Severity of Spring Black Stem on Alfalfa Cultivars in Ohio

L. H. RHODES, Associate Professor, Department of Plant Pathology, and D. K. MYERS, Associate Professor, Department of Agronomy, The Ohio State University and Ohio Agricultural Research and Development Center, Columbus 43210

ABSTRACT

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Spring black stem (SBS) severity differed among alfalfa cultivars in the field at three locations in Ohio in 1983 and 1984. Of the 10 cultivars represented at all locations, Classic and Vernal had the lowest disease severity rating (DSR). First- and second-harvest yields were negatively correlated with DSR. In a greenhouse study of selected cultivars, dry-matter yields were reduced 20% by artificial inoculation with *Phoma medicaginis* var. *medicaginis*, even though plants not subjected to artificial inoculation also became infected. The cultivar Vernal, classed resistant under field conditions, had the highest DSR in the greenhouse. Saranac AR, classed intermediate in the field, had the lowest DSR and no yield reduction resulting from artificial inoculation in the greenhouse.

Spring black stem (SBS) of alfalfa (*Medicago sativa* L.) caused by *Phoma medicaginis* Malbr. & Roum. var. *medicaginis* Boerema (*P. m.* var. *medicaginis*) is prevalent each year in Ohio alfalfa fields. Characteristic symptoms include foliar and stem lesions, defoliation, reduced plant growth, and death of small shoots. When infection is severe, stand loss may occur. Such damage has been shown to cause yield losses as high as 44% in controlled greenhouse experiments (5).

Although the disease was first reported in the United States nearly 80 yr ago (9), relatively little is currently known about variation in susceptibility to SBS among cultivars of alfalfa. Early studies showed that *Medicago* species and selections differed in their reactions to SBS (6,8) and that resistance to SBS in alfalfa populations could be increased through selection and breeding (7). Recently, Angevain (1) reported differences in SBS severity among several European cultivars and selections. However, few alfalfa cultivars used in the United States are designated resistant to SBS. Gray (4) found no significant differences in SBS severity among 11 alfalfa cultivars at six locations in Wyoming. The recommended alfalfa host differentials, Ramsey (resistant), Ranger (susceptible), and Lahonton (susceptible) (3), were included in these evaluations.

Results of preliminary field observations in Ohio suggested that SBS severity differed among alfalfa cultivars subjected to similar environmental and cultural conditions and that such differences were reflected in cultivar yields. The purpose of this investigation, therefore, was to determine the reactions of alfalfa cultivars to infection by *P. m.* var. *medicaginis* in the field and the effect of SBS on survival and growth of selected cultivars under controlled environmental conditions.

MATERIALS AND METHODS

Field study. Field plots were established in Ohio at Jackson (August 1980), Wooster (April 1981), and South Charleston (July 1981). Plots 1.8 × 6.1 m were planted to a single cultivar at 5.5 kg of seed per hectare with a row spacing of 18 cm. At each location, plots were arranged in a randomized complete block design with four replicates. Rainfall at each location from 1 April (approximate time of initiation of spring growth) until SBS ratings were made in 1983 and 1984, respectively, was 25.1 and 12.3 cm at Jackson, 21.0 and 18.6 cm at South Charleston, and 22.5 and 21.6 cm at Wooster.

Because SBS is usually not severe during the seeding year, field ratings of disease severity were not made until at least the second production year after planting. In mid- to late May, immediately before each year's first harvest, disease severity was rated on a scale of 1–5, where 1 = no disease to a trace of infection (a few small lesions occurring on some leaves and stems, no defoliation); 2 = light infection (many lesions on leaves and stems, some defoliation); 3 = moderate infection (stem lesions coalescing to cover most of lower 15 cm of stem, lower stems largely defoliated with remaining leaves heavily infected); 4 = heavy infection (lower stems of all plants blackened by coalescing stem lesions, lower stems defoliated, some shoots killed); and 5 = severe infection (all plants blackened by coalescing stem lesions, plants obviously stunted, many shoots killed). Disease severity ratings (DSR) in three randomly selected 0.1-m^2 areas of each plot were averaged to give a single plot rating. Data were subjected to analysis of variance, and Duncan's multiple range test was used to determine differences among cultivar means.

