S. botryosum* ($-0.76$, $P \leq 0.01$) and *P. m.* var. *medicaginis* ($-0.59$, $P \leq 0.05$). In all but two cases, incidence of *S. botryosum* and *P. m.* var. *medicaginis* was positively related with correlation of 0.41 ($P \leq 0.15$) and 0.40 ($P \leq 0.15$) at Units 1 and 9, respectively, in 1985 and 0.62 ($P \leq 0.05$) and 0.59 ($P \leq 0.05$) at these two locations in 1986. At Plymouth, occurrence of these two pathogens was not related, whereas at Salisbury, there was a negative correlation ($-0.82$, $P \leq 0.10$) for their occurrence. In 1986, the presence of *C. medicaginis* was not related significantly with the occurrence of the other three leaf spot pathogens.

## DISCUSSION

Leaf spots of alfalfa in North Carolina are caused by at least four fungi. The presence of two or more pathogens in an individual leaflet was common throughout both growing seasons and at all locations. Seasonal differences in the pathogens present were detected, however.

*L. briosiana* was the only leaf spot pathogen of alfalfa present continuously at all locations studied. Previous reports have suggested that *L. briosiana* occurs under cool and wet weather conditions (8,15,19,22). During the 1985 and 1986 growing seasons in North Carolina, however, occurrence of *L. briosiana* was not limited to cool and wet weather conditions. Frequency of occurrence of *L. briosiana* was positively influenced by mean maximum and mean minimum temperatures in 1985, when mean weekly maximum temperatures were rarely at or above 30 C. Weekly mean maximum temperatures above 30 C during 1986 apparently affected *L. briosiana* adversely, since detection of this fungus decreased after these warmer temperature periods (Figs. 5 and 6). Frequency of *L. briosiana* detected in alfalfa leaves generally decreased from day 160 to day 220 in 1986 at both locations when weekly mean maximum temperatures were $\geq$30 C. Also, this trend was observed at the four locations in 1985, although fewer sample periods had mean maximum temperatures $\geq$30 C (Figs. 1–4). This apparent negative effect of mean maximum temperatures $\geq$30 C on *L. briosiana* is consistent with the temperature limits found in vitro for growth of this fungus (13; C. L. Campbell, *unpublished*).

The seasonal presence of *S. botryosum* was associated primarily with cooler temperatures in the spring and in the fall. Although occurrence of this fungus was negatively related to frequency of rain and mean rainfall, this relationship may not always hold and may be a limitation of the seasons studied, since rain was more frequent during periods of warmer temperatures. *P. m.* var. *medicaginis* did not show a consistent pattern of

**Table 1.** Correlations[a] between occurrence of *Leptosphaerulina briosiana*, *Stemphylium botryosum*, *Phoma medicaginis* var. *medicaginis*, and *Cercospora medicaginis* detected in leaflets[b] of the alfalfa cultivar Arc on V-8 juice agar or after incubation in a moist chamber and weather parameters[c] 1, 2, or 3 wk before sampling leaflets at Unit 1 (U1) and Unit 9 (U9) in Wake County and at Plymouth (Ply) and Salisbury (Sal), North Carolina, in 1985 and 1986

