Evaluation of *Penicillium bilaji* Inoculation and Copper and Zinc Fertilization in Relation to Crop Yield and Nutrient Uptake.

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Abstract

Growth chamber and field experiments were conducted on three low micronutrient alkaline soils from Northeastern Saskatchewan to test the efficacy of PB-50 inoculation and phosphorus (P), copper (Cu) and zinc (Zn) fertilization in relation to plant yield and nutrient uptake responses. Field experiments involving wheat (*Triticum aestivum* 'Katepwa') and peas (*Pisum sativum* 'Titan') showed no yield or P uptake as a result of PB-50 seed inoculation or P_2O_5 fertilization for three locations. Fertilization of wheat with $CuSO_4$ and peas with $ZnSO_4$ produced increases in seed and total plant uptake of Cu and Zn. A yield increase from 2722 kg/ha to 3682 kg/ha (40 to 54 bu/ac) as a result of Cu fertilization was observed for Katepwa wheat on Nipawin Soil (DTPA Cu = 0.1 µg/g). No other yield increases were observed for Cu or Zn fertilization on the other soils. Growth chamber experiments involving wheat (*Triticum aestivum* 'Park') and navy beans (*Phaselous vulgaris* L.) produced results similar to field experimentation. Increased Cu uptake in wheat for $CuSO_4$ fertilized treatments was observed for all three soils. Cu fertilization in the Nipawin soil showed a yield increase in wheat relative to control. Wheat seed inoculation with PB-50 produced no yield increases and minor nutrient uptake differences. Increased yields and Zn uptake in beans for $ZnSO_4$ fertilized treatments was observed in all three soils. Seed inoculation of navy bean with PB-50 produced variable yield increases and nutrient uptake differences in two of three soils.

Introduction

Extensive research has been conducted to examine the nature and occurrence of micronutrient deficiencies in cropping systems. Early assessment of the micronutrient status of Saskatchewan soils found that most of the province's soils were adequately supplied with copper (Cu) and zinc (Zn) (Stewart, 1969). Recent findings suggest the opposite. For example, Kruger et al.(1985) suggest that Cu deficiencies in Saskatchewan are likely concentrated in the soils of the light transition of grey and brownish grey podzolic soils. Karamanos et al.(1983) found significant yield increases with Zn fertilization of some Saskatchewan soils.

Soluble Cu and Zn fertilizers are the usual method of correcting Cu and Zn deficiencies. These sources are expensive and inefficient because the majority of the applied element is immobilized in insoluble fractions of the soil (Kabata-Pendias and Pendias, 1984; Tisdale et al., 1984). Plant uptake of Cu and Zn is dependant on soluble levels in the rhizosphere.

Microbial metabolism and the release of root exudates enhance the solubility and availability of metals in the rhizosphere by mediating pH and complexation reactions (Barber and Lee, 1974; Kucey, 1988; Mench et al., 1988). For example, Linehan et al. (1989) observed seasonally-increased micronutrient concentrations in the rhizosphere of barley (*Hordeum vulgare* L.). They attributed this observation to the biological production of ligands which mobilized insoluble forms of micronutrients in the rhizosphere.

Root exudates produced by higher plants are soluble organic compounds ranging from simple sugars to complex vitamins and complex and mucilages (Rovira et al., 1979). These compounds may promote the dissolution of rhizosphere metals by forming soluble metal complexes (Bromfied, 1958; Godo and Reisenaur, 1980; Olsen and Brown, 1980; Uren, 1984; Curl and Truelove, 1986; Merckx et al., 1986; Mench et al., 1988). Low molecular weight root exudates have acidic functional groups which dissociate and react with metals to form strong metal complexes. For example, metal chelation/complexation by root exudates may be induced by dissociation of the functional groups of organic acids (eg., citrate, malate), amino acids (eg., glutamate, lysine) and HO- hydroxyl and phenolic groups associated with each exudate (Mench et al., 1988).