Yield determinations were made by harvesting a strip 0.9 × 6 m in the center of each plot. The forage was weighed immediately after cutting. Samples from throughout the plot were dried for moisture content determinations, and all forage weights were expressed on a dry-weight basis. Correlation and linear regression analyses were used to determine the relationship between DSR and yield.

Greenhouse study. On the basis of field data, five cultivars that showed differences in degree of SBS severity were selected for a greenhouse study. The cultivars Vangard and Hi-phy were classed susceptible, Saranac AR intermediate, and Vernal and Classic resistant. Twenty seeds of each cultivar were planted in sterilized peat, perlite, and soil medium (1:1:1) in 15-cm plastic pots. Plants in each of 15 replicate pots were thinned to 10 per pot 2 wk after seedling emergence, and pots were arranged on the greenhouse bench in a completely randomized design. After growing for 12 wk in the greenhouse, plants were clipped 6 cm above the soil line.

Two weeks after clipping, plants were inoculated with a conidial suspension of *P. m.* var. *medicaginis* as follows: Five monoconidial isolates of *P. m.* var. *medicaginis* from four locations in Ohio and one in Wisconsin were maintained on acidified potato-dextrose agar for 4 wk. Conidial inoculum was decanted from *P. m.* var. *medicaginis* cultures that had been flooded with sterile distilled water for 10–15 min. Concentrations were adjusted to 10^6 conidia per milliliter for each isolate (2). Inocula of all isolates were then combined and sprayed onto stem and leaf surfaces with an atomizer pressurized at 0.35 kg/cm^2. Each set of 10 plants was sprayed for 20 sec.

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Table 1. Severity of spring black stem on alfalfa cultivars at Jackson, OH, in 1983 and 1984

Cultivar	Disease severity rating[w] 1983[x]	1984[x]
Classic[y]	1.85 a[z]	1.00 a
Dekalb 120	2.00 ab	1.75 b
WL 318	2.00 ab	1.58 b
Pioneer 524	2.25 ab	1.58 b
Apollo[y]	2.25 ab	1.75 b
WL 313[y]	2.25 ab	1.50 b
Vernal[y]	2.25 ab	1.33 ab
Pioneer 531	2.33 ab	1.58 b
Dekalb 130	2.35 ab	1.50 b
Raidor[y]	2.43 ab	1.33 ab
Saranac AR[y]	2.50 ab	1.50 b
Trident[y]	2.53 ab	1.58 b
Vangard[y]	2.58 ab	1.83 b
Vancor[y]	2.58 ab	1.58 b
Hi-phy[y]	2.58 ab	1.58 b
G 7505	2.75 b	1.83 b

[w] Disease severity rated on a scale of 1–5, where 1 = none to trace, 2 = light, 3 = moderate, 4 = heavy, and 5 = severe. Each figure represents the mean of three subsamples in each of four replicates.
[x] Ratings made 20 May 1983 and 17 May 1984.
[y] Cultivar represented at all locations.
[z] Means in a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Inoculated plants and a corresponding number of uninoculated control plants were placed in a mist chamber that supplied an overhead mist for 6 sec at 6-min intervals for 10 hr each day. Plants were kept under these conditions for 2 wk after inoculation, then removed to a greenhouse bench and watered daily with overhead irrigation for 2 wk. This pattern of alternating incubation under mist chamber conditions and greenhouse conditions for 2-wk intervals was repeated, resulting in a total incubation period of 8 wk after inoculation. Throughout this time, air temperatures in the greenhouse and mist chamber were set to maintain a maximum of 21 C during daylight hours and a maximum of 18 C during the dark period.

