

Phytophthora Seed Rot and Seedling Blight of Sainfoin (*Onobrychis viciifolia*)

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ABSTRACT

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Phytophthora megasperma was recovered from damped-off seedlings of sainfoin (*Onobrychis viciifolia*) grown in field soil naturally infested with *P. megasperma*. In tests conducted in the greenhouse with several sterile growing media, seed and very young seedlings were readily colonized and killed by *P. megasperma*, whereas older plants were unaffected. The Canadian cultivar Nova and the Montana State University experimental line Bozeman showed a moderate level of seedling resistance. Seedling resistance was also shown in several *O. viciifolia* plant introductions. The highest resistance (85% seedling survival) occurred in PI 229613 from Iran. Treating seed with the fungicide metalaxyl increased seedling survival by 47% in field soil naturally infested with *P. megasperma*.

Sainfoin (*Onobrychis viciifolia* Scop.) is a relatively new forage crop in the United States. It has a long history of cultivation in Europe (4) and is a potentially valuable forage species for North America. Its success in the United States has been limited primarily because of the rapid decline of stands attributed to disease. Several pathogens have been identified as causal agents or potential causal agents of diseases in irrigated sainfoin (2,9,11,13-15,21,22). Others undoubtedly exist and await discovery. Although several cultivars have been developed for North America (3,7,16,17,20), none have been bred for disease resistance.

Gray (10) reported sainfoin to be susceptible to isolates of *Phytophthora megasperma* Drechs. recovered from alfalfa in Arizona. Tests were conducted in the greenhouse with a pasteurized sand-soil mixture. When seeds were inoculated at planting, stand loss after 8 wk was 99% (10). Similar results were also obtained with a Wyoming isolate of *P. megasperma* from alfalfa (11). Although not reported, *Phytophthora* root rot (PRR) failed to develop on sainfoin when 1-yr-old plants were inoculated with the same Wyoming isolate and observed after 6 mo. Failure of the Wyoming isolate to cause disease was assumed to be due to some external factor rather than the lack of pathogenicity of the fungus. *P. megasperma* has not been reported to attack sainfoin in the field.

In 1982, studies were initiated in Wyoming to determine if sainfoin was attacked by *P. megasperma* in the field. The feasibility of using host resistance as a means of control was also investigated.

In this paper, we report the susceptibility of sainfoin to *P. megasperma* in naturally infested field soil; the evaluation of available cultivars, several experimental lines, and the world collection of *O. viciifolia* for resistance to *P. megasperma*; and the effect of plant age on susceptibility.

MATERIALS AND METHODS

Field study. In May 1982, four observation plots of sainfoin (cultivar Eski) were established in north central Wyoming in an area previously planted to alfalfa and naturally infested with *P. megasperma*. The test was established to determine if sainfoin was susceptible to *P. megasperma* in the field. The test was adjacent to and was planted on the same day as an alfalfa forage yield trial. Sainfoin plots were 2.3 m wide (15 rows spaced 15.2 cm apart) × 4.6 m long. Ten randomly selected plants per plot were removed in the fall of 1982 and 1983 and observed for symptoms of PRR. Isolations were made from suspect roots on a *Phytophthora*-selective medium (5). Symptoms of PRR were observed and isolation of *P. megasperma* made from an equal number of nearby alfalfa plants. Soil was collected from each of the four sainfoin plots and bioassayed for *P. megasperma* (19).

Greenhouse studies with naturally infested field soil. Symptoms of PRR were present on mature alfalfa but not on sainfoin plants removed from the field plots. Because seed germination of cultivar Eski was relatively high (85%) and *P. megasperma* was recovered from soil in all plots, we speculated that seedling damping-off caused by *P.*

megasperma may have been responsible for the rapid loss of sainfoin stands. Therefore, soil was collected from the sainfoin plots and taken to the greenhouse in Laramie to see if the damping-off phase of the disease could be reproduced. Some of the soil was placed in flats, seeded with sainfoin (Eski), and kept moist to encourage the development of *P. megasperma*. Seedlings that showed symptoms of damping-off and seeds that failed to emerge were removed, thoroughly washed, disinfected in a 0.5% solution of NaOCl for 3 min, floated in sterile water, and observed after several days for reproductive structures of *P. megasperma*.

