Effects of Plant Debris on Intensity of Leaf Spot Diseases, Incidence of Pathogens, and Growth of Alfalfa

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ABSTRACT

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When leaf spot diseases are severe, early harvest of alfalfa (*Medicago sativa*) has been recommended to preempt defoliation, preserve yield and quality, and prevent accumulation of pathogen-infested leaf debris. Effects of debris on incidence of all leaf spots taken together (percentage of leaves with lesions caused by species of *Leptosphaerulina*, *Stemphylium*, *Phoma*, and/or *Cercospora*), frequency of leaf spot pathogens (percentage of diseased leaflets infected by each pathogen), and yield were evaluated during five periods of alfalfa growth in each of 2 yr among untreated plots (10.0 × 4.1 m) and plots in which naturally infested debris was either removed or added immediately after each harvest. Effects of treatments on disease incidence and on frequencies of leaf spot pathogens were small or absent during most periods of alfalfa growth. Mean disease incidences in debris-removed, untreated, and debris-added plots were 17, 22, and 23%, respectively, in 1987, and 30, 32, and 33%, respectively, in 1988. Frequency of *Leptosphaerulina* sp. on diseased leaflets, but not other leaf spot pathogens, was increased significantly by adding debris and was reduced significantly by removing debris in each year. Environment had a much greater effect on disease incidence and pathogen frequency than levels of debris. Under moist conditions, disease incidence increased rapidly. There was no evidence of a decrease in yield due to increased levels of debris and disease in any period of alfalfa growth. During hot and dry periods in both years, dry-matter yield was reduced by removing debris, apparently because soil moisture was reduced. Thus, under these or similar growing conditions, early harvest may not be a useful method of reducing yield losses caused by leaf spot diseases.

Leptosphaerulina trifolii (Rost.) Petr., *Phoma medicaginis* Malb. & Roum., *Stemphylium botryosum* Wallr. (teleomorph *Pleospora herbarum* (Pers. ex Fr.) Rab.), and *Cercospora medicaginis* Ell. & Ev. cause leaf spot diseases of alfalfa (*Medicago sativa* L.) in North Carolina (9,35–37,39). Methods available to control these diseases are limited. Alfalfa cultivars that are highly resistant to leaf spot diseases are not available, although severity of the diseases varies among cultivars under field conditions (36). Foliar fungicides currently are not registered for use on alfalfa for forage in the United States.

Leaf spot diseases increase defoliation of alfalfa and reduce yield and quality of forage (5–9,15,16,20,23,33,37,40–42). More diseased leaves may be lost during cutting, drying, and bailing than before harvest (33). In North Carolina, harvest at the 10% bloom stage of crop development generally is recommended (24). Early harvest, for example at the bud stage, may preempt leaf loss when leaf spot diseases are severe (3,13,14,25). In addition, because infested leaf debris on the surface of soil may serve as a reservoir of leaf spot pathogens, early harvest may reduce debris-borne inoculum and the intensity of leaf spot diseases in subsequent periods of alfalfa growth (14,18,25).

Effects of infested debris on intensity of leaf spot diseases have not been evaluated critically. Renfro and Sprague (28) spread naturally infested debris on the surface of soil to initiate epidemics in field experiments, but they did not compare levels of disease among plots with high and low levels of debris. In North Carolina, level of debris was correlated positively with intensity of leaf spot

diseases in the field (37,38). However, high levels of debris may have been the consequence, rather than the cause, of high levels of disease. Von Chong (38) found no evidence of a consistent relationship between levels of debris or defoliation and density of airborne spores of *Leptosphaerulina* sp. or *Stemphylium* sp. Although leaf spot diseases reduce yield, growth of alfalfa, measured by height of the canopy, was correlated positively with both level of debris and severity of disease during some periods of alfalfa growth (37,38). Thus, relationships among debris, leaf spot diseases, and growth of alfalfa remain unclear. The objective of this study was to evaluate the effect of plant debris on intensity of leaf spot diseases, incidences of leaf spot pathogens, and growth of alfalfa.

MATERIALS AND METHODS

Experimental design. In August 1986, a seed bed was prepared in a field of 52 × 62 m near Raleigh, NC. Ground limestone was incorporated into the Cecil clay soil at a rate of 1,800 kg/ha. Phosphorous, potassium, and boron then were applied as a topdressing at 22, 65, and 3.5 kg/ha, respectively. Five areas of 50.0 × 12.3 m were marked within the field. Each area was divided along its length into three strips (50.0 × 4.1 m). The alfalfa cultivars Raidor, Dekalb 120, and Florida 77 were assigned randomly to the strips in each area. On 15 September 1986, seed was inoculated with *Rhizobium* sp. and was sown in the strips using a seed drill. Strips consisted of 24 rows spaced 0.17 m apart. All cultivars were seeded at a rate of 40 kg/ha and at a depth of 20 mm.

