RESPONSE OF ALFALFA IN THE ESTABLISHMENT YEAR TO INOCULATION WITH THE PHOSPHATE-SOLUBILIZING FUNGUS *Penicillium bifaii* (*Provide*®).

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ABSTRACT

This study was conducted to determine if alfalfa plants inoculated with the phosphate-solubilizing soil fungus *Penicillium bilaii* are able to source otherwise unavailable forms of soil P. A total of ten field trials were established throughout the major alfalfa producing regions of Saskatchewan in 1994 to determine the effects of inoculating alfalfa seed on the early season vegetative growth and P uptake, and forage yield response of alfalfa over a range of soil and climatic conditions. Increased alfalfa dry matter production, P uptake, and forage yield responses were observed following inoculation with *P. bilaii,* most likely the result of increased P availability from the solubilization of otherwise unavailable soil P. *Penicillium bilaii* is registered for use in Canada on wheat, canola, pea, and lentil under the trade name ***Provide@.***

INTRODUCTION

Many soils are deficient in phosphorus (P), and P is probably the most universally applied nutrient among alfalfa crops. Inadequate P nutrition can cause reduced alfalfa forage yields, quality and stand longevity. As P plays a vital role in plant growth, it is essential that alfalfa crops obtain an adequate supply of P for maximum forage yield and quality.

The availability of soil P for uptake by plants is restricted by precipitation of solution P with soil cations and adsorption to the surface of soil particles (Barber, 1984). These rapid soil fixation reactions result in low use efficiency of P fertilizer by plants, typically ranging from 10% to 25% in the year of application (Spinks and Barber, 1947). Consequently, a large proportion of inorganic soil P exists in forms which are not immediately available for plant uptake.

Kucey (1983) identified an isolate of ***Penicillium*** bilaii (ATCC Strain No. 2085 1, previously reported as ***P. bilaji)*** capable of solubilizing P from rock P at rates two to four times greater than other fungal or bacterial isolates, grew actively on a wide range of nutrient sources, and maintained P solubilization activity at temperatures as low as 4°C. Inoculation with this strain increased the vegetative growth, P uptake, and grain yield of wheat (Kucey, 1987, 1988; Asea et al., 1988; Chambers and Yeomans, 1990, 1991; Gleddie et al., 1991), bean (Kucey, 1987), pea (Gleddie, 1993), and canola (Kucey and Leggett, 1989; Keyes, 1990; Gleddie et al., 1993 j. No information is available regarding the efficacy of ***P. bilaii*** for enhancing alfalfa growth by increasing P availability and uptake. Accordingly, the objective of this study was to determine the effects of inoculating alfalfa with the phosphate-solubilizing fungus ***P. bilaii*** on the early season growth, P uptake, and forage yield response of alfalfa over a range of soil and climatic conditions.

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METHODS

Field trials were established at eight locations in 1994 by Philom Bios Inc. and Agriculture and Agri-Food Canada (AAFC). Research sites were located in the brown, dark brown, black, and grey soil zones of Saskatchewan. Available P levels (as determined by modified Kelowna method, Plains Innovative Labs, Saskatoon, Saskatchewan) in the top 30 cm of soil ranged from 8 to 26 kg P ha^{-1}.

Philom Bios Trials

Philom Bios Inc. established pure stands of alfalfa in small-plot trials at six locations in Saskatchewan in 1994. Four trials were seeded in the spring and two in the fall.

Each trial was arranged in a randomized complete-block design with ten blocks per treatment and ten treatments per block. Treatments included 'Algonquin' alfalfa inoculated with *Rhizobium meliloti* versus alfalfa inoculated with P. *bilaii* plus *R. meliloti.* Both alfalfa treatments were planted with 0, 20, 40, 60, and 80 kg P_2O_5 ha^{-1} rates of P fertilizer. Phosphate fertilizer treatments, applied as triple-super-phosphate (TSP; 0-45-0), were broadcast and incorporated prior to seeding.

Prior to seeding, basal treatments of N, K, S, or micronutrients were broadcast at each field site according to soil test recommendations to ensure adequate nutrient supply and to highlight crop responses to P. Ethalfluralin was applied at each site for weed control. Basal nutrient and ethalfluralin applications were incorporated to a depth of 8 cm with a 1.5-m wide rototiller just prior to application of the fertilizer P treatments, and then cross-incorporated a second time in the prevailing plot direction. Plots were subsequently sprayed with foliar herbicides or hand-weeded as needed to remove any weeds not eliminated by ethalfluralin.

