

Assessing resistance to spring black stem and leaf spot of alfalfa caused by *Phoma* spp.

H. Wang¹, S. F. Hwang^{1,4}, K. F. Chang², B. D. Gossen³, G. D. Turnbull¹, and R. J. Howard²

¹Alberta Research Council, Vegreville, Alberta, Canada T9C 1T4; ²Crop Diversification Centre South, Brooks, Alberta, Canada T1R 1E6; ³Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, Saskatchewan, Canada S7N 0X2. Received 10 February 2003, accepted 3 September 2003.

Wang, H., Hwang, S. F., Chang, K. F., Gossen, B. D., Turnbull, G. D. and Howard, R. J. 2004. **Assessing resistance to spring black stem and leaf spot of alfalfa caused by *Phoma* spp.** Can. J. Plant Sci. **84**: 311–317. The disease reaction of alfalfa (*Medicago sativa*) cultivars to spring black stem was evaluated in field trials and greenhouse experiments. In field trials, differences in cultivar reaction to leaf spot (predominantly spring black stem) were observed in 9 of 16 station years. The reaction of certain cultivars was consistent across most trials, but other cultivars were quite variable. Under controlled conditions, one isolate each of *Phoma sclerotoides* and *P. exigua* produced symptoms on alfalfa leaves that were similar to those caused by *P. medicaginis*. These results indicate that *P. medicaginis* is not the only pathogen responsible for symptoms of spring black stem on alfalfa in the prairie region. In a detached-leaf study, one isolate each of *P. medicaginis*, *P. sclerotoides* and *P. exigua* produced leaf lesions on all 18 alfalfa cultivars assessed. Disease incidence in Absolute, Algonquin, Pickseed 3006 and Anik (*M. sativa* subsp. *falcata*) was lower than in 630 and AC Blue J. Inoculation of eight selected cultivars using a range of spore concentrations under controlled conditions showed a similar pattern; all three isolates produced leaf lesions on all eight cultivars. Inoculation with conidial suspensions of *P. medicaginis* resulted in a lower disease incidence on Absolute than on Beaver.

Key words: *Medicago sativa*, *Medicago sativa* subsp. *falcata*, *Phoma medicaginis*, *P. sclerotoides*, *P. exigua*, detached leaves.

Wang, H., Hwang, S. F., Chang, K. F., Gossen, B. D., Turnbull, G. D. et Howard, R. J. 2004. **Résistance à la tige noire printanière et à la tache des feuilles induite par *Phoma* sp. chez la luzerne.** Can. J. Plant Sci. **84**: 311–317. Les auteurs ont évalué la résistance des cultivars de luzerne (*Medicago sativa*) à la tige noire printanière dans le cadre d'essais aux champs et en serre. Dans le premier cas, les cultivars n'ont pas réagi de la même façon à la tache des feuilles (surtout la tige noire printanière) neuf années-stations sur seize. Si la réaction de certaines variétés était uniforme dans la majorité des essais, elle variait considérablement dans d'autres cas. En milieu contrôlé, un isolat de *Phoma sclerotoides* et de *P. exigua* présentaient chacun des symptômes similaires à ceux de *P. medicaginis* sur les feuilles de luzerne. On en déduit que *P. medicaginis* n'est pas le seul pathogène à l'origine de la tige noire printanière dans la région des Prairies. Lors d'une étude sur des feuilles détachées du plant, des isolats de *P. medicaginis*, de *P. sclerotoides* et de *P. exigua* ont tous produit des lésions sur les feuilles des 18 cultivars examinés. L'incidence de la maladie était plus faible chez les variétés Absolute, Algonquin, Pickseed 3006 et Anik (*M. sativa* ssp. *falcata*) que chez les cultivars 630 et AC Blue J. L'inoculation d'une série de concentrations de spores à huit cultivars dans des conditions contrôlées a donné des résultats analogues. Les trois isolats ont provoqué des lésions sur les feuilles des huit cultivars. L'inoculation d'une suspension de conidies de *P. medicaginis* a entraîné une plus faible incidence de la maladie chez Absolute que chez Beaver.

