Assessment of Resistance to Leaf Spot Diseases Among Alfalfa Cultivars in North Carolina Fields

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ABSTRACT

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Experimental plots were sampled destructively and leaves on individual stems were evaluated visually for leaf spot severity to assess differences in disease level among 16 alfalfa cultivars. Samples were collected from sites in Rowan and Washington counties during 1983 and in Rowan, Sampson, Wake, and Washington counties during 1984. Disease severity was estimated for each leaf on a stem. Total length, length of defoliation up the stem, number of intact and defoliated nodes, and number of abscised main stem leaves were measured for each stem. Significant differences were detected among cultivars in disease severity, maximum disease on a stem, and percentage of nodes with abscised main stem leaves. A cultivar by location interaction was not detected. Florida 77 appeared to be more susceptible than most other cultivars, whereas Raidor had a higher level of resistance to leaf spot diseases than other cultivars. Stability analysis indicated that Raidor has the most stable disease resistance. High correlations ($r \geq 0.70$) were detected among several of the variables measured. Cluster analysis was used to group cultivars on the basis of disease severity and percentage of defoliated nodes.

Numerous fungi cause leaf spots on alfalfa (*Medicago sativa* L.) (4). The most common leaf-spotting pathogens in North America include *Leptosphaerulina briosiana* (Poll.) Graham & Luttrell, *Phoma medicaginis* Malbr. & Roum. var. *medicaginis* Boerema, *Pseudopeziza medicaginis* (Lib.) Sacc., *Stemphylium botryosum* Wallr., and *Cercospora medicaginis* Ell. & Ev. These pathogens reduce forage quality by causing the production of harmful metabolites (1,6,9) and by contributing to defoliation, which removes the most nutritious part of the forage—the leaf (5,9). Leaf spots also reduce yields through defoliation, reduced photosynthesis, decreased growth, and reduced stand vigor and longevity (8,12,15).

Some alfalfa cultivars contain a degree of resistance to specific leaf spots (11); however, information on leaf spot resistance in most cultivars is lacking. Effective resistance to leaf spot caused by *L. briosiana* is not reported for any commercial alfalfa cultivars (4), and information on the expression of leaf spot resistance in the field is limited.

The primary objective of this study was to evaluate differences in leaf spot severity and several other variables related to disease resistance among alfalfa cultivars. A further objective was to examine how these other variables are related to disease severity.

MATERIALS AND METHODS

Experimental design and sampling procedures. Sixteen alfalfa cultivars in the official forage cultivar trial conducted by J. P. Mueller and J. T. Green (Department of Crop Science, North Caroline State University) were evaluated in fields located in Rowan and Washington counties in North Carolina during the 1983 growing season and in Rowan, Washington, Wake, and Sampson counties during 1984 (Table 1). Evaluations were made in several growth cycles during each growing season (Table 2).

Presence of specific leaf spot pathogens was determined visually or by isolation from selected leaves. Visual observations were performed at ×70 magnification on leaves incubated in moist

TABLE 1. Alfalfa cultivars evaluated for leaf spot severity at four locations in North Carolina in 1983 and 1984

Cultivar	Source
Apollo	North America Plant Breeders
Arc	USDA
Cimarron	Great Plains
Classic	Farmers Forage Research
Florida 77	Pioneer
HiPhy	Farmers Forage Research
Raidor	Northrup-King
Shenandoah	Great Plains
Southern Special	Waterman-Loomis
Vancor	Northrup-King
Weevlchek	Farmers Forage Research
WL 311	Waterman-Loomis
WL 318	Waterman-Loomis
DeKalb 120	DeKalb Co.
DeKalb 130	DeKalb Co.
Pioneer 531	Pioneer Seed Co.

TABLE 2. Sampling dates for evaluation of leaf spot severity on 16 alfalfa cultivars in four counties in North Carolina

Location[z]	Planting date (day/mo/yr)	Sampling dates	
		Year	Day/Month
Washington	21/9/81	1983	5/7, 3/8
		1984	3/7, 3/8
Rowan	3/9/82	1983	2/6, 6/7, 4/8, 13/10
		1984	25/4, 31/5, 25/6, 30/7
Wake	31/8/83	1984	31/5, 27/6, 27/7
Sampson	7/9/83	1984	11/4, 20/7

[z] County in which field was located.

