Impact of Leaf Spot Diseases on Yield and Quality of Alfalfa in North Carolina

C. LEE CAMPBELL, Associate Professor, and JAMES A. DUTHIE, Graduate Assistant, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616

ABSTRACT

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Plants of alfalfa (*Medicago sativa*) grown in a test field in 1986 and 1987 were harvested five times each year. Leaf spot diseases on the plants were attributable to *Leptosphaerulina briosiana, Stemphylium botryosum, Phoma medicaginis*, and *Cercospora medicaginis*. The particular fungi present varied from harvest to harvest. Application of chlorothalonil (at rates equivalent to 0, 0.65, 1.3, 2.6, or 5.2 kg a.i./ha) to mature, healthy alfalfa plants in the greenhouse did not affect amount of dry matter accumulated during 4 wk of regrowth. In the field, mean disease severity (percentage of leaf area diseased) at all harvests in 1986 was consistently ≤5% in plots sprayed weekly with chlorothalonil (1.3 kg a.i./ha) but ranged from 5 to 14% in unsprayed plots. In 1987, final disease severity was consistently ≤3% in sprayed plots but ranged from 3 to 8% in unsprayed plots. Dry matter yield was 5.3–34.8% less in unsprayed plots than in sprayed plots at the five harvests in 1986 and 12.9–37.2% less in 1987. Annual differences in yield between sprayed and unsprayed plots were 18.6% (1.69 Mg/ha) in 1986 and 24.8% (2.64 Mg/ha) in 1987. Percentages of total digestible nutrients and acid detergent fiber did not differ in alfalfa harvested from treated or untreated plots. Mean adjusted crude protein did not differ with respect to fungicide treatment or harvest in 1986 but was greater in unsprayed plots (22.1%) than in plots sprayed with fungicide (20.6%) in 1987.

Leaf spot diseases occur throughout the entire growing range of alfalfa (*Medicago sativa* L.). The fungi that cause these diseases vary with geographic region and, in some cases, seasonally. In North Carolina, four species are the predominant leaf spot pathogens: *Leptosphaerulina briosiana* (Pollacci) J. H. Graham & Luttrell, *Phoma medicaginis* Malbr. & Roum., *Stemphylium botryosum* Wallr. (teleomorph *Pleospora herbarum* (Pers.:Fr.) Rabenh.), and *Cercospora medicaginis* Ellis & Everh. Another species, *Pseudopeziza medicaginis* (Lib.) Sacc., occurs only rarely. The frequency of occurrence of these pathogens varies seasonally. *L. briosiana* is generally present throughout the growing season; *P. medicaginis* is present in the spring and fall but generally absent in the summer; *S. botryosum* is present intermittently, generally at low levels; and *C. medicaginis* occurs from mid-June to October (16).

Leaf spot diseases reduce yield and quality of harvested alfalfa and can also reduce stand life. Reductions in dry matter yield of 5–44% caused by leaf spot diseases have been reported from Alberta (5.7%) and Ontario (8.4%), Canada (1,2); Victoria, Australia (40%) (9); Canterbury, New Zealand (16%) (5); and in the United States from California (15.5%) (15), Illinois (17.6–20.6%) (4), Iowa (44%) (11), Kansas (14–28.3%) (19), and Minnesota (10.5–42%) (17,18). Percentage loss is usually measured relative to fungicide-sprayed controls and can vary among harvests according to the presence of specific pathogens during certain growth cycles (17,19). Among the quality factors of alfalfa hay that are affected by leaf spot diseases are the content of crude protein (3,8,10,13), carbohydrates (8), and carotene (19). Digestibility (10) and animal intake and weight gain (7) may also be affected. Reductions in dry matter yield, however, may not affect concentration of protein in hay (15,19).

Although information is available on losses in yield and quality due to leaf spot diseases for many alfalfa-growing regions, no such loss estimates have been made in North Carolina or in the southeastern United States. With the variation in pathogen occurrence at different times of the year and the four or five harvests made between May and September each year, the leaf spot disease mixture in North Carolina results in an epidemiologically different pathosystem from those in other geographic areas of North America. Our purpose was, therefore, to obtain estimates of losses in yield and quality of alfalfa in North Carolina, paying particular attention to the variation in the possible losses in different alfalfa growth cycles.