Mots clés: *Medicago sativa*, *Phoma medicaginis*, *P. sclerotoides*, *P. exigua*, feuilles détachées

Foliar diseases cause substantial yield and quality losses wherever alfalfa (*Medicago sativa* L.) is grown (Stuteville and Erwin 1990). Spring black stem is an important foliar disease in North America, and is the most common foliar disease of alfalfa on the Canadian prairies (Gossen and May 1996; Wang et al. 1999, 2000, 2002; Gossen et al. 2002). Symptoms of spring black stem are quite distinctive; small, dark purple lesions develop on leaves, crown buds, stems, flowers and pods. As the spots enlarge and coalesce, affected leaves turn yellow and abscise prematurely. Stem lesions may coalesce to girdle and kill young stems.

The causal agent of spring black stem is *Phoma medicaginis* Malbr. & Roum. in Roum. (syn. *P. herbarum*

Westend. var. *medicaginis* Fuckel). The only other species of *Phoma* known to attack alfalfa in this region is *Phoma sclerotoides* G. Preuss ex Sacc. (syn. *Plenodomus meliloti* Markova-Letova), which causes brown root rot (Berkenkamp et al. 1991; Hwang and Flores 1987; Gossen 1989, 1990). However, isolations from alfalfa stems and leaves often yield other *Phoma* spp. (Gossen unpublished), which were not considered to be pathogens.

Breeding for resistance to spring black stem has been undertaken (Elgin et al. 1988) and a standardized test has been developed for use under greenhouse conditions (Fox et al. 1992). Also, isolates of *P. medicaginis* differed in their ability to produce lesions or chlorosis on detached leaves and petioles (Gray et al. 1990). However, information about the cultivar reactions to spring black stem is limited. Rhodes and Myers (1986) reported variation in disease severity among 10 alfalfa cultivars in Ohio, but Gray (1983) found

⁴To whom correspondence should be addressed (e-mail: hwang@arc.ab.ca).

Table 1. Foliar disease severity (predominantly spring black stem) in selected alfalfa cultivars assessed in 1993 and 1994, in trials seeded at Saskatoon, SK in 1989, 1990 and 1991, and at Outlook, SK in 1987 and 1990

Cultivar	1990 test			Overall mean	1987 test	1989 test	1991 test
	Saskatoon		Outlook		Outlook	Saskatoon	Saskatoon
	1993	1994	1994		1994	1993	1994
OAC Minto	66ab	48a	30ab	48	33a	17b	
Peace	74a	26b	38a	46			27bc
Spredor II	66ab	30b	38a	45	16b		15c
Beaver	61abcd	38ab	35ab	45	21ab	13b	28a
Impact	58abcd	31b	34ab	41		17b	
Multileaf	61abcd	23b	38a	41			
Anchor	60abcd	28b	26ab	38	19ab	15b	20abc
Vernal	66ab	24b	23b	38	28ab	12b	16bc
Rangelander	58abcd	26b	26ab	37			
Trumpetor	44def	30b	30ab	35			23bcd
Heinrichs	47cdef	26b	28ab	34			20abc
Apica	39ef	24b	30ab	31	19ab		22abc
Rambler	35f	24b	28ab	29	19ab	7b	16bc
Sure	35f	23b	28ab	29			
Barrier					23ab	29a	23bcd

a-c Means in a column followed by the same letter do not differ based on Duncan's multiple range test at $P \leq 0.05$.

no differences among 11 cultivars in Wyoming. Harding (1972) reported differences in reaction to spring black stem among cultivars in a small study in Saskatchewan, but no detailed studies have been made on the reactions of alfalfa cultivars adapted to the Canadian prairies.

Studies were initiated to investigate the economic impact and management of foliar diseases on forage and seed alfalfa. The current report focuses on: (i) cultivar reaction to spring black stem in field trials, (ii) disease reaction of 18 cultivars to three *Phoma* spp. under controlled conditions, and (iii) the effect of spore concentration on symptom development of foliar diseases caused by *Phoma* spp. in greenhouse tests.