TABLE 3. Means of leaf spot or related variables measured from four stems from each of 16 alfalfa cultivars on 17 sample dates at four locations

County	Sampling date (day/mo/yr)	Leaf spot severity	Maximum severity per stem	Nodes with abscised main stem leaves (%)	Abscised nodes (%)	Defoliated length (%)
Washington	5/7/83	3.8	19.3	41.5	22.0	31.6
	3/8/83	0.6	1.7	8.5	7.9	20.9
	3/7/84	1.4	4.4	7.6	2.4	7.3
	3/8/84	1.8	6.8	25.7	15.3	30.5
Rowan	2/6/83	12.1	47.0	37.1	23.9	...[z]
	6/7/83	7.2	34.7	35.5	23.8	37.4
	4/8/83	3.0	10.0	14.5	8.3	26.0
	13/10/83	2.4	8.5	38.6	9.2	21.2
	25/4/84	2.5	10.7	34.9	15.4	17.4
	31/5/84	4.5	18.8	36.6	19.2	27.5
	25/6/84	3.2	12.2	22.6	13.4	22.1
	30/7/84	2.0	8.2	19.6	10.8	21.2
Wake	31/5/84	5.0	14.6	36.5	23.7	41.4
	27/6/84	1.7	7.9	18.7	11.5	25.1
	27/7/84	3.1	14.7	23.7	14.4	29.1
Sampson	11/4/84	0.9	4.9	31.2	19.5	19.1
	20/7/84	3.1	12.2	19.5	10.9	22.1

[z]Defoliated length was not measured for first sample (2/6/83).

TABLE 4. Leaf spot pathogens observed on alfalfa leaf samples from two North Carolina counties in 1983 and four North Carolina counties in 1984[z]

County	Date (day/mo/yr)	*Leptosphaerulina briosiana*	*Stemphylium botryosum*	*Cercospora medicaginis*
Washington	5/7/83	x	x	
	3/8/83	x	x	
	3/7/84			x
	3/8/84	x		x
Rowan	6/7/83	x	x	
	4/8/83	x		
	25/6/84	x		
	30/7/84	x		x
Wake	27/6/84	x	x	
	27/7/84	x		x
Sampson	20/7/84	x		x

[z]An x indicates the presence of the specific pathogen on sampled leaves as determined by isolation on agar media or observations of leaves incubated in moist chambers. Isolations were not made from samples taken before the dates indicated.

chambers for about 5 days at room temperature. Isolations were performed by collection of ejected ascospores of *L. briosiana* on acid water agar or by surface disinfestation (10% sodium hypochlorite, 1 min) and subsequent plating of diseased leaf tissue on acid water agar.

The experimental design for the field plots was a randomized complete block at each location, with one plot per cultivar in each block. Plots consisted of three parallel, 6.1-m rows planted on 23-cm centers and separated from other plots by 30 cm. Four replications of 16 cultivars were evaluated at each location.

Four stems were sampled systematically from the center row of each plot and were transported to the laboratory on ice for storage at 4 C until evaluation. Total length, length from the base to the first foliated node, number of defoliated nodes, presence or absence of the main stem leaf at a node, and number of foliated nodes were recorded for each stem. Leaf spot severity was evaluated visually for each leaf on a stem. A rating key based on a Horsfall-Barratt type scale was used (7). Disease severity for each stem was calculated by averaging the severity estimates from individual leaves.

Statistical analysis. Several variables related to disease resistance or affected by disease level were analyzed: disease severity rating, maximum disease level among individual leaves on a stem, percentage of defoliated nodes, percentage of nodes with abscised main stem leaves, and percentage of stem defoliated (length from base of stem to first intact node divided by total stem length).

Correlations were calculated to investigate the relationship among the variables measured for each stem. The correlations were estimated based on the means for each cultivar on a sampling date, resulting in 16 data points on each of 17 sampling dates. Percentage of diseased stem length was not measured on the first sampling date (2 June 1983); therefore, correlations involving this variable involved only 16 samples.