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MATERIALS AND METHODS

Assay of chlorothalonil effect on alfalfa growth. A greenhouse study was conducted to determine any possible effects of chlorothalonil (Bravo 500) on the growth of alfalfa. Plants of alfalfa cultivars Arc and Raidor were grown in clay pots (15.25 cm in diameter, 1,800 mm^3 in volume) filled with pasteurized loam soil and sand (3:1, v/v). At 16 wk, the plants were trimmed to a height of 8–9 cm above the soil surface. Each plant was sprayed once a week for 5 wk with 2.5 ml of chlorothalonil at 0, 75, 150, 300, or 600 μg a.i./ml. These rates represent approximately 0, 0.5, 1, 2, or 4 times the application rate in the field study, where chlorothalonil was applied to each 3 × 3 m plot at 1.3 kg a.i./ha. Two plants per cultivar per treatment were included in each replication. One week after the last fungicide application, plants were cut to a height of 8–9 cm. The number of stems per plant was recorded, and the harvested material was dried and weighed. The 2 × 5 factorial experiment was conducted as a randomized complete-block design with four replications.

Field plot establishment. In early September 1984, a field (57 × 81 m) was broadcast-planted with alfalfa cultivar Arc at 56.8 kg of *Rhizobium*-inoculated seed per hectare. The field was treated before planting with 3.4 kg a.i./ha of EPTC (Eptam 7EC) for control of weeds. After seeding, and again in the springs of 1986 and 1987, the field received applications of a 0-9-27 fertilizer (227 kg/ha) and boron (3.4 kg/ha). Near the end of March of both years, one application of either carbofuran (Furadan 4F, 0.57 kg a.i./ha) or chlorpyrifos (Lorsban 4EC, 1.13 kg a.i./ha) was made for control of alfalfa weevil (*Hypera postica* Gyllenhal). During 1985, the field was managed as a commercial production field with five harvests. During 1986, no herbicides were applied. In early March 1987, metribuzin (Sencor 4F, 0.57 kg a.i./ha) was applied to control weeds.

In March 1986, 24 3 × 3 m plots were established in a 3 × 8 arrangement with a north-south spacing between plots of 6 m and east-west spacing between plots of 12 m. Twelve plots were selected randomly to receive weekly applications of chlorothalonil during the two growing seasons, and 12 other plots served as untreated controls. Fungicide-treated plots were sprayed with chlorothalonil (0.59 mg a.i. in 300 ml water per plot) each

Monday from 17 March 1986 to 29 September 1986 and from 23 March 1987 to 21 September 1987. This resulted in an application rate equivalent to 1.3 kg a.i./ha.

Seasonal occurrence of leaf spot pathogens. Immediately before each harvest (except harvest III in 1986), two or three trifoliolate leaves of alfalfa with leaf spot symptoms were collected from an area adjacent to the central 1 × 1 m portion of each plot. Leaves from each of the two treatments were bulked, and 60 or 100 diseased leaflets were selected arbitrarily from each treatment to determine the relative frequency of each leaf spot fungus. Leaflets were surface-disinfested for 15–20 sec in 0.263% sodium hypochlorite and sterile distilled water, rinsed in sterile distilled water, and then placed on moistened filter paper in a petri dish, six or seven leaflets to a dish. Dishes were incubated for 5–10 days at room temperature (about 22–24 C) with alternating 12-hr periods of supplemental light supplied by two cool-white fluorescent tubes (Philips F96T12/GW) suspended 27 cm above the plates. After incubation, leaflets were examined at ×40 magnification under a stereo dissecting microscope. Characteristic fruiting structures were used to identify fungal pathogens present.

Table 1. Analysis of variance to determine the effect of alfalfa cultivar and fungicide treatment on dry weight per plant and number of stems per plant after 30 days of regrowth in the greenhouse

Source	df	Mean squares: Dry weight per plant	Mean squares: Stems per plant
Block	3	3.31**[x]	2.66
Cultivar (C)[y]	1	13.72**	12.66
Fungicide treatment (F)[z]	4	0.16	1.32
F × C	4	0.58	4.81
Residual	27	11.59	5.15

[x] ** = Probability of a greater $F \leq 0.01$.
[y] Cultivars used were Arc and Raidor.
[z] Each plant was sprayed once per week for 5 wk with 2.5 ml of a chlorothalonil solution at 0, 75, 150, 300, or 600 μg a.i./ml.

