

Evidence for the heritability of resistance to brown root rot of alfalfa, caused by *Phoma sclerotoides*

C.R. Hollingsworth, F.A. Gray, and R.W. Groose

Abstract: Brown root rot (BRR) of alfalfa, caused by *Phoma sclerotoides*, a low-temperature pathogen, is associated with winterkill in the contiguous United States. Identified in Canada during the 1920s, BRR has been identified from legume roots in four western Canadian provinces. Some Canadian cultivars exhibit BRR resistance, although only one was consciously selected for the trait. Heritability of BRR resistance was evaluated in this study in which container-grown alfalfa plants were inoculated with *P. sclerotoides*. Plants that were evaluated are progeny (cycle 1) of one generation of selection and intermating of healthy plants drawn from six diverse North American alfalfa populations (five commercial cultivars, one experimental population) that survived exposure to *P. sclerotoides* soil infestation for several winters. These were evaluated together with plants grown from seed of the original unselected populations (cycle 0), and putative check cultivars 'Multi-plier' (BRR susceptible) and 'Peace' (BRR resistant). Plants were rated for disease severity for a total of 4 test years. Cycle 1 plants exhibited a reduced level of disease severity compared with cycle 0 plants ($P < 0.05$), indicating heritability of BRR resistance in alfalfa. Also, especially important for future alfalfa breeding efforts, this study demonstrates that 'Multi-plier' and 'Peace' are useful checks for screening BRR resistance.

Key words: *Phoma sclerotoides*, brown root rot of alfalfa, BRR, *Medicago sativa*, heritability.

Résumé : Causée par le *Phoma sclerotoides*, un champignon pathogène frigophile, la pourriture brune de la luzerne (PB) est associée à la mortalité hivernale dans la partie limitrophe des États-Unis. Identifiée au Canada dans les années 1920, la PB a été identifiée sur des racines de légumineuses dans quatre provinces de l'Ouest canadien. Quelques cultivars canadiens possèdent de la résistance à la PB, quoique seulement un fut intentionnellement sélectionné pour ce caractère. L'héritabilité de la résistance à la PB fut évaluée dans la présente étude au cours de laquelle de la luzerne en contenants fut inoculée avec le *P. sclerotoides*. Les plantes qui furent évaluées sont la descendance (cycle 1) d'une génération de sélection et d'un croisement entre les plantes saines issues de six différentes populations nord-américaines de luzerne (cinq cultivars commerciaux, une population expérimentale) et qui ont survécu à une exposition à un sol infecté par le *P. sclerotoides* pendant plusieurs hivers. Ces populations furent évaluées conjointement avec des plantes issues de graines de la population originale non sélectionnée (cycle 0) et avec les cultivars témoins putatifs 'Multi-plier' (sensible à la PB) et 'Peace' (résistant à la PB). Les plantes furent classées selon l'intensité de la maladie lors des 4 années de l'expérience. Le degré de maladie visible était plus faible chez les plantes du cycle 1 que chez les plantes du cycle 0 ($P < 0,05$), ce qui témoigne d'une héritabilité de la résistance à la PB chez la luzerne. De plus, ce qui est particulièrement important pour les futurs efforts d'amélioration de la luzerne, la présente étude démontre que 'Multi-plier' et 'Peace' sont des témoins utiles pour la sélection génétique pour la résistance à la PB.

Mots clés : *Phoma sclerotoides*, pourriture brune de la luzerne, PB, *Medicago sativa*, héritabilité.

Introduction

Brown root rot (BRR) of alfalfa (*Medicago sativa* L.), caused by *Phoma sclerotoides* G. Preuss ex Sacc. (syn. *Plenodomus meliloti* Dearn. & G.B. Sanford), was recently reported in Wyoming, Montana, and Idaho, USA (Mikkelsen 1997; Hollingsworth and Gray 1999; Larsen et al. 2002; Hollingsworth et al. 2002, 2003).

Known in Canada since the 1920s, the pathogen causes rot on roots of susceptible forage legumes (*Trifolium* spp., *Melilotus* spp., and *Medicago sativa*) and eventually severs taproots (Sanford 1933; Davidson 1990). Brown root rot is

Accepted 8 December 2004.

C.R. Hollingsworth,^{1,2} F.A. Gray, and R.W. Groose. Plant Sciences, Department 3354, University of Wyoming, 1000 East University Avenue, Laramie, WY 82071, USA.

¹Corresponding author (e-mail: holli030@umn.edu).

²Present address: University of Minnesota, Northwest Research and Outreach Center and Department of Plant Pathology, 2900 University Avenue, Crookston, MN 56716, USA.

associated with widespread winter mortality of plants in established alfalfa stands. The fungus is active during winter months but tends towards dormancy during summer. Sanford (1933) identified *in vitro* minimum, optimum, and maximum temperatures for fungal growth of 0, 15–16, and 27 °C, respectively. He postulated that *P. sclerotioides* is an abundant native saprobe in Canadian prairies that becomes opportunistically pathogenic to susceptible forage legumes on cultivated land. Our surveys (op. cit.) suggest that this may be true for alfalfa in the Intermountain West and perhaps across the upper tier of the United States.