Phosphate-solubilizing rhizosphere microbes solubilize inorganic phosphorus and micronutrient compounds in soil by excreting organic acids into their environment (Kucey, 1988). Analyses of culture filters of these microbes have revealed the production of the following acids: lactate, glycolate, citrate, 2-ketogluconate, malate, oxalate, malonate, tartarate and succinate (Fig. 1) (Sperber, 1958; Louw and Webley, 1959; Duff et al., 1963; Taha et al., 1969; Banik and Dey, 1982). The quantities of acid produced by such microbes may equal > 5 % of the total carbohydrate consumed by the organism (Banik and Dey, 1982).

Organic acids produced by rhizosphere microbes may dissociate insoluble Cu and Zn compounds in soil (Kucey, 1988). This process is believed to occur through a three step process: i) lower rhizosphere pH, ii) chelate/complex metals and iii) initiate exchange reactions in the growth environment (Sperber, 1958; Molla and Chowdhury, 1984; Asea et al., 1988). Such micronutrient - organic chelates may be of great importance in the release of metal nutrients in the rhizosphere (Stevenson and Ardakani, 1972; Linehan et al., 1989).

Berrow et al. (1982) concluded that 2-ketogluconate isolated from a culture of *Erwinium* species was able to extract the some amount of Cu, Mn, Mo, Ni, and Zn from soil as concentrated EDTA and DTPA solutions. Kucey (1988) showed that the addition of 0.05 M EDTA to solutions containing insoluble Cu and Zn had the same effects on solubilization effect as inoculation with *Penicillium bialji*. In addition, *P. bilaji* inoculation of wheat (*Triticum aestivum* Chester) increased plant uptake of Cu and Zn from soil in greenhouse and field experiments. A plant growth promoting isolate of *Pseudomonas putida* inoculated onto clover was shown to increase plant uptake of Fe, Cu, Al, Zn, Co, and Ni (Meyer and Linderman, 1986).

Penicillium bilaji is a naturally occuring soil fungus which is the 'active ingredient' in a seed inoculant termed PB-50™. This organism can mobilize insoluble micronutrient and phosphate soil fractions by secreting various organic acid metabolites in the rhizosphere (Kucey, 1988). Inoculation with *P. bilaji* and fertilization with inexpensive insoluble Cu and Zn sources may represent an alternative to more expensive Cu and Zn fertilizer methods. Field and growth chamber experiments were conducted on three low micronutrient alkaline soils to meet three test objectives, evaluation of: PB-50 seed inoculation, Cu and Zn fertilization, and P fertilization in the field.

Materials and Methods

Growth Chamber

Growth chamber experiments were established in 10 x 15 cm columns to determine the efficacy of PB-50 seed inoculation and Cu and Zn fertilization in relation to plant yield and nutrient uptake. Wheat (*Triticum aestivum* 'Park') and navy beans (*Phaselous vulgaris* L.) were grown on three low micronutrient alkaline soils (Table 1) from Northeastern Saskatchewan to serve this purpose. Soil was collected from the Ah horizon (0 - 15 cm) of each soil and was dried, ground, and sieved through a 2 mm polypropylene screen before use in the columns.

Table 1 Basic properties of the soils used in experimentation.

Site	Texture	pH	Cond.	Concentration (µg/g)							
			(dS/m)	NO_3	P	K	SO_4	Cu*	Zn*	Fe*	Mn*
Snowden	Loam	8.0	0.3	5.6	15	134	11	0.4	0.4	22	3.0
Nipawin	Sand	8.0	0.3	2.2	14	70	7	0.1	0.6	17	3.0
Aylsham	Loam	8.1	0.4	8.3	19	82	10	0.3	0.9	26	6.7

* DTPA extractable levels

The Snowden, Nipawin, and Aylsham soils are mapped under the Smeaton-comp, Whitefox, and Weirdale soil associations respectively. Each soil is relatively low in Cu and Zn and would receive recommendation for Cu and Zn application for sensitive crops (SSTL, 1989).