Carbofuran (Furadan 4F, Mobay Chemical Corp., Kansas City, MO) was applied at a rate of 0.57 kg/ha on 19 March 1987 and on 23 March 1988 to control alfalfa weevil (*Hypera postica* Gyllenhal). Phosphorous, potassium, and boron were applied as a topdressing at rates of 17, 51, and 3.4 kg/ha, respectively, on 25 February 1988. Metribuzin (Sencor 4F, Mobay Chemical Corp.) was applied at a rate of 0.57 kg/ha on 9 March 1988 to control winter annual weeds.

Only strips of cultivar Raidor were used in this experiment. On 4 May 1987, each strip was divided across its length into five plots (10.0 × 4.1 m). An area 1.36 m (8 rows) wide at the center of each plot was divided into subplots. In 1987, the area was 4.0 m long and was divided into eight subplots. In 1988, the area was 6.0 m long and was divided into 12 subplots (Fig. 1A). In both years, each subplot (1.00 × 0.68 m) consisted of four sub-subplots (Fig. 1B). A sub-subplot was centered on a row and measured 1.00 × 0.17 m.

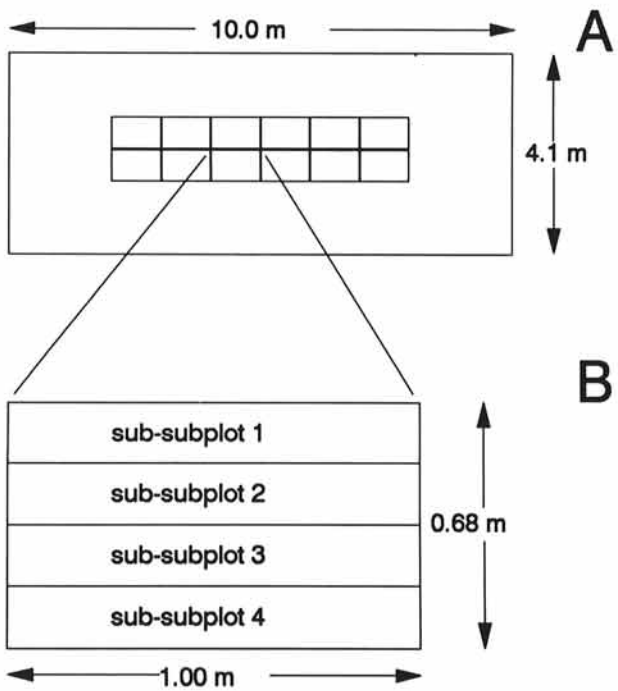

Fig. 1. A, Position of 12 subplots (1.00 × 0.68 m) within each plot in 1988; and **B,** position of four sub-subplots (1.00 × 0.17 m) within each subplot.

Five treatments were assigned randomly to the plots in each area initially and maintained for the two growing seasons. In one treatment, organic debris on the surface of the soil was removed with a gasoline-powered leaf blower-vacuum. Debris consisted primarily of abscised leaves. Debris from the five replicate plots was collected, mixed thoroughly, and divided into five portions of roughly equal volume. One portion was distributed evenly over the surface of the soil in each plot assigned to the second treatment. Debris treatments were imposed within 3–5 days after each harvest and, therefore, five times in each year. A third treatment consisted of untreated plots with natural levels of debris. In plots assigned to the fourth and fifth treatments, chlorothalonil (Bravo 500, Fermenta ACS Corp., Mentor, OH) was applied weekly during the first 2 wk of each growth period or during the entire growth period. Only the first three treatments are considered and reported in this paper.

In 1987, harvests were made on days of the year 128 (8 May), 161 (10 June), 195 (14 July), 229 (17 August), and 272 (29 September). In 1988, harvests were made on days of the year 133 (12 May), 167 (15 June), 201 (19 July), 232 (19 August), and 271 (27 September). In each year, harvests were numbered I to V, and periods of alfalfa growth were numbered I to VI. Periods I to V preceded harvest I to V, respectively. Period VI followed harvest V. The crop was cut when plants reached the early (1–10%) bloom stage, except at harvest IV in 1987 when plants were cut at the early bud stage.