Each individual treatment plot was 1.5 m by 10.0 m and consisted of six treatment rows spaced 15 cm apart. Each plot was bordered by two guard rows of uninoculated alfalfa to eliminate any potential edge effects. Each complete trial was also bordered by untreated guard plots to eliminate edge effects.

Trials were seeded with a small-plot double-disc drill specially constructed to minimize contamination between inoculant treatments. Alfalfa was planted at a rate of 10 kg ha^{-1} and at depths ranging from 0.5 to 1.5 cm, depending upon soil moisture conditions at each location.

For spring-seeded trials, plant samples were collected from each plot for; (1) early season dry matter (DM) production at the 3 trifoliate leaf stage, and (2) forage yield at the 10% bloom stage. For fall-seeded trials, samples were collected only at the 3 trifoliate leaf stage. All above-ground biomass was collected from adjacent 1-metre square areas within each treatment plot at the 3 trifoliate sampling date. At the 10% bloom stage, alfalfa was harvested at approximately a 5-cm cutting height from adjacent 3-metre square areas within each treatment plot. At each sampling time, plants were dried, weighed and ground. Subsamples of the dried, ground plant biomass were analyzed for P and N concentration.

Agriculture and Agri-Food Canada Trials

Large-plot alfalfa field trials were established by Agriculture and Agri-Food Canada at two Saskatchewan locations in 1994. Two trials were established at each location; one without a companion crop and one using wheat as a companion crop.

Each large-plot trial was arranged in a randomized complete-block design with four blocks and six treatments per block. Treatments included alfalfa inoculated with *R. meliloti* versus alfalfa inoculated with *P. biluii* plus *R. meliloti*. Both alfalfa treatments were planted with 0, 38, and 75 kg P_2O_5 ha^{-1} of P fertilizer, applied as side-banded mono-ammonium phosphate (11-55-o). These rates are equivalent to one-half and one times the recommended P application rate.

Alfalfa was direct-seeded at both locations at a rate of 10 kg ha^{-1} using a 4-m wide air drill with 23-cm row spacing. The individual treatment plots measured 4 m by 75 m. To determine the impact of a companion crop on the response of alfalfa to *P. biluii*, the main trial experiment was repeated on an adjacent area at each site using 'Katepwa' wheat sown at right angles to the alfalfa, at a rate of 45 kg ha-'. Weeds were controlled post-emergence using diclofop methyl and 2,4-DB.

Alfalfa plants were sampled at three dates over the growing season for; (1) early season DM production at 5 weeks after emergence; (2) forage yield at the 10% bloom stage; and (3) forage yield immediately after the first killing frost. At five weeks after emergence, all above-ground biomass was collected from three randomly selected 1 -metre squares within each treatment plot. At the 10% bloom stage, alfalfa was harvested at a 5 cm cutting height from three randomly selected 1-metre squares within each treatment plot. After the first killing frost, alfalfa was harvested at a 5 cm cutting height from two randomly selected 15-m^2 areas within each treatment plot. At each sampling time, plants were dried, weighed and ground. Subsamples of the ground material were analyzed for P and N concentration.

Data Analyses

The primary objective of the field research was to evaluate the effects of inoculation with *P. bilaii* over a wide range of soil and climatic conditions. As such, only combined location analyses are presented in the Results section. Analysis of variance was performed using the General Linear Model procedure of the Statistical Analysis System package (SAS Institute Inc., 1988). Combined analysis of variance was calculated as outlined by Gomez and Gomez (1984). Single degree-of-freedom contrasts were used to statistically compare treatment means.

Response data collected from trials containing the same number of treatments were used to calculate combined location analyses. Therefore, Philom Bios data is presented in the Results separately from AAFC data. Agriculture and Agri-Food Canada trials were analyzed separately according to alfalfa grown as pure stand or companion crop. Response data from the two forage yield dates in AAFC trials are presented as a mean of the two harvest dates and locations.

RESULTS

Application of P fertilizer or inoculation with ***P. bilaii*** significantly ($P \leq 0.01$) increased alfalfa DM production when measured at the three trifoliate leaf stage in Philom Bios trials (Table 1). The significant ($P \leq 0.10$) P-quadratic by ***P. bilaii*** interaction for DM indicates that response to ***P. biluii*** was greatest with the lower rates of P fertilizer. The highest level of DM response occurred with ***P. biluii*** inoculation and a P application rate of 60 kg P_2O_5 ha-‘.

