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Effect of Seed Treatments and Root Pathogens on Seedling Establishment and Yield of Alfalfa, Birdsfoot Trefoil and Sweetclover

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Abstract: Soil-borne fungal pathogens can reduce stand density in alfalfa (*Medicago sativa*), birdsfoot trefoil (*Lotus corniculatus*) and yellow sweetclover (*Melilotus officinalis*) by reducing seedling establishment and subsequent stand longevity. Fungicide seed treatments containing metalaxyl and fludioxonil were examined in inoculated greenhouse and field tests to determine their efficacy against seedling blight and root rot caused by *Fusarium avenaceum* and *Rhizoctonia solani*. Inoculation increased disease severity and reduced establishment, especially in field trials inoculated with *Rhizoctonia solani*. Under controlled conditions, fludioxonil (alone or in a formulation with metalaxyl) was effective against either pathogen in inoculated trials-seed treatment consistently increased seedling survival and reduced root rot on all three forage species. However, seed treatment had little impact on subsequent forage yield under field conditions. Metalaxyl alone was not efficacious. In fields with high pathogen populations, application of fludioxonil seed treatment on forage legume species could improve seedling establishment substantially.

Key words: *Medicago sativa*, *Lotus corniculatus*, *Melilotus officinalis*, *Fusarium avenaceum*, *Rhizoctonia solani*, fungicide seed treatment

INTRODUCTION

Strong seedling establishment and growth in forage crops are critical to achieve the crop density required to out-compete weeds and produce uniform high forage yields. Damping-off and seedling blight caused by soil-borne pathogens can cause substantial reductions in seedling stands of forage legume crops on the northern prairies (Couture and Coulman, 2003; Gossen, 2003). These early losses can also result in reduced stand longevity. For instance, *Rhizoctonia solani* Kühn and *Fusarium* sp. can reduce stand establishment and the longevity of alfalfa (*Medicago sativa* L.) (Gossen, 1994a, 1998; Hwang *et al*., 1989; Hwang and Flores, 1987; Reeleder, 1982; Wang *et al*., 1999). These pathogens affect the stand by reducing seedling establishment, removing feeder rootlets and thereby reducing plant productivity (Hancock, 1985) and killing plants weakened by other stresses.

Fungicide seed treatment is a simple and inexpensive method for reducing seedling blight and is widely used in many crops that are propagated from seed. However, fungicide seed treatments in forage crops like alfalfa are used infrequently on the northern prairies because of a perceived high cost/benefit ratio (Leath, 1991; Summer, 2000). The few fungicides that are registered for seed treatments in forage legumes, (i.e., metalaxyl) are used to control seedling damping-off caused by *Pythium* sp.

The objective of this study was to assess the impact of several fungicide seed treatments on seedling establishment of alfalfa, birdsfoot trefoil (*Lotus corniculatus* L.) and yellow sweetclover (*Melilotus officinalis* (L.) Lam.), under controlled environment conditions and in field trials under conditions of high disease pressure with fusarium and rhizoctonia seedling blight diseases.

MATERIALS AND METHODS

Inoculum: Two aggressive isolates of *Rhizoctonia solani* and two of *Fusarium avenaceum* (Fr.) Sacc. (Hwang *et al*., 1989) were selected for the study. Inoculum was produced as described previously (Hwang, 1988). Briefly, a mixture of oat and wheat seeds (1:1, v:v) was soaked in water for 24 h, autoclaved in Hi-Patch® bags (Western Biologicals, Aldergrove, BC) and then

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inoculated with a specific isolate and incubated at 20-22°C. After incubation, the infested grain was air-dried and ground to a powder using a Thomas-Wiley laboratory mill (Arthur H. Thomas Co., Philadelphia, PA) with a mesh size of 3 mm. The inoculum of *R. solani* provided an initial population density of 2×10^4 cfu g^{-1} and *F. avenaceum* had an initial population density of 3×10^6 cfu g^{-1}. Aliquots of sterile grain were also dried and stored for use as the noninoculated controls.