Collection of data. Amount of debris on the surface of soil, level of defoliation, height of alfalfa shoots, and intensity of all leaf spot diseases taken together were monitored during periods II to VI in 1987 and periods I to V in 1988. During each period, sampling began when new leaves appeared after harvest and was repeated at 2- to 4-day intervals. On each sampling date, measurements of each variable were made in every sub-subplot in each of two subplots selected randomly from each plot.

Debris was measured as the percentage of the area of the soil surface that was covered by plant debris (debris cover). Rating scores from 0 to 11 corresponded to percentages of 0, 1.5, 4.5, 9.0, 18.5, 37.5, 62.5, 81.5, 91.0, 95.5, 98.5, and 100, respectively. Defoliation was measured as the number of leaves per main stem that had been abscised, averaged visually over all main stems. A main stem was the central stem of a shoot that originated at or near the crown. Height of canopy was measured as the distance from the surface of the soil to the top of the canopy. Incidence of disease was measured as the percentage of leaves in the canopy with symptoms of leaf spot. Incidence was measured with a rating scale with scores from 0 to 10 corresponding to percentages of 0, 1, 5, 10, 25, 50, 75, 90, 95, 99, and 100, respectively.

The number of main stems per unit area (stand density) was assessed once during each period. Counts were made approximately 20 days before harvest in one sub-subplot in each of two subplots in each plot. Subplots and sub-subplots were selected randomly. Dry matter yield was measured at harvests II to V in 1987 and at each harvest in 1988. Alfalfa in all subplots in each plot was cut at a height of approximately 7 cm, placed in a cotton bag, dried at 50 C for 24–72 hr, and then weighed. Alfalfa in the remainder of the field was harvested with a flail-chop harvester (Carter Mfg. Co., Brookston, IN) and discarded.

Frequency of leaf spot pathogens was assessed twice during each period of alfalfa growth. Plots were sampled after harvest, when lesions first appeared on new leaves, and again immediately before harvest. On each sampling date, diseased leaflets were selected arbitrarily from within the subplots at the center of each plot. In 1987 and 1988, eight and 16 leaflets, respectively, per plot were sampled. Leaflets were transported on ice, stored for up to 48 h at 5 C, surface disinfested, and placed with abaxial surfaces down on moistened filter paper in petri dishes. For surface

disinfestation, leaflets were dipped in 75% ethanol for 30 sec, immersed in 0.53% NaOCl for 1 min, and rinsed in four changes of tap water.

Petri dishes, each containing eight leaflets, were sealed with Parafilm and incubated for 5–7 days at room temperature (approximately 22 C) under cool-white fluorescent lights (65 μmol $m^{-2}s^{-1}$) with a day length of 14 h. Each leaflet was examined under a stereo dissecting microscope at ×40 magnification. Fungal leaf spot pathogens sporulating on the adaxial surfaces of leaflets were identified. Reproductive structures were transferred periodically to microscope slides and examined at ×100 magnification to confirm identifications. For each plot, the frequency of a pathogen was calculated as the percentage of leaflets on which it occurred.

Air temperature, relative humidity, rain, leaf wetness, and soil moisture were monitored with electrical sensors located near the center of the experimental field. Output of sensors was recorded using microprocessor-based data loggers (model CR21, Campbell Scientific, Inc., Logan UT). Air temperature and relative humidity were sampled once each hour using a relative humidity probe (model 201, Campbell Scientific), which comprises a thermistor and a sulphonated polystyrene humidity sensor. The probe was aspirated for 7 min before sampling and was shielded from radiation and rain with an apparatus described by Davis et al (10). Two probes were deployed within the canopy 6 m apart at a height of 15 cm above the soil surface. Total daily rain was measured with a tipping-bucket rain gauge (model RG2501, Campbell Scientific). The duration of leaf wetness within the canopy was recorded each hour using three electrical impedance grid sensors (model CEL-WFD, Campbell Scientific) deployed 10 m apart and at a height of 15 cm. The surface of each sensor was coated with green latex paint (12). Output of sensors at the wet-dry transition point was determined in the field by visual inspection of leaves as dew evaporated.

During periods III, IV, and V in 1988, soil moisture also was measured with gypsum block electrical resistance sensors (model 5201, Soilmoisture Equipment Corp., Santa Barbara, CA) buried to a depth of 20 cm at the center of each plot. Sensor output was sampled at 2- to 4-day intervals. Six sensors were calibrated with a pressure plate apparatus (model 1500, Soilmoisture Equipment). Sensor output in soil removed from experimental plots was sampled at soil matric potentials of 0, −10, −30, −50, −90, −110, −130, −150, −190, −250, and −300 kPa. A calibration curve for each sensor was determined by polynomial regression. Curves for the six sensors were similar, and therefore a single curve was developed to calibrate all sensors.