Three analyses of variance were performed on each variable. The first was an unbalanced analysis based on all sampling dates at all locations. Because of computational limitations and problems with interpreting interaction effects from an unbalanced analysis, only effects necessary for the test of the cultivar main effect were calculated. The second and third analyses were performed to estimate the importance of the cultivar by location or cultivar by location-year interactions. The data for these analyses were balanced by using two sampling dates for each location. The second analysis, used to evaluate the cultivar by location interaction, was based on 1984 data only, with two sampling dates at each of four locations. The third analysis, used to evaluate the cultivar by location-year interaction, was based on both 1983 and 1984 data, with six location-years each containing two sampling dates. Mean separations were performed where appropriate using the Waller-Duncan k-ratio t test ($k = 100$) (14).

Stability analysis (2,3,10) was performed on the disease severity data to estimate the stability of the disease resistance of a cultivar over different environments. The analysis is based on the regression model:

$$Y_{ij} = \mu_i + b_i I_j + d_{ij}$$

where Y_{ij} = mean disease severity of the ith cultivar in the jth environment, μ_i = mean disease severity of the ith cultivar over all environments, b_i = stability parameter estimated by the regression coefficient of the ith cultivar on the environmental index, I_j = the environmental index calculated as the mean disease severity in the jth environment minus the grand mean (all cultivars over all environments), and d_{ij} = deviation from regression of the ith cultivar in the jth environment. Several parameters have been suggested as indicators of stable resistance in a cultivar: mean disease severity; the stability parameter, b_i; and the r^2 value for the regression. Values of the stability parameter, b_i, near zero indicate that the level of resistance expressed in a cultivar is relatively insensitive to the environment in which the cultivar is grown. Values of r^2 near one indicate that μ and I_j account for most of the variation observed. A high r^2 value is important because the stability parameter value is meaningful only if the model provides a good fit to the data.

A cluster analysis using the SAS procedure CLUSTER (13) with the centroid option was performed to group cultivars based on

disease severity and percentage of defoliated nodes. These two variables were selected because they were relatively uncorrelated and because both disease severity and defoliation may be factors of leaf spot resistance.

RESULTS

Mean disease severity ranged from 0.6% for the 3 August 1983 sampling date at Washington County to 12.1% for 2 June 1983 at Rowan County (Table 3). Average maximum disease severity on a stem ranged from 1.7% to 47.0% and corresponded to the same dates, 3 August 1983 in Washington County and 2 June 1983 in Rowan County. Percentage of nodes with abscised main stem leaves ranged from 7.6% for the 3 July 1984, Washington County sampling date to 41.5% for the 5 July 1983, Washington County date. Percentage of defoliated nodes ranged from 2.4% (3 July 1984, Washington County) to 23.9% (2 June 1983, Rowan County), and percentage of defoliated length ranged from 7.3% (3 July 1984, Washington County) to 41.4% (31 May 1984, Wake County).

Three pathogens contributed to the leaf spots observed in the present study: *L. briosiana, S. botryosum,* and *C. medicaginis* (Table 4). *L. briosiana* was present in all but one of the samples from which isolations were made. *S. botryosum* was seen less frequently, usually during late June and July, and *C. medicaginis* was observed mainly in the later part of summer. *Phoma medicaginis* occurred rarely.

Most of the correlations among measured variables were significant (Table 5). The highest correlations were found for average leaf spot severity vs. maximum severity among leaves on a stem, percentage of defoliated nodes vs. percentage of nodes with abscised main stem leaves, percentage of defoliated nodes vs. percentage of defoliated length, stem length vs. number of nodes per stem, stem length vs. number of leaves per stem, and number of nodes per stem vs. number of leaves per stem.