Inoculum density: To assess the impact of inoculum density on disease severity, inoculum was combined with steam-pasteurized soil mix (1:1, loam: peat moss) in ratios of 1:10,000, 1:5,000, 1:2,000, 1:1,000, 1:500 1:200, 1:100, 1:50 and 1:10 (w:w) to produce a range of inoculum densities between 0.5 and 10^5 cfu g^{-1} of soil mixture for *F. avenaceum* and between 3×10^{-2} and 10^4 cfu g^{-1} of soil mixture for *R. solani*. A treatment of noninfested cornmeal-sand inoculum in pasteurized soil mixture (1:200, w:w) was included as a control.

Small plastic cups (450 mL tuffcups, Georgia-Pacific, Dixie Business, Norwalk, CT) containing pasteurised soil mixture were used in the study. Ten cups of alfalfa cv. Beaver, birdsfoot trefoil cv. Leo, or a blended seed lot of yellow sweetclover were planted per treatment, with 10 seeds per cup. At seeding, 5 mL of the diluted pathogen inoculum was placed at the same level as the seed in each cup. Pots were arranged on a greenhouse bench in a randomized complete block design. The greenhouse was maintained at 20/16°C day/night with a 16 h photoperiod. A light intensity of 250-300 µmol $m^{-2}sec^{-1}$ was provided by a mixture of cool-white fluorescent and incandescent bulbs. Seedling establishment per pot was counted 4 weeks after seeding. The experiment was repeated.

Fungicide greenhouse assessments: The efficacy of fungicide seed treatments against seedling blight caused by *R. solani* or *F. avenaceum* on alfalfa, trefoil and sweetclover was evaluated in greenhouse studies. Seed was treated in a Hege II small batch seed treater (Hege Equipment Inc., Colwich, KS) using seed-treatment fungicides supplied by Syngenta Crop Protection Canada, Inc., Portage la Prairie, MB. In 2003, the treatments included: fludioxonil (Maxim 480) at 0.025 and 0.050 g a.i./kg seed, metalaxyl-M (Apron XL) at 0.150 g a.i./kg seed and fludioxonil at 0.150 g a.i./kg seed combined with metalaxyl at 0.025 g a.i. Non-treated seed was used for the inoculated and non-inoculated controls. When the experiment was repeated in 2005, three treatments were added: metalaxyl-M at 0.075 g a.i./kg seed and a formulated product containing fludioxonil+metalaxyl-M (Apron Maxx, 0.73% fludioxonil, 1.1% metalaxyl-M) at 0.0625 and 0.125 g a.i./kg seed.

Two concentrations of inoculum (1:20 and 1:100) were assessed for each pathogen in each trial (1.5×10^5 and 3×10^4 cfu g^{-1} sand mix for *F. avenaceum*; 10^4 and 2×10^3 cfu g^{-1} sand mix for *R. solani* for the 1:20 and 1:100 concentrations, respectively). Non-inoculated controls were inoculated with the same amount of clean sand. The methods were as described previously, except that the alfalfa cv. Algonquin was used in place of the cv. Beaver in the trial in 2003. Pots were arranged in a completely random design in a greenhouse and watered daily. Each trial consisted of 10 replications with 10 seeds per replication (pot). Seedling emergence and establishment were counted at 7 and 21 days after seeding. Disease incidence and severity, post-emergence damping-off (%) and seed decay (%) were recorded at 21 days after seeding. Disease severity was assessed on a scale of 0 to 4, where 0 = no disease, 1 = small lesions on root, 2 = large lesions, 3 = lesions covering more than ½ of the root circumference and 4 = lesions occupying more than ½ of the root cross-section and plants wilted, stunted or dead. Each treatment was examined visually for phytotoxicity associated with fungicide application. Seedlings were cut off at the soil level and dried. Seedling dry weight was recorded. Total disease incidence was calculated as the percentage of seedlings that had seedling blight, died following emergence, or failed to emerge (based on 10 seeds per pot). The trial was repeated.