The remaining soil was used in an experiment in which the fungicide metalaxyl (Ridomil 2E) was applied as a seed treatment to measure loss from *P. megasperma*. Metalaxyl has selective activity against *Phytophthora* and *Pythium* spp. The experimental design was a randomized complete block (RCB) with four replicates. Seeds of the cultivar Remont were planted in furrows (50 seeds per furrow, four furrows per flat spaced 10 cm apart) in aluminum flats containing either autoclaved or nonautoclaved field soil. Seeds were either treated in an aqueous solution of metalaxyl (10 g of seed placed in 50 ml of distilled water [DW] containing 3.2 µl a.i. of metalaxyl) or in DW alone (untreated control) for 10 min, then removed and allowed to dry overnight. Two rows were treated and two were left untreated per flat. Before seeding, 50 cm³ of soil was collected from each flat and bioassayed for *P. megasperma*. Live plant counts were taken 15 wk after planting to determine the long-term effects of the seed treatment on stand establishment.

Greenhouse studies with artificially infested soil. Inoculum preparation. Inoculum of *P. megasperma* (alfalfa isolate) was grown in 0.95-L medicine bottles containing 160 ml of a liquid medium (200 ml of Campbell's V-8 juice and 800 ml of DW per liter). Two 0.5-cm plugs from 2-wk-old stock cultures of *P. megasperma*, maintained on cornmeal agar, were placed in each upright culture bottle containing the V-8 juice medium. After 5 days, the bottles were shaken vigorously to fragment the mycelium and placed in a horizontal position for 9 days. To prepare inoculum, mycelial mats were removed from the culture bottles (one mat per bottle), washed, and blended in DW. Inoculum was prepared at a ratio of one mycelial mat to 238 ml of DW. The

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volume of inoculum varied among experiments. The inoculum rates, which varied for the first and third experiments, are given under "Evaluation of sainfoin for resistance."

Evaluation of sainfoin for resistance.

Two experiments were conducted to evaluate available sainfoin cultivars and several experimental lines for seedling resistance to *P. megasperma* similar to that expressed in alfalfa (1,10,12,13,18). Sainfoin cultivars used in both tests and their releasing agencies were Eski and Remont, Montana State University; Renumex, New Mexico State University; and Melrose and Nova, Canada Department of Agriculture. Experimental lines Bozeman, Creston, and W-40 from Montana State University were also tested. The PRR-resistant alfalfa cultivar Agate and the susceptible cultivar Saranac were included in the first experiment, and the cultivar Saranac, in the second experiment. Entries were seeded in 30-cm rows in redwood flats (60 × 42.5 × 8 cm) containing 1:1 (v/v) Metro Mix 200 (W. R. Grace & Co., Cambridge, MA) and autoclaved river bottom sand. After planting, flats were maintained in the greenhouse at day/night temperatures of 27/13 ± 2 C and watered twice a day to maintain a high soil moisture conducive to infection and disease development. Fluorescent lamps were used to extend the day length to 12 hr. The experimental design was an RCB with six replicates.

In the first experiment, inoculum was incorporated into the soil before seeding. Soil was removed from six flats, and 1 L of inoculum was incorporated into each flat. Six flats received 1 L of DW and served as uninoculated controls. Inoculum consisted of mycelial mats from six 2-wk-old cultures in 6 L of DW. Plant counts were taken after 8 wk.

Less than desirable seedling kill was obtained in the first experiment, when inoculum had been incorporated into the soil before seeding. Therefore, a second experiment was conducted in which inoculum was placed in the seed furrow (25 ml per furrow) just before planting. Location of *Phytophthora* inoculum has been shown previously to affect the degree of seedling mortality in alfalfa seedlings (10). Plant counts were taken after 6 wk.

