WHEAT RESPONSE TO PB-50 (*Penicillium bilaji*), A PHOSPHATE-SOLUBILIZING INOCULANT

G. L. Hnatowich[1], S. C. Gleddie[2] and D. R. Polonenko[2]

1. Saskatchewan Wheat Pool, Product Development, P.O. Box 1461, #207 - 112 Research Drive, Saskatoon, Saskatchewan, Canada S7K 3P7

2. Philom Bios Inc., #104 - 110 Research Drive, Saskatoon, Saskatchewan, Canada S7N 3R3

ABSTRACT

Phosphorus is a key plant nutrient which is rapidly precipitated and made unavailable for plant use when added to soil as fertilizer. Every soil contains a number of microorganisms which act to solubilize fixed phosphate making it available for plant uptake. One such microorganism, a fungus called *Penicillium bilaji*, demonstrated superior phosphate solubilizing ability in liquid media. In greenhouse and field trials established in 1985, 1986, and 1987, bran inoculated with *P. bilaji* applied in-furrow consistently increased phosphate availability and uptake by crop plants. *P. bilaji* was subsequently registered in Canada under the trade name PB-50™. Trials were established at 38 locations in 1988 and 1989 to examine the effect of seed inoculated *P. bilaji* on wheat yield over increasing rates of phosphate fertilizer. In general, *P. bilaji* treatments exhibited significant yield increases over the lower check rates of phosphate fertilizer. As phosphate fertilizer rates approached recommended levels, yield differences due to *P. bilaji* tended to decrease.

OVERVIEW

Phosphorus is one of the key nutrients which are essential for plant growth and development. However, the availability of this nutrient for uptake by plants and microorganisms is restricted by its tendency to precipitate with certain cations (e.g., Ca^{++}, Mg^{++}, Fe^{+++}, Al^{+++})(Barber, 1984) or to become tightly bound to or within soil particles (Tisdale and Oades, 1982). These forms of phosphorus are relatively insoluble and unavailable for uptake by plants. Because of their extreme reactivity, plant-available phosphates represent only a small portion of the total phosphorus present in soils (Barber 1984). Consequently, in most agricultural soils, fertilizer phosphate application is required to supplement native soil phosphate in order to supply crop phosphate demands and to produce the required crop yields (Stewart and Sharpley, 1987).

The roles of microorganisms in the release of precipitated phosphates into soluble plant available forms are well documented. Research with fungi and bacteria isolated from soils and root regions indicate that a wide range of microorganisms have the capacity to solubilize precipitated inorganic phosphates (Kucey, et al., 1989). Researchers led by Dr. Reg Kucey at the Agriculture Canada Research Station in Lethbridge, Alberta, isolated from soil a number of fungi and bacteria capable of solubilizing precipitated inorganic phosphate (Kucey, 1983). One of these, an isolate of the fungus *Penicillium bilaji*, was unique in the efficiency with which it solubilized rock phosphate in liquid media.

Bran inoculated with P. bilaji applied in-furrow was shown to increase phosphate availability and uptake by wheat and canola in greenhouse and field trials established in 1985, 1986, and 1987.

Under field conditions, wheat yield and P uptake of P. bilaji plus rock phosphate (RP) was equivalent to increases due to the addition of monoammonium phosphate (MAP) at an equivalent rate of P. Yield and P uptake was not affected when RP was applied in the absence of P. bilaji (Kucey, 1987). Wheat dry matter production and total P uptake in turn coincided with a significant increase in phosphate solubilizing fungi in the rhizosphere of inoculated plants (Kucey, 1988). In greenhouse trials, the addition of P at 20 mg/Kg soil as RP plus P. bilaji resulted in P uptake by canola equivalent to that obtained from MAP at the same rate of P. Under field conditions, canola dry matter and P uptake of inoculated plants at half the rate of MAP was equal to that absorbed from the full rate of MAP by uninoculated plants (Kucey and Leggett, 1989).

The efficacy of P. bilaji has been demonstrated on phosphate responsive soils and registered in Canada under the trade name PB-50™ for in-furrow application with wheat and canola. However, the formulation was not practical for large scale field use. Successful liquid fermentation of P. bilaji has enabled the replacement of the in-furrow bran formulation with a water soluble, dry powder, seedcoat formulation.

OBJECTIVES

The objectives of the 1988 and 1989 research program were to determine the efficacy of the P. bilaji seedcoat formulation on wheat over a wide range of soil and climatic conditions. In this paper, we report the results of field trials established with wheat in 1988 and 1989 across the three prairie provinces.

MATERIALS AND METHODS

Trials to assess the efficacy and performance of the P. bilaji seedcoat formulations on wheat were established by Philom Bios, the Saskatchewan Wheat Pool and Westco Fertilizers Limited at 38 locations across the three prairie provinces in 1988 and 1989.