Current BRR disease control strategies are mostly limited to proper management of the alfalfa stand to minimize winter stress and crop rotation with spring-sown small grains to reduce inoculum density. Choice of alfalfa cultivar may also reduce disease severity, because some Canadian cultivars exhibit BRR resistance and, in Canada, forage yield may be related to BRR resistance (Tsukamoto 1965; Berkenkamp et al. 1991). However, Canadian cultivars are strongly fall dormant as an adaptation to short growing seasons. Because BRR has become more widely identified in North America, alfalfa producers need cultivars with BRR resistance in higher yielding germplasm adapted to longer growing seasons.

The objective of this research was to determine whether resistance to BRR is a heritable trait. Disease severity responses of progeny of plants from six diverse alfalfa populations exposed to *P. sclerotioides* in the field for 6 years were compared with plants grown from remnant seed of the same six original populations that were maintained in storage.

Materials and methods

On 12 October 1998, 20 mature, healthy-appearing 6-year-old alfalfa plants from each of six entries ('Alpine', 'Heinrichs', 'OK49', 'Ranger', SC3801, and 'Webfoot MPR' (120 plants)) were dug from a BRR-infested field located in Sweetwater County in southwestern Wyoming. A University of Wyoming alfalfa forage yield trial had been established within that field in July 1992, which, at planting, included 15 entries: 11 cultivars (9 US, and 2 Canada) and 4 experimental lines (2 US, and 2 Canada), ranging from very fall dormant to moderately fall dormant types (fall dormancy categories 1–5). The six entries chosen for the present study represent the full range of fall dormancy categories included in the original planting. They are also a well-differentiated cross section of six historic germplasms that have contributed to modern winterhardy, multiple-pest-resistant alfalfa cultivars (Table 1; Barnes et al. 1977; Lawrence and Irvine 1982; Caddel et al. 1992; Gray et al. 2004). Five entries are registered cultivars; one entry, SC3801, is an experimental line developed by Agriculture and Agri-Food Canada, Swift Current, Saskatchewan (P.G. Jefferson, personal communication).

Plants from the six entries were transplanted and maintained in the greenhouse at the University of Wyoming, Laramie, Wyoming (Albany County). In 1999, plants were hand-pollinated every 1 to 3 d. Plants were randomly mated within entries (e.g., 'Alpine' plants crossed only with other 'Alpine' plants, 'Heinrichs' plants crossed only with other

'Heinrichs' plants, and so on). An equal number of seed from each of the 20 plants within each entry were bulked to produce six different cycle 1 (C1) seed lots.

In addition to C1 plants, experiments included six cycle 0 (C0) entries (Table 1), and the cultivars 'Peace' and 'Multiplier' as, respectively, BRR-resistant and BRR-susceptible checks. 'Peace' was developed at Fort Vermillion, Alberta, in soils where *P. sclerotioides* is common and has exhibited a high level of BRR resistance in Canada (McKenzie et al. 1981; Berkenkamp et al. 1991). 'Multiplier' is the cultivar in which BRR was first observed in the lower US in 1996 in a severely decimated stand near Farson, Wyoming (Hollingsworth and Gray 1999).

Disease response experiment 1 (1999–2001)

On 1 September 1999, seed from C0 and C1 entries, 'Peace', and 'Multiplier' were hand scarified and placed on moistened Whatman® No. 4 filter paper circles in covered glass Petri dishes to germinate. Dishes were placed in an environmental chamber maintained at 22 °C without light. Water was added as needed to keep filter paper moistened.

Jiffy-7® (Jiffy Products of America, Inc., Norwalk, Ohio) pellets made from compressed sphagnum peat were floated on water at room temperature until fully imbibed and extended. Peat pots were then dipped into a Captan™ fungicide solution (4 g Captan™ 50% wettable powder in 1 L water). The fungicide treatment was used to restrict algal and fungal growth on pots during initial seedling establishment and growth.

When radicles were at least 1 cm in length, one seedling was transplanted into each peat pot. Plants were maintained within a growth chamber (22 °C under continuous light) and were rotated to different shelves daily to equalize light exposure. After 8 weeks, each pot was sprinkled with approximately 0.01 g of *Sinorhizobium meliloti* (*Rhizobium meliloti*) (Nitragin Company, Milwaukee, Wisconsin), placed into a Tall One Treepot™ (Stuewe and Sons, Inc., Corvallis, Oregon; volume, 3.79 L; depth, 36 cm) within a pasteurized soil mix (1:1, sand – native soil, v/v), and transferred to a greenhouse bench (21 °C, 15-h photoperiod maintained by artificial lighting).