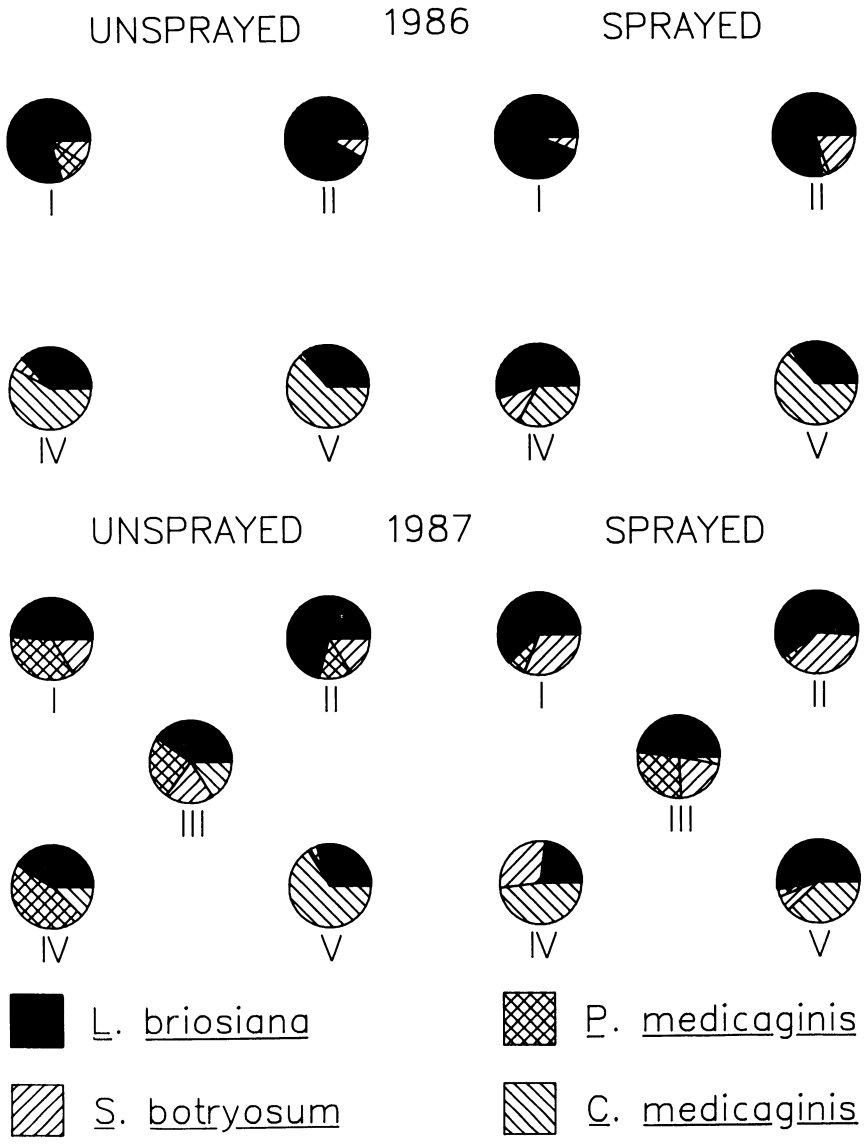

Fig. 1. Incidence of four leaf spot pathogens on symptomatic, surface-disinfested leaves of alfalfa (from unsprayed and chlorothalonil-sprayed plots) in 1986 and 1987 expressed as a proportion of total identifications made after 5–10 days of incubation in a moist chamber. Roman numerals indicate harvests. No samples were taken in harvest III, 1986.

Determination of host growth, disease, dry matter yield, and quality. Canopy height and defoliation (height from the ground surface to the first intact leaf node) were measured weekly in the central 1 × 1 m area of each plot. Disease severity (percentage of total leaf area with disease symptoms) was estimated using the Horsfall-Barratt scale (6) in each of four 0.5 × 0.5 m subplots within the central area of each plot.