Field trials were arranged in a split-plot experimental design. The effects and interactions of the subplot factors, the check and the P. bilaji seed treatment, were compared over the mainplot factors, which were rates of phosphate fertilizer application (0, 10, 20, 30 Kg/ha P_2O_5). Prior to seeding, supplemental nitrogen fertilizer was applied over all trial locations as required to ensure adequate nutrient supply and to highlight response to phosphate. Triple-super phosphate (0-45-0) was added in-furrow as the mainplot phosphate treatments. P. bilaji was applied to wheat seed at rates calculated to give 10^3 - 10^4 colony forming units per seed. All trials were seeded with small plot seed drills and harvested with small-plot combines.

Subplot treatment mean yields were separated using multiple location paired-comparison T tests.

RESULTS AND DISCUSSION

Combined location analysis for 38 separate trials is shown in Table 1. Within uninoculated phosphate fertilizer check treatments a mean yield response of 138 Kg/ha occurred with the addition of 10 Kg/ha P_2O_5. As would be expected, incremental yield increases declined with further phosphate additions. Within inoculated P. bilaji treatments, grain yield was increased with the application of 10 Kg/ha P_2O_5. Additional fertilizer applications had no further influence on grain yield.

TABLE 1: Effect of Penicillium bilaji on the yield of wheat. Multiple year data summary for 38 locations.

Phosphate Applied (Kg/ha P_2O_5)	Mean Yield (Kg/ha) PHOSPHATE CHECK	Mean Yield (Kg/ha) P. bilaji TREATMENT	YIELD DIFFERENCE (P. bilaji minus Phosphate)	[1]Statistical Significance (Prob T >|T|)
0	2771	2827	56	0.01
10	2909	2958	49	0.04
20	2930	2962	32	0.15
30	2962	2948	(14)	0.54

[1] Paired-comparison T-test for P. bilaji response. Values denote the level of significance of the observed differences between PB-50 treatment and phosphate check yields for each rate of applied phosphate.

The addition of P. bilaji alone ($P<0.01$) and with 10 Kg/ha P_2O_5 ($P<0.04$) significantly increased grain yield when compared to uninoculated controls. As the rate of phosphate fertilizer applied increased, the yield response to inoculation decreased. It would appear that at higher rates of fertilization the crop was able to obtain adequate levels of phosphate for optimum plant growth.

The combined site analysis was separated into trials established on low to medium residual phosphate soils (<20 Kg P/ha) and those established on high residual phosphate soils (>20 Kg P/ha).

The combined location analysis for 28 trials established on low to medium phosphate soils (Table 2) indicates that within uninoculated treatments yield continued to increase as the rate of phosphate fertilizer increased. Within inoculated treatments, yield did not increase beyond levels obtained with 10 Kg/ha P_2O_5. Treatments inoculated with P. bilaji increased the mean grain yield of wheat without added phosphate ($P<0.004$) and of plants receiving 10 Kg/ha P_2O_5 ($P<0.04$) and 20 Kg/ha P_2O_5 ($P<0.08$).

TABLE 2: Multiple year data summary. Trials established on low to medium phosphate soils[2] at 28 locations.

Phosphate Applied (Kg/ha P_2O_5)	Mean Yield (Kg/ha) PHOSPHATE CHECK	Mean Yield (Kg/ha) *P. bilaji* TREATMENT	YIELD DIFFERENCE (*P. bilaji* minus Phosphate)	[1]Statistical Significance (Prob T >|T|)
0	2581	2657	76	0.004
10	2765	2824	59	0.04
20	2797	2841	44	0.08
30	2839	2815	(24)	0.31

[1] Paired-comparison T-test for *P. bilaji* response. Values denote the level of significance of the observed differences between PB-50 treatment and phosphate check yields for each rate of applied phosphate.

[2] Low to medium phosphate soils: 0 - 20 lb available P/acre.

Results of trials established on high residual phosphate soils are outlined in Table 3. Neither phosphate fertilization nor *P. bilaji* induced a yield response. It is believed that the high levels of soil phosphate in effect precluded yield responses to phosphate fertilizer and inoculation with *P. bilaji*. The lack of response to *P. bilaji* under high soil phosphate conditions is not unexpected. The relative benefits of phosphate solubilizing fungi have been observed to decrease as phosphate availability increases (Ross, 1971).

TABLE 3: Multiple year data summary. Trials established on high phosphate soils[2] at 10 locations.

Phosphate Applied (Kg/ha P_2O_5)	Mean Yield (Kg/ha) PHOSPHATE CHECK	Mean Yield (Kg/ha) *P. bilaji* TREATMENT	YIELD DIFFERENCE (*P. bilaji* minus Phosphate)	[1]Statistical Significance (Prob T >|T|)
0	3313	3312	(1)	0.97
10	3282	3309	26	0.59
20	3280	3280	ND	1.0
30	3298	3312	16	0.73

[1] Paired-comparison T-test for *P. bilaji* response. Values denote the level of significance of the observed differences between PB-50 treatment and phosphate check yields for each rate of applied phosphate.

[2] High phosphate soils: >20 lb available P/acre.