On 6 December 1999, upper tap roots of 3-month-old plants were inoculated with barley grain infested with the 'Berg' Wyoming No. 2 isolate of *P. sclerotioides* (American Type Culture Collection MYA-295). Soil and peat pot material were pulled away from one side of upper root systems. Two barley grain inocula were placed directly on each tap root approximately 2 cm below the soil surface. Inocula were held in place with a ball of cotton (Sanford 1933), and soil was replaced. Inoculated plants were then returned to the greenhouse bench. Viability of *P. sclerotioides* was confirmed by placing infested barley grain on full-strength Difco® potato dextrose agar in five Petri dishes (3 inocula/dish).

One week after inoculation, greenhouse temperatures were lowered and supplemental lighting was turned off to promote winter dormancy of plants. Plants were watered sparingly, and greenhouse temperatures were maintained at 16 °C day : 10 °C night for 2 weeks, then reduced to 10 °C day : 7 °C night for the following 2 weeks. Vegetative growth slowed as plants became more dormant. After 4 weeks, plant

Table 1. Percent contribution of six historic germplasms to six alfalfa entries evaluated for brown root rot resistance in the cycle 0 and cycle 1 generations in the present study.

Entry	Origin	FDC ^a	falcata	Ladak	varia	Turkistan	Flemish	Chilean
'Heinrichs'	Canada	1	25	16	54	5	—	—
SC3801	Canada	1	—	—	—	—	100	—
'Alpine'	USA	2	15	15	10	20	35	5
'Ranger'	USA	3	—	10	45	45	—	—
'Webfoot.MPR'	USA	4	10	8	45	6	30	1
'OK49' ^b	USA	5	—	—	—	1	1	95

^aFall dormancy categories.^b3% germplasm unknown.

foliage was cut to approximately 4 cm above crowns. On 13 January, pots were placed outside on top of a debris-free soil surface in metal racks. Straw bales of winter wheat were pushed snugly against racks on all four sides of the test, and loose straw was forced into gaps around bales, racks, and pots to limit air circulation during winter.

Three stainless-steel dial thermometers with stem lengths of 15 cm were inserted into the soil of three pots at an approximate depth of 11 cm. Soil temperatures were recorded each morning. During their first 4 weeks outside, soil temperatures in pots averaged -2.9 °C, and ranged from 1 °C on 16 January to -11 °C on 1 February. Pots were covered with approximately 10 cm of snow 2 weeks after being placed outside (weather data presented in appendices to Hollingsworth 2002). Frequent snow events maintained a fairly complete soil cover for about 5 weeks.

Plants remained outside during the remainder of the experiment (during two winter seasons). Pots were watered daily as needed during spring, summer, and fall, and soil temperatures were recorded daily (data not shown). Forage was clipped at 10%–50% bloom three times in 2000 (16 June, 25 July, 22 August) and twice in 2001 (27 June, 3 August).

The experiment was a completely randomized design (CRD) with approximately 30 plants per entry. An analysis of variance was performed separately for each experiment year, and Fisher's protected LSD mean comparisons were used (PROC GLM, SAS Institute Inc. 1988). Orthogonal contrasts were conducted to determine individual and combined disease severity differences between C0 and C1 entries.

Root symptoms from all potted plants were rated once yearly (year 1, May 2000, plant age 8 months, 5 months postinoculation; year 2, June 2001, plant age, 21 months, 18 months postinoculation). Intact cotton was recovered from upper plant roots by sliding soil and root mass out of pot tops approximately 13 cm and gently working soil aggregates free from root tissues. Exposed roots were rinsed with tap water and visually examined for BRR symptoms. A disease severity rating (DSR) of 1–5 was assigned for each root system: 1, no disease symptoms; 2, slight root symptoms such as epidermal tissue discoloration; 3, moderate symptoms with defined lesions; 4, severe symptoms such as tissue rot; and 5, acute symptoms such as plant death. After rating root symptoms, recovered cotton pieces

and soil were replaced, and potted plants were maintained outside as before.

Disease response experiment 2 (2000–2002)

Alfalfa seed were prepared for germination on 27 June 2000; 2 months earlier than experiment 1. This was done so potted plants could be transferred outside to harden and become dormant naturally. Seed from the same entries (C0 and C1 populations, 'Peace' and 'Multi-plier') were germinated in Petri dishes as previously described.

Seedling growth medium was changed for experiment 2 because of difficulties in removing experiment 1 plant roots from the Jiffy-7[®] sphagnum peat material prior to inoculation. Instead, seedlings with a radicle growth of at least 1 cm were transplanted into a fungicide-free, pasteurized soil mixture (1:1, sand – Laramie native soil, v/v) contained within Ray Leach Cone-tainers[™] (Stuewe and Sons, Inc.; volume, 164 mL; diameter, 3.8 cm; depth, 21 cm). While transplanting, approximately 0.01 g *S. meliloti* was sprinkled onto seedling roots, and pots were placed on greenhouse benches (21 °C day : 15 °C night, natural photoperiod). After the first 2 weeks, plants were fertilized weekly in irrigation water (Peter's Professional General Purpose 20:20:20, The Scotts Co., Marysville, Ohio).