Plots were harvested five times per year when the average plant growth stage was 5–10% bloom. Harvests, designated by Roman numerals, occurred on days 122, 156, 191, 233, and 274 of 1986 and days 126, 161, 195, 229, and 272 of 1987. The central 1 × 1 m portion of each plot was cut by hand with hedge shears to a height of 10–12 cm. The cut alfalfa was removed, placed in muslin bags, dried in a forced-air forage drying oven at 40 C for 24–48 hr, and then weighed. At harvests II, III, and IV in 1986 and at all harvests in 1987, a subsample of dried alfalfa was selected arbitrarily after weighing from each of six randomly chosen treated and untreated plots. These subsamples were sent to the Forage Testing Laboratory of the North Carolina Department of Agriculture for quality analysis.

Environmental data. We obtained mean maximum and minimum temperatures and total rainfall for each week during the 1986 and 1987 growing seasons from the monthly NOAA climatological data summaries for North Carolina. We used the data for reporting station Raleigh 4SW, which is located approximately 5 km from the experimental site.

Data analysis. For the greenhouse study, effects of cultivar and fungicide rate were evaluated by conducting a separate analysis of variance for both variables (number of stems per plant and plant dry weight) used to measure plant growth. Data were analyzed as a randomized complete-block design with combinations of cultivar and fungicide treatments arranged factorially.

For each year of the field study, each

response variable was analyzed separately as a split plot in time (14), with whole plots consisting of fungicide treatments (sprayed or unsprayed) and split plots consisting of harvest numbers I–V. All effects were assumed to be random except for fungicide treatment (F), which was assumed to be fixed. Appropriate divisors for F tests were chosen by determining the variance components of each mean square.

All analyses of variance were conducted using PROC GLM of the Statistical Analysis System (12). Model assumptions were checked by visual inspection of plots of the residuals against predicted values. Coefficients of variation were calculated for each overall mean.

RESULTS

Effect of chlorothalonil on growth of alfalfa. There was no evidence that chlorothalonil had an effect on the growth of alfalfa (Table 1). Dry weights of plants, but not the number of stems per plant, varied significantly between cultivars. Mean dry weights for plants of cultivars Arc and Raidor were 2.69 and 1.52 g, respectively. The mean number of stems per plant was 8.1.

Incidence of leaf spot pathogens. At harvests I and II in both years, *L. briosiana* was observed most frequently on diseased leaves. *S. botryosum* and *P. medicaginis* occurred less frequently (Fig. 1). No leaf samples were taken for harvest III in 1986, but all four pathogens were observed at harvest III in 1987. *L. briosiana* and *C. medicaginis* were both observed frequently at harvest IV. *S. botryosum* and *P. medicaginis* occurred infrequently, except at harvest IV in 1987 (Fig. 1).

Environment, plant growth, defoliation, and disease. Total monthly rainfall exceeded the 30-yr average in April and September 1986 and in August 1987 and was below average in all other months of the study (Table 2). Except for April 1987, mean monthly temperatures exceeded the 30-yr average (Table 2).

The final proportion of defoliation (distance from soil surface to first intact leaf divided by canopy height, in centimeters) differed ($P = 0.01$) among harvests but not between sprayed and unsprayed plots in 1986 (Table 3). Mean proportional defoliation at harvest was 0.47, 0.44, 0.24, and 0.41 for harvests I, III, IV, and V, respectively, but was only 0.02 at harvest II (Fig. 2). In 1987, there was an interaction ($P = 0.0001$) between fungicide and harvest effects for final proportion of defoliation. Less defoliation was observed in sprayed plots than in unsprayed plots at harvests I (0.05 vs. 0.19), II (0.41 vs. 0.53), and V (0.04 vs. 0.31) (Fig. 2). At harvest III, the proportions of defoliation were similar and low (0.02 vs. 0.06). No defoliation was observed before harvest IV.