The third experiment was conducted to evaluate mature sainfoin plants for resistance to PRR. The experimental design was an RCB with four replicates. Entries included the eight sainfoin entries used in the first two experiments and the alfalfa cultivar Agate. All entries were seeded in 15.2-cm clay pots, eight pots per entry, containing autoclaved soil and thinned to three plants per pot. Soil was a Rock River Series, a fine loamy Borollic Haplargid obtained from the University of Wyoming Agricultural Experiment Station Farm located near Laramie.

When plants were 9 wk old, four of the eight pots of each entry were inoculated. Three holes 10 cm deep were made in the soil and extended downward to the root zone to facilitate proper placement of the inoculum, which consisted of mycelial mats from 18 bottles macerated in 4.5 L of DW. Each plant received 60 ml of inoculum. Saucers were placed under the pots to maintain a high soil moisture. Twelve weeks after inoculation, when plants were 21 wk old, plants were removed and roots were washed and examined for symptoms of PRR. Roots from randomly selected plants in the inoculated group were plated out on the *Phytophthora*-selective medium (5) to determine if *P. megasperma* was present.

The fourth experiment was conducted to evaluate plant introductions of sainfoin for seedling resistance to *P. megasperma*. The world collection of *O. viciifolia* (147 entries) was obtained from the USDA, ARS, Regional Plant Introduction Center at Pullman, WA. Entries were seeded in 25-cm rows in plastic flats, nine rows per flat (52 × 26 × 6 cm) containing the same growing medium used in the first two experiments (total of 17 flats for the 147 entries). Flats were either inoculated with *P. megasperma* at the time of seeding or left uninoculated. Because of limited seed, 34 flats were inoculated (two replicates) and 17 flats served as uninoculated controls (total of 51 flats). The number of seeds per row was the same for the inoculated and uninoculated treatments within an entry but varied among entries. Twenty-five milliliters of inoculum was poured in each furrow just before seeding. Furrows were then closed and flats watered thoroughly. Flats were maintained as in the first two experiments. Live plant counts were taken after 4 wk and percent survival determined.

Effect of plant age on susceptibility. Because seed and seedling blight had been observed in the first three experiments conducted in the greenhouse and was suspected to have occurred in the field study, and because PRR symptoms on older plants had not been observed, we hypothesized that only seeds and very young seedlings of sainfoin were susceptible. Therefore, an experiment was conducted to study the relationship of plant age and susceptibility to *P. megasperma*. The experimental design was an RCB with a split-plot arrangement (three replicates). Two sainfoin cultivars, Eski and Remont, and five plant age inoculations were included. Entries were planted in two rows with five seeds per row in 25.4-cm clay pots containing an autoclaved 1:1 (v/v) sand-soil mixture. The soil was described previously in the third experiment under "Evaluation of sainfoin for resistance." The 0-wk age group was inoculated at the time of planting. The remaining plant age groups were inoculated 1, 2, 4, and 6 wk after

planting by pouring the inoculum in a 2.5-cm-deep furrow made adjacent to the plant row. Thirty milliliters of inoculum was applied in each furrow. After inoculation, furrows were closed and sealed with a light sprinkling. DW was poured in furrows of control pots. Pots were watered twice a day to promote disease development. Plant counts were taken weekly for 6 wk. After 12 wk, plants were removed, roots were washed and observed for root rot, and isolations of randomly selected plants were made on the selective medium. Fifty cubic centimeters of soil was collected from the upper 5 cm of soil in each pot and bioassayed for *P. megasperma* (19).