| Weeks before sampling | | Maximum temperature | | | | Minimum temperature | | | | Mean rainfall | | | | Frequency of rain | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | U1 | U9 | Ply | Sal | U1 | U9 | Ply | Sal | U1 | U9 | Ply | Sal | U1 | U9 | Ply | Sal |
| *L. briosiana* | | | | | | | | | | | | | | | | | |
| 1985 | 1 | – | 0.44++ | – | 0.99** | – | 0.57* | – | 0.81++ | – | – | 0.98** | – | – | – | 0.86++ | – |
| | 2 | – | 0.44++ | – | 0.98** | – | 0.64** | – | 0.93* | – | 0.43+ | – | 0.76+ | – | – | – | – |
| | 3 | – | 0.51++ | – | 0.98** | – | 0.65** | – | 0.97* | 0.41+ | – | – | – | – | – | 0.85++ | – |
| 1986 | 1 | – | – | | | – | – | | | -0.52++ | – | | | – | – | | |
| | 2 | – | – | | | – | – | | | – | – | | | – | – | | |
| | 3 | – | -0.60++ | | | – | -0.61++ | | | – | – | | | – | – | | |
| *S. botryosum* | | | | | | | | | | | | | | | | | |
| 1985 | 1 | -0.76++ | -0.63** | -0.97** | – | -0.77** | -0.66** | -0.91* | -0.78+ | -0.51** | – | – | – | – | – | | |
| | 2 | -0.75** | -0.61** | -0.99** | – | -0.79** | -0.69** | -0.88* | – | – | -0.43+ | – | -0.78++ | – | – | | |
| | 3 | -0.79** | -0.63** | -0.98** | – | -0.80** | -0.69** | -0.86* | – | -0.52* | – | 0.98** | – | – | – | | |
| 1986 | 1 | -0.61* | – | | | -0.63* | – | | | -0.54++ | – | | | -0.46+ | – | | |
| | 2 | -0.47+ | – | | | -0.57* | – | | | -0.47+ | – | | | -0.93++ | – | | |
| | 3 | – | -0.80** | | | -0.54++ | -0.81** | | | -0.84++ | – | | | -0.61* | -0.55++ | | |
| *P. m.* var. *medicaginis* | | | | | | | | | | | | | | | | | |
| 1985 | 1 | – | – | – | – | – | -0.42+ | – | 0.74+ | -0.42+ | – | – | – | – | – | -0.73+ | – |
| | 2 | – | – | – | – | – | – | – | – | – | – | – | 0.88* | – | – | – | 0.81++ |
| | 3 | – | -0.42+ | – | – | – | -0.41+ | – | – | – | – | – | – | – | – | – | – |
| 1986 | 1 | – | -0.56++ | | | – | -0.54++ | | | – | – | | | – | – | | |
| | 2 | – | -0.46+ | | | – | -0.49+ | | | – | – | | | – | – | | |
| | 3 | – | -0.50+ | | | – | -0.55+ | | | – | – | | | – | – | | |
| *C. medicaginis* | | | | | | | | | | | | | | | | | |
| 1986 | 1 | 0.47+ | – | | | 0.50++ | – | | | – | 0.44+ | | | – | – | | |
| | 2 | – | – | | | – | – | | | – | – | | | – | – | | |
| | 3 | – | – | | | – | – | | | – | – | | | – | – | | |

[a] For Unit 1 and Unit 9 in Wake County, $n = 14$ in 1985 and 12 in 1986; for Plymouth and Salisbury (sampled in 1985 only), $n = 5$ where $n$ is the number of sampling dates. – = Not significant; ** = $P = 0.01$, * = $P = 0.05$, ++ = $P = 0.10$, and + = $P = 0.15$.

[b] One leaflet was selected arbitrarily for each of 25 alfalfa stems at Unit 1 and Unit 9 or 50 alfalfa stems at Plymouth and Salisbury for pathogen isolation. Stems were collected at 2-wk intervals at Unit 1 and Unit 9 and at 4-wk intervals at Plymouth and Salisbury.

[c] Maximum temperature = mean of the daily maximum temperature, minimum temperature = mean of the daily minimum temperature, mean rainfall = average daily rainfall, and frequency of rain = number of days with rain for the 7-, 14-, and 21-day period before the sampling date.

occurrence in this study but was detected irregularly throughout both growing seasons. The time of detection of *C. medicaginis* in our study agrees with previous reports (2,3) where leaf spot symptoms caused by *C. medicaginis* appeared in mid-June and persisted until the end of the growing season. The initial occurrence of *C. medicaginis* at both locations in 1986 also coincided with the first period of mean maximum temperatures above 30 C (Figs. 5 and 6).

*L. briosiana* occurred at the highest frequency among those fungal pathogens detected each growing season and is probably the most important leaf spot pathogen of alfalfa in North Carolina. However, *S. botryosum, P. m.* var. *medicaginis*, and *C. medicaginis* also were detected in alfalfa leaf tissue and probably play a critical role in causing the leaf spot diseases responsible for yield losses in North Carolina. There is not a clear-cut and unique seasonal distribution of each of these leaf spot pathogens. Rather, their presence overlaps during the growing season, indicating that these alfalfa leaf spot pathogens are not mutually exclusive in the niche they can occupy. The correlation coefficients for pathogen occurrence suggest that the weather conditions favorable for occurrence of *L. briosiana* are not equally favorable for the other three pathogens observed. Environmental factors that favored the occurrence of *S. botryosum* also generally favored the occurrence of *P. m.* var. *medicaginis* but not the occurrence of *C. medicaginis*. It would be interesting to examine the nature of potential interactions among the pathogens in the alfalfa leaf spot complex in a further attempt to better understand the epidemiology of these diseases.