Fungicide field assessments: Field experimental plots were established on May 19, 2003 and June 4, 2004 at Vegreville, Alta., on a black chernozemic sandy loam soil. Identical field plots were established on May 28, 2003 and June 3, 2004 at Edmonton, Alta., on a black chernozemic clay loam soil. The trials were repeated at Brooks, Alta. on May 12, 2005, on a brown chernozemic loam soil. Each of the three forage crops was grown in a separate test. Each trial was laid out in a split-plot design with four replications. Main plots consisted of inoculation with *F. avenaceum* or *R. solani*. The inoculum was mixed with the seed just prior to planting at a rate of 20 mL/row based on previous inoculation tests. The converted inoculum concentrations for field trials were 1.2×10^6 cfu mL^{-1} grain inoculum for *F. avenaceum* and 1.2×10^4 cfu mL^{-1} grain inoculum for *R. solani*. The subplot treatments consisted of nine seed treatments: (1) nontreated seed in non-inoculated soil; (2) non-treated seed in inoculated soil; (3) fludioxonil at 0.025 g a.i./kg seed; (4) fludioxonil at 0.050 g a.i./kg seed; (5) metalaxyl-M at 0.075 g a.i./kg seed; (6) metalaxyl at 0.150 g a.i./kg seed; (7) metalaxyl+fludioxonil at 0.15+0.025 g a.i./kg seed; (8) Apron Maxx (combination of metalaxyl-fludioxonil) at 0.0625 g a.i./kg seed; and (9) Apron Maxx at 0.1250 g a.i./kg seed. Treatments (5), (8) and (9) were not evaluated in 2003.

Each subplot consisted of four rows, 3 m in length, with a 25 cm inter-row spacing. Seeds were planted about 1 cm deep at a rate of 0.1 g of seeds m^{-1}. There was 0.6 m between plots and 2 m between replicates. To assess the phytotoxicity of seed treatments, an additional non-inoculated control was added in main plots in 2004 and 2005, in which all treatments were seeded without the artificial inoculation. Seedling emergence and establishment (presented as seedling survival) were counted at 7 and 21 days after emergence. Seedling vigour was assessed on a 1-5 scale based on relative row fullness in each plot, where 1 = less than 30%, 2 = 30-50%, 3 = 50-70%, 4 = 70-90% and 5 = 90-100%. To calculate dry matter yield, the fresh weight of the harvested forage was taken, the moisture content of about 1 kg was determined and then the weight of the harvested forage was adjusted to a dry-weight basis.

Data analysis: Analyses were conducted using SAS software (version 9.1.3, SAS Institute Inc., Cary, NC). Data were assessed with the Mixed Model procedure for the analysis of variance using the restricted/residual maximum likelihood method and where appropriate, Tukey's Honestly Significant Difference (HSD) test at p<0.05 was used for means separation (Littell *et al*., 1996; Verbeke and Molenberghs, 1997). Seed treatments were designated as a fixed effect and the Satterthwaite option was used to calculate the degrees of freedom for each estimate. In the greenhouse experiments, the two inoculum levels for each pathogen were assigned as random effects. In the field experiments, data for seedling establishment and vigour from the five experiments were combined where year and location were treated as random effects. However, data from the three years were separated when analyzing forage yield, since some experiments exhibited extremely large coefficients of variation (≥20%) that interfered with the combined analysis. Linear regression was used to determine the relationships between seedling survival and density in the inoculum density study using SigmaPlot 2001 software (SPSS Inc., Chicago, IL).