Six weeks later, approximately one seedling per entry was transplanted into a Tall One Treepot[™] (50 plants per entry total). Two barley grain inocula were placed directly on plant roots during transplanting. Approximately 675 plants were inoculated and afterward returned to greenhouse benches. Inocula viability was confirmed as previously described. After 2 weeks, on 28 August, plants were placed outside. A straw bale perimeter barrier was used to protect plants during the winter months as described earlier. Plants remained outside for the remainder of the experiment (two winter seasons) and were watered as needed. Forage was clipped at 10%–50% bloom, twice in 2001 (27 June, 3 August) and once in 2002 prior to experiment completion (19 June).

The experiment was a CRD with approximately 40 plants in each entry. Statistical analyses were as in experiment 1. Root symptoms from each plant were rated yearly (year 1, July 2001, plant age, 12 months, 11 months postinoculation; year 2, March 2002, plant age, 20 months, 19 months post-

Table 2. Yearly disease severity ratings (DSR) of brown root rot for cycle 0 (C0) and cycle 1 (C1) alfalfa entries.

Entry	DSR experiment 1		DSR experiment 2	
	2000	2001	2001	2002
'Ranger' _{C1}	2.3 ab	3.7 def	1.3 a	1.7 abc
'Peace' ^a	2.3 ab	2.8 ab	1.4 a	1.4 a
SC3801 _{C1}	2.9 cdef	2.9 abc	1.6 ab	1.7 ab
'Heinrichs' _{C0}	2.1 a	2.6 a	1.8 abcd	2.0 abcd
'Alpine' _{C1}	3.0 def	3.4 cde	1.9 bcde	1.9 abcde
SC3801 _{C0}	2.4 abc	3.4 bcd	2.0 bcdef	1.9 abcd
'Heinrichs' _{C1}	2.6 abcde	3.0 abc	2.1 bcdef	1.6 ab
'Webfoot.MPR' _{C1}	3.1 ef	3.7 def	2.2 cdef	2.1 cdef
'Ranger' _{C0}	2.5 abcd	3.8 def	2.2 cdef	2.1 bcde
'OK49' _{C1}	3.3 f	3.8 def	2.4 def	2.2 cde
'Webfoot.MPR' _{C0}	2.7 bcdef	4.0 ef	2.4 ef	2.5 ef
'Alpine' _{C0}	2.8 bcdef	4.3 f	2.4 ef	2.3 def
'OK49' _{C0}	3.0 cdef	4.2 f	2.4 ef	2.5 ef
'Multi-plier' ^a	2.5 abcd	4.0 ef	2.5 f	2.8 f
Mean	2.7	3.5	2.0	2.0
LSD _(0.05)	0.6	0.6	0.6	0.6

Note: Disease severity ratings: 1, no disease symptoms; 2, slight root symptoms such as epidermal tissue discoloration; 3, moderate symptoms with defined lesions; 4, severe symptoms such as tissue rot; 5, acute symptoms such as plant death. Means in the same columns followed by the same letter were not significantly different at $P = 0.05$ using Fisher's protected LSD mean comparisons.

^aCycle 0 plants from 'Peace' and 'Multi-plier' were used as the resistant and susceptible control cultivars, respectively.

inoculation) as previously described. Disease severity rating criteria remained the same across experiments and test years. Pot soil was replaced, and plants were maintained outside as before.

Results

Disease response experiment 1: year 1 (late-winter 2000)

Roots exhibited slight to severe disease symptoms (DSR 2–4), but 16 of 499 plants (3.2%) died from BRR (DSR 5; 2-'Alpine'_{C0}, 2-'Alpine'_{C1}, 4-'OK49'_{C0}, 3-'OK49'_{C1}, 1-'Ranger'_{C0}, 1-SC3801_{C0}, 2-'Webfoot.MPR'_{C0}, 1-'Heinrichs'_{C1}) during the first winter. Disease severity differences among entries were significant ($P = 0.0005$; Table 2). 'Peace', 'Multi-plier', 'Heinrichs'_{C0}, 'Ranger'_{C0}, SC3801_{C0}, 'Heinrichs'_{C1}, and 'Ranger'_{C1} had the least severe symptoms, while 'Alpine'_{C0}, 'OK49'_{C0}, 'Webfoot.MPR'_{C0}, 'Alpine'_{C1}, 'OK49'_{C1}, SC3801_{C1}, and 'Webfoot.MPR'_{C1} had the most severe symptoms. Disease severity results were similar between BRR-resistant 'Peace' and BRR-susceptible 'Multi-plier'.

Orthogonal contrasts determined that differences between SC3801_{C0} and SC3801_{C1} entries were significant ($P = 0.10$), and grouped C1 entries were more diseased than C0 entries ($P = 0.0117$).