MATERIALS AND METHODS

Cultivar Reaction in the Field

Tests were established under irrigation at Outlook, SK in 1987, 1989, and 1990 (two tests) and under dryland conditions at Saskatoon, SK in 1989, 1990 (two tests), and 1991, with 20–30 genotypes in each test. The tests were laid out in a randomised complete block design with five replicates. Each plot was seeded at a rate of 2 kg ha⁻¹ and consisted of four rows, 6-m long, with 0.3-m row spacing. Forage harvests were made at 10% bloom. For more detailed information on the cultivars, methods, and production data, see Gossen et al. (1993). In 1993 and 1994, disease assessments were made just prior to the second harvest (late July to early August). Whole-plot disease ratings were made at the middle height of the canopy using the Horsfall-Barrett scale (0 = no disease, 11 = all foliage affected) and converted to percentage leaf area affected (Horsfall and Barrett 1945). Pathogen identification was based on visual examination of symptoms, with occasional isolation on media to confirm pathogen identification. In all, 16 test years of data were collected. Six of the studies assessed alfalfa genotypes prior to registration as cultivars (tests seeded in different years included different lines), and two of the studies (seeded at Saskatoon and Outlook in 1990) assessed the performance of cultivars that were registered in Canada at that time.

Cultivar Reaction Under Controlled Conditions

Three *Phoma* isolates were used in the study: an isolate of *P. medicaginis* from an infected alfalfa leaf collected in the Peace River region of Alberta in 1999, one of *P. sclerotoides* isolated from an infected alfalfa leaf from the Peace River region in 1998, and one of *Phoma exigua* Desm. (syn. *P. solanicola* Prill. & Delacr.) isolated from an infected alfalfa leaf collected near Camrose, AB in 2000. Cultures were single-spored and grown on potato dextrose agar (PDA) at 20–25°C under a 9-h photoperiod for 2 wk. Detached-leaf assays were used to assess the pathogenicity of the three *Phoma* spp. on alfalfa seedlings grown under greenhouse conditions. Eighteen alfalfa cultivars were assessed; 13 cultivars with standard root morphology (tap-root): Absolute, AC Blue J, Algonquin, Anchor, Arrow, Beaver, Peace, Pickseed 3003, Proleaf, Saranac, Ultrastar, 630 and 20-20; four creeping-rooted cultivars: Drylander, Heinrichs, Prowler I and Runner; and Anik, a cultivar of *M. sativa* subsp. *falcata*. Each isolate × cultivar combination was assessed in an individual humidity chamber, constructed using two layers of damp filter paper at the bottom of a 10-cm diameter Petri dish, covered by a pre-sterilized metal screen. Petri dishes were incubated in the chamber at 15–20°C under fluorescent growth lights (230 μE m⁻² s⁻¹ intensity) with a 12-h photoperiod. In each chamber, two compound leaves from 3–4-wk-old seedlings were placed on the screen. Mycelial disks were cut from the margin of 2-wk-old cultures using a 5-mm cork borer. Two agar disks (5-mm diameter) from a 2-wk-old culture were placed on each compound leaf. Lesion development was monitored daily, and lesion diameters were measured 4, 8 and 12 d after inoculation. There were three replicates per test and the assay was repeated once.

Spore Concentration

The impact of spore concentration of the three *Phoma* spp. on detached leaves of Absolute, Algonquin and Peace was assessed in humidity chambers (described previously). *Phoma* isolates were grown as described above, and spore suspensions were made by adding an aliquot of sterile water

Table 2. Foliar disease severity (primarily spring black stem) in 1993 and 1994 at Saskatoon, SK, and Outlook, SK, in regional adaptation tests of registered cultivars, established in 1990

Cultivar	Saskatoon		Outlook		Overall mean
	1993	1994	1993	1994	
OAC Minto	48a	43a	23a	19ab	29
Heinrichs	30bcde	35ab	34a	13ab	28
Algonquin	42ab	39ab	26a	15ab	27
89-03-06	33bcd	30abc	33a	19ab	27
Anchor	32bcd	30abc	22a	22a	25
Beaver	37abc	30abc	24a	22a	25
Champ	15fgh	30abc	27a	15ab	24
Vernal	28bcdef	23bcd	29a	19ab	24
Citation	30bcde	31abc	21a	13ab	23
Arrow	23defgh	31abc	24a	13ab	23
Maxim	24defg	26abcd	21a	23a	23
Peace	25defgh	34abc	23a	13ab	23
Rambler	34abcd	28abc	26a	15ab	23
Edge	23defgh	30abc	20a	15ab	22
Grimm	28cdef	30abc	22a	13ab	22
Sparta	39abc	34abc	19a	11b	21
Trumpetor	26cdefg	30abc	21a	13ab	21
Rangelander	28bcdef	26abcd	24a	9b	20
VW-34-2	18efgh	24bcd	23a	13ab	20
LSP	13gh	24bcd	22a	11b	19
Apica	23defgh	26abcd	21a	11b	19
Barrier	24defg	17cd	24a	15ab	19
Excalibur	21defgh	26abcd	17a	13ab	19
HSP	21defgh	26abcd	19a	13ab	19
Anik	9h	11d	26a	15ab	17