Four treatments were established for the copper experiment involving park wheat: control, *P. bilaji* inoculation, $CuSO_4$, and *P. bilaji* + $CuSO_4$. Similar treatments were imposed in the zinc experiment involving navy beans: control, *P. bilaji* inoculation, $ZnSO_4$, and *P. bilaji* + $ZnSO_4$. Each source of Cu or Zn was added so that an equivalent of 10.00 g of Cu or Zn per 1.5 kg soil was added for each Cu or Zn treatment. The experiment was established on a complete randomized block design and each treatment was replicated four times.

Approximately 1.5 kg of soil was used in each column. An application of plant nutrients was applied to each column to ensure that only the test element would limit fertility (Table 2).

Table 2 Nutrient sources added to growth chamber soils.

Source	Soil Level (g/kg)	
NH_4NO_3	N = 13.3	
$Ca(H_2PO_4)_2$	P = 5.3	Ca = 3.5
KCl	K = 26.7	
$CaCl_2{\cdot}6H_2O$	Ca = 8.0	
$MgSO_4{\cdot}7H_2O$	Mg = 0.7	S = 0.9
$FeSO_4{\cdot}7H_2O$	Fe = 0.67	S = 0.4
$MnSO_4{\cdot}4H_2O$	Mn = 0.53	S = 0.1
$Na_2B_4O_7{\cdot}10H_2O$	B = 0.07	
$Na_2MoO_4{\cdot}2H_2O$	Mo = 0.08	
$ZnSO_4{\cdot}7H_2O$ (wheat only)	Zn = 0.66	S = 0.3
$CuSO_4{\cdot}5H_2O$ (beans only)	Cu = 0.66	S = 0.3

Seeds inoculated with *P. bilaji* were plated on potato dextrose agar (0.5 % v/v rosebengal and tetrachlorocycline and 0.5 % and 1.0 % w/v K_2HPO_4 and $CaCl_2$ respectively) to assay the population of *P. bilaji* on the seed surface. Plates were counted based on a dilution series from 10^2 to 10^4 colony forming units (CFU's) per seed.

Each column was watered daily with distilled deionized water according to weight so that the soil was always between 60 and 90 % field capacity. Eight wheat seeds and four bean seeds were seeded at 3 and 4 cm depths respectively into moist soil. The wheat and pea seedlings were thinned to 4 and 2 plants per column respectively two weeks after seeding. A 16 hour photoperiod was used at diurnal temperatures of 22 ± 1 °C and 16 ± 1 °C respectively. The growth chamber light intensity was 175 $\mu E \bullet s^{-1} \bullet m^{-2}$ at the soil surface for the duration of the experiment.

The plants were harvested by cutting 2 cm above the soil surface 84 and 95 days after seeding for beans and wheat respectively. Each sample was dried at 60 °C for 1 week and the total plant mass values were determined. The plant samples were then thrashed by hand and the seed and straw masses were measured. Seed and straw samples were ground in a cyclone mill with a nickel impeller and digested according to Thomas et al. (1967). Analysis of plant uptake of P, Cu, and Zn was conducted using an ICP plasma spectrometer.

All statistical mass and nutrient uptake values were generated from analysis of variance with a protected least significant difference at 95% significance according to Steel and Torrie (1980).

Field

Field trials were established during the summer of 1990 on the sites where the growth chamber soil was sampled in Northeastern Saskatchewan. A completely randomized block design was employed for experimentation. The plot dimensions were 1.05 meters in width and 12 meters in length (12.50 m^2 area) All Cu and Zn fertilizer calculations were based on these dimensions and a 0.15 m depth.

The Cu and Zn treatments imposed were identical to those in growth chamber experimentation. The Cu and Zn fertilizers were applied at a 15 kg/ha rate by broadcasting on the soil surface and incorporating with a light harrowing. Additional phosphorus treatments were established at each site to determine the efficacy of phosphorus fertilization and PB-50 seed inoculation. The P source used was triple super phosphate (0-46-0). These treatments were: no P_2O_5, no P_2O_5 + PB-50, 1/2 P_2O_5, 1/2 P_2O_5 + PB-50, full P_2O_5, and full P_2O_5 + PB-50. The P_2O_5 rates on all sites were as follows: 1/2 = 10 kg/ha and full = 20 kg/ha. In each case, the P was seed placed. Each treatment was replicated five times.