Disease severity at harvest was generally greater in 1986 than in 1987. There was a significant interaction between fungicide treatment and harvest for final disease severity in both years (Table 3). Disease severity was greater in unsprayed plots than in sprayed plots (except at harvest IV in 1987) (Fig. 2), but the

Table 2. Mean monthly temperature and rainfall and departure from 30-yr mean values during the 1986 and 1987 alfalfa growing season[x]

		Temperature (C)		Rain (cm)	
Month	Days of year	Mean	Departure	Total	Departure
1986					
March	60–90	11.7	+2.1	5.5	− 5.1
April	91–120	17.1	+1.8	2.4	− 6.2
May	121–151	21.1	+1.5	6.2	− 5.1
June	152–181	25.9	+2.6	5.4	− 4.6
July	182–212	28.2	+2.8	10.0	− 2.8
August	213–243	25.2	+0.2	30.5	+18.8
September	244–273	22.1	+0.4	1.7	− 8.0
1987					
March	60–90	10.2	+0.6	8.3	− 2.4
April	91–120	14.2	−1.1	13.0	+ 4.4
May	121–151	21.2	+1.6	3.3	− 8.0
June	152–181	25.2	+1.9	3.1	− 6.9
July	182–212	27.1	+1.7	11.8	− 1.0
August	213–243	26.6	+1.6	6.4	− 5.2
September	244–273	22.9	+1.3	18.3	+ 8.6

[x] Data for NOAA reporting station Raleigh 4SW, located approximately 5 km from study site.

Table 3. Effect of fungicide (chlorothalonil) treatment (F) and harvest (H) on final disease severity, defoliation, dry matter yield, and three measures of forage quality---acid detergent fiber (ADF), total digestible nutrients (TDN), and adjusted crude protein (ACP)

		Disease (%)[t]		Defoliation (%)		Yield (g/m^2)		ADF		TDN		ACP	
Year	Source	df	MS	df	MS	df	MS	df	MS	df	MS	df	MS
1986	F	1	32.52	1	31.51	1	34,375	1	12.3	1	5.4[u]	1	0.1
	Rep (F)	...	...[v]	22	15.50	22	57,930	10	11.3	...[v]	...	...[v]	...
	H	3[w]	5.20	3[w]	125.68**[x]	4	99,431	2	214.7	2	91.6	2	27.0
	F × H	3	0.63**	3	...[v]	4	2,360*	2	25.6	2	11.2	2	9.1*
	Residual	88	0.14	66	10.19	88	796	20	8.1	30	3.9	30	2.8
	Total	95		95		119		35		35		35	
	CV (%)		14.8		20.1		17.1		9.3		3.0		8.8
1987	F	1	13.82	1	1,053.38	1	84,960	1	41.5	1	17.7	1	32.3**[y]
	Rep (F)	...	...[v]	...[w]	...[v]	22	2,808	10	9.7	10	4.2	...[v]	...
	H	4	2.59	3[w]	1,710.28	4	145,123	4	63.0	4	26.8	4	117.8**[z]
	F × H	4	1.97**	3	140.71**	4	7,407**	4	11.6	4	4.9	...	...[v]
	Residual	110	0.03	88	12.85	88	500	40	7.0	40	3.0	54	2.7
	Total	119		95		119		59		59		59	
	CV (%)		9.3		35.6		12.0		14.3		2.6		2.6

[t] Values were square-root transformed before analysis.
[u] Appropriate divisor for F test was mean square for F × H.
[v] Variation due to this effect was not significant at the 25% level. Therefore, the sum of squares was pooled with the residual sum of squares to estimate error variance.
[w] Harvests II and IV were excluded from the analysis because values for the most observations (and, therefore, their error variances) were unusually low.
[x] * = Probability of a greater $F \leq 0.05$, ** = probability of a greater $F \leq 0.01$.
[y] Appropriate divisor for F test was mean square for Rep (F).
[z] Appropriate divisor for F test was residual mean square.

