Assessment of Alfalfa Cultivar Reaction to Anthracnose in Controlled and Field Environments

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ABSTRACT

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Fifty-two alfalfa cultivars were evaluated for reaction to *Colletotrichum trifolii* (anthracnose) by a standardized seedling assay and during natural epidemics. An anthracnose index was calculated for each cultivar by dividing the number of stems with foliar symptoms per square meter by the number observed for Saranac (the susceptible standard cultivar) and multiplying by 100. Cultivar ranks based on this anthracnose index were significantly correlated with rankings based on percent resistant seedlings determined in the standardized seedling assay. There was a significant and inverse linear relationship between percent resistant seedlings and the $\ln(x+1)$ transformation of the anthracnose indices for cultivars at all locations. Anthracnose indices from the three field assessments were significantly correlated with one another and effectively differentiated cultivar reactions to *C. trifolii*. Anthracnose indices recorded for the cultivars Maris Kabul, Vista, Thor, Trumpetor, and Vertus were higher than expected based on results from the seedling assay and lower than expected for the cultivars WL-313, Apollo II, and Baker. The latter phenotypic reaction is suggestive of a type of field or adult-plant resistance not measured by the current protocol of the seedling assay. These results establish the importance of evaluating the reaction of alfalfa cultivars to anthracnose under both controlled conditions in the seedling stage and in the field.

Anthracnose of alfalfa (*Medicago sativa* L.), caused by *Colletotrichum trifolii* Bain & Essary, was first described in 1906 (3) and is now a prevalent disease in many regions of the world (2,6,9,10,14). *C. trifolii* infects stems and crowns resulting in the reduction of shoot numbers, crown bud numbers, and overall crown size and health (11). Anthracnose has been implicated as a major cause of mid- and late-summer yield reduction and plant mortality in many regions of the United States and the world (4,5,10,14).

Resistant alfalfa cultivars are important for the control of anthracnose (5). However, the occurrence of three physiological races of *C. trifolii* in the United States (1,9,13,17) indicates the need for regional cultivar evaluation in the presence of the locally occurring populations of the pathogen. Resistance to *C. trifolii* is reported to be conditioned by single genes (8), although other genetic mechanisms have been proposed (12). Historically, methods to evaluate alfalfa populations for resistance to *C. trifolii* under controlled environments have been more quantitative relative to methods for field evaluations. Elgin and Ostazeski (7) reported that alfalfa germ plasm can be evaluated for anthracnose resistance by inoculating 14-day-old alfalfa seedlings and scoring them as living (resistant) or dead (susceptible) 14 days later. This method has become the standard method for determining the level of resistance to anthracnose in alfalfa populations. The methods previously reported for evaluation of alfalfa populations in the field have used a subjective rating of the intensity of anthracnose symptoms on a whole-plot basis (4,5). Devine and McMurtrey (5) differentiated the reactions of selected or nonselected alfalfa populations for resistance to *C. trifolii* in natural epidemics using a subjective rating.

Alfalfa cultivars need to be characterized for resistance to *C. trifolii* in order to predict their performance when planted in areas with a high potential for anthracnose. The objectives of this study were to develop a standardized method to objectively measure incidence of anthracnose in the field and to determine the relationship between cultivar reactions in a standardized seedling assay and field assessments.

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MATERIALS AND METHODS

Fifty-two alfalfa cultivars of commercial and public origin were evaluated for reaction to *C. trifolii* in both controlled and field environments. The cultivars Saranac (susceptible), Saranac AR (race 1 and 2, resistant), and Arc (race 1, resistant) were used as check cultivars for the evaluation of alfalfa lines for resistance to anthracnose (7).

Seedling assay. The method of Elgin and Ostazeski (7) was used to evaluate alfalfa cultivars for resistance to *C. trifolii* in a controlled environment. Fifty seeds of each cultivar were planted in flats (30 × 60 cm) containing a mixture of steamed sand and muck soil (1:1, v/v). Seeds of each cultivar were placed in single furrows 5 mm deep and 2 cm apart, then covered with soil. Flats were overseeded and thinned to 50 per row 7 days after planting. Each row represented one of six replications of each cultivar. Cultivars were randomized within flats and the flats were arranged in a randomized complete block design. Seedlings were grown in a greenhouse at 24 ± 2 C and were provided supplemental fluorescent light for 16 hr per day.

Six isolates of *C. trifolii* race 1 (14,16), originally recovered from alfalfa stems collected in Wisconsin, were used in this study. Isolates were cultured on potato-dextrose agar at 24 C. Conidia were harvested by rinsing 10-day-old sporulating colonies with sterile distilled water. Final inoculum was adjusted using a hemacytometer so that it consisted of an equal number of conidia from each isolate. A 10^6 conidia per milliliter

suspension was sprayed onto 14-day-old seedlings until runoff. Flats of inoculated seedlings were immediately covered for 3 days with a clear polyethylene sheet to maintain high humidity. Fourteen days after inoculation seedlings were scored as dead or alive and survivors were regarded as resistant. Cultivars were characterized by the percentage of resistant plants.