RESULTS

Field study. None of the sainfoin plants removed on August 1982 or September 1983 had PRR symptoms, and all root isolations were negative. Eleven percent of the alfalfa plants in 1982 and 26% in 1984 had typical symptoms of PRR, and *P. megasperma* was readily isolated from diseased roots. Although initial stand counts were not taken, sainfoin stands were poor (<50%) by August of the seeding year. Because germination of the Eski seed used in the test was 85%, we suspected that seedling damping-off caused by *P. megasperma* may have been a factor. *P. megasperma* was recovered by the seedling bioassay from soil collected from each of the four sainfoin plots.

Greenhouse studies with naturally infested field soil. When flats containing the field soil seeded with cultivar Eski were observed after 2 wk, several seedlings showed symptoms of damping-off. Cotyledons were chlorotic and shriveled and the unifoliate petiole wilted. Affected seedlings had an overall grayish green color. When removed, the entire root below the soil surface was discolored. Many of the seeds failed to emerge. When diseased seeds and seedlings were floated in sterile water, sporangia and oospores typical for *P. megasperma* (6) developed after 2–3 days. Seeds and seedlings infected with *P. megasperma* were rinsed in sterile water, blotted dry, and placed on a *Phytophthora*-selective medium (5). *P. megasperma* was eventually isolated but not without considerable effort because of the presence of an aggressive *Pythium* sp. In addition, *Rhizoctonia solani* was also recovered from several seedlings plated on potato-dextrose agar. The pathogenicity of the *Pythium* sp. and *R. solani* on sainfoin was not determined.

A difference in seedling survival of 47% occurred between the metalaxyl-treated and untreated control in the nonautoclaved soil (mean plant counts of 28 and 15, respectively), indicating loss caused by *P. megasperma* and other Pythiaceae fungi. Differences between the treated and untreated seeds in the

autoclaved soil were not significant (mean plant counts of 38 and 34, respectively), indicating the seed treatment was not phytotoxic. *P. megasperma* was recovered by seedling bioassay from soil in all four nonautoclaved flats but not from soil in the four autoclaved flats.

Greenhouse studies with artificially infested soil. *Evaluation of sainfoin for resistance.* Seedling mortality of sainfoin and alfalfa was much greater when *P. megasperma* was placed in the furrow before seeding than when incorporated into the soil (Table 1). A higher percentage of plants died from pre-emergence damping-off than from postemergence damping-off in both tests. In the first experiment, the PRR-resistant alfalfa (cultivar Agate) had significantly less seedling mortality ($P = 0.05$) than the susceptible cultivar Saranac. Sainfoin entries performed similarly in both tests. Cultivar Nova had the lowest seedling mortality (best resistance), whereas cultivar Eski had the highest.

None of the sainfoin plants inoculated when 9 wk old had PRR symptoms after 12 wk. *P. megasperma* was not isolated from any of the randomly selected roots. Typical PRR symptoms did occur in the PRR-resistant alfalfa cultivar Agate (12.5% diseased plants). These results are similar to those obtained in the field study.

Many of the plant introductions had poor seed germination. Therefore, percent seedling mortality was calculated only for entries with 10 or more plants in their uninoculated treatment (90 of the 147 entries). The 57 entries with poor seed germination include 170582, 201511, 201512, 201865, 204594, 204595, 205200, 205201, 206458, 206459, 206577, 223389, 225728, 228156, 228289, 228352, 228402, 234644, 234823, 236486, 237089, 239957, 239958, 239959, 239960, 250024, 251669, 251840, 258769, 258770, 258772, 258773, 258775, 258776, 258778, 259493, 259494, 263158, 273784–273786, 273788, 273790, 273791, 302936–302938, 306693, 311469, 311470, 313060, 318602, 319059–319062, and 319713. Seedling mortality within the 90 entries ranged from 15 to 100% (Table 2). The Iranian entry PI 229613 had the lowest mortality (15%). The next best entries were PI 368034 from Turkey, with 38% mortality, and PI 110397 from the Soviet Union, with 63%. The remaining 87 entries had 79% mortality or higher.