a-h Means in a column followed by the same letter do not differ based on Duncan's multiple range test at $P \leq 0.05$.

to a culture and rubbing the surface of the culture with a glass rod to release the conidia. The suspension was filtered through two layers of cheesecloth to remove mycelial fragments. Conidial concentration was quantified using a hemacytometer, then adjusted to 10^4 , 10^5 , 10^6 or 10^7 conidia mL^{-1} by adding sterile distilled water plus 0.5% (vol/vol) Tween 80 (polyoxyethylene sorbitan monooleate) (EM Science, Gibbstown, NJ). Spores were inoculated onto detached leaves in a chamber using an H-set airbrush (Paasche Airbrush Company, Harwood Heights, IL) at 100 kPa.

The number of viable spores that landed on leaves was estimated by spraying the same volume of each suspension onto dishes of water agar. Spore germination was determined for each isolate by counting the number of germinating spores in four replications of 100 spores each, using a compound microscope. Germ tube length was determined by measuring 10 germ tubes in each of six replications. Spore germination and germ tube length were recorded after 12, 24 and 36 h of incubation.

The impact of spore concentration on the efficacy of inoculation with *P. medicaginis* was assessed in more detail on seedlings of Absolute, Algonquin, Anchor, Beaver, Heinrichs, Peace, Ultrastar and 20–20. Five to ten seeds were planted in a soil mix (1:1, loam:peat moss) in 350-mL pots (8-cm diameter foam cups). After emergence, each pot was thinned to 3–5 seedlings, and maintained in a greenhouse (15–25°C, $270 \mu\text{E m}^{-2} \text{s}^{-1}$ light intensity and 12-h photoperiod) for 3 wk. Spore suspensions of *P. medicaginis* at 10^4 , 10^5 , 10^6 and 10^7 conidia mL^{-1} plus 0.5% Tween 80 were prepared. Seedlings were inoculated with 0.75 mL of suspension per pot, applied with an airbrush. All the seedlings

inoculated with the same spore concentration were incubated together in a $145 \times 85 \times 90$ cm moist chamber, enclosed with polyethylene film for 2 mo. Spring black stem development was assessed at 15-d intervals. Assessments of disease incidence and severity (percentage leaf area affected) were made on 20 leaves from the upper portion of the plant and 20 lower leaves in each plot, using assessment diagrams for spring black stem (James 1971). The percentage leaf area with lesions was recorded as 1, 5, 20, 50, 70 or 80% based on these diagrams, and mean severity was calculated for each rating date. There were five replicates of each cultivar \times concentration combination, and the study was repeated.

Data Analysis

The disease reaction of cultivars in each field trial was assessed using analysis of variance (SAS Institute, Inc., Cary, NC), and differences among means were assessed using Duncan's multiple range test at $P \leq 0.05$ if the main effects were significant. Data in the controlled environment trials were analysed using the mixed model procedure in analysis of variance. The Satterthwaite option was applied to determine the correct degrees of freedom for each estimate in the mixed model. Treatment means were compared using Tukey's Honestly Significant Difference (HSD) test when main effects were significant ($P \leq 0.05$). Prior to analysis, a $\log(X + 1)$ transformation was applied to the data from the excised leaf assay to improve homogeneity of variance, but the untransformed means are used in the tables for ease of presentation. The relationships between spore concentration and disease level in the detached-leaf study were assessed using linear regression.