Disease response experiment 1: year 2 (winter 2000–2001)

Surviving alfalfa plants from experiment 1 were again exposed to winter conditions in Laramie, Wyoming. Snow cover was established in late October, and periodic snowfall

events kept pots covered with snow for several months (data not shown).

Alfalfa roots exhibited increased disease severity after the second test year compared with the first (Table 2). An additional 143 plants died (DSR 5; 21-'Alpine'_{C0}, 7-'Alpine'_{C1}, 3-'Heinrichs'_{C0}, 4-'Heinrichs'_{C1}, 13-'OK49'_{C0}, 14-'OK49'_{C1}, 8-'Ranger'_{C0}, 13-'Ranger'_{C1}, 6-SC3801_{C0}, 11-SC3801_{C1}, 14-'Webfoot.MPR'_{C0}, 9-'Webfoot.MPR'_{C1}, 6-'Peace', 14-'Multi-plier'), bringing the 2-year total to 159 (32%). Disease severity differences among entries were significant ($P < 0.0001$); 'Peace', 'Heinrichs'_{C0}, 'Heinrichs'_{C1}, and SC3801_{C1} had the least severe symptoms, while 'Multi-plier', 'Alpine'_{C0}, 'OK49'_{C0}, 'Ranger'_{C0}, 'Webfoot.MPR'_{C0}, 'OK49'_{C1}, 'Ranger'_{C1}, and 'Webfoot.MPR'_{C1} had the most severe symptoms (Table 2). 'Multi-plier' exhibited significantly more disease symptoms than 'Peace'.

Orthogonal contrasts determined that differences between 'Alpine'_{C0} and 'Alpine'_{C1} entries were significant ($P = 0.01$), while contrasts from the five remaining entry pairs were not (Table 3). Unlike year 1, grouped C0 entries were significantly more diseased than grouped C1 entries ($P = 0.02$).

Disease response experiment 1: overall summary

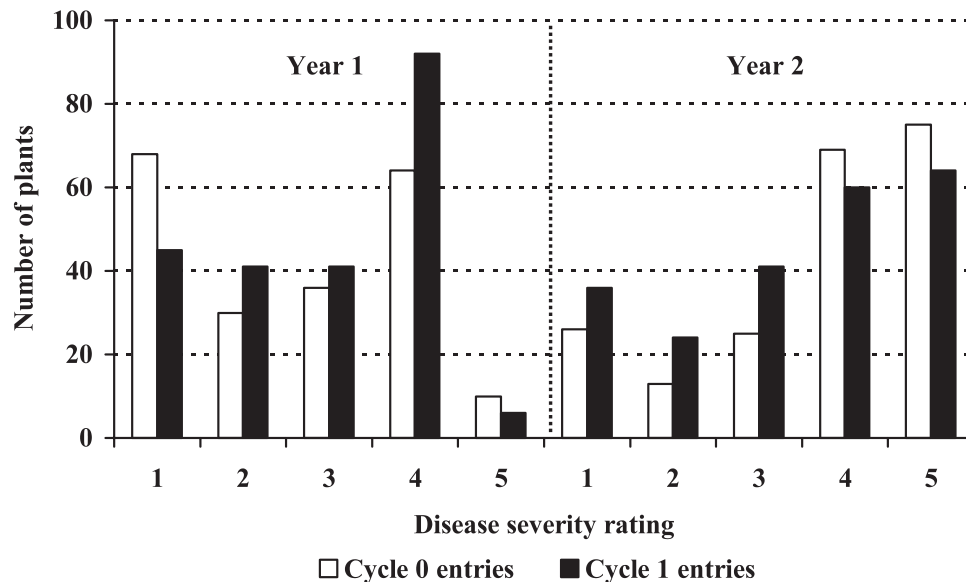
Brown root rot disease symptoms increased substantially during 2001 compared with 2000 (Fig. 1). Fewer second-year plants appeared healthy (DSR 1) compared with first-year disease severity ratings. Conversely, there were more C1 plants with slight to moderate symptoms (DSRs 2 and

Table 3. Percent increase (+), or decrease (–), in disease severity ratings of cycle 1 (C1) alfalfa entries compared with cycle 0 (C0) germplasm resulting from root inoculations of *Phoma sclerotioides*, causal organism of brown root rot.

Entry	Experiment 1		Experiment 2	
	Year 1, 2000	Year 2, 2001	Year 1, 2001	Year 2, 2002
'Alpine'	+7.5	–19.5***	–19.8*	–14.5
'Heinrichs'	+17.2	+11.2	+8.0	–21.3
'OK49'	+11.1	–9.1	–2.9	–9.7
'Ranger'	–7.5	–3.1	–40.6***	–19.9
SC3801	+19.4*	–13.4	–21.0	–11.2
'Webfoot.MPR'	+12.7	–8.3	–11.1	–16.2

Note: Orthogonal contrasts conducted on corresponding pairs of C0 and C1 entries across test years 1 and 2, where *, **, and *** denote significant differences in disease severity ratings at $P < 0.10$, $P < 0.05$, and $P < 0.01$, respectively.