Inoculation with ***P. biluii*** or P application significantly ($P \leq 0.05$) increased P uptake by alfalfa at the three trifoliate leaf stage (Table 1). Phosphate application and ***P. biluii*** inoculation were additive in effect on P uptake as no significant P by ***P. biluii*** interactions occurred. The highest P uptake occurred with ***P. biluii*** inoculation at the 60 kg P_2O_5 ha-’ P application rate.

Table 1. Dry matter production and phosphorus uptake at the three trifoliate leaf stage for pure stand alfalfa inoculated with *Penicillium biluii* **at five phosphate levels, in field trials established by Philom Bios Inc., in 1994’.**

Phosphate	Inoculant	Dry Matter	P Uptake
		3 trifoliate	
kg P_2O_5 ha^{-1}		kg ha“	kg P ha^{-1}
0	*R. meliloti*	*44.4*	*0.15*
	P. bilaii + *R. meliloti*	*47.8*	*0.16*
20	*R. me!iloti*	*51.2*	0.18
	P. bilaii + *R. meliloti*	*54.4*	*0.19*
40	*R. meliloti*	*50.8*	*0.18*
	P. bilaii + *R. meliloti*	*56.3*	*0.20*
60	*R. meliloti*	*53.5*	*0.19*
	P. bilaii + *R. meliloti*	*60.3*	*0.21*
80	*R. meliloti*	*55.7*	*0.20*
	P. bilaii + *R. meliloti*	*54.6*	*0.20*
LSD (P<=0.05):		5.2	0.02
Contrast:	Phosphate (0)	46.1	0.15
	Phosphate (20)	52.8	0.18
	Phosphate (40)	53.6	0.19
	Phosphate (60)	56.9	0.20
	Phosphate (80)	55.2	0.20
	Phosphate (P)-linear	**	**
	P-quadratic	**	**
	P. bilaii (Pb)	*54.7* **	*0.19* *
	vs no *P. bilaii*	*51.1*	*0.18*
	P-linear x *Pb*	*NS*	*NS*
	P-quadratic x *Pb*	t	NS

1 Mean of six trial locations.

†, *, **, NS: Significant at P<=0.10, P<=0.05, P<=0.01, and nonsignificant, respectively.

Phosphate fertilizer applications significantly ($P \leq 0.0$ 1) increased DM production and P uptake of pure stand alfalfa five weeks after emergence in AAFC trials (Table 2). Alfalfa DM production and P uptake responses to P. ***biluii*** inoculation were greater at the zero and mid application rates of P, but lower at the highest application rate of P, as indicated by the significant ($P \leq 0.10$) P by ***P. bilaii*** interactions. The highest levels of DM production and P uptake occurred at the highest P application rate.

Inoculation with ***P. biluii*** significantly ($P \leq 0.01$) increased alfalfa DM production and P uptake at five weeks after emergence when grown with a companion crop (Table 2).

Table 2. Dry matter production and phosphorus uptake five weeks after emergence for pure stand or companion crop alfalfa inoculated with or without *Penicillium biluii* at three P fertilization levels, in trials established by Agriculture and Agri-Food Canada, in 1994.’

Phosphate	Inoculant	------ Pure Stand Alfalfa ------		--- Companion Crop Alfalfa ---	
		Drv Matter	P Uptake	Drv Matter	P Uptake
(kg P_2O_5 ha-‘)		kg ha^{-1}	kg P ha^{-1}	kg ha^{-1}	kg P ha^{-1}
0	*R. meliloti*	513	1.75	69.1	0.21
	P. hilaii + R. meliloti	**553**	**1.85**	**92.6**	**0.27**
38	*R. meliloti*	**692**	**2.24**	**75.4**	**0.24**
	P. bilaii + R. meliloti	*802*	*2.49*	*96.0*	*0.30*
75	*R. meliloti*	*878*	*2.72*	*77.1*	*0.24*
	P. bilaii + R. meliloti	*762*	*2.42*	*87.0*	*0.28*
LSD ($P<0.05$)		119	0.33	21.5	0.07
Contrast:	Phosphate (0)	533	1.80	80.8	0.24
	Phosphate (38)	747	2.36	85.7	0.27
	Phosphate (75)	820	2.57	82.1	0.26
	Phosphate (P)-linear	**	**	NS	NS
	P-quadratic	†	†	NS	NS
	P. bilaii (Pb)	706	2.26	91.8 **	0.28 **
	vs. no *P. bilaii*	*694*	*2.23*	*73.9*	*0.23*
	P-linear x *Pb*	†	†	*NS*	*NS*
	P-quadratic x *Pb*	*	†	NS	NS

[1] Mean of two trial locations.