After the 8-wk period, each group of plants was rated according to the rating system previously described for the field study. Plants were clipped 6 cm above the soil surface. The forage was dried at 70 C for 48 hr and weighed. Plants were returned to the greenhouse bench for an additional 5 wk, then a second harvest was made and the forage treated similarly. Data were subjected to analysis of variance, and Duncan's multiple range test was used to determine differences between treatment means.

RESULTS

Field Study. SBS severity differed among cultivars (Tables 1–3). Of the 10 cultivars represented at all locations, Classic and Vernal had the lowest DSR. Many individual plants within these cultivars were uninfected or had only a few small lesions.

A functional relationship between DSR and yield was shown by linear regression analysis (Fig. 1), with a significant negative correlation ($r = -0.57$, $P = 0.01$) between DSR and first cutting yield. Second cutting yield was also negatively correlated ($r = -0.46$, $P = 0.01$) with SBS severity.

Considering only the 10 cultivars represented at all locations, SBS severity was greater in 1983 (DSR = 2.6) than in 1984 (DSR = 1.6). SBS severity was greater at Wooster (DSR = 2.4) than at Jackson or South Charleston (DSR = 2.0 and 1.9, respectively). There were no significant cultivar × year, cultivar × location or year × location interactions for DSR.

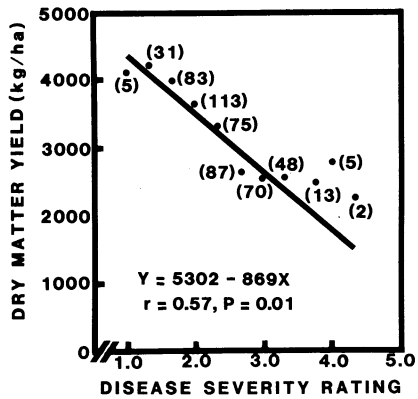

Fig. 1. Relationship of first-harvest dry-matter yield of alfalfa to severity of spring black stem. Points show the average yield for a given plot rating; numbers in parentheses denote number of plots receiving that rating. Disease severity was rated as follows: 1 = no disease or trace, 2 = light infection, 3 = moderate infection, 4 = heavy infection, and 5 = severe infection.

Table 2. Severity of spring black stem on alfalfa cultivars at South Charleston, OH, in 1983 and 1984

Cultivar	Disease severity rating[w] 1983[x]	1984[x]
Vernal[y]	1.83 a[z]	1.25 ab
Apollo[y]	2.08 ab	1.41 abc
Classic[y]	2.15 ab	1.08 a
Pioneer 526	2.15 ab	2.00 e
Marathon	2.22 abc	1.41 abc
Honeoye	2.30 abc	1.25 ab
Epic	2.40 abc	1.33 abc
Saranac AR[y]	2.40 abc	1.50 abc
G 7730	2.50 abc	1.58 abc
Raidor[y]	2.50 abc	1.41 abc
Trident[y]	2.55 abc	1.41 abc
Oneida	2.65 abc	1.58 abc
Vancor[y]	2.75 abc	1.42 abc
Pioneer 532	2.80 abc	1.91 de
WL 312	2.80 abc	1.42 abc
WL 313[y]	2.83 bcd	1.25 ab
Vangard[y]	3.15 cde	1.75 bcd
Cimarron	3.30 de	1.83 de
Hi-phy[y]	3.40 e	1.92 de

[w] Disease severity rated on a scale of 1–5, where 1 = none to trace, 2 = light, 3 = moderate, 4 = heavy, and 5 = severe. Each figure represents the mean of three subsamples in each of four replicates.
[x] Ratings made 12 May 1983 and 23 May 1984.
[y] Cultivar represented at all locations.
[z] Means in a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Greenhouse study. Although inoculated

Table 3. Severity of spring black stem on alfalfa cultivars at Wooster, OH, in 1983 and 1984