RESULTS

Inoculum density: There was no repetition×inoculum density interaction for either pathogen (Fig. 1), so data from the two repetitions were combined for subsequent analyses. Standard error of the estimates averaged 0.6 (0.4-0.8) for *F. avenaceum* and 1.0 (0.8-1.1) for *R. solani* across the three forage species. The relationship between inoculum density and seedling establishment for each crop and pathogen was described

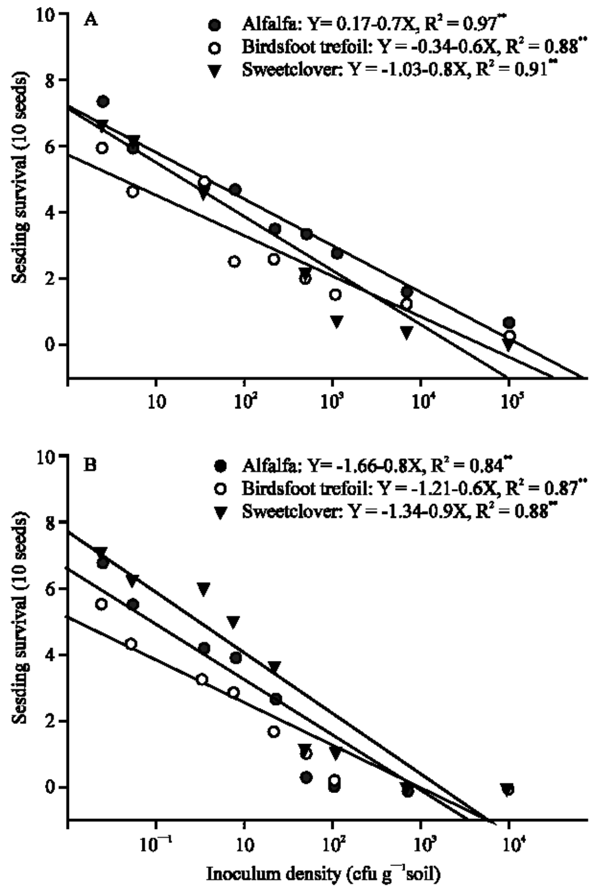

Fig. 1: Effect of inoculum concentration of (A) *Fusarium avenaceum* or (B) *Rhizoctonia solani* on seedling survival of alfalfa, birdsfoot trefoil and sweetclover under controlled environmental conditions (solid lines calculated using linear regression).

by a linear equation (Y = a + bX), where Y represents establishment (%), X = inoculum density, a = intercept and b = slope. For all forage species and pathogens, increasing inoculum concentration reduced seedling establishment, with R^2 values ranging from 0.84 to 0.97 (P<0.001).

Fungicide greenhouse assessments: In 2003 (the initial trial), only seedling survival data were collected and there were fewer treatments than in 2005, so seedling survival was separated from the 2005 data. Data collected from the 2005 trials on seed rot and total disease is presented in Table 2. Inoculation with *F. avenaceum* reduced seedling survival and increased the percentage of seed rot in the inoculated control compared with the non-inoculated control for all three crops (Table 1 and 2). Fludioxonil at the low rate consistently increased seedling

Table 1: Impact of fungicide seed treatments and inoculation with *Fusarium avenaceum* and *Rhizoctonia solani* on seedling establishment (10 seeds planted per pot) of (A) alfalfa, (B) birdsfoot trefoil and (C) sweet clover under controlled conditions.