Wheat (*Triticum aestivum* 'Katepwa') and peas (*Pisum sativum* 'Titan') were seeded at 4 and 5 cm depths respectively with a small plot seed drill. The seeding rates for wheat and peas were 108.5 and 156.8 g/plot (86.8 and 125.4 kg/ha) respectively. Both wheat and peas were inoculated with commercial PB-50 according to label recommendations. The *P. bilaji* inoculated seeds were assayed for viable colonies using the same method as in growth chamber experimentation. All of the pea seeds were inoculated with a commercial strain of *Rhizobium leguminosarum* to ensure N_2 fixation. Blanket applications of plant nutrients were applied to each plot to meet fertilizer recommendations. The pea plots did not receive nitrogen fertilizer.

During the course of the field experiment, the plots at all three sites were maintained for weed and insect control and visual yield and vigor ratings were taken. Each plot was harvested by cutting 4 m^2 samples from each plot. The Snowden, Nipawin, and Aylsham sites were harvested 105, 103, and 103 days after seeding respectively. The plant yield for each plot was determined after 14 days of drying. The samples were then thrashed and the seed yield was measured from each plot. Seed and straw samples were ground, digested, and analyzed for P, Cu, and Zn uptake according to the procedures used in growth chamber experimentation.

All statistical mass and nutrient uptake values were generated from analysis of variance with a protected least significant difference at 95% significance according to Steel and Torrie (1980).

Results and Discussion

The results of the plate counts for the growth chamber and field experiments (Table 3) indicate that in each experiment the seeds had viable *P. bilaji* on the seed surface. The navy bean seeds from the Snowden and Nipawin growth chamber studies were not assayed properly, so CFU numbers could not be obtained, but *P. bilaji* colonies were observed on the respective plates.

Table 3 Plate count results of *P. bilaji* inoculated seeds from growth chamber and field experiments.

Soil	Crop	Count (CFUs/seed)	
		Growth Chamber	Field
Snowden	Beans/Peas	nd	3270
	Wheat	5100	nd
Nipawin	Beans/Peas	nd	500
	Wheat	5100	1823
Aylsham	Beans/Peas	2400	350
	Wheat	5100	3316

Growth Chamber

Park wheat yields from growth chamber experimentation (Table 4) show variable responses to *P. bilaji* inoculation anc $CuSO_4$ fertilization. The Nipawin soil showed yield (Table 4) and Cu uptake (Table 5) responses to $CuSO_4$ additions because it was deficient in

Table 4. Mean plant mass (g/column) values of Park wheat grown on Nipawin, Snowden, and Aylsham Soils*.

Treatment	Plant Mass (g/column)		
	Nipawin	Snowden	Aylsham
Control	5.277_b	8.704_a	7.012_a
P. bilaji	5.899_b	7.222_b	6.613_a
$CuSO_4$	6.158_b	7.567_b	6.427_a
P. bilaji + $CuSO_4$	7.449_a	8.224_{ab}	6.817_a

* Values with different letters are significantly different (95%)

Cu (DTPA Cu = 0.1 µg/g) (Table 1). Snowden and Aylsham soils had higher Cu levels (DTPA Cu = 0.4 and 0.3 µg/g respectively) (Table 1) and therefore less of a response to applied Cu. *P. bilaji* inoculation without Cu fertilization for the three soils produced no yield or Cu uptake increases relative to control (Tables 4 and 5). *P. bilaji* inoculation with Cu addition increased yield and Cu uptake values for Park wheat grown on the Nipawin soil. Park wheat yield responses appear to be related to increases in Cu uptake. Yield responses are variable and dependant on the Cu status of the soil.

Table 5. Mean plant copper uptake (µg/column) values of Park wheat grown on Nipawin, Snowden, and Aylsham Soils*.