Analysis of data. In each year, debris cover, disease incidence, defoliation, canopy height, yield, stand density, soil moisture, and frequencies of leaf spot pathogens each were compared among treatments and among periods of alfalfa growth by profile analysis (17), a form of multivariate analysis of variance. Effects of treatments but not periods were assumed to be independent.

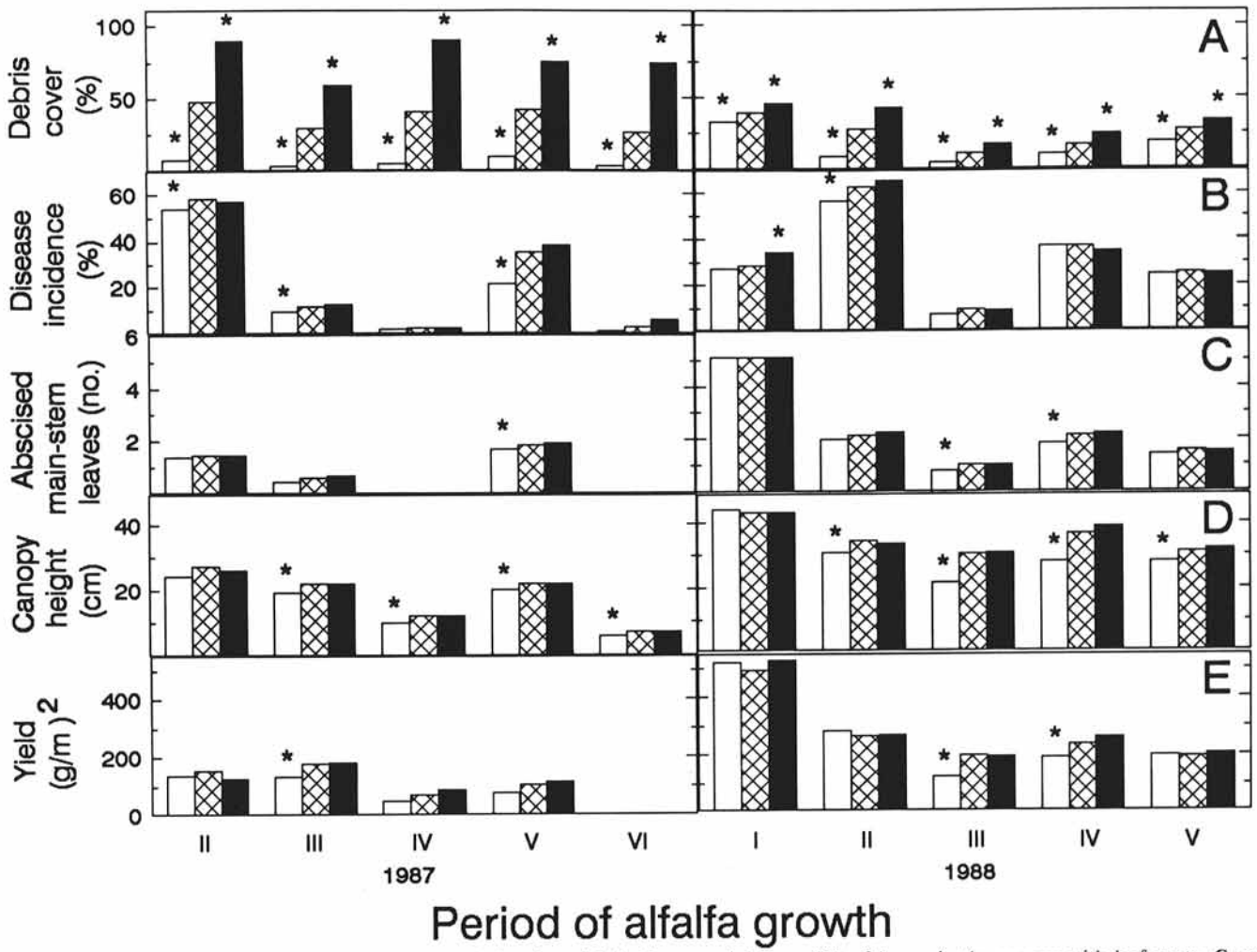

Fig. 2. A, Mean of area of the soil surface (%) covered by plant debris, **B,** mean incidence (%) of leaves in the canopy with leaf spots, **C,** mean number of abscised main-stem leaves, **D,** mean height (cm) of the alfalfa canopy, and **E,** mean dry-matter yield (g/m^2) for each of 10 periods of alfalfa (cultivar Raidor) growth in 1987 and 1988. Treatments included removal of debris from plots (empty bars), control plots with natural levels of debris (crosshatched bars), and addition of debris to plots (solid bars). Roman numerals indicate successive periods within each year. Profile analyses indicated that a significant interaction between period of alfalfa growth and each response variable was present in each year. Therefore, means for debris-removed and debris-added plots were each compared with means for control plots within each period. An asterisk indicates that the treatment mean differed significantly ($P \leq 0.05$) from the mean for untreated plots.

Effects of treatments in each period of growth were evaluated if the interaction between treatments and periods was significant ($P \leq 0.05$). If the interaction was not significant, then mean effects of treatments over all periods in each year were compared. In both cases, effects of removing or adding debris were assessed by comparing means for debris-removed or debris-added plots, respectively, to the mean for untreated plots using single df linear contrasts ($P \leq 0.05$).

During each period of growth, effects of treatments on debris cover, disease incidence, defoliation, and canopy height were summarized by the area under the curve, calculated for each variable by the trapezoidal rule (32), and by the mean of each variable calculated over all sampling dates within each period. Rating scores for debris cover and disease incidence were converted to percent values before calculation of area under the curve and variable means. Results from the two methods were similar. Only results for growth period means are presented because units are more readily interpreted.