Field evaluations for anthracnose resistance. Fifty cultivars evaluated in the seedling assay were planted at two locations within the University of Wisconsin Research Station at Arlington in 1982, and 24 cultivars (22 of the 50 plus two additional ones, Spectrum and Eagle) were planted at a third location at Arlington in 1984. Cultivars were evaluated for 2–3 location years. In 1982 (locations 1 and 2), plots consisted of rows of each cultivar that were 4.6 m long and spaced 0.76 m apart, planted with 5 g of seed. In 1984 (location 3), plots were 1 m wide and 6.1 m long, planted with 20 g of seed in five rows, 15 cm apart. A randomized complete block design with four replications was used for each field experiment.

The anthracnose reaction of each cultivar was expressed on a relative basis to Saranac (susceptible standard) by calculating an anthracnose index. Cultivars were evaluated by enumerating individual stems per plot with foliar symptoms typical of those caused by *C. trifolii* (chlorotic shoots, frequently

Table 1. Reaction of 50 alfalfa cultivars to anthracnose

Cultivar	% Resistant plants[a]	Anthracnose index[b] Location 1 (1983)	Location 2 (1983)	Location 3 (1985)	Mean of locations
Saranac	1	100	100	100	100
Maris Kabul	1	155	100	···	127
Peak	2	87	16	···	52
Honeoye	2	91	44	···	67
Iroquois	2	90	78	79	82
Vista	3	119	107	···	113
Tempo	3	71	45	···	58
Blazer	3	43	27	26	32
Riley	4	64	7	···	36
Thor	4	123	55	84	87
WL-315	4	83	31	12	42
Epic	5	59	22	···	41
Valor	5	79	29	···	54
Perry	5	75	35	···	55
WL-313	6	32	13	10	18
Oneida	6	76	31	···	54
DK 120	6	97	49	48	65
Phytor	6	88	85	···	87
Vernal	7	56	55	···	56
Wrangler	7	72	5	···	39
Baker	7	19	7	···	13
Agate	9	33	40	···	37
Apollo II	10	17	7	11	12
Classic	12	49	24	···	37
Futura	12	44	25	18	29
Answer	12	37	29	···	33
Apollo	15	43	25	···	34
Trident	16	52	7	···	30
Decathalon	17	33	15	8	19
Drummor	18	33	40	12	28
Preserve	18	31	25	···	28
Magnum	20	35	24	7	22
Duke	22	25	5	7	12
Advantage	23	24	15	1	13
Trumpetor	24	84	40	23	49
DK 135	25	35	18	···	27
Excalibur	26	43	29	···	36
Mercury	26	15	5	···	10
Vertus	28	99	29	55	61
Endure	29	28	5	1	11
Expo	30	32	15	···	24
Emerald	31	41	27	···	34
Armor	32	20	9	10	13
Raidor	36	56	18	···	37
Atlas	46	5	11	1	7
Olympic	46	25	7	···	16
Vancor	51	36	18	7	20
Saranac AR	54	37	22	5	21
WL-316	59	7	7	4	6
Arc	71	20	7	···	14
Cultivar $\bar{X}$	18	54	30	24	
LSD ($P = 0.05$)	8	36	29	22	

[a] Percentage of resistant plants as determined by the standardized seedling assay.
[b] Anthracnose index = (no. of symptomatic stems/m^2 for each cultivar divided by no. of symptomatic stems/m^2 for Saranac at that location) × 100. Saranac had 7.7, 5.5, and 7.3 symptomatic stems/m^2 at locations 1, 2, and 3, respectively.