On the basis of Tables 1 and 2, it is apparent that the largest yield response to inoculation occurs when phosphate fertilizer is not applied. Presuming this yield response occurs due to increased phosphate availability, it appears P. bilaji is able to solubilize soil phosphate fractions which are unavailable to the uninoculated check. Within inoculated treatments, optimum yield occurred with the addition of 10 Kg/ha P_2O_5; additional fertilization did not induce yield responses. It is hypothesized that with inoculation, yield was sustained through solubilization of soil phosphate and increased solubilization of commercial fertilizer. Previous results indicate that P. bilaji was able to solubilize soil and added phosphate sources (Asea et al., 1988).

The mode of action of P. bilaji is, at present, not fully understood. The fungi is known to be able to solubilize relatively unavailable forms of soil phosphate. It is believed that P. bilaji acts to solubilize inorganic phosphate by acidification of the surrounding media. Acidification occurs through the production of organic acid metabolites (Kucey manuscript in preparation). However, this may not be the sole mechanism of action. Several studies on phosphate solubilizing organisms have demonstrated a lack of correlation between the ability of organisms to solubilize phosphate and a reduction in media pH (Surange, 1989, Gaur et al., 1973). Tracer studies found that P. bilaji was capable of releasing greater amounts of phosphate from rock phosphate than that released by 0.1 N HCl applied to obtain equivalent media pH levels (Asea, et al., 1988). Therefore, it is probable that organic acids produced by P. bilaji are also acting through calcium chelation thereby altering the chemical equilibrium between solid and solution phosphate and shifting reactions to favor the latter.

In summary, field trials established with wheat in 1988 and 1989 have demonstrated that P. bilaji increased phosphate availability and uptake as evidenced by positive yield responses. Although cross-comparison statistical analysis has not yet been completed, it is of interest to note that yields obtained with the P. bilaji treatment at 10 Kg/ha P_2O_5 were equivalent to those obtained with the uninoculated controls at 20 and 30 Kg/ha P_2O_5.

ACKNOWLEDGEMENTS

In addition to Philom Bios, the Saskatchewan Wheat Pool and Westco Fertilizers Limited, research on P. bilaji has been conducted by Dow-Elanco Canada, the University of Saskatchewan, the University of Alberta, the University of Manitoba, and Agriculture Canada Research Stations at Lethbridge, Beaverlodge, Indian Head, Swift Current, and Saskatoon. The strong contribution of all members of this collaborative team is warmly appreciated. We are also pleased to acknowledge the financial support of Western Economic Diversification Canada and Saskatchewan Science and Technology.

RESEARCH IN PROGRESS

Additional research underway includes the following:

1. Evaluation of the efficacy of P. bilaji on other crops such as winter wheat, fall rye, barley, oats, flax, canola, corn, and forages.

2. Evaluate interactions which may occur between P. bilaji and Rhizobium spp. when applied on pulse crops.

3. Identification of mechanisms pertaining to P. bilaji's mode of action.

LITERATURE CITED

Asea, P.C.A., R.M.N. Kucey and J.W.B. Stewart. 1988. Inorganic phosphate solubilization by two Penicillium species in solution culture and soil. Soil Biol. Biochem. 20:459-464.

Barber, S.A. 1984. Soil nutrient bioavailability. Wiley-Interscience, John Wiley and Sons, N.Y.

Gaur, A.C., M. Madan and K. P. Ostral. 1973. Solubilization of phosphatic compounds by native microflora of rock phosphate. Indian J. Exp. Biol. 11:427-429.

Kucey, R.M.N. 1983. Phosphate-solubilizing bacteria and fungi in various culturated and virgin Alberta soils. Can. J. Soil Sci. 63:671-678.

Kucey, R.M.N. 1987. Increased phosphorus uptake by wheat and field beans inoculated with a phosphorus-solubilizing Penicillium bilaji strain and with vesicular-arbuscular mycorrhizal fungi. Appl. Environ. Microbiol. 53:2699-2703.

Kucey, R.M.N. 1988. Effect of Penicillium bilaji on the solubility and uptake of P and micronutrients from soil by wheat. Can. J. Soil Sci. 68:261-270.

Kucey, R.M.N. and M.E. Leggett. 1989. Increased yields and phosphorus uptake by Westar canola (Brassica napus L) inoculated with a phosphate-solubilizing isolate of Penicillium bilaji. Can. J. Soil Sci. 69:425-432.

Kucey, R.M.N., H.H. Janzen and M.E. Leggett. 1989. Microbially mediated increases in plant-available phosphorus. Adv. Agron. 42:199-228.

Ross, J.P. 1971. Effect of phosphate fertilization on yield of mycorrhizal and nonmycorrhizal soybean. Phytopathology. 61:1400-1403.

Stewart, J.W.B. and A.N. Sharpley. 1987. Controls on dynamics of soil phosphorus and sulfur. p. 101-121. In soil fertility and organic matter as critical components of production systems. R.F. Follett, J.W.B. Stewart, and C.V. Cole. SSSA Spec. Pub. no. 19.

Surange, S. 1985. Comparative phosphate solubilizing capacity of some soil fungi. Curr. Sci. 54:1134-1135.

Tisdale, J.M. and J.M. Oades. 1982. Organic matter and water-stable aggregates in soils. J. Soil Sci. 33:141-163.