Table 3. Mean lesion size on detached leaves of 18 alfalfa cultivars 8 d after inoculation with 3 *Phoma* spp. ($n = 6$)

Cultivar	Lesion size (mm diameter)			Overall mean
	<i>P. medicaginis</i>	<i>P. sclerotoides</i>	<i>P. exigua</i>	
<i>Tap-rooted</i>				
Absolute	2.5c	2.3bcd	3.3d	2.7
Algonquin	2.7c	3.1abcd	2.9d	2.9
Pickseed 3006	2.9c	2.2cd	3.8cd	4.0
Peace	6.7ab	3.7abcd	1.8d	3.6
Saranac	3.7bc	4.1abcd	4.3cd	4.2
Arrow	4.4bc	4.8abc	4.3cd	4.8
Proleaf	5.7ab	4.9abc	3.5cd	4.8
Anchor	4.3bc	5.0abc	5.8abc	5.2
20-20	6.0ab	4.7abcd	4.4cd	5.4
Ultrastar	5.8ab	5.5ab	5.6abc	5.6
Beaver	5.3bc	5.5ab	6.4ab	5.9
AC Blue J	7.6ab	5.5ab	6.3ab	6.5
630	8.2a	6.2a	7.3a	6.9
<i>Creeping-rooted</i>				
Drylander	5.1bc	5.4abc	6.1ab	5.4
Heinrichs	5.1bc	5.1abc	6.4ab	5.4
Prowler I	6.8ab	4.2abcd	6.0abc	5.6
Runner	6.9ab	5.8a	4.9bcd	5.8
<i>Medicago sativa</i> subsp. <i>falcata</i>				
Anik	3.2c	1.4d	3.3d	2.5
SE	0.53	0.54	0.37	

a-d Means in a column followed by the same letter do not differ based on Tukey's HDS test at $P \leq 0.05$. Data were transformed for analysis, but unadjusted data presented.

RESULTS

Cultivar Reaction in the Field

In the six alfalfa genotype tests, analyses of variance identified differences ($P \leq 0.05$) among the alfalfa lines in 6 of 12 site-years in ratings taken in 1993–1994. Data are presented only for cultivars included in at least three of the six assessments where differences were identified (Table 1). In the two tests of registered cultivars (seeded in 1990), there were differences among the cultivars in 3 of 4 site years over the same period (Table 2). At Saskatoon, spring black stem was by far the dominant disease, and almost all of the lesions were caused by this disease. At Outlook, the disease complex was dominated by spring black stem, but common leaf spot [*Pseudopeziza medicaginis* (Lib.) Sacc.] and other pathogens were present and may have confounded some of the assessments.

Several cultivars exhibited a consistent reaction across most sites and years. For example, spring black stem severity was consistently high in OAC Minto, lower in Apica and very low in Anik (*M. sativa* subsp. *falcata*) with the exception at Outlook in 1993 and 1994 (Tables 1 and 2). However, other lines did not exhibit such a consistent pattern. For example, Peace and Rambler are widely separated in the Saskatoon 1990 test assessed in 1993 in Table 1 (high and low severity, respectively), but occur together (moderate severity) in all other tests (Tables 1 and 2).

Cultivar Reaction under Controlled Conditions

Results from the two repetitions were pooled because an analysis of variance of the combined data from both tests did not detect a significant cultivar \times isolate \times test interac-

tion. Cultivar reactions to the three *Phoma* spp. are presented separately because there was an interaction ($P \leq 0.05$) between cultivar and *Phoma* spp. (Table 3). All three *Phoma* spp. were pathogenic on detached alfalfa leaves and induced lesions with similar symptoms. The cultivars Absolute, Algonquin, Pickseed 3006 and Anik (*M. sativa* subsp. *falcata*) exhibited some resistance to all three isolates, based on smaller lesion size. AC Blue J and 630 were highly susceptible to all three isolates and consistently developed large lesions. Peace was resistant to *P. exigua*, but susceptible to *P. medicaginis*. Ultrastar and Runner were susceptible to *P. sclerotoides* and *P. medicaginis*. Lesion size increased ($P \leq 0.05$) with increasing incubation period; mean lesion size across the three *Phoma* isolates was 1.9 ± 1.1 mm at 4 d, 4.8 ± 2.5 mm at 8 d and 7.6 ± 2.7 mm at 12 d after inoculation.