Treatment	Copper Uptake (µg/column)		
	Nipawin	Snowden	Aylsham
Control	34.02_c	57.75_a	37.83_a
P. bilaji	38.76_{bc}	48.34_a	38.81_a
$CuSO_4$	36.88_{bc}	52.65_a	35.03_a
P. bilaji + $CuSO_4$	52.02_a	54.90_a	43.21_a

* Values with different letters are significantly different (95%)

Navy beans showed yield responses to $ZnSO_4$ additions in the Nipawin and Snowden soils and no response in the Aylsham soil. This may be the result of low Zn levels in Nipawin and Snowden soils (DTPA Zn = 0.6 and 0.4 µg/g respectively) relative to higher levels in the Aylsham soil (DTPA Zn = 0.9 µg/g) (Table 1). *P. bilaji* inoculation produced yield increases in the three test soils. Inoculation without $ZnSO_4$ addition produced yield increases in Nipawin and Aylsham soils. Inoculation with $ZnSO_4$ addition gave yield increases in Nipawin and Snowden soils (Table 6).

Table 6. Mean plant mass (g/column) values of Navy beans grown on Nipawin, Snowden, and Aylsham Soils*.

Treatment	Plant Mass (g/column)		
	Nipawin	Snowden	Aylsham
Control	3.932_c	9.303_b	6.538_b
P. bilaji	4.882_a	10.379_{ab}	8.419_a
$ZnSO_4$	4.075_{bc}	10.434_{ab}	6.973_b
P. bilaji + $ZnSO_4$	5.282_a	12.192_a	7.295_b

* Values with different letters are significantly different (95%)

Navy bean yield increases (Table 6) are related to increased plant uptake of Zn in the Nipawin and Snowden soils but not in the Aylsham soil (Table 7). In all cases, $ZnSO_4$ fertilization produced greater plant uptake of Zn relative to control treatments (Table 7). *P. bilaji* seed inoculation without $ZnSO_4$ addition led to Zn uptake increases in the Nipawin and Aylsham soils relative to control (Table 7). *P. bilaji* inoculation with $ZnSO_4$ produced the highest Zn uptake in Navy bean relative to all other treatments (Table 7).

In general, Navy beans showed more consistent yield and Zn uptake responses to $ZnSO_4$ addition and *P. bilaji* inoculation compared to Park wheat responses to $CuSO_4$ addition and *P. bilaji* . The Navy beans may have been more sensitive to soluble Zn levels than Park wheat was to soluble Cu levels.

In these experiments, soil fertility for both wheat and beans was in the following order: Snowden > Aylsham > Nipawin. These experiments also showed that *P. bilaji* inoculation and $CuSO_4$ / $ZnSO_4$ additions did not ensure yield increases because responses were variable. Yield responses to Cu were observed in low Cu Nipawin soil and Zn responses were observed in low Zn Nipawin and Snowden soils. Aylsham soil was not responsive to Cu or Zn additions because it was not deficient in Cu or Zn.

Table 7. Mean plant zinc uptake (µg/column) values of Navy beans grown on Nipawin, Snowden, and Aylsham Soils*.

Treatment	Zinc Uptake (µg/column)		
	Nipawin	Snowden	Aylsham
Control	69.01_b	145.50_b	102.51c
P. bilaji	109.61_a	172.16_b	135.75_b
$ZnSO_4$	112.33_a	268.19_a	149.68_a
P. bilaji + $ZnSO_4$	142.40_a	303.60_a	163.61_a

* Values with different letters are significantly different (95%)

The majority of the native and fertilizer Cu and Zn in the high pH test soils should exist in insoluble alkaline fractions. If *P. bilaji* solubilization is occuring in the rhizosphere of treated plants, it is likely occuring through an acidification mechanism.

Field

Katepwa wheat yields from field experimentation show variable response to $CuSO_4$ addition and no response to *P.bilaji* seed inoculation. The Nipawin site showed a yield increase to $CuSO_4$ fertilization, but no response to *P.bilaji* (Table 8). Yield increases in the Nipawin site correspond to increased Cu uptake in $CuSO_4$ amended plots (Table 9). The Snowden site showed no yield or Cu uptake response to $CuSO_4$ or *P.bilaji* treatments (Tables 8 and 9). The Aylsham site showed no significant yield responses to $CuSO_4$ or *P.bilaji* (Table 8), but showed increases in Cu seed uptake from both amendments (Table 9). This indicates that some external factor, not the Cu and *P.bilaji* treatments, affected Aylsham wheat yields.