The F test for the cultivar effect in the overall analysis had a significance level of 0.06 for leaf spot severity and 0.05 for maximum severity among leaves on a stem (Table 6). The F test for percentage of nodes with abscised main stem leaves in the 1984 balanced analysis was significant at the 0.06 level, but all other variables indicated nonsignificant cultivar effects. The cultivar by location effect was nonsignificant for all variables. Leaf spot severity and percentage of nodes with abscised main stem leaves were significant for the cultivar effect (P = 0.02 and 0.03, respectively) in the 1983/1984 balanced analysis. None of the cultivar by location-year interaction effects was significant.

TABLE 5. Correlations among variables measured in assessment of leaf spot severity on alfalfa[y]

Variable	Maximum severity	Defoliated nodes (%)	Nodes with main stem leaves (%)	Defoliated length (%)	Stem length	Nodes per stem	Leaves per stem
Average leaf spot severity	0.91[z] **	0.54 **	0.53 **	0.61 **	0.11	0.05	0.09
Maximum severity	...	0.59 **	0.57 **	0.51 **	0.22 **	0.22 **	0.28 **
Defoliated nodes (%)		...	0.78 **	0.75 **	0.45 **	0.21 *	0.24 **
Nodes with abscised main stem leaves (%)			...	0.49 **	0.37 **	0.28 **	0.29 **
Defoliated length (%)				...	0.38 **	0.07 **	0.09
Stem length					...	0.80 **	0.81 **
Nodes per stem						...	0.89 **

[y] Based on mean values for each of 16 cultivars from 17 sampling dates (n = 272), except correlations involving percentage of defoliated length, which are based on 16 cultivars from 16 sampling dates (n = 256).
[z] ** = Significant at P = 0.01, * = significant at P = 0.05.

TABLE 6. Combined analyses for leaf spot severity, maximum leaf spot severity per stem, percentage of defoliated nodes, percentage of abscised main stem leaves, and percentage of defoliated length for 16 alfalfa cultivars at four locations in North Carolina

		Leaf spot severity			Maximum severity			Defoliated nodes (%)			Abscised main stem leaves (%)			Defoliated length (%)		
Analysis[x]	Source	MS[y]	df[y]	prob[y]	MS	df	prob	MS	df	prob	MS	df	prob	MS	df	prob
Overall	Cultivar	0.360	15	0.06	0.365	15	0.05	0.00551	15	0.33	0.00985	15	0.23	0.00599	15	0.40
1984	Error	0.198	45		0.191	45		0.00472	45		0.00746	45		0.00532	45	
balanced	Cultivar	0.210	15	0.19	0.211	15	0.26	0.00527	15	0.18	0.0102	15	0.06	0.00449	15	0.59
	Error	0.150	45		0.165	45		0.00370	45		0.00563	45		0.00511	45	
	Cult*Loc[z]	0.226	96	0.71	0.275	112	0.93	0.00700	134	0.32	0.0104	130	0.54	0.0101	142	0.56
	Error	0.250	253		0.356	157		0.00645	143		0.0106	143		0.0104	161	
1983/1984	Cultivar	0.348	15	0.02	0.318	15	0.12	0.00463	15	0.21	0.0118	15	0.03	0.00460	15	0.62
balanced	Error	0.166	75		0.208	75		0.00350	75		0.00614	75		0.00543	75	
	Cult*Loc-Yr[z]	0.240	149	0.51	0.329	172	0.79	0.00664	221	0.50	0.0111	207	0.72	0.0117	255	0.59
	Error	0.241	233		0.371	234		0.00664	239		0.0120	244		0.0121	259	

[x] Overall analysis is for 17 sample dates (see Table 1). The 1984 analysis is for two sampling dates (day/mo) per location during 1984 (Washington, 3/7 and 3/8; Rowan, 25/6 and 30/7; Wake, 27/6 and 27/7; Sampson, 11/4 and 20/7). The 1983/1984 analysis is for six location-years, with two sampling dates each (same as in 1984 plus Washington, 5/7/83 and 3/8/83; Rowan, 6/7/83 and 4/8/83). All effects are random except cultivar (fixed).
[y] Mean squares (ms), degrees of freedom (df) or effective degrees of freedom, and probability (prob) of a greater F based on F test or approximate F test (see note z).
[z] Mean squares for the interaction effects are sums of two mean squares from the analysis of variance. The degrees of freedom given are effective ones based on the formula given by Satterthwaite and the probabilities are from the approximate F test (14).