Fig. 1. Disease severity ratings of alfalfa with brown root rot (caused by *Phoma sclerotioides*). First and second test year comparisons for experiment 1 (1999–2001) cycle 0 and cycle 1 entries. Disease severity ratings: 1, no disease symptoms; 2, slight root symptoms such as epidermal tissue discoloration; 3, moderate symptoms with defined lesions; 4, severe symptoms such as tissue rot; 5, acute symptoms such as plant death.



3) compared with C0 plants during both test years. The number of C1 plants with severe root symptoms (DSR 4) was especially large during the first test year, but was less than the C0 total after year 2. Few plants died (DSR 5) in test year 1, but many died during year 2. The overall disease trend was for increasing symptom severity throughout the 2-year period.

Disease response experiment 2: year 1 (winter 2000–2001)

Forty-four of 672 plants died (DSR 5) during the first winter (7%) from BRR (DSR 5; 6-‘Alpine’_{C0}, 1-‘Alpine’_{C1}, 5-‘Heinrichs’_{C0}, 2-‘Heinrichs’_{C1}, 4-‘OK49’_{C0}, 5-‘OK49’_{C1}, 6-‘Ranger’_{C0}, 2-SC3801_{C0}, 4-‘Webfoot.MPR’_{C0}, 3-‘Webfoot.MPR’_{C1}, 1-‘Peace’, 5-‘Multi-plier’). Disease severity differences among entries were significant ($P < 0.001$, Table 2). ‘Peace’, ‘Heinrichs’_{C0}, ‘Ranger’_{C1}, and SC3801_{C1} had the least severe symptoms, while ‘Multi-plier’, ‘Alpine’_{C0}, ‘OK49’_{C0},

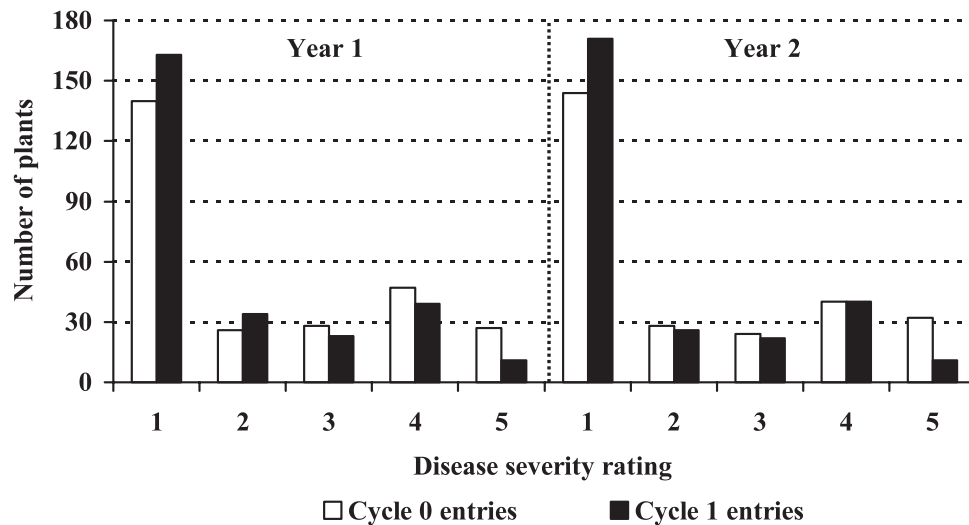
‘Ranger’_{C0}, SC3801_{C0}, ‘Webfoot.MPR’_{C0}, ‘Heinrichs’_{C1}, ‘OK49’_{C1}, and ‘Webfoot.MPR’_{C1} had the most severe symptoms. ‘Multi-plier’ exhibited significantly more disease symptoms than ‘Peace’.

Orthogonal contrasts determined ‘Ranger’_{C0} had significantly more severe disease than ‘Ranger’_{C1} ($P = 0.002$; Table 3). Grouped C0 entries were significantly more diseased than grouped C1 entries ($P = 0.005$).

Disease response experiment 2: year 2 (winter 2001–2002)

Root disease severities increased, decreased, or remained the same compared with first test year results. Five plants died (DSR 5; 3-‘Alpine’_{C0}, 1-SC3801_{C0}, 1-‘Webfoot.MPR’_{C0}) during the second winter, bringing the 2-year total to 49 (7%). Disease severity differences among entries were significant ($P < 0.001$; Table 2). ‘Peace’, ‘Heinrichs’_{C0}, SC3801_{C0}, ‘Alpine’_{C1}, ‘Heinrichs’_{C1}, ‘Ranger’_{C1}, and SC3801_{C1} had the

Fig. 2. Disease severity ratings of alfalfa with brown root rot (caused by *Phoma sclerotioides*). First and second test year comparisons for experiment 2 (2000–2002) cycle 0 and cycle 1 entries. Disease severity ratings are the same as in Fig. 1.



least severe symptoms, while ‘Multi-plier’, ‘Alpine’_{C0}, ‘OK49’_{C0}, ‘Webfoot.MPR’_{C0}, and ‘Webfoot.MPR’_{C1} had the most severe symptoms. Disease severity differences among ‘Peace’ and ‘Multi-plier’ were significant. As in year 1, grouped C0 entries were more diseased than grouped C1 entries ($P = 0.0051$).