Table 8. Mean seed mass ($kg \cdot ha^{-1}$) values of Katepwa wheat grown on Nipawin, Snowden, and Aylsham Soils*.

Treatment	Seed Mass (kg/ha)		
	Nipawin	Snowden	Aylsham
Control	2722_b	4616_a	3470_a
P. bilaji	2661_b	3887_a	2620_b
$CuSO_4$	3682_a	4213_a	2682_b
P. bilaji + $CuSO_4$	3536_a	4349_a	2795_{ab}

* Values with different letters are significantly different (95%)

As with the growth chamber wheat experiments, a positive yield response to Cu was only observed on the Nipawin soil with low Cu levels.

Table 9. Mean values of seed copper uptake ($mg/4m^2$) of Katepwa wheat grown on Nipawin,Snowden, and Aylsham soils *.

Treatment	Copper Uptake ($mg/4m^2$)		
	Nipawin	Snowden	Aylsham
Control	0.492_b	4.969_a	0.734_b
P. bilaji	1.118_{ab}	4.278_a	1.369_a
$CuSO_4$	2.067_a	5.007_a	1.555_a
P. bilaji + $CuSO_4$	1.280_{ab}	5.008_a	1.509_a

* Values with different letters are significantly different (95%)

Katepwa wheat from the Nipawin and Aylsham sites showed no yield responses to the various P rates (Table 10). The Snowden site showed a yield increases for the 1/2 rate P_2O_5 treatments, but no response to the full P_2O_5 rate. The yield results for this site suggest that the full P_2O_5 rate had the same effect on yield as the control (no P_2O_5) treatment. The wheat at this site produced a relatively flat response to *P.bilaji* seed inoculation (Table 10).

Table 10. Mean seed mass ($kg{\bullet}ha^{-1}$) values of Katepwa wheat grown on Nipawin, Snowden, and Aylsham soils*.

Treatment	Seed Mass (kg/ha)		
	Nipawin	Snowden	Aylsham
No P_2O_5	3128_a	3972_b	2903_a
No P_2O_5 + *P. bilaji*	3272_a	4218_b	2860_a
1/2 P_2O_5	3214_a	5198_a	3088_a
1/2 P_2O_5 + *P. bilaji*	2996_a	4462_{ab}	2644_a
Full P_2O_5	3682_a	4213_b	2682_a
Full P_2O_5+ *P. bilaji*	3536_a	4349_{ab}	2795_a

* Values with different letters are significantly different (95%)

No differences in wheat seed P uptake were observed for the Nipawin site (Table 11). Differences in P uptake were observed in the Snowden and Aylsham sites. The 1/2 P_2O_5 treatment yielded the highest seed P uptake for both Snowden and Aylsham wheat. In both sites, the full P_2O_5 treatments had the same effect on seed P uptake as the control (no P_2O_5) treatment (Table 11).

Table 11. Mean values of seed phosphorus uptake ($g/4m^2$) of Katepwa wheat grown on Nipawin,Snowden, and Aylsham soils *.

Treatment	Phosphorus Uptake ($g/4m^2$)		
	Nipawin	Snowden	Aylsham
No P_2O_5	4.999_a	5.258_b	4.448_{ab}
P. bilaji + No P_2O_5	5.197_a	5.897_b	4.608_a
1/2 P_2O_5	5.249_a	7.442_a	4.662_a
P. bilaji + 1/2 P_2O_5	4.767_a	5.902_b	3.196_b
Full P_2O_5	5.842_a	5.822_b	4.125_{ab}
P. bilaji + Full P_2O_5	5.160_a	6.197_{ab}	4.265_{ab}

* Values with different letters are significantly different (95%)

An explanation for the lack of a trend in yield or seed P uptake for the various treatments may relate to the high P levels of these soils (Table 1) and inherent spatial variability of soil P.