Cultivar	Disease severity rating[v] 1983[w]	1984[w]
Nugget	2.03 a[x]	1.75 ab
Hi-Ton 70	2.25 ab	1.83 ab
C/W-6306	2.25 ab	1.83 ab
Pioneer 526	2.28 abc	1.66 ab
Vernal (VCC)[y]	2.28 abc	1.75 ab
Vernal[z]	2.35 abc	1.75 ab
Classic[z]	2.43 abc	1.83 ab
Raidor[z]	2.47 abc	2.08 ab
Marathon	2.50 abc	1.75 ab
Dekalb 120	2.50 abc	1.83 ab
Iroquois	2.50 abc	1.67 ab
Baker	2.50 abc	1.66 ab
Hi-phy[z]	2.60 abc	2.17 b
Peak	2.65 abc	1.83 ab
O's Gold Nordic Brand	2.68 abc	2.00 ab
NK 80-334	2.75 bcd	1.91 ab
Epic	2.75 bcd	2.08 ab
Weevlchek	2.75 bcd	1.50 a
O's Gold Nordic +3	2.78 bcd	1.58 ab
Pioneer 531	2.78 bcd	1.75 ab
NK 80-335	2.83 bcd	2.08 ab
Oneida	2.83 bcd	1.66 ab
Dekalb 130	2.85 bcd	1.91 ab
Riley	2.85 bcd	1.75 ab
WL-311	2.90 bcd	2.16 b
C/W-69	2.93 bcd	2.00 ab
Trident[z]	2.93 bcd	2.08 ab
Saranac AR[z]	2.93 bcd	1.83 ab
Hi-Ton 30	3.00 cde	1.83 ab
C/W-62	3.00 cde	1.92 ab
C/W-61	3.00 cde	1.58 ab
Pioneer 532	3.00 cde	2.00 ab
Pioneer 524	3.00 cde	1.83 ab
VR-50	3.00 cde	1.91 ab
WL-318	3.00 cde	2.08 ab
PH 2121	3.00 cde	1.75 ab
A-54	3.03 def	2.00 ab
WL-312	3.03 def	2.08 ab
WL-313[z]	3.08 efg	1.58 ab
WL-315	3.10 efg	1.92 ab
Apollo[z]	3.10 efg	2.08 ab
Cimarron	3.15 efg	2.00 ab
Armor	3.15 efg	1.91 ab
Vancor[z]	3.25 fg	1.92 ab
Duke	3.25 fg	1.92 ab
A-57	3.25 fg	2.00 ab
Arc	3.25 fg	1.92 ab
Vangard[z]	3.30 fg	1.58 ab
WL-221	3.33 g	1.83 ab

[v] Disease severity rated on a scale of 1–5, where 1 = none to trace, 2 = light, 3 = moderate, 4 = heavy, and 5 = severe. Each figure represents the mean of three subsamples in each of four replicates.
[w] Ratings made on 24 May 1983 and 24 May 1984.
[x] Means in a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[y] Seed obtained from Central Alfalfa Improvement Council.
[z] Cultivar represented at all locations.

Table 4. Severity of spring black stem on selected alfalfa cultivars in the greenhouse

Cultivar	Inoculated[w]				Uninoculated[w]			
	Disease severity rating[x]	Plant dry weight (g)[y]			Disease severity rating[x]	Plant dry weight (g)[y]		
		Harvest 1	Harvest 2	Total		Harvest 1	Harvest 2	Total
Saranac AR	2.67 a[z]	4.59 a	4.20 a	8.79 a	2.93 a	4.03 bc	3.11 bc	7.14 cd
Classic	3.33 ab	4.61 a	3.58 a	8.19 a	2.53 a	6.58 a	4.86 a	11.44 a
Hi-phy	3.40 b	3.49 ab	3.90 a	7.39 a	2.87 a	5.11 b	3.84 ab	8.95 bc
Vangard	3.53 bc	3.96 a	3.28 a	7.24 a	2.80 a	4.72 bc	5.10 a	9.82 ab
Vernal	4.13 c	2.37 b	1.56 b	3.93 b	3.67 b	3.52 c	1.94 c	5.46 d