Florida 77, Arc, and WL 318 had the highest disease severity levels and Raidor had the lowest severity level (Table 7); when mean separations were performed on rating scale values, however, no cultivar was in a group by itself. Cultivar rankings in Table 7 differ slightly from those based on percentage of disease severity because mean percentage disease severity is the average of values on a linear scale, whereas mean rating scale value is the average of values from a logarithmic scale. DeKalb 120, WL 311, and Classic had the highest values for percentage of nodes with abscised main stem leaves, and Florida 77, Pioneer 531, and Raidor had the lowest (Table 8). Again, no cultivar was in its own grouping.

Estimates of the stability analysis regression parameter, b, ranged from 0.67 for Raidor to 1.32 for DeKalb 130 (Table 9). All regression parameter estimates differed significantly from zero. S_d^2 values, which represent the variance about the regression line from the stability analysis, ranged from 0.83 for Raidor to 5.36 for Arc. Values of r^2 ranged from 0.68 (Apollo) to 0.84 (Vancor, WL 311).

The cluster analysis based on leaf spot severity and percentage of defoliated nodes indicated four groupings of cultivars: Florida 77, Raidor, DeKalb 120 and Classic, and all other cultivars. Florida 77 was separated because of a high value for leaf spot severity and a low value for percentage of defoliated nodes. Raidor was grouped separately on the basis of low leaf spot severity, and DeKalb 120 and Classic had higher levels of percentage of defoliated nodes than did other cultivars.

TABLE 7. Mean separations for leaf spot severity from analysis of 16 alfalfa cultivars combined over four locations during 1983 and 1984

Cultivar[w]	Mean severity[x]	Mean rating value[y]	Grouping[z]
Florida 77	4.2	1.88	A
Arc	4.1	1.77	A B
WL 318	3.8	1.77	A B
Apollo	3.5	1.73	A B
WL 311	3.5	1.73	A B
Vancor	3.5	1.73	A B
DeKalb 130	3.8	1.72	A B
Southern Special	3.9	1.72	A B
Cimarron	3.3	1.71	A B
Classic	3.4	1.67	A B C
Pioneer 531	3.2	1.66	B C
DeKalb 120	3.1	1.65	B C
Shenandoah	3.3	1.64	B C
HiPhy	3.1	1.61	B C
Weevlchek	3.2	1.60	B C
Raidor	2.7	1.49	C

[w] Cultivar effect was significant at $P = 0.06$.
[x] Mean percentage of disease severity for cultivar over all sampling dates.
[y] Mean disease rating value for cultivar over all sampling dates; mean separations are based on these values. Values were calculated as an arithmetic mean of rating scale values where 0 = 0%, 1 = 1%, 2 = 2%, 3 = 5%, 4 = 11%, 5 = 23%, 6 = 50%, and 7 = 75% of leaf tissue with leaf spot symptoms.
[z] Mean separation based on Waller-Duncan k-ratio t test ($k = 100$).

DISCUSSION

Disease severity levels among the sampling dates ranged from relatively low (0.9% on 11 April 1984, Sampson County) to relatively severe (12.1% on 2 June 1983, Rowan County). Both of these values appear low when compared with severity measures from other pathosystems, but they are based on an average of all leaves on a stem. Defoliation of highly diseased leaves and growth of new, healthy leaves limit the maximum level of disease attainable on a stem. Thus, an average severity level of 12% represents relatively severe alfalfa leaf spot.