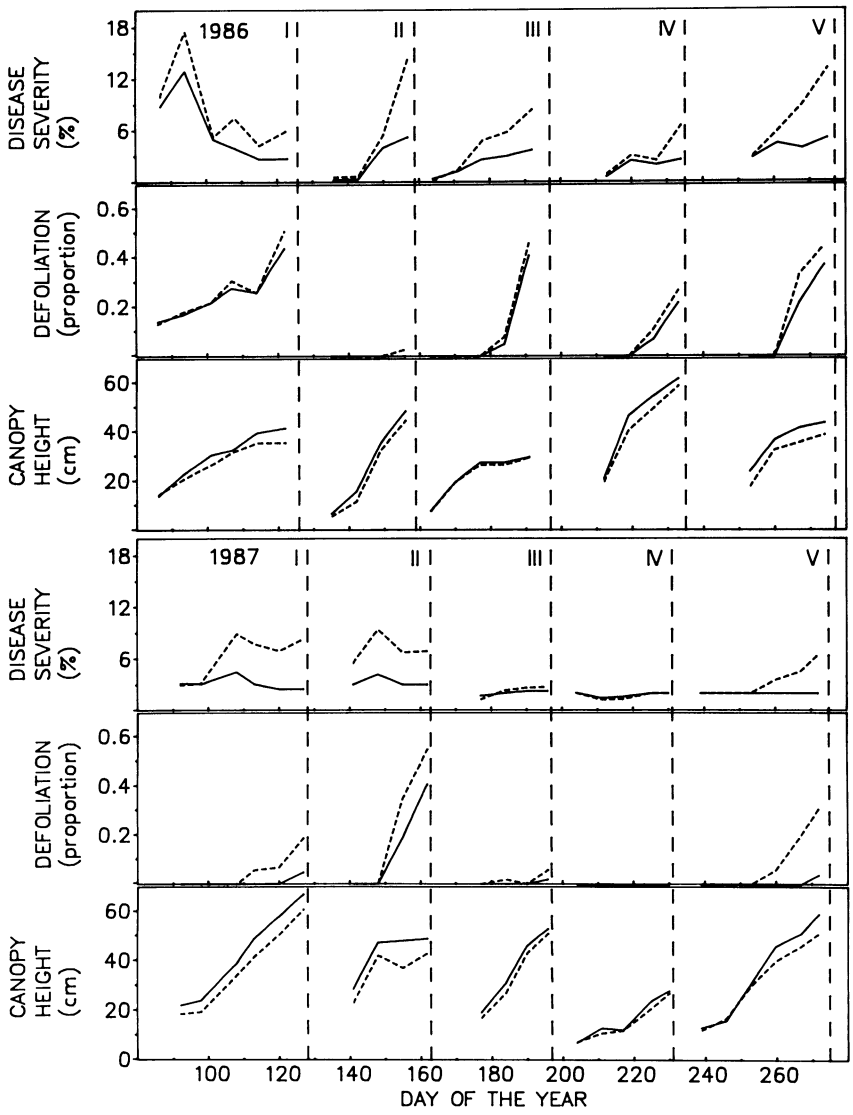

Fig. 2. Disease severity, defoliation (centimeters from soil surface to first intact leaf/centimeters of canopy height), and canopy height during epidemics of alfalfa leaf spot diseases in 1986 and 1987. Roman numerals indicate growth periods or harvest. Broken lines show data obtained from plots sprayed weekly with chlorothalonil (1.3 kg a.i./ha); solid lines show data obtained from unsprayed plots.

Table 4. Mean alfalfa yield in chlorothalonil-sprayed and unsprayed plots, yield loss, and relative contribution of yield loss at each harvest to annual total yield loss due to leaf spot diseases in 1986 and 1987

Harvest	Mean yield in sprayed plots (Mg/ha)	Mean yield in unsprayed plots (Mg/ha)	Yield loss at this harvest (%)[y]	Yield loss as percent of annual yield[z]
1986				
I	1.59	1.28	19.5	3.4
II	2.47	2.34	5.3	1.4
III	1.34	1.13	15.7	2.3
IV	2.56	1.91	25.4	7.2
V	1.12	0.73	34.8	4.3
Total	9.08	7.39	...	18.6
1987				
I	3.27	2.42	26.0	8.0
II	2.42	1.52	37.2	8.5
III	2.02	1.76	12.9	2.4
IV	0.72	0.61	15.3	1.0
V	2.21	1.69	23.5	4.9
Total	10.64	8.00	...	24.8

[y] Calculated for each harvest as: 100 × [(sprayed plot yield − unsprayed plot yield)/sprayed plot yield].
[z] Calculated for each harvest as: 100 × [(sprayed plot yield − unsprayed plot yield)/total annual yield in sprayed plots].

magnitude of the difference varied among harvests. Final disease severity was less than 5% in sprayed plots at all harvests.