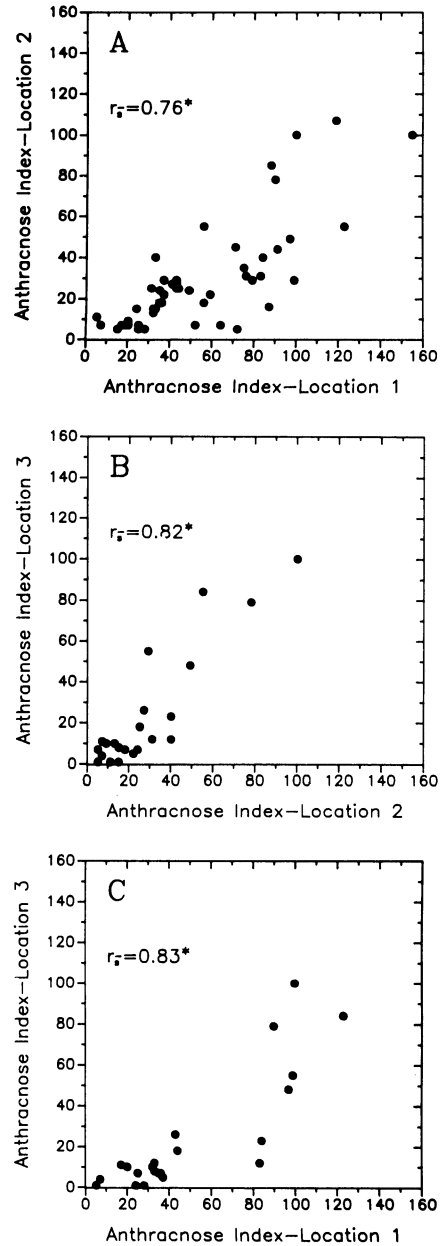

Fig. 1. Comparison of the anthracnose indices of 50 cultivars. The 50 cultivars were identical at locations 1 and 2, and 22 of the 50 cultivars were evaluated at location 3. Spearman's rank correlations of anthracnose index ranks were significant ($P = 0.05$) between: **(A)** locations 1 and 2 ($r_s = 0.76$); **(B)** locations 2 and 3 ($r_s = 0.82$); and **(C)** locations 1 and 3 ($r_s = 0.83$). Spearman's rank correlation coefficients (r_s) were significant ($P = 0.05$), as indicated by asterisks.

exhibiting shepherd's crooked stems) and conversion of the quantity to a per-square-meter basis. The anthracnose index was calculated for each cultivar by dividing the number of symptomatic stems per square meter by the number of observed per square meter for Saranac and multiplying by 100. For nine cultivars at location 3, an additional method to estimate the incidence of anthracnose was used. Fifty stems were sampled from four sites (0.1 m^2 each) within each plot and pooled into a composite sample. The 200 stems per plot were visually assessed for the presence or absence of stem lesions typical of anthracnose and data were expressed as percent lesioned stems per cultivar.

Statistical tests. Differences among cultivar means and the relationship between measures of disease incidence were determined statistically by analysis of variance, Fischer's least significant difference test, linear regression analysis, and Spearman's rank correlation test (15).

RESULTS AND DISCUSSION

Seedling assay. The percentage of resistant seedlings for the 52 cultivars ranged from a low of 1% for Saranac and Maris Kabul to over 50% for Vancor, Saranac AR, WL-316, and Arc (Tables 1 and 2). The results of this study compare very closely to those of Elgin and Ostazeski (7) for the cultivars common to both studies. The high level of resistance expressed by both Saranac AR and Arc confirm that the isolates of *C. trifolii* used were race 1 (1,7). Race 2 of *C. trifolii* has not been recovered from alfalfa in the Midwest (9).

Field assessment of anthracnose incidence. Maximum incidence of foliar symptoms was observed during the third harvest period of the second stand year at all locations. Incidence of anthracnose was minimal before and after that time at all locations (*data not presented*). Conversion of the data from the number of stems with foliar symptoms per square meter to an anthracnose index allowed for a direct comparison of cultivar reaction relative to Saranac at all locations (Tables 1 and 2). The incidence of anthracnose was greater at location 1 compared with location 2 in 1983, but similar disease was measured between locations 2 (1983) and 3 (1985) (Table 1). At location 1 the incidence of anthracnose on Maris Kabul, Vista, and Thor exceeded that of Saranac, but at location 2 only Vista had greater incidence than Saranac. Significant ($P = 0.05$) Spearman's rank correlation coefficients of $r_s = 0.76$, 0.82, and 0.83 between the ranks of cultivars at locations 1 and 2, 2 and 3, and 1 and 3, respectively, provided confidence that the rank assigned to cultivars based on the anthracnose index was consistent among locations within and between years (Fig. 1A–C). This approach is a useful method to differentiate cultivars for reaction to anthracnose (Tables 1 and 2). Anthracnose indices were more variable among locations for cultivars that had a low percentage of resistant seedlings determined by the seedling assay (Fig. 1A–C; Table 1).

Table 2. Comparison of variables used to measure anthracnose incidence in the field at location 3

Cultivar	% Resistant plants[a]	Symptomatic stems/m^2	Anthracnose index[b]	Lesioned stems (%)
Saranac	1	7.3	100	55
Spectrum	2	3.8	52	26
Blazer	3	1.9	26	15
Thor	4	6.1	84	49
Duke	22	0.5	7	8
Armor	32	0.7	10	7
Eagle	33	0.0	0	2
Atlas	46	0.1	1	3
Saranac AR	54	0.4	5	3
LSD ($P = 0.05$)	8	1.6	22	11

[a] Percentage of resistant plants as determined by the standardized seedling assay.
[b] Anthracnose index = (no. of symptomatic stems/m^2 for each cultivar divided by no. of symptomatic stems/m^2 for Saranac at that location) × 100.