In each analysis, residuals were plotted against predicted values. For incidences of leaf spot pathogens, residual plots indicated that variances were heterogeneous. Therefore, these data were arcsine transformed (21). For the remaining variables, distinct patterns were absent in residuals plots, and data were not transformed. All data were analyzed with the Statistical Analysis System (31).

RESULTS

Effects of treatments on debris cover, disease incidence, defoliation, canopy height, and yield (Figs. 2A–2E, respectively) varied significantly among periods of alfalfa growth in each year. Debris cover (Fig. 2A) in untreated plots was always significantly greater than in debris-removed plots and was always significantly lower than in debris-added plots. Debris cover tended to be greater in 1987 than in 1988.

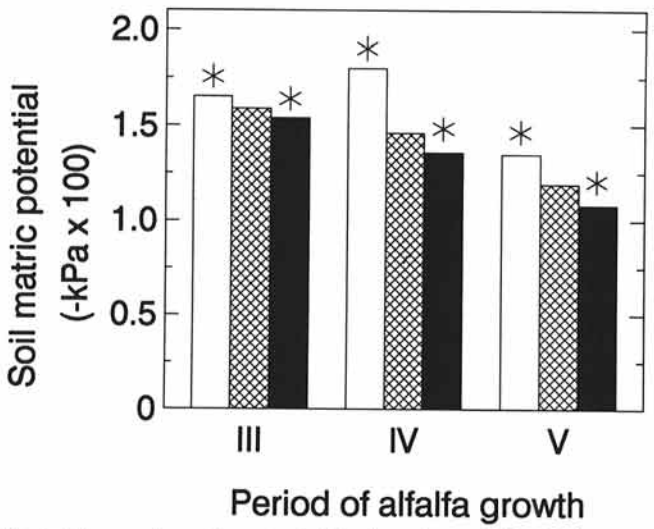

Fig. 3. Mean soil matric potential in plots from which debris on the surface of soil was removed (empty bars), in control plots with natural levels of debris (crosshatched bars), and in plots to which debris was added (solid bars) during three periods of alfalfa growth in 1988. An asterisk indicates that the treatment mean differed significantly ($P = 0.05$) from the mean for untreated plots.

Disease incidence, defoliation, canopy height, and yield varied considerably among periods of alfalfa growth; however, differences among treatments were usually small or absent. Over all sampling dates, mean disease incidence in debris-removed, untreated, and debris-added plots was 17, 22, and 23%, respectively, in 1987 and 30, 32, and 33%, respectively, in 1988. Disease incidence was greatest during period II in both 1987 and 1988 (Fig. 2B). In each of these periods, incidence was reduced significantly by removing debris, but the effect was small. There was no evidence of an effect of adding debris. Levels of disease were intermediate during period V in 1987 and periods I, IV, and V in 1988. Incidence was reduced significantly by removing debris in only one of these periods (period V in 1987). Incidence was increased significantly by adding debris in only one of these periods (period I in 1988). During the remaining periods, incidence was low, and no differences among treatments were detected. Weather, particularly moisture, appeared to have a large effect on incidence. During periods with high and low incidence, duration of leaf wetness and duration of relative humidity greater than 90% (Table 1) tended to be high and low, respectively.