The variables measured for each stem fall into three groups: measures of disease severity (percentage of disease severity, maximum disease severity on a stem), defoliation (percentage of nodes with abscised main stem leaves, percentage of defoliated nodes, percentage of stem length defoliated), and growth (stem length, number of nodes on a stem, number of leaves on a stem). Most of the correlations among the measured variables were significant and all were positive, but the highest correlations were

TABLE 8. Mean separations for percentage of nodes with abscised main stem leaves on 16 alfalfa cultivars from six locations during 1983 and 1984

Cultivar	Abscised main stem leaves (%)[y]	Grouping[z]
DeKalb 120	24.7	A
WL 311	24.6	A B
Classic	23.9	A B C
Vancor	23.4	A B C D
HiPhy	23.2	A B C D
Weevlchek	23.1	A B C D
Apollo	23.0	A B C D
DeKalb 130	22.6	A B C D
Southern Special	22.5	A B C D
WL 318	22.2	A B C D
Shenandoah	22.1	A B C D
Arc	22.1	A B C D
Cimarron	21.1	A B C D
Raidor	20.4	B C D
Pioneer 531	19.9	C D
Florida 77	19.3	D

[y] Percentage of nodes with missing main stem leaf.
[z] Mean separation based on Waller-Duncan k-ratio t test.

TABLE 9. Stability parameters for leaf spot severity on 16 alfalfa cultivars at four locations in North Carolina during 1983 and 1984

Cultivar	Disease severity	Stability parameters[y]			
		b	$se(b)^2$	S_d^2	r^2
Florida 77	4.2	1.10	0.087	3.63	0.72[z]
Arc	4.1	1.23	0.106	5.36	0.69
Southern Special	3.9	1.26	0.098	4.58	0.72
WL 318	3.8	1.28	0.081	3.11	0.80
DeKalb 130	3.8	1.32	0.100	4.83	0.73
Vancor	3.5	0.96	0.053	1.34	0.84
WL 311	3.5	0.95	0.052	1.28	0.84
Apollo	3.5	0.83	0.073	2.54	0.68
Classic	3.4	0.93	0.077	2.74	0.71
Shenandoah	3.3	1.08	0.088	3.69	0.71
Cimarron	3.3	0.86	0.050	1.18	0.83
Pioneer 531	3.2	0.88	0.060	1.72	0.77
Weevlchek	3.2	0.94	0.059	1.66	0.80
DeKalb 120	3.1	0.85	0.056	1.48	0.79
HiPhy	3.1	0.81	0.067	2.17	0.69
Raidor	2.7	0.67	0.042	0.83	0.80

[y] Parameter b is the regression parameter from stability analysis, $se(b)$ is the standard error of b, and S_d^2 is the variance about the regression line from the stability analysis.
[z] Coefficient of determination for regression from the stability analysis.

among measures within a group (Table 5), indicating that it is generally not necessary to measure all of these variables. The highest correlation was between disease severity and maximum disease on a stem, and these variables responded similarly in overall analysis of variance. The defoliation measures had lower correlations, especially the correlation between percentage of nodes with abscised main stem leaves and percentage of defoliated length. The ideal measure of defoliation would be the number of abscised leaves; since this is extremely difficult to assess on alfalfa, however, an alternative is needed. The percentage of defoliated nodes was related to the other two measures of defoliation and may be the best measure if a single measure is to be used.

Differences were seen among the cultivars investigated, several occurring consistently enough to allow some generalizations about the cultivars involved. Because there was no significant interaction between cultivar and location or location-year, the generalizations concerning cultivar resistance should hold for all locations investigated. Florida 77 generally had higher levels of disease than the other cultivars present in the studies. Arc and WL 318 also showed high levels of disease severity. Raidor was more resistant to the leaf spot diseases than other cultivars and was the most stable cultivar. Percentage of nodes with abscised main stem leaves followed a pattern different from that of disease severity: both Raidor and Florida 77 had low percentages, and Arc and WL 318 had intermediate values. This may indicate a weakness in the use of disease severity as the sole variable for separating cultivars. Certain cultivars, such as Florida 77, may tolerate greater disease levels than other cultivars before defoliation occurs. These cultivars may be incorrectly interpreted as being less resistant if disease severity is the only variable considered.

A method of incorporating disease severity and defoliation into a single value would be useful but would require a more complete understanding of the relationship between defoliation and disease severity. Directly combining severity and defoliation data would, however, ignore the fact that some defoliation occurs even when leaf spot diseases are not present. Controlled experiments to assess the effect of disease on defoliation and the amount of naturally occurring defoliation for different cultivars would be useful in developing such a combined disease index.

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