Spore Concentration

In the detached-leaf assay, conidia on water agar plates were counted for each isolate \times concentration combination. Spore density was 0.2 conidia mm^{-2} at 10^4 conidia mL^{-1} , $2\text{--}4$ conidia mm^{-2} at 10^5 , $27\text{--}34$ conidia mm^{-2} at 10^6 , and $104\text{--}154$ conidia mm^{-2} at 10^7 . Spore germination was over 90% for all three isolates after 24 h of incubation. Germ tubes were longer ($P \leq 0.05$) at 24 h of incubation in *P. sclerotoides* (mean of 50 ± 14.9 μM) than in *P. medicaginis* (37 ± 12.5 μM) or *P. exigua* (25 ± 11.8 μM). The period of incubation increased germ tube length, but did not affect germination frequency. In the assay, similar small, dark purple lesions were observed on leaves inoculated with each of the three *Phoma* spp. Symptom incidence ($P \leq 0.05$) and severity ($P = 0.002$) increased as the spore

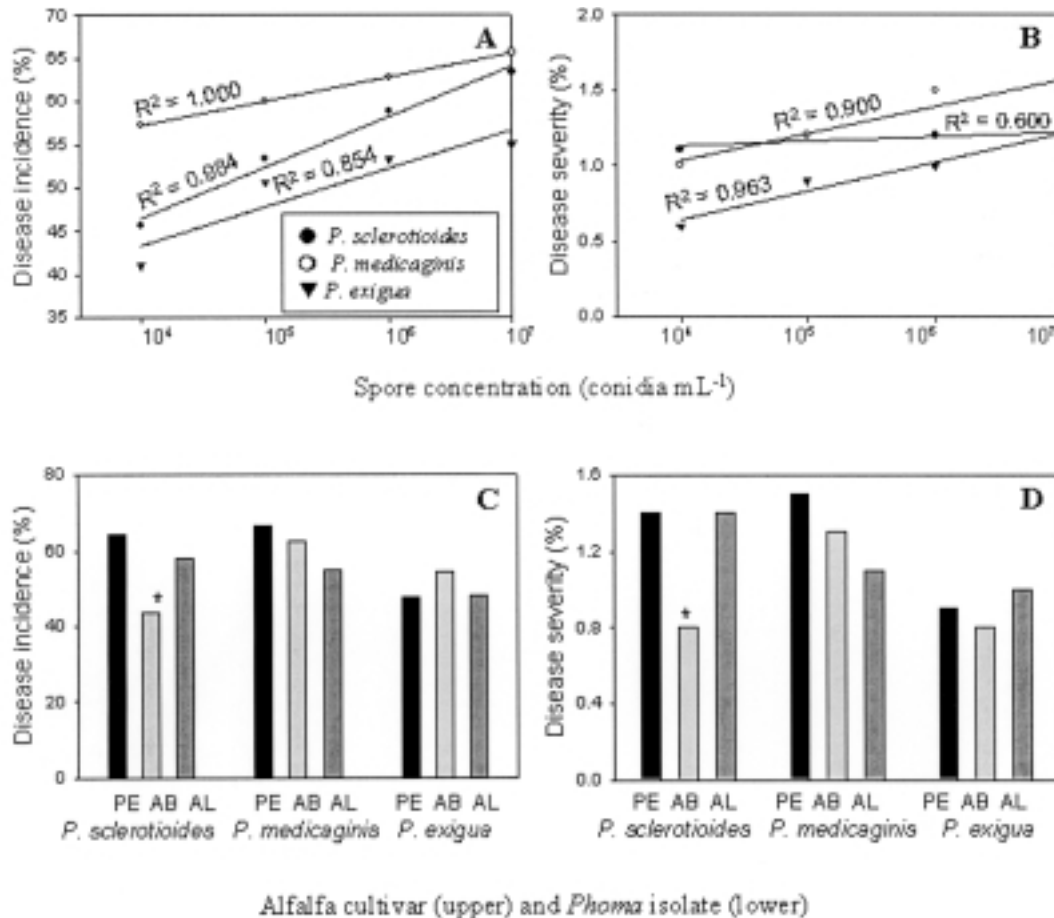


Fig. 1. Reaction of detached leaves of 3 alfalfa cultivars [Absolute (AB), Algonquin (AL), Peace (PE)] to inoculation with conidial suspensions of three *Phoma* spp. at various concentrations. Data were combined across cultivars to show the impact of spore concentration on (a) disease incidence and (b) disease severity, and across concentrations to show the interaction of *Phoma* isolate and cultivar on (c) disease incidence and (d) disease severity. (*Different at $P \leq 0.05$, $n = 5$ for each isolate \times concentration \times cultivar combination.)