†, *, **, NS: Significant at P<=0.10, P<=0.05, P<=0.01, and nonsignificant, respectively.

Applications of P fertilizer or inoculation with ***P. biluii*** significantly ($P \leq 0.0$ 1) increased alfalfa forage yield and N uptake measured at 10% bloom (Table 3). As indicated by nonsignificant P by ***P. bilaii*** interactions, forage yield and N uptake responses to P application and ***P. bilaii*** were additive in effect. The highest level of forage yield and N uptake response occurred with ***P. bilaii*** inoculation at the highest P application rate.

Table 3. Forage yield and nitrogen uptake at the 10% bloom stage for pure stand alfalfa in the establishment year inoculated with or without *Penicillium bilaii at five* phosphate levels, in field trials established by Philom Bios Inc., in 1994[1].

Phosphate	Inoculant	Forage Yield	N Uptake
		10% bloom	
kg P_2O_5 ha^{-1}		kg ha“	kg N ha^{-1}
0	*R. meliloti*	2816	76.2
	P. bilaii + *R. meliloti*	2826	76.1
20	*R. meliloti*	3041	83.8
	P. bilaii + *R. meliloti*	*3083*	*84.6*
40	*R. meliloti*	*3006*	*83.9*
	P. bilaii + *R. meliloti*	*3175*	*86.8*
60	*R. meliloti*	*3166*	*87.7*
	P. bilaii + *R. meliloti*	*3320*	*91.8*
80	*R. meliloti*	*3273*	*88.9*
	P. bilaii + *R. meliloti*	*3398*	*91.7*
LSD (P<=0.05):		153	5.1
Contrast:	Phosphate (0)	2821	76.2
	Phosphate (20)	3062	84.2
	Phosphate (40)	3091	85.4
	Phosphate (60)	3243	89.8
	Phosphate (80)	3336	90.3
	Phosphate (P)-linear	**	**
	P-quadratic	**	*
	P. bilaii (Pb)	*3160* **	*86.2* †
	vs no *P. bilaii*	*3060*	*84.1*
	P-linear x *Pb*	*NS*	*NS*
	P-quadratic x *Pb*	*NS*	*NS*

1 Mean of four trial locations.

†, *, **, NS: Significant at P<=0.10, P<=0.05, P<=0.01, and nonsignificant, respectively.

Combined across both forage yield dates, P application or ***P. bilaii*** inoculation significantly (P≤0.0 1) increased alfalfa forage yield when grown as a pure stand (Table 4). Phosphate application and ***P. bilaii*** inoculation were not additive in effect on forage yield in pure stand alfalfa, as indicated by the significant (P≤0.05) P-linear by ***P. bilaii*** interaction. Alfalfa forage yield responses to ***P. bilaii*** inoculation were greater at the lower P application rates than with the highest P application rate .

Phosphate application significantly (P≤0.0 1) increased N uptake by pure stand alfalfa (Table 4). Nitrogen uptake response to ***P. bilaii*** inoculation was greater when used without P fertilizer than in combination with the highest P application rate, as indicated by the significant ($P \leq 0.05$) P-linear by ***P. bilaii*** interaction.

Combined across both forage yield dates, P application or ***P. bilaii*** inoculation significantly ($P \leq 0.0$ 1) increased alfalfa forage yield and N uptake when grown with a companion crop (Table 4). Phosphate application and ***P. bilaii*** inoculation were additive in effect on forage yield and N uptake, as indicated by the nonsignificant P by ***P. bilaii*** interactions. The greatest forage yield and N uptake occurred with ***P. bilaii*** inoculation at the highest rate of P.

Table 4. Forage yield and nitrogen uptake in the establishment year for pure stand or companion crop alfalfa inoculated with or without *Penicillium bilaii at* three phosphate fertilization levels, in field trials established by Agriculture and Agri-Food Canada, in 1994.'