Inoculum and seed treatment	Rate (g a.i./kg)	Seedling survival in 2003 A	B	C	Seedling survival in 2005 A	B	C
F. avenaceum							
Non-inoc. control	-	6.3a	6.4a	8.7a	9.1a	7.5a	4.7ab
Inoculated control	-	1.8c	3.6b	4.3b	5.4d	5.2c	2.5c
Fludioxonil (F)	0.025	6.1a	6.5a	8.0a	6.8bc	7.1ab	4.1b
	0.050	6.7a	6.0a	7.8a	7.7b	8.1a	5.8a
Metalaxyl (M)	0.075	nd	nd	nd	3.9e	6.1bc	5.2ab
	0.150	3.1b	3.3b	4.0b	4.0e	5.3c	6.0a
M+F	0.150+0.025	6.1a	5.9a	7.7a	6.2cd	7.9a	4.8ab
Apron Maxx (M-F)	0.0625	nd	nd	nd	6.5c	7.7a	4.1b
	0.1250	nd	nd	nd	7.0bc	8.0a	5.6a
R. solani							
Non-inoc. control	-	7.8a	6.7a	8.2a	8.5a	7.8a	2.9a
Inoculated control	-	7.2a	6.3a	7.8a	0.1d	0.1e	0.5d
Fludioxonil (F)	0.025	8.0a	6.2a	8.5a	4.0c	3.2c	1.9a-c
	0.050	7.7a	7.6a	9.0a	5.5b	3.8bc	2.4a
Metalaxyl (M)	0.075	nd	nd	nd	0.0d	0.0e	0.8cd
	0.150	7.2a	6.8a	7.6a	0.3d	0.0e	1.2b-d
M+F	0.150+0.025	6.5a	6.3a	8.0a	4.3c	2.0d	2.3ab
Apron Maxx (M-F)	0.0625	nd	nd	nd	3.9c	2.0d	2.1ab
	0.1250	nd	nd	nd	5.5b	4.4b	2.5a

Values within a column and inoculation followed by the same letters do not differ based on Tukey's HSD test at $p \leq 0.05$, nd = not done

Table 2: Effect of fungicide seed treatments and inoculation with *Fusarium avenaceum* and *Rhizoctonia solani* on seedling blight and root rot of (A) alfalfa, (B) birdsfoot trefoil and (C) sweet clover under controlled environmental conditions.

Inoculum and seed treatment	Rate (g a.i./kg)	Seed rot (%) A	B	C	Total disease (%) A	B	C
F. avenaceum							
Non-inoc. control	-	3d	23cd	40bc	na	na	na
Inoculated control	-	37b	41a	57a	84ab	89a	90a
Fludioxonil (F)	0.025	22c	23cd	47ab	69c	62bc	76a-c
	0.050	16c	15d	31cd	69c	65b	64c
Metalaxyl (M)	0.075	47a	29bc	36b-d	87a	68b	75bc
	0.150	47a	34ab	24d	82ab	67b	67bc
M+F	0.150+0.025	21c	16d	29cd	80ab	54c	80ab
Apron Maxx (M-F)	0.0625	23c	19d	46ab	74bc	67b	90a
	0.1250	22c	17d	28cd	79abc	62bc	81ab
R. solani							
Non-inoc. control	-	3e	19e	58d	na	na	na
Inoculated control	-	100a	99a	91a	100a	100a	99a
Fludioxonil (F)	0.025	45c	57c	71bc	86b	84b	94abc
	0.050	31d	48cd	64cd	85b	82bc	96abc
Metalaxyl (M)	0.075	100a	100a	89a	100a	100a	98ab
	0.150	97a	100a	84ab	100a	100a	90bc
M+F	0.150+0.025	46c	70b	65cd	85b	89b	88c
Apron Maxx (M-F)	0.0625	54b	70b	72bc	85b	83bc	94a-c
	0.1250	35d	41d	59cd	84b	76c	97ab

Values within a column and inoculation followed by the same letters do not differ based on Tukey's HSD test at $p \leq 0.05$, na = not assessed

survival and reduced seed rot and total disease incidence over the inoculated control. However, this difference was a non-significant effect for seed rot and total disease incidence in sweetclover. The high rate of fludioxonil did not produce a significant improvement over the low rate except for establishment and seed rot of sweetclover in 2005. Metalaxyl plus fludioxonil and Apron Maxx generally improved seedling establishment, but only slightly reduced total disease incidence in alfalfa and sweetclover when the trial was repeated. Metalaxyl alone generally did not reduce disease or increase establishment (Table 1 and 2).