There was no evidence that defoliation (number of abscised main stem leaves) differed consistently between debris-added and untreated plots during any period (Fig. 2C). Defoliation was significantly lower in debris-removed plots than in untreated plots only during period V in 1987 and periods III and V in 1988. In one of these periods (period IV in 1988), the difference in defoliation was significant even though incidence of diseased leaves was similar. No defoliation was observed during periods IV and VI in 1987.

Growth of alfalfa was reduced by removing debris but was unaffected by adding debris. In debris-removed plots, canopy height was reduced significantly during each period except period II in 1987 and period I in 1988 (Fig. 2D), and yields were reduced significantly at harvests after period III in 1987 and periods III and IV in 1988 (Fig. 2E). Effects of removing debris were greatest when soil moisture was low and air temperature was high. There was no evidence of an effect of adding debris on canopy height or yield during any period. In debris-added and untreated plots, mean canopy height was 19.2 and 19.0 cm, respectively, in 1987, and 35.7 and 35.4 cm, respectively, in 1988. Mean yield for debris-added and untreated plots was 127 and 126 g/m^2, respectively, in 1987 and 287 and 271 g/m^2, respectively, in 1988. Soil moisture was significantly lower in debris-removed plots than in untreated plots and was significantly greater in debris-added plots than in untreated plots during periods III, IV, and V in 1988 (Fig. 3).

Density of the shoots varied significantly among periods of alfalfa growth, but there was no evidence of an effect of treatments. Mean stand density was 507, 614, 401, 361, and 381 shoots/m^2 during periods II to VI, respectively, in 1987, and 359, 519, 408, 362, and 351 during periods I to V, respectively, in 1988. Stand density in debris-removed, untreated, and debris-added plots was 476, 446, and 435 shoots/m^2, respectively, in 1987, and 423, 405, and 371 shoots/m^2, respectively, in 1988.

Frequency of each pathogen on diseased leaflets varied significantly among periods of alfalfa growth (Fig. 4) and among treatments (Fig. 5), but there was no evidence of an interaction. In both years, the most frequent leaf spot pathogen was *L. trifolii*. It was detected during all periods of alfalfa growth and was observed on more than 50% of diseased leaflets during each period

TABLE 1. Moisture variables and air temperature during periods of growth of alfalfa (cultivar Raidor) in 1987 and 1988

	Period of alfalfa growth, 1987					Period of alfalfa growth, 1988				
	II	III	IV	V	VI	I	II	III	IV	V
Total rainfall (mm)	47	99	50	115	79	80	57	50	104	112
Mean air temperature (C)	22	25	27	22	12	15	21	27	27	22
Mean soil matric potential (−kPA × 100)	0.5	1.3	1.2	1.2	1.1	0.4	1.0	1.6	1.8	12.5
Mean duration of leaf wetness (hr/day)	9.5	6.2	4.4	7.6	11.3	8.3	10.6	5.8	8.1	7.9
Mean duration of RH > 90% (hr/day)	13.4	11.9	7.0	10.5	12.0	14.9	11.8	7.2	13.6	18.3

except periods III, IV, and V in 1988. In both years, frequency of *L. trifolii* on diseased leaflets decreased from period II to period V. *S. botryosum* occurred during all periods except period V in 1987 and period V in 1988. In 1987, *P. medicaginis* was detected rarely except during period V. In 1988, *P. medicaginis* was most frequent on diseased leaflets during period III when few leaves were diseased (Fig. 1B). In each year, *C. medicaginis* was most common during period V. In 1987, it was rare or absent during other periods. In 1988, frequency of *C. medicaginis* on diseased leaflets increased from periods I to V.

Over all periods, effects of treatments on frequency of leaf spot pathogens on diseased leaflets were small (Fig. 5). Frequency of *L. trifolii* on diseased leaflets was reduced significantly by removing debris in both years but was increased significantly by adding debris only in 1988. There was no evidence that frequencies of other pathogens were affected by adding debris. Frequency of *S. botryosum* on diseased leaflets differed among treatments by less than 2% in each year. In 1988, frequency of *P. medicaginis* on diseased leaflets was significantly greater in debris-removed plots than in untreated plots. In 1987, *C. medicaginis* was detected on fewer diseased leaflets in debris-removed plots than in untreated plots, but its frequency did not differ among treatments in 1988.