Phosphate	Inoculant	------ Pure Stand Alfalfa ------		--- Companion Crop Alfalfa ---	
		Forage Yield	N Uptake	Forage Yield	N Uptake
(kg P_2O_5 ha^{-1})		kg ha^{-1}	kg N ha^{-1}	kg ha^{-1}	kg N ha^{-1}
0	*R. meliloti*	1527	51.3	369	13.7
	P. bilaii + R. meliloti	*1866*	*61.6*	*480*	*17.4*
38	*R. meliloti*	*2047*	*72.5*	*451*	*16.6*
	P. bilaii + R. meliloti	223 1	*73.9*	*534*	*19.8*
75	*R. meliloti*	*2499*	*83.3*	*493*	*18.2*
	P. bilaii + R. meliloti	*2509*	*82.4*	*597*	*22.0*
LSD (P<=0.05)		214	7.9	83	3.2
Contrast:	Phosphate (0)	1697	56.5	425	15.6
	Phosphate (38)	2139	73.2	493	18.2
	Phosphate (75)	2504	82.9	545	20.1
	Phosphate (P)-linear	**	**	**	**
	P-quadratic	NS	NS	NS	NS
	P. bilaii (Pb)	2202 **	72.6	537 **	19.7 **
	vs. no ***P. bilaii***	*2024*	*69.0*	*438*	*16.2*
	P-linear x ***Pb***	*	*	*NS*	*NS*
	P-quadratic x ***Pb***	*NS*	*NS*	*NS*	*NS*

1 Mean of two trial locations and two forage harvest dates.
†, *, **, NS: Significant at P<=0.10, P<=0.05, P<=0.01, and nonsignificant, respectively.

DISCUSSION

Inoculation with ***P. bilaii*** increased the early season vegetative growth and P uptake, and forage yield of alfalfa in this study. These results are in agreement with previous reports of increased P uptake and yield by wheat, canola, bean, and pea following inoculation with ***P. bilaii*** **(Kucey,** 1987, 1988; Kucey and Leggett, 1989; Chambers and Yeomans, 1990, 1991; Keyes, 1990, Gleddie et al., 1991, 1993; Gleddie, 1993).

As the soils used in these studies were generally P-deficient, any increase in P availability (from P application or inoculation with P. ***bilaii)*** resulted in increased DM production and P uptake, providing other factors were not limiting plant growth. Increases in early season P uptake following P. ***biluii*** inoculation or P application were similar in magnitude to the level of DM response in these trials. Thus, increased DM production was the primary factor influencing P uptake by alfalfa.

Increased P uptake observed in P. ***biluii*** inoculated plants is most likely the result of solubilization of otherwise unavailable soil P. As tracers were not used in these studies, the source of additional P in P. ***biluii*** inoculated plants cannot be determined. Solubilization of rock P by P. ***biluii*** has been demonstrated in solution culture, most likely by the production of organic acids which acidify the surrounding media (Kucey, 1983; Asea et al., 1988). Previous studies using ^{32}P indicated that plants inoculated with P. ***biluii*** are able to utilize native soil P sources which are otherwise unavailable to control plants (Chambers and Yeomans, 1990; 1991) as well as added rock P (Asea et al., 1988).

Further indirect evidence that inoculation with P. ***biluii*** increases the availability of soil P is provided by comparisons of P. ***biluii*** inoculated plants to plants which received greater amounts of P fertilizer alone. Increasing soil available P by adding P fertilizer increased P uptake and forage yield of alfalfa. Inoculation with P. ***biluii*** generally resulted in equivalent P uptake and forage yield responses to those obtained by using the next increment of P fertilizer alone. Thus, when used with the lower P application rates, inoculation with P. ***bilaii*** caused increases in soil P availability equivalent to the increases in soil P availability obtained by adding an additional 20 kg P_2O_5 ha^{-1}.

Overall, inoculation with P. ***bilaii*** increased the early season growth and P uptake, and forage yield of alfalfa in the establishment year over a range of soil and climatic conditions. It appears that P. ***bilaii*** is able to increase P availability and uptake by alfalfa by solubilization of otherwise unavailable soil P. ***Penicillium bilaii*** is registered in Canada for use on wheat, canola, pea, and lentil under the trade name ***Provide***®.

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