Effect of plant age on susceptibility. Live plant counts taken after 12 wk indicated plant mortality occurred only in the inoculated, 0-wk age group. Eski and Remont had 96 and 85% mortality, respectively; most died from preemergence damping-off. Typical PRR symptoms were not present on any of the surviving plants, and *P. megasperma* was not recovered in root isolations. *P. megasperma* was recovered from soil in

Table 1. Reactions of sainfoin and alfalfa after exposure to *Phytophthora megasperma* either incorporated into the soil or placed in the seed furrow before planting¹

Inoculum incorporated in soil		Inoculum placed in seed furrow	
Entry	Seedling mortality after 8 wk (%)	Entry	Seedling mortality after 6 wk (%)
Alfalfa		Alfalfa	
Saranac (susceptible)	74.7 a	Saranac	99.0 a
Agate (resistant)	46.5 b
Sainfoin		Sainfoin	
Eski	33.3 bc	Eski	98.0 ab
Renumex	25.8 cd	Renumex	86.2 abc
Remont	19.7 cd	W-40	85.6 abcd
W-40	18.2 cd	Creston	83.6 abcde
Creston	17.3 cd	Remont	82.4 abcdef
Bozeman	13.8 cd	Melrose	82.4 abcdefg
Melrose	7.2 d	Nova	63.0 h
Nova	6.3 d	Bozeman	61.2 h

¹ Values represent percent seedling mortality (percent of control) and are the mean of six replicates. Mean values followed by the same letter(s) within a column do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

Table 2. Seedling reactions of the world collection of *Onobrychis viciifolia* to *Phytophthora megasperma*

Plant introduction ^a	Origin	Live plant count after 4 wk		Seedling mortality ^c (%)
		Uninoculated ^b	Inoculated ^b	
110397 ^d	Soviet Union	38	14	63
110400	Turkey	12	1	92
110404	Soviet Union	42	2	96
167236	Turkey	13	0	100
170583	Turkey	22	1	98
170585	Turkey	10	0	100
171725	Turkey	15	1	97
171726	Turkey	14	0	100
178988	Turkey	11	0	100
182247	Turkey	12	0	100
186520	Spain	13	0	100
192993	Spain	13	0	100
192994	Spain	17	1	94
192995	Spain	10	1	95
200872	Turkey	11	0	100
205202	Turkey	22	0	100
212241	Washington	15	0	100
227038	Iran	14	0	100
227373	Iran	33	4	88
229612 ^d	Iran	10	1	90
229613*	Iran	27	23	15
234822	Switzerland	24	2	94
243227	Iran	16	1	94
251160	Yugoslavia	11	0	100
258767	Soviet Union	22	0	100
258768	Soviet Union	13	2	89
258771	Soviet Union	11	2	82
258774	Soviet Union	22	2	93
258777	Soviet Union	13	0	100
259491	United Kingdom	13	0	100
259492	United Kingdom	22	1	96
263159	Soviet Union	17	0	100
273787	Soviet Union	12	3	80
273789	Soviet Union	15	1	93
302939	Spain	13	2	88
311467	Spain	16	0	100
311468	Spain	21	5	79
311471	Spain	12	1	92
313046	Spain	27	2	93
313047	Germany	22	3	89
313048	Soviet Union	29	1	97
313049	Poland	15	0	100
313050	Soviet Union	37	1	97
313051	Switzerland	29	5	83
313052	Soviet Union	23	1	98
313053	Soviet Union	12	1	96

(continued on next page)

10 of 15 inoculated pots of the cultivar Remont and from five of 15 pots of the cultivar Eski when the experiment was terminated. It was not recovered from soil in any of the uninoculated pots of either cultivar.