Inoculation with *R. solani* reduced seedling survival and seed rot in the inoculated control compared with the non-inoculated control in 2005, but did not affect seedling survival in the 2003 trial. Fludioxonil (low rate), metalaxyl plus fludioxonil and Apron Maxx increased seedling establishment and reduced seed rot in all three crops in 2005, while only metalaxyl plus fludioxonil and Apron Maxx significantly reduced total disease incidence in alfalfa and birdsfoot trefoil. The treatment with metalaxyl plus fludioxonil was the only one that significantly reduced total disease incidence in sweetclover. There was a trend for the three forage species to benefit from

Table 3: Effects of fungicidal seed treatments on seedling establishment (plant m^{-2}) and seed vigour (1-5) of (A) alfalfa, (B) birdsfoot trefoil and (C) sweet clover in field trials inoculated with *Fusarium avenaceum* and *Rhizoctonia solani* over five station years in Alberta in 2003-2005

Inoculum and seed treatment	Rate (g a.i./kg)	Seedling survival A	Seedling survival B	Seedling survival C	Seedling vigour A	Seedling vigour B	Seedling vigour C
F. avenaceum							
Non-inoc. control	-	83a	49a	67a	4.6a	4.1a	4.2a
Inoculated control	-	42c	32bc	36b	3.2a	2.8a	2.8a
Fludioxonil (F)	0.025	63bc	41a-c	52b	4.2a	3.6a	3.4a
	0.050	64b	40a-c	49b	4.0a	3.8a	3.7a
Metalaxyl (M)	0.075	48c	29c	47b	3.9a	2.8a	3.4a
	0.150	48c	32bc	39b	4.0a	3.4a	3.2a
M+F	0.150+0.025	72ab	42a-c	57b	4.4a	3.1a	3.6a
Apron Maxx (M-F)	0.0625	70ab	45ab	54b	4.3a	3.8a	3.6a
	0.1250	68ab	50a	58b	4.2a	3.7a	3.5a
R. solani							
Non-inoc. control	-	86a	52a	63a	4.4a	4.1a	4.2a
Inoculated control	-	11c	7b	9d	1.3b	1.0c	1.2d
Fludioxonil (F)	0.025	42b	20b	33c	3.6a	1.8bc	2.8bc
	0.050	52b	27b	41bc	3.8a	2.3b	3.3a-c
Metalaxyl (M)	0.075	6c	6b	10d	1.7b	1.1c	1.4d
	0.150	11c	8b	11d	1.8b	1.3c	1.5d
M+F	0.150+0.025	49b	18b	44bc	3.8a	2.1bc	3.3a-c
Apron Maxx (M-F)	0.0625	38b	21b	32c	3.4a	2.0bc	2.6c
	0.1250	58ab	26b	50b	4.0a	2.8b	3.7ab

Values within a column and inoculation treatment followed by the same letters do not differ based on Tukey's HSD test at $p \leq 0.05$

Table 4: Effects of fungicide seed treatments on forage yield (t ha^{-1}) of (A) alfalfa, (B) birdsfoot trefoil and (C) sweetclover in field trials inoculated with *Fusarium avenaceum* and *Rhizoctonia solani* across five station years in Alberta, 2003-2005