Stems with foliar symptoms of anthracnose were readily visible throughout the canopy, but there was concern as to how this measure of disease incidence was related to the incidence of stems with anthracnose lesions. Although the observed number of stems with foliar symptoms per square meter was less than the number of lesioned stems (Table 2), the rank of each cultivar was highly correlated ($r_s = 0.99$, $P = 0.01$) for each measure of disease incidence.

Comparison of results from seedling assay and field assessment. In general at all locations, the anthracnose index decreased as the percentage of resistant plants measured by the seedling assay increased (Table 1; Fig. 2A–C). The cultivar rank based on seedling assay results was significantly correlated ($P = 0.05$) with the rank based on anthracnose indices recorded at each location ($r_s = -0.70$, -0.62, and -0.71 for locations 1, 2, and 3, respectively). The relationship between percent resistant plants and anthracnose indices was significantly nonlinear. However, anthracnose indices were transformed using the $\ln(x + 1)$ transformation and were plotted versus percent resistant plants; highly significant linear relationships were obtained (Fig. 2A–C). Regression lines and confidence belts ($P = 0.05$) are presented in Figure 2A–C. Although variables used to measure disease incidence were significantly correlated, the reactions of several cultivars whose transformed anthracnose index values lie outside of the 95% confidence belts can be discussed. WL-313, Baker, and Apollo II are examples of cultivars that had anthracnose indices significantly lower than the other cultivars with a similar or greater percentage of resistant plants (Table 1). This host reaction to *C. trifolii* is suggestive of a type of field or adult-plant

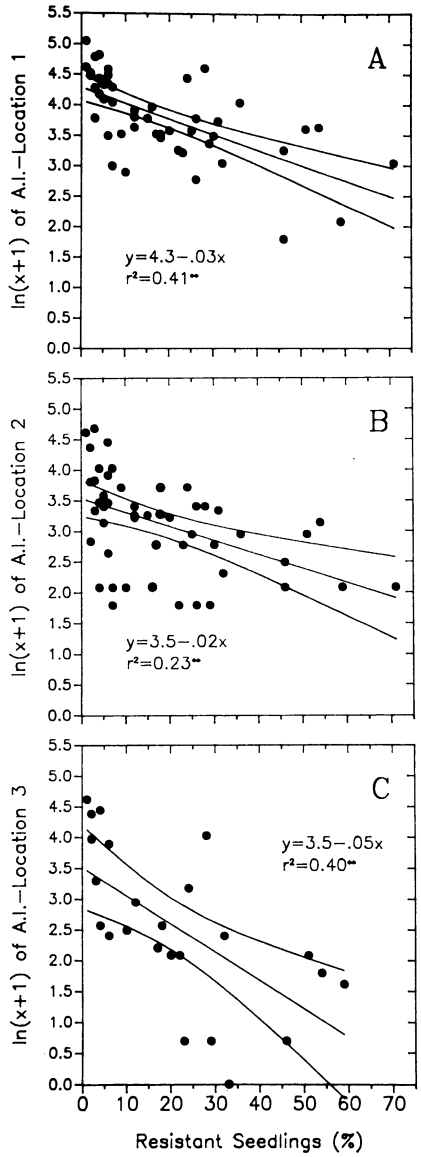

Fig. 2. Relationship between the percent of resistant plants as determined by the seedling assay and the $\ln(x + 1)$ transformation of the anthracnose indices of 50 cultivars evaluated at **(A)** location 1 and **(B)** location 2; **(C)** 24 cultivars evaluated at location 3. The area between the upper and lower curves represents the 95% confidence belt of the regression lines. Coefficients of determination (r^2) were highly significant ($P = 0.01$), as indicated by asterisks.

resistance (18) not measured by the current standardized seedling assay and merits further investigation. Conversely, the anthracnose indices of Maris Kabul, Vista, Thor, Trumpetor, and Vertus were significantly higher than predicted from results of the seedling assay (Table 1; Fig. 2A–C). These cultivars have been derived from predominantly Flemish germ plasm. Although no direct evidence is available, they may lack adult-plant resistance that we postulate was present in WL-313, Baker, and Apollo II.

Differences observed among cultivar reactions may be due to unrecognized strains of the pathogen or to environmental conditions that may have altered the host phenotype. The reactions of Trumpetor and Vertus are particularly troublesome because this represents the first report of a substantial difference between results of the seedling assay and a field assessment in the Midwest. These results indicate the importance of evaluating the reaction of alfalfa cultivars to anthracnose under both controlled and field conditions and the need to monitor local populations of *C. trifolii* for variability in pathotypes.

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