DISCUSSION

Effects of pathogen-infested plant debris on development of epidemics of leaf spot diseases of alfalfa were much smaller than effects of environment in this study. Although statistically significant effects of debris on incidence of leaf spot diseases and on frequency of pathogens could be detected during some periods of alfalfa growth, incidence was high under moist conditions, even when levels of debris were low, and incidence was low under dry conditions, even when levels of debris were high. Thus, weather appeared to have an overwhelming effect on development of epidemics. These results are consistent with previous reports of effects of environment on development of leaf spot diseases. Large differences, in intensity of leaf spot diseases and in frequency of leaf spot pathogens, among periods of alfalfa growth are common in North Carolina (9,36,37) and elsewhere (3,8,16,29,30,42). Disease intensity increases rapidly under favorable weather conditions (9,29,30,37).

Several factors may account for the absence of a large effect of debris on intensity of leaf spot diseases, although such factors were not investigated. First, debris-borne inoculum may have little effect on development of leaf spot diseases if the efficacy of inoculum of individual leaf spot pathogens is limited by weather. Effects of moisture, temperature, and light intensity on virulence of leaf spot pathogens, lesion formation, and efficacy of inoculum under controlled conditions have been well documented (1,2,4,11,19, 22,27,34). Second, weather may have a greater influence on levels of inoculum than the amount of pathogen-infested debris per

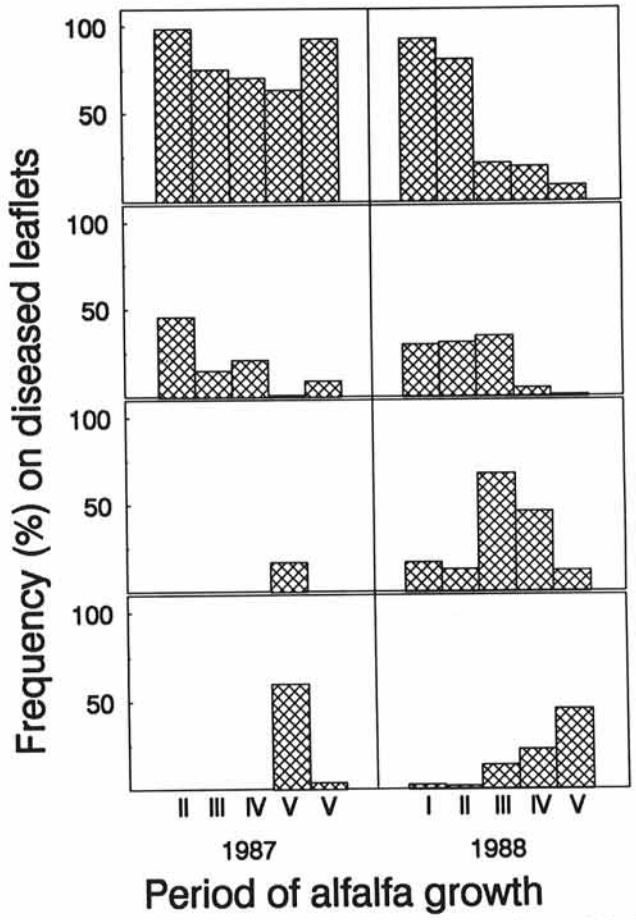

Fig. 4. Mean frequency (%) of: **A,** *Leptosphaerulina trifolii*, **B,** *Stemphylium botryosum*, **C,** *Phoma medicaginis*, and *Cercospora medicaginis* on diseased leaflets during each of 10 periods of alfalfa (cultivar Raidor) growth in 1987 and 1988. Roman numerals indicate successive periods in each year.