Treatment	Rate (g a.i./kg)	2003 A	2003 B	2003 C	2004 A	2004 B	2004 C	2005 A	2005 B	2005 C
F. avenaceum										
Non-inoc. control	-	0.51a	0.31a	0.41b	1.31a	0.15a	0.61a	4.87a	1.68ab	11.19a
Inoculated control	-	0.36a	0.19a	0.22c	0.73b	0.14a	0.26c	4.67a	1.26b	9.00bc
Fludioxonil (F)	0.025	0.47a	0.34a	0.50ab	0.93b	0.14a	0.35bc	4.33a	1.78ab	9.74ab
	0.050	0.57a	0.45a	0.58a	0.98ab	0.21a	0.42abc	4.34a	1.90a	9.01bc
Metalaxyl (M)	0.075	-	-	-	1.05ab	0.14a	0.26c	4.13a	1.52ab	7.82b
	0.150	0.51a	0.11a	0.35bc	0.78b	0.15a	0.28c	4.10a	1.78ab	9.55bc
M+F	0.150+0.025	0.64a	0.43a	0.66a	1.07ab	0.23a	0.50ab	4.45a	1.78ab	8.79bc
Apron Maxx (M-F)	0.0625	nd	nd	nd	1.05ab	0.27a	0.34bc	4.33a	1.55ab	7.59c
	0.1250	nd	nd	nd	1.31a	0.24a	0.51ab	4.60a	2.08a	8.12bc
R. solani										
Non-inoc. control	-	0.41a	0.14a	0.72ab	1.18a	0.41a	0.60a	5.73a	1.62a	8.90ab
Inoculated control	-	0.13a	0.11a	0.21c	0.22c	0.02b	0.13c	0.86c	0.42d	2.00c
Fludioxonil (F)	0.025	0.51a	0.11a	0.51abc	0.89ab	0.05b	0.30b	3.39b	0.75bcd	6.82ab
	0.050	0.63a	0.27a	0.84a	0.80b	0.05b	0.34b	4.43ab	0.95bc	7.87ab
Metalaxyl (M)	0.075	nd	nd	nd	0.16c	0.02b	0.14c	1.27c	0.41d	1.32d
	0.150	0.20a	0.09a	0.23bc	0.25c	0.02b	0.19bc	1.11c	0.68cd	1.01d
M+F	0.150+0.025	0.65a	0.20a	0.70abc	0.79b	0.03b	0.31b	4.27b	0.86bc	8.12ab
Apron Maxx (M-F)	0.0625	nd	nd	nd	0.76b	0.05b	0.35b	3.86b	1.11b	4.47bc
	0.125	nd	nd	nd	1.00ab	0.13b	0.34b	3.55b	0.89bc	8.93a

Values within a column and inoculation treatment followed by the same letters do not differ based on Tukey's HSD test at $p \leq 0.05$, nd = not done

the higher rate of fludioxonil and Apron Maxx treatments; significant improvement over the low rate was observed for both fungicides in seedling survival and seed rot in alfalfa and the high rate of Apron Maxx increased seedling survival and decreased seed rot in birdsfoot trefoil (Table 1 and 2). Metalaxyl alone did not reduce the effect of inoculation with *R. solani*.

No phytotoxicity was noted in these tests. Also, there were no differences among seed treatments when they were seeded in non-inoculated soil (data not presented). There were no visible disease symptoms on roots in the non-inoculated controls.

Fungicide field assessments: The impact of fungicide seed treatments in the field trials varied among seed treatments and forage crops (Table 3 and 4). When compared to the non-inoculated treatments, seedling establishment, seedling vigour and forage yield were not affected by fungicide treatment and no phytotoxicity was noted (data not presented).

Inoculation with *F. avenaceum* reduced ($p<0.05$) seedling establishment in all three crop species. Forage yield was generally reduced in the inoculated control compared to the non-inoculated control, but the reduction was not always significant (Table 4).

Fludioxonil, metalaxyl plus fludioxonil and Apron Maxx generally improved seedling establishment compared to the inoculated control, with the greatest improvement observed in alfalfa. Fungicide treatment occasionally resulted in increased forage yields, i.e., fludioxonil on sweetclover in 2003 and on birdsfoot trefoil in 2005, metalaxyl plus fludioxonil on sweetclover in 2003 and 2004 and the high rate of Apron Maxx on alfalfa and sweetclover in 2004 and on birdsfoot trefoil in 2005. Also, stand density and yield were occasionally greater at the high rate of Apron Maxx and fludioxonil compared to the low rate. However, fungicide treatment did not have a consistent impact on forage yield in these tests. Seedling vigour was not affected by fungicide treatment. Metalaxyl alone did not provide effective control of *F. avenaceum* (Table 3 and 4).