Yield quantity and quality. At each harvest, less dry matter was obtained from unsprayed plots than from plots sprayed with chlorothalonil (Table 4). The fungicide treatment × harvest interaction was significant in both years (Table 3). In 1986, yield was 5.3–34.8% less in unsprayed plots than in sprayed plots over the five harvests; in 1987, yield was 12.9–37.2% less (Table 4). Loss (difference between yield in sprayed and unsprayed plots) at each harvest, expressed as a percentage of total annual yield, depended on the contribution of a specific harvest to annual yield. Loss varied from 1.4 to 7.2% in 1986 and from 1.0 to 8.5% in 1987 (Table 4). Total annual dry matter loss was 18.6% in 1986 and 24.8% in 1987.

The percentage of total digestible nutrients and acid detergent fiber in harvested alfalfa did not differ with respect to fungicide treatment or harvest in either year (Table 3). The percentage of mean adjusted crude protein did not differ with respect to fungicide treatment or harvest in 1986. Averaged across fungicide treatment, adjusted crude protein measured 25.8, 21.2, 20.8, and 23.0% at harvests II, III, IV, and V, respectively; no quality analysis was performed at harvest I. In 1987, adjusted crude protein differed between fungicide-sprayed and unsprayed plots and among harvests (Table 3). Mean adjusted crude protein over all harvests was 22.1% in unsprayed plots and 20.6% in sprayed plots. Averaged over fungicide treatments, mean adusted crude protein was 22.8, 17.5, 19.8, 25.9, and 20.8% at harvests I, II, III, IV, and V, respectively.

DISCUSSION

The alfalfa leaf spot disease mixture in 1986 and 1987 was similar to that reported by Von Chong and Campbell (16). The growth periods before harvests I and II each year were dominated by leaf spots caused by *L. briosiana*. The period before harvest III was transitional with regard to pathogen species composition. The two growth periods preceding harvests IV and V were characterized by leaf spots caused primarily by a mixture of *C. medicaginis* and *L. briosiana*. At harvests when *P. medicaginis* was observed, its incidence was generally lower in sprayed than in unsprayed plants.

Loss as a percentage of annual yield totaled 18.6% (1.69 Mg/ha) in 1986 and 24.8% (2.64 Mg/ha) in 1987. This percentage loss is similar to that reported from California (15), Illinois (4), Iowa (11), Kansas (19), and Minnesota (17,18). Rainfall was lower and temperatures were higher than average, however, during most months of the study (Table 2). These conditions were not conducive

to optimum growth of alfalfa in North Carolina or to the development of severe epidemics of leaf spot diseases. Disease was generally more severe in 1986 than in 1987, with the greatest overall amount of disease observed early in the period before harvest I in 1986 (Fig. 2). The yield losses estimated during our study are representative of years with weather conditions similar to those we encountered, but such estimates may be conservative for years in which temperatures and precipitation are nearer the 30-yr mean and in which more disease could be expected to develop.

Leaf spots caused by *Pseudopeziza medicaginis* can reduce forage quality, particularly with regard to protein content (10,13), although this reduction is not observed universally (15). Diseases caused by other leaf spot pathogens generally are not reported to affect quality of harvested forage (7,19), although quality reductions can occur at high (>80%) disease levels (8). In our study, no changes in quality of harvested alfalfa were observed in 1986. In 1987, however, mean adjusted crude protein over all harvests was slightly higher in unsprayed plots (22.1%) than in fungicide-sprayed plots (20.6%). This apparent decrease in forage quality in sprayed plots may be due to lower levels of defoliation.

When considering yield loss caused by leaf spot diseases in alfalfa, the overall annual loss is an important and useful measure. Because the yield is accumulated over multiple harvests, however, the contribution of an individual harvest to annual yield and that harvest's contribution to the annual loss should be examined. Yield loss percentages from individual harvests can provide valuable information about the role of specific leaf spot diseases or disease mixtures in reducing yield. However, using only the loss figures from individual harvests may give a distorted picture of yield loss. For example, losses at harvest IV and V in 1986 were 25.4 and 34.8%, respectively, which seems to indicate a higher loss at harvest V. When these losses were corrected for the contribution that each harvest made to the annual yield, however, the losses as a percent of annual yield at harvests IV and V were 7.2 and 4.3%, respectively. The greater actual loss in megagrams per hectare was at harvest IV.

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