[w] Plants inoculated with 6×10^6 spores per 10 plants. Uninoculated refers to plants that became infected through natural dissemination of inoculum in the greenhouse.
[x] Disease severity rated on a scale of 1–5, where 1 = none to trace, 2 = light, 3 = moderate, 4 = heavy, and 5 = severe.
[y] Dry weights are means of 15 replicates, each with 10 plants.
[z] Means in a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

plants showed symptoms somewhat earlier than uninoculated plants, by the conclusion of the experiment, plants in both inoculated and uninoculated treatments were infected. Overall, inoculation significantly increased DSR and reduced first-harvest yields.

The cultivar Vernal, classed resistant on the basis of field ratings, was most severely infected under the conditions imposed in the greenhouse, with the highest DSR and lowest yields (Table 4). Many plants of this cultivar were killed. Within the inoculated treatment, Saranac AR had the lowest numerical DSR; however, it was not significantly different from the mean rating for Classic.

DISCUSSION

Ratings of SBS severity indicate that differences exist between cultivars in degree of disease development in the field. Because all cultivars are infected to some extent and considerable variability exists in symptom development even within a single cultivar, SBS may be overlooked as an important yield-loss factor. However, analysis of data from nearly 1,600 individual observations indicates a substantial reduction in yield with increasing SBS severity.

Although a significant correlation between SBS severity and yield was obtained, the correlation coefficient (−0.57) indicates that other factors also contribute to yield differences. Susceptibility of a given cultivar to diseases other than SBS could add considerably to variation. For example, the cultivar Hi-phy is resistant to Phytophthora root rot, whereas Classic is susceptible to this disease. According to results of field ratings, the opposite is true for these cultivars with respect to SBS. Thus, when Phytophthora root rot is a factor, higher yields are likely with Hi-phy even though SBS severity may be greater.

Inherent yield potential may also conflict with an attempt to attribute yield-loss effects to SBS. SBS development is favored by the cool, humid microclimate that occurs under a dense alfalfa canopy. Rapidly growing (and thus high-yielding) cultivars that quickly develop a dense canopy may be more prone to infection by *P. m.* var. *medicaginis*. Our observations suggest that this phenomenon occurs frequently. The opposite effect of reduced SBS severity in thinned stands, which have greater light penetration and air circulation, may also be a confounding factor. Further studies under controlled conditions are needed to clarify the relationship between canopy density, SBS severity, and forage yield.

Earliness of spring growth may also affect SBS severity. Vernal and other hardy alfalfa cultivars may break dormancy as late as 7–10 days after Flemish types such as Saranac AR. This may provide a means for escape from early inoculations and thus may account for the low level of SBS observed in the field on Vernal.

Finally, cultivars with a large proportion of highly susceptible plants may show a reduction in the level of SBS severity over a period of several years. Susceptible plants are killed or weakened by *P. m.* var. *medicaginis* and gradually disappear from the stand, leaving only those with some degree of resistance. Conversely, some moderately resistant (or tolerant) cultivars may sustain constant or increasing levels of disease over the life of the stand.

Although greenhouse inoculations provided severely infected plants for cultivar evaluation, results were dissimilar to those predicted from field ratings. Several factors could have contributed to these differences. For example, plant age may have influenced disease severity. Plants used for the greenhouse study were 14 wk old at the time of inoculation, whereas plants evaluated in the field were at least 2 yr old. Also, the possible escape from early infection by the cultivar Vernal, as suggested before, could not occur in the greenhouse, where all cultivars were inoculated simultaneously.

Results of this study indicate that SBS is a important factor in alfalfa production in Ohio and that SBS resistance should be considered in programs of cultivar development.

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