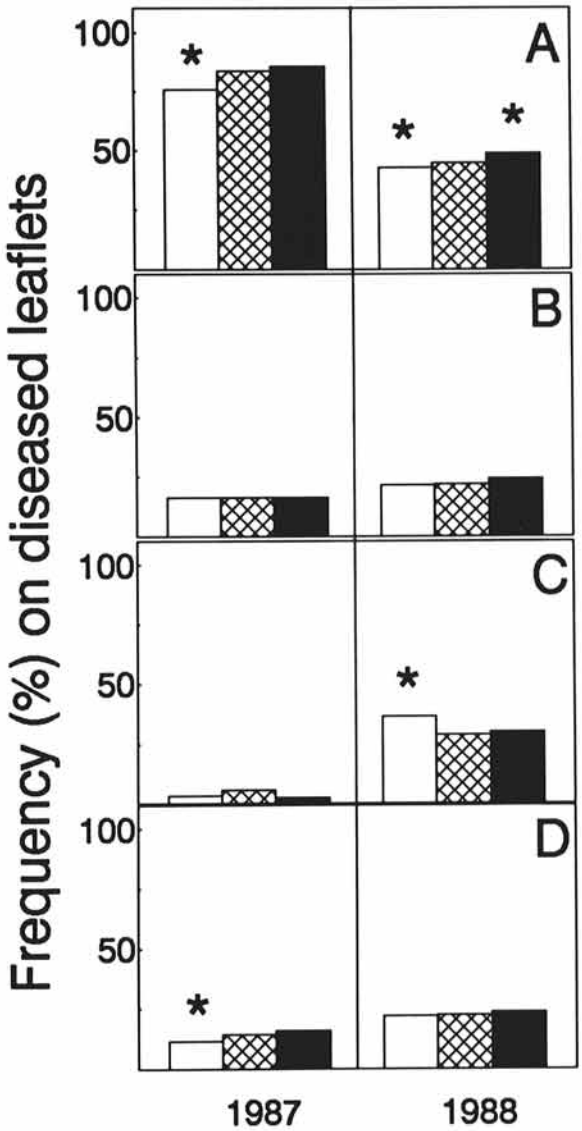

Fig. 5. Mean frequency (%) of: **A,** *Leptosphaerulina trifolii*, **B,** *Stemphylium botryosum*, **C,** *Phoma medicaginis*, and **D,** *Cercospora medicaginis* on diseased leaflets in plots of alfalfa (cultivar Raidor) from which plant debris was removed (empty bars), in control plots with natural levels of debris (crosshatched bars), and in plots to which debris was added (solid bars) during 1987 and 1988. An asterisk indicates that the treatment mean differed significantly ($P \leq 0.05$) from the mean for untreated plots.

se. For example, ascospores of *Leptosphaerulina* sp. are ejected from pseudothecia in dead leaves only when leaves are wet (11). Third, in addition to debris, reservoirs of inoculum include stubble that is not removed at harvest or alfalfa and other host plants in nearby fields. *L. trifolii*, *S. botryosum*, *P. medicaginis*, and *C. medicaginis* are readily isolated from stem and crown tissues of alfalfa and are able to infect a variety of legume species (2,14,28). Fourth, secondary inoculum may arise from leaves infected during the current period of alfalfa growth. Latent period for leaf spot caused by *L. trifolii* is approximately 14 days (26), and secondary spread of *C. medicaginis* is rapid under favorable weather conditions (2). Thus, even though levels of primary inoculum of one or more leaf spot pathogens may be reduced by removing debris, the epidemiological importance of such a reduction apparently is small.

Effects of treatments on intensity of all leaf spot diseases taken together were investigated in this study although incidences of individual leaf spot pathogens were measured on diseased leaflets. A similar approach has been taken in previous studies (7-9,35,36,38). However, frequency of leaf spot pathogens on diseased leaflets may not reflect their abundance in the entire alfalfa canopy if the proportion of diseased leaflets fluctuates. In this study, the proportion of all leaves in the canopy infected by a pathogen could be estimated by assuming that incidence of the pathogen in the diseased leaflets was similar to its incidence on diseased leaves or that the incidence of diseased leaves was similar to the incidence of diseased leaflets. Thus, the abundance of a pathogen on all leaves was, approximately, the product of its incidence on diseased leaflets and the incidence of diseased leaves.

Because effect of pathogen-infested debris on intensity of leaf spot diseases was small, early harvest of alfalfa to reduce levels of pathogen-infested debris does not appear to be justified, at least under environmental conditions similar to those in this study. Furthermore, yield of alfalfa may be influenced directly by levels of debris on the surface of soil. Preliminary evidence suggested that soil moisture was reduced when debris was removed from plots and, therefore, growth and yield of alfalfa also were reduced. In this case, the mulching effect of debris apparently had a greater overall effect on yield than did disease.

When leaf spot diseases of alfalfa are severe, early harvest, for example at the bud stage, may preempt defoliation and reduce losses in forage yield and quality at the current harvest. In North Carolina, harvest at early bloom is recommended to maximize yield of high-quality components of forage and to maintain plant vigor (24). If disease increases rapidly during the early stages of vegetative growth and if harvest is delayed until the early bloom stage, defoliation, and therefore yield and quality loss, may be extensive. Further research is needed to evaluate relationships among foliar infections by leaf spot pathogens, defoliation both before and during harvest, stage of crop development, and yield and quality of alfalfa forage.

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