Disease response experiment 2: overall summary

Brown root rot symptoms were compared across years. Overall, the number of C1 symptomless plants (DSR 1) was greater than C0 plants during first and second test years (Fig. 2). More C1 plants exhibited slight root rot symptoms (DSR 2) compared with C0 plants during the first test year, but the trend was reversed the second year. Numbers of plants with moderate, severe, and acute disease symptoms (DSRs 3, 4, and 5), were greater for C0 than with C1 entries in both years, with one exception. Second-year DSR 4 totals were equal for C0 and C1 entries. Overall, fewer plants died (DSR 5) in experiment 2 than in experiment 1.

Discussion

Brown root rot disease symptoms on plant roots were more severe in experiment 1 than in experiment 2. Plants in experiment 1 exhibited advancing disease symptoms during both test years, while plants in experiment 2 did not.

Seedlings from experiment 1 were transplanted to peat pots. At inoculation, roots were difficult to uncover, resulting in plant injury. While Cormack (1934) and Sanford (1933) determined that plant infection by *P. sclerotioides* is not dependent on root injury, it may have resulted in additional plant stress at a time when seedlings were vulnerable. Stress caused from root injury, pathogen inoculation, and a winter exposure period that appeared to support fungal growth may have contributed, at least in part, to increased disease severity in experiment 1. Plant root injury was minimized in experiment 2 by using a sand–soil mixture that fell

away from roots readily, leaving plant tissues intact during inoculation and again later during rating.

Experiment 1 plants were planted during late fall, and later winter-hardened in the greenhouse. Experiment 2 plants were germinated in June of 2000 and transferred outside during late summer to allow plants time to harden naturally. This outside acclimation period may also have reduced stress for plants in experiment 2.

The severity of BRR symptoms on susceptible hosts was variable across years and is suspected to be tied to environmental conditions (Davidson 1990). Moderation of soil temperatures and increased soil moistures during the second year of experiment 1, provided by a late-October snowstorm, may have produced optimum conditions for disease development.

While seedling stress factors and snow cover were likely responsible, in part, for disease progression, the addition of cotton adjacent to roots may have also played a role. Cotton balls were colonized by *P. sclerotioides* before plant root symptoms were visible. A large number of pycnidia were produced on the cotton as it degraded. *Phoma sclerotioides* appeared to utilize cotton as a food source before invading plant roots. After cotton was colonized, *P. sclerotioides* appeared to infect roots in direct contact with the cotton rather than the much smaller surface area in contact with barley seed inocula.

Experiment 1 plants had a thick layer of organic material from peat pots as well as a ball of cotton at the inoculation site. These provided an abundance of organic substrate for fungal colonization. The materials in peat pots may have promoted fungal growth. These conditions were absent for plants in experiment 2, which were grown in a sand–soil medium. The soil organic fraction was substantially reduced in experiment 2. Plants were inoculated with two barley grains infested with *P. sclerotioides* and covered with cotton as in experiment 1. The fungus colonized the cotton during the first winter exposure period. When upper root systems were uncovered to rate for disease severity following the

first winter, most of the cotton was not recoverable because of degradation.

Brown root rot symptoms developed on susceptible plants in experiment 2 during the first winter. However, more resistant plants may not have been exposed to adequate disease pressure over a sufficient period of time, and thus failed to develop disease symptoms.

These experiments were designed to determine whether selection for BRR resistance occurred in a production alfalfa field in Wyoming. As a group, C1 entries exhibited less severe disease symptoms than C0 entries after the second test year in each experiment.

'Peace' alfalfa, a Canadian cultivar selected for BRR resistance, performed well across experiments and test years. It was consistently the second most resistant entry in three of four test years, and was not significantly different from the most resistant entry. 'Peace' had slightly more severe disease symptoms than 'Heinrichs'_{C0} during both test years of experiment 1, and more than 'Ranger'_{C1} during the first test year of experiment 2. However, it had least severe disease during the second test year of experiment 2.

A relationship between BRR resistance and fall dormancy levels may exist. Of the alfalfa entries tested, very fall-dormant entries ('Heinrichs', 'Peace', and SC3801) exhibited more BRR resistance than less dormant entries. Plants with sufficient root stores of nonstructural carbohydrates and proteins (Smith 1961; Volenec et al. 1996) may be better prepared to survive severe and extended winters in the presence of *P. sclerotioides*. This is a worthy topic for future research efforts.