concentration increased (Figs. 1a and 1b) for all three *Phoma* species. There were differences among pathogen species ($P \leq 0.001$) for both disease incidence and severity, and there was an isolate \times concentration interaction ($P \leq 0.001$) for both disease incidence and severity. Inoculation with *P. medicaginis* generally produced more disease incidence than *P. sclerotoides* ($P \leq 0.03$), and *P. exigua* produced the lowest disease incidence and severity ($P \leq 0.001$) compared with *P. medicaginis*, and significantly low disease severity ($P \leq 0.001$) compared with *P. sclerotoides*. When assessed across the three *Phoma* spp., both disease incidence and severity differed among cultivars ($P \leq 0.01$), and there was a cultivar \times isolate interaction ($P \leq 0.001$). There were no differences among cultivars in the mean response to *P. medicaginis* or *P. exigua* (Figs. 1c and 1d), but Absolute had lower disease incidence and severity ($P \leq 0.001$) than Algonquin and Peace when inoculated with *P. sclerotoides*. No interaction was observed between cultivar and spore concentration.

In the seedling inoculation study, disease incidence and severity increased over time for all eight cultivars, and all inoculation concentrations showed a similar trend consequently, so only one rating (30 d after inoculation) is presented in Table 4.

The results from the two repeated experiments were combined since there was no significant interaction between cultivar and experiment. There was no stem infection observed, and differences among cultivars were smaller than in the detached-leaf assay. Seedlings of 'Beaver' exhibited the highest disease incidence (60%) and Absolute the lowest (53%), but there were no differences in severity among cultivars. As in the detached-leaf assay, symptom expression increased ($P \leq 0.001$) with inoculum concentration. The highest inoculum concentration (10^7 conidia mL⁻¹) resulted in 66% disease incidence, but mean severity was low (6.7%). Also, disease incidence and severity were higher ($P \leq 0.001$) on the lower leaves than the upper leaves (Table 4). There was no interaction between cultivar and spore concentration in analysis of variance.

DISCUSSION

Observations of disease reactions among cultivars under field conditions supported previous reports that some resistance to spring black stem is present in alfalfa. For example, Apica and Anik (*M. sativa* subsp. *falcata*) exhibited lower severity than 'OAC Minto' in most field tests, though some of them were not statistically significant. However, these

Table 4. Disease incidence and severity on leaves of eight alfalfa cultivars inoculated with *Phoma medicaginis* at various concentrations in a controlled environment and assessed 30 d after inoculation ($n = 5$)

Treatment	Disease incidence (%)	Disease severity (%)
<i>Cultivar</i>		
Absolute	53.2c	3.2a
Algonquin	54.5bc	3.7a
Heinrichs	56.0bc	4.1a
Anchor	56.0bc	3.4a
Peace	54.6bc	3.6a
Ultrastar	56.6b	3.9a
20–20	57.5ab	3.9a
Beaver	60.3a	4.0a
SE	2.66	0.73
<i>Inoculum concentration (conidia mL⁻¹)</i>		
10 ⁴	46.9d	1.5d
10 ⁵	54.3c	2.8c
10 ⁶	56.7b	3.9b
10 ⁷	66.4a	6.7a
SE	2.62	0.72
<i>Plant part</i>		
Upper leaves	45.4b	2.1b
Lower leaves	61.5a	5.4a
SE	2.60	0.71

a–c Means in a column and category followed by the same letter do not differ based on Tukey's HSD test at $P \leq 0.05$.

studies also highlighted the problems inherent in field tests to assess cultivar reaction to spring black stem, including highly variable levels of disease, inconsistencies among tests (even at the same test site), and the confounding effects of other foliar pathogens. Since leaf spot diseases on alfalfa generally occur as part of a disease complex, and the relative importance of the components of the complex is associated with changing weather conditions over time, so the presence of several pathogens represents the most intractable problem associated with field testing. These problems prompted a search for techniques that would allow rapid and consistent assessment of differences in cultivar reaction under controlled conditions.