Titan peas grown at the Snowden and Aylsham sites showed no yield response to $ZnSO_4$ additions or *P.bilaji* seed inoculation. The application of $ZnSO_4$ produced higher seed Zn uptake values at both sites. *P.bilaji* inoculation had no significant effect on seed uptake at either site (Table 12).

Table 12. Mean seed mass ($kg{\bullet}ha^{-1}$) and seed zinc uptake ($g/4m^2$) values of Titan peas grown on Nipawin, Snowden,and Aylsham soils*.

Treatment	Seed Mass (kg/ha)		Zinc uptake ($g/4m^2$)	
	Snowden	Aylsham	Snowden	Aylsham
Control	3246_a	3354_a	23.507_b	24.654_b
P. bilaji	3345_a	3264_a	20.173_b	27.404_{ab}
$ZnSO_4$	3090_a	2951_a	43.095_a	33.400_a
P. bilaji + $ZnSO_4$	2884_a	3229_a	37.511_a	26.791_{ab}

* Values with different letters are significantly different (95%)

The P_2O_5 and *P.bilaji* treatments had no significant effect on seed yield or P uptake at the Snowden and Aylsham sites (Table 13). This non-response could be attributed to the relalitively high P levels of the Snowden and Aylsham soils (P = 15 and 19 µg/g respectively) (Table 1).

Table 13. Mean seed mass ($kg{\bullet}ha^{-1}$) values of Titan peas grown on Nipawin, Snowden, and Aylsham soils*.

Treatment	Seed Mass (kg/ha)		Phosphorus uptake ($g/4m^2$)	
	Snowden	Aylsham	Snowden	Aylsham
No P_2O_5	2977_a	3335_a	4.104_a	5.335_a
No P_2O_5 + *P. bilaji*	3012_a	3873_a	4.156_a	5.962_a
1/2 P_2O_5	3222_a	3447_a	4.794_a	5.459_a
1/2 P_2O_5 + *P. bilaji*	2951_a	3243_a	4.314_a	5.151_a
Full P_2O_5	3090_a	2951_a	4.288_a	4.729_a
Full P_2O_5+ *P. bilaji*	2884_a	3229_a	4.033_a	5.012_a

* Values with different letters are significantly different (95%)

Conclusions

Variable responses of wheat and beans to Cu, Zn, and *P.bilaji* treatments were observed in growth chamber experiments. Additions of Cu and Zn increased plant yields only in soils with low levels of each nutrient. The Nipawin soil was low in both nutrients and produced yield increases in park wheat and navy beans as a result of Cu and Zn additions. The Snowden soil had low Zn levels and showed yield increases in Navy beans with the addition of Zn to soil. Seed inoculation with *P.bilaji* produced variable responses. Inoculation without Cu or Zn addition produced produced only one positive yield result (Aylsham Navy bean). Seed inoculation with Cu and Zn soil fertilization produced yield and Cu/Zn increases in Nipawin Park wheat and Navy beans and Snowden Navy beans.

Katepwa wheat grown on the Nipawin field site showed yield and Cu uptake increases from Cu fertilization because this site had very low Cu levels and no effect of *P.bilaji* inoculation. The Aylsham site showed a decrease in yield with Cu addition and *P.bilaji* inoculation, but an increase in seed uptake of Cu with both treatments. The Snowden site showed no response to *P.bilaji* or Cu. The Snowden site showed yield and seed P uptake increases for the 1/2 P_2O_5 treatments. The full P_2O_5 treatments had the same effect as the no P_2O_5 treatments. This may be related to a toxic effect of the higher P level on plant growth. The Nipawin and Aylsham sites showed no yield response to the P_2O_5 treatments or PB-50 seed inoculation and no trends in P uptake as a result of the various treatments.

Titan wheat grown on the Snowden and Aylsham field sites showed no yield or P uptake responses to the P_2O_5 treatments or PB-50 seed inoculation. The level of $NaHCO_3$-extractable P may have been too high in these soils to induce yield responses.

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