DISCUSSION

Field and greenhouse tests showed that only seed and young seedlings of sainfoin (less than 1 wk old) are susceptible to attack by *P. megasperma*. This is strikingly different from alfalfa, which is susceptible at any age (8). Apparently, *P. megasperma*-infected seedlings either die within 1–2 wk or fully recover from the infection, because root isolations of older plants from field and greenhouse tests

were all negative and root rot symptoms were not observed in older plants. Although the disease was not observed on sainfoin grown in the field, it was observed on seedlings grown in the greenhouse in nonautoclaved field soil naturally infested with *P. megasperma*. The seed rot and seedling blight phase of the disease should be expected to occur in the field, and when environmental conditions are optimal for infection and disease development, moderate to severe stand failure may occur.

Although researchers have not consciously selected sainfoin for resistance to *P. megasperma*, the cultivar Nova and the experimental line Bozeman displayed a moderate to good level of seedling

resistance, 65 and 63%, respectively (mean of two experiments). A comparable level of seedling resistance was shown in the *O. viciifolia* PI 368034 from Turkey (62%). The highest level of resistance occurred in PI 229613 from Iran (85%). The combination of resistance shown in Nova and Bozeman and in the two plant introductions suggests the level of resistance in populations of sainfoin can be increased or occurs naturally and that control with host resistance appears feasible.

The fungicide metalaxyl was chosen because of its selective activity against *Phytophthora* and *Pythium* spp. A loss in seedling stand of 47% (difference between metalaxyl-treated and untreated seed) occurred when seeds were grown in field soil naturally infested with *P. megasperma*. Both *P. megasperma* and an unidentified *Pythium* sp. were identified on diseased seedlings. Although the pathogenicity of the *Pythium* sp. on sainfoin was not determined, stand loss was most likely attributable to both fungi. Metalaxyl provided a good level of control against seed rot and seedling blight, and its use as a means of control warrants further investigation.

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Table 2. (continued from preceding page)

Plant introduction ^a	Origin	Live plant count after 4 wk		Seedling mortality ^c (%)
		Uninoculated ^b	Inoculated ^b	
313054	Soviet Union	25	2	94
313055	Soviet Union	15	0	100
313056	Norway	22	2	93
313057	Soviet Union	27	1	98
313058	Soviet Union	17	2	91
313059	Germany	21	1	98
313061	Soviet Union	21	1	95
313062	Soviet Union	29	5	83
313063	Soviet Union	33	4	88
313064	Italy	31	2	95
313065	Soviet Union	36	6	85
313066	Bulgaria	21	3	88
314099	Soviet Union	10	0	100
316296	Soviet Union	21	1	98
318604	Switzerland	20	0	100
318605	Switzerland	20	0	100
318606	Switzerland	14	1	93
319058	Spain	1	0	100
338651	Morocco	19	1	97
368034 ^d	Turkey	17	11	38
368035	Turkey	32	2	94
368036	Turkey	34	4	90
372828	Czechoslovakia	14	1	93
372829	Czechoslovakia	30	4	88
372830	Czechoslovakia	20	3	85
372831	Czechoslovakia	29	6	79
372832	Czechoslovakia	30	8	75
372833	Czechoslovakia	43	8	83
372835	Czechoslovakia	38	5	88
380948	Iran	48	7	85
380949	Iran	42	10	76
383713	Turkey	41	6	87
383714	Turkey	24	2	84
383715	Turkey	14	0	100
383716	Turkey	34	1	97
383717	Turkey	29	1	96
400305	Rhodesia	17	0	100
400306	Rhodesia	26	6	79
401419	Unknown	11	1	91
401467	Romania	22	2	93
401468	Romania	36	4	89
401715	Soviet Union	31	2	94
440575	Soviet Union	53	6	89
440576	Soviet Union	43	3	94

^aFifty-seven of the 147 entries had poor seed germination and are not included.

^bValues under the "uninoculated" column are the actual number of live plants remaining. Values under the "inoculated" column are the mean of two replicates and are rounded off to the nearest whole number.

^cPercent seedling mortality was calculated before rounding off and only for those entries with 10 or more plants in the uninoculated treatment.

^dIntroductions showing a promising level of resistance.

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