Under controlled environment conditions, leaf spot symptoms were visible at 7–10 d after inoculation on all alfalfa cultivars inoculated with spore suspensions of *P. sclerotoides*, *P. medicaginis*, or *P. exigua*. The symptoms were small, dark purple to black spots on the leaves. There were no visible differences in symptoms produced on leaves by the three species. We conclude that more than one *Phoma* species can cause the leaf spot phase of spring black stem, which has previously been solely attributed to *P. medicaginis*. *Phoma sclerotoides* causes brown root rot of alfalfa (Stuteville and Erwin 1990), but these results indicated that it also causes foliar disease symptoms that are very similar to those of *P. medicaginis*. The foliar phase of this disease may be important for long distance spread of the pathogen, because infected leaflets are much more likely to be moved (i.e., carried by wind, in hay, etc.) to new locations than are infected roots. This may be one of the factors that contributed to the introduction of brown root rot into the high mountain areas of Wyoming in recent years (Hollingsworth et al. 2003).

This study is the first to report *P. exigua* on alfalfa. *Phoma exigua* is a soil-borne fungus that is weakly pathogenic, primarily on dicotyledonous plants (Morgan-Jones and Burch 1988). Various foliar diseases caused by *P. exigua* have been reported in recent years, such as crown necrosis on yellow starthistle (*Centaurea solstitialis* L.) in Russia (Widmer et al. 2002), foliar necrosis on dandelion (*Taraxacum officinale* F. H. Wigg.) in Canada (Brebaum and Boland 1999), and stem canker on cotton (*Gossypium hirsutum* L.) in the United States (Koenning et al. 2000).

No stem infections were observed in the greenhouse trials, despite the fact that infected plants were retained for 2 mo after inoculation. Also, disease severity levels on leaves were low, even where disease incidence was high. A previous study reported that isolates of *P. medicaginis* differ in aggressiveness (Gray et al. 1990). It is possible that the isolates employed in this study were not highly aggressive, but more work is required to assess a wider range of isolates from the three species.

Differences in resistance among cultivars to spring black stem (Harding 1972; Rhodes and Myers 1986; Fox et al. 1992) and brown root rot (Berkenkamp et al. 1991) have previously been reported in alfalfa. No highly resistant lines are known, but the resistance that is available is moderately heritable (Elgin et al. 1988). In the present study, a detached-leaf assay identified resistance in several alfalfa cultivars that are adapted to Canadian conditions, such as Absolute, Algonquin, Pickseed 3006 and Anik (*M. sativa* subsp. *falcata*). The results from Anik are consistent with those from other studies, in which lines with *M. sativa* subsp. *falcata* in their background carry improved resistance to a range of diseases (Wilcoxson et al. 1977; Gossen 1998; Gossen et al. 1992).

In the moist chamber study, disease severity on the inoculated seedlings was relatively low, and differences were not as pronounced as in the detached-leaf study, but the performance of most cultivars from disease incidence was comparable with observations from the detached-leaf studies (Table 4). For instance, Beaver had a high incidence of disease in the moist chamber study and large lesions in the detached-leaf study, while Absolute exhibited low incidence and small lesions in these studies. However, Algonquin, which had relatively small disease lesions in the study using colonized agar discs, developed more disease following spore inoculation. The detached-leaf screening procedure needs to be evaluated against disease levels from natural epidemics under field conditions.

The seven cultivars that were evaluated in the field and, using detached leaves, a strong positive association was observed for five of these cultivars, i.e., Beaver, Anchor and Heinrichs displayed high disease levels in all trials, while Anik and Arrow had low levels of disease. The results for Peace and Algonquin were not as clear. Peace exhibited highly variable results, while Algonquin was relatively resistant to *Phoma* infection in the greenhouse study and susceptible in the field study. A previous study also reported that some cultivars that were susceptible under greenhouse conditions were quite resistant in the field (Rhodes and Myers 1986), which indicates that confirmation of con-

trolled environment assessments with field testing is particularly important in this host-pathogen system. However, inoculation of detached leaves using spore suspension or agar disk may develop into an economically feasible means to screen alfalfa cultivars for resistance to *Phoma* spp.

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