Inoculation with *R. solani* reduced ($P<0.05$) seedling establishment and vigour (Table 3). Also, inoculation generally reduced forage yield, except for alfalfa and birdsfoot trefoil in 2003 (Table 4). All of the fungicide seed treatments containing fludioxonil improved seedling survival and vigour over the inoculated control. The only exception was birdsfoot trefoil, where the difference in seedling survival was not significant. These treatments consistently exhibited numerically higher forage yield than the inoculated control, but differences were not significant for any specific treatment. The high rates of Apron Maxx and fludioxonil often improved seedling establishment, seedling vigour and forage yield over the low rate. For example, the high rate of Apron Maxx increased seedling establishment of sweetclover from 32 seedlings m^{-2} to 50 seedlings m^{-2} and increased seedling vigour from 2.6 to 3.7. The efficacy of metalaxyl plus fludioxonil was comparable to the low rate of Apron Maxx. Metalaxyl alone did not provide control of *R. solani*.

DISCUSSION

This study showed that both *F. avenaceum* and *R. solani* can reduce seedling emergence and stand establishment of alfalfa, birdsfoot trefoil and sweetclover under field conditions. Both pathogens caused substantial reductions in stand density and yield, but their impact varied substantially from year to year and from site to site. Both pathogens have previously been reported to affect forage yield by increasing plant stress that contributes to stand decline (Hwang *et al*., 1989; Hwang and Flores, 1987; Reeleder, 1982).

Birdsfoot trefoil exhibited relatively low rates of seedling survival in the field study. It is less competitive against weeds and therefore more difficult to establish (Rhykerd *et al*., 2000) than the other two crops. In contrast, sweetclover establishes quickly under favourable conditions and produced the highest forage yield in 2005.

The grain inocula of *Fusarium* and *Rhizoctonia* applied in this study were very aggressive according to the inoculum density studies under greenhouse conditions. The same inocula also resulted in significant levels of infection in all field experiments. However, there was a general failure for the *Rhizoctonia* inoculum in the 2003 greenhouse trials, where none of the inoculated treatments resulted in significant reduction of seedling survival. This failure was believed to be the result of poor quality in a specific batch of grain inoculum.

Seed treatment with metalaxyl alone did not affect seedling survival of the three forage species under controlled environment or field conditions. Metalaxyl is an effective fungicide against seedling diseases caused by *Pythium* sp. It was included in the study to assess the impact of background levels of *Pythium* sp. on seedling establishment and yield. However, metalaxyl did not improve establishment or yield, so we conclude that populations of *Pythium* sp. at these sites were low and were not an important factor in the field studies.

Fludioxonil is a protectant fungicide used as a seed treatment on a range of crops for control of damping-off and root rot caused by *Rhizoctonia* sp. In our study, fludioxonil consistently reduced losses in seedling survival and establishment caused by *R. solani* and *F. avenaceum*. A high rate of fludioxonil occasionally improved seedling survival relative to the low rate in the greenhouse study, but no differences were observed between the two rates in the field trials. However, few differences in forage yield were detected, despite the positive impact of seed treatment on seedling establishment.

Hancock (1993) reported that systemic seed treatments protect seedlings after emergence, when they are especially vulnerable to attack by soil-borne pathogens. However, compensatory growth of plants adjacent to gaps in the stand caused by damping-off and root rot may have reduced the impact of poor establishment on subsequent yield. In an earlier study, seed treatment had no impact on alfalfa establishment in non-inoculated treatments (Gossen, 1994b). However, recent studies indicate that the importance of *R. solani*, *F. avenaceum* and other seedling blight pathogens is increasing on the northern prairies (Chang *et al*., 2004; Gossen and Derksen, 2003; Hwang *et al*., 2000, 2002). This is likely associated with the dramatic increase in the acreage of highly susceptible crop species such as canola and grain legumes in this region over the last 25 years. Improving seedling establishment has the potential to increase yield, but may also extend the productive life of forage stands, which would allow producers to amortize the cost of stand establishment over a longer period. Fungicide seed treatment of forage legume crops may be warranted where populations of soil-borne pathogens are high, seed vigour is low, or where stand uniformity is especially important, such as for certified seed production.

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