In summary, this research indicates that natural selection for BRR resistance occurred in a *P. sclerotioides*-infested field in Wyoming over 6 years. We conclude that BRR resistance is a heritable trait in alfalfa. This research has formed the basis for a standard test for BRR resistance in alfalfa (Gray et al. 2004).

Acknowledgements

The authors thank Alan Gray for establishing the 1992 alfalfa forage yield trial from which selections were made and for providing seed from each of the original six entries. Thanks are extended to Drs. Deborah Samac and Paul Peterson for their critical review of this manuscript. Financial support for this project was provided by the University of Wyoming Agricultural Experiment Station Competitive Grants Program, USDA Western Regional Integrated Pest Management Competitive Grants Program, and the Sweetwater County, Wyoming, Weed and Pest District Board.

References

Barnes, D.K., Bingham, E.T., Murphy, R.P., Hunt, O.J., Beard, D.F., Skrdla, W.H., and Teuber, L.R. 1977. Alfalfa germplasm

- in the United States: Genetic vulnerability, use, improvement, and maintenance. USDA Agric. Res. Serv. Tech. Bull. 1571.
- Berkenkamp, B., Bittman, S., and McCartney, D. 1991. Resistance of alfalfa cultivars to brown root rot. *Can. J. Plant Sci.* 71: 211–213.
- Caddel, J.L., Berberet, R.C., Shelton, K.T., and Zarrabi, A.A. 1992. Registration of 'OK 49' alfalfa. *Crop Sci.* 32: 280.
- Cormack, M.W. 1934. On the invasion of roots of *Medicago* and *Melilotus* by *Sclerotinia* sp., and *Plenodomus meliloti* D., and S. *Can. J. Res.* 11: 474–480.
- Davidson, J.G.N. 1990. Brown root rot. In *Compendium of alfalfa diseases*. 2nd ed. Edited by D.L. Stuteville and D.C. Erwin. American Phytopathological Society Press, St. Paul, Minn. pp. 29–31.
- Gray, F.A., Hollingsworth, C.R., Groose, R.W., Reedy, C.J., and Larsen, R.C. 2004. Brown root rot resistance. *Phoma sclerotioides* G. Preuss ex Sacc. www.naaic.org.
- Hollingsworth, C.R. 2002. Assessing heritability of brown root rot (*Phoma sclerotioides*) resistance and forage yield in nine alfalfa (*Medicago sativa* ssp. *sativa*) populations. Ph.D. thesis, University of Wyoming, Laramie, Wyoming, USA. pp. 86–120.
- Hollingsworth, C.R., and Gray, F.A. 1999. First report of brown root rot on alfalfa caused by *Phoma sclerotioides* in the continental United States. *Plant Dis.* 83: 1071.
- Hollingsworth, C.R., Gray F.A., Groose, R.W., and Mims, C.W. 2002. Morphological responses of Canadian and USA isolates of *Phoma sclerotioides* to different growth media, temperatures and light. *Mycotaxon*, 81: 331–339.
- Hollingsworth, C.R., Gray, F.A., Koch, D.W., Groose, R.W., and Heald, T.E. 2003. Distribution of *Phoma sclerotioides* and incidence of brown root rot of alfalfa in Wyoming, USA. *Can. J. Plant Pathol.* 25: 215–217.
- Larsen, R.C., Hollingsworth, C.R., Vandemark, G.J., Gritsenko, M.A., and Gray, F.A. 2002. A rapid method using PCR-based SCAR markers for detection and identification of *Phoma sclerotioides*, the cause of Brown root rot disease of alfalfa. *Plant Dis.* 86: 928–932.
- Lawrence, T., and Irvine, R.B. 1982. 'Heinrichs' alfalfa. *Can. J. Plant Sci.* 62: 805–808.
- McKenzie, J.S., Pankiw, P., and Siemens, B. 1981. Peace alfalfa. *Can. J. Plant Sci.* 61: 473–474.
- Mikkelsen, M. 1997. Summary of plant diseases diagnosed on commercial and yard and garden plants in 1996. Montana State University, Plant Disease Clinic, Bozeman, Mont.
- Sanford, G.B. 1933. A root rot of sweet clover and related crops caused by *Plenodomus meliloti* Dearness & Sanford. *Can. J. Res. Sect. C*, 8: 337–348.
- SAS Institute Inc. 1988. SAS/STAT user's guide, version 6.03. SAS Institute Inc., Cary, N.C.
- Smith, D. 1961. Association of fall growth habit and winter survival in alfalfa. *Can. J. Plant Sci.* 41: 244–251.
- Tsukamoto, J.Y. 1965. Phenotypic characteristics of alfalfa tolerant to brown root rot. *Can. J. Plant Sci.* 45: 197–198.
- Volenec, J.J., Ourry, A., and Joern, B.C. 1996. A role for nitrogen reserves in forage regrowth and stress tolerance. *Physiol. Plant.* 97: 185–193.