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## LITERATURE CITED


1. Anonymous. 1985. Climatological data in North Carolina. Vol. 90, Nos. 3–9. National Oceanic and Atmospheric Administration, National Climatic Data Center, Asheville.
2. Baxter, J. W. 1956. Cercospora black stem of alfalfa. Phytopathology 46:398-400.
3. Berger, R. D., and Hanson, C. H. 1963. Pathogenicity, host parasite relationships, and morphology of some forage legume Cercosporae, and factors related to disease development. Phytopathology 53:500-508.
4. Borges, O. L., Stanford, E. H., and Webster, R. K. 1976. The host-pathogen interaction of alfalfa and *Stemphylium botryosum*. Phytopathology 66:749-753.
5. Brigham, R. D. 1959. Effect of Cercospora disease on forage quality of alfalfa. Agron. J. 51:365.
6. Broscious, S. C., and Kirby, H. W. 1988. Economic evaluation of fungicides for control of foliage diseases of alfalfa. Phytopathology. In press.
7. Campbell, C. L. 1985. Disease losses in North Carolina: Alfalfa. In: Estimates of crop losses in North Carolina due to plant diseases and nematodes. C. E. Main and S. M. Nusser, eds. N.C. State Univ. Dep. Plant Pathol. Spec. Publ. 4. 152 pp.
8. Elliot, A. M., and Wilcoxson, R. D. 1964. Effect of temperature and moisture on formation and ejection of ascospores and on survival of *Leptosphaerulina briosiana*. Phytopathology 54:1443-1447.
9. Graham, J. H., Kreitlow, K. W., and Faulkner, L. R. 1972. Diseases. Pages 497-526 in: Alfalfa Science and Technology. C. H. Hanson, ed. American Society of Agronomy, Madison, WI. 812 pp.
10. Hanson, C. H. 1965. Foliar diseases and forage quality. Pages 1209-1213 in: Proc. Int. Grassland Congr. 9th.
11. Higgins, U. J. 1972. Role of the phytoalexin medicarpin in three leafspot diseases of alfalfa. Physiol. Plant Pathol. 2:289-300.
12. Leath, K. T., Shenk, J. S., and Barnes, R. F. 1974. Relation of foliar disease to quality of alfalfa forage. Agron. J. 66:675-677.
13. Martinez, E. S., and Hanson, E. W. 1963. Factors affecting growth, sporulation, pathogenicity, and dissemination of *Leptosphaerulina briosiana*. Phytopathology 53:938-945.

14. Renfro, B. L., and Kernkamp, M. F. 1963. Fungi isolated from black stem of alfalfa and the influence of temperature on lesion formation and disease severity. Phytopathology 53:774-777.
15. Roberts, D. A., Horner, E. S., Prine, G. M., and Johnson, F. A. 1984. An epidemic of lepto leaf spot in alfalfa caused by *Leptosphaerulina briosiana*. Plant Dis. 68:732.
16. SAS User's Guide. 1985. Statistics. Version 5. SAS Institute, Inc., Cary, NC. 956 pp.
17. Stoessl, A. 1982. Biosynthesis of phytoalexins. Pages 133-180 in: Phytoalexins. J. A. Bailey and J. W. Mansfield, eds. John Wiley & Sons, New York. 334 pp.
18. Sundheim, L. 1972. Role of toxins in the leafspot disease of alfalfa caused by *Leptosphaerulina briosiana*. Pages 423-424 in: Phytoalexins in Plant Disease. Proc. NATO Advanced Study Inst., Pugnochiuso, Italy. R. K. S. Wood, A. Ballio, and A. Graniti, eds. Academic Press, London. 530 pp.
19. Sundheim, L., and Wilcoxson, R. D. 1965. *Leptosphaerulina briosiana* on alfalfa: Infection and disease development, host parasite relationships, ascospore germination, and dissemination. Phytopathology 55:546-553.
20. Thal, W. M., and Campbell, C. L. 1987. Sampling procedures for determining severity of alfalfa leaf spot diseases. Phytopathology 77:157-162.
21. Thal, W. M., and Campbell, C. L. 1987. Assessment of resistance to leaf spot diseases among alfalfa cultivars in North Carolina fields. Phytopathology 77:964-968.
22. Willis, W. G., Stuteville, D. L., and Sorensen, E. L. 1969. Effects of leaf and stem diseases on yield and quality of alfalfa forage. Crop Sci. 9:637-640.
23. Wilcoxson, R. D., and Bielenberg, O. 1972. Leaf disease control and yield increase in alfalfa with fungicides. Plant Dis. Rep. 56:286-289.
24. Wilcoxson, R. D., Bielenberg, O., and Bissonnette, H. L. 1973. Yield of alfalfa hay increased by control of foliar diseases. Plant Dis. Rep. 57:353-354.