THE UNIVERSITY OF MANITOBA

INFLUENCE OF A COMMERCIAL FUNGAL INOCULANT (PB-50) ON PLANT NUTRIENT AVAILABILITY AND CROP GROWTH

by

JARRETT W. CHAMBERS

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ABSTRACT

Research has indicated that rhizosphere microorganisms can affect the plant availability of phosphates, through the production of organic acids. The fungi genera (including Penicillium sp.) are more efficient than bacteria in solubilizing insoluble forms of calcium phosphates.

Field and growth chamber experiments were conducted to determine the influence of a commercial fungal inoculant, PB-50 (Penicillium bilaji), on the time of initiation of phosphorus (P) uptake over a range of P fertilizer rates on soils of different pH, and on the dry matter and grain yields of wheat and flax.

In the field studies, PB-50 inoculated wheat enhanced early season uptake of P immediately after emergence. In 1989, increases in dry matter production early in the season were obtained with PB-50 treatments, whereas P concentrations were not consistently increased. In contrast, inoculated wheat enhanced plant P concentration and dry matter production for the majority of the sampling times and sites in 1990. As the rate of P fertilization increased, the response to PB-50 increased, in terms of plant P concentration and dry matter production. However, at the latter sampling times, more responses to PB-50 inoculation were observed. By maturity, the benefits of PB-50 had dissipated, as only the Wellwood-89 site reported a significant grain yield increase to the addition of PB-50. It was evident that the benefits of PB-50 were independent of the soil pH, but rather dependent on the responsiveness of the soil to P fertilizer.

In growth chamber experiments, PB-50 treated wheat and flax

increased the contribution from the soil P fraction. This enhancement was correlated to an enhanced early season (1 week) total P uptake that persisted to maturity. The increased nutrient status of the wheat plants was accompanied by larger dry matter and grain yields. Similar to the field experiments, at the higher rates of P fertilizer, the benefits of PB-50, in terms of plant P concentration and dry matter production, were increased. The two soils reported similar responses to the application of PB-50 when assessing the plants P concentration, dry matter and grain yield.

All of the plant samples accumulated in the field and growth chamber experiments were analyzed for Ca, Mg, Fe and Al. The relationship between P fertilizer and PB-50 on the plant nutrient concentrations was investigated, as well as the comparison of these nutrients on different pH soils. For all the experiments and sampling times, both field and growth chamber, varying the rate of P fertilizer did not significantly affect the Ca, Mg, Al and Fe concentrations in wheat and flax. In addition, PB-50 inoculation produced inconsistent responses that did not constitute any beneficial effects to the plant. Variations in plant nutrient status did exist for the different pH soils, but were a result of the characteristics and reaction of the soil, rather than from the introduction of PB-50.

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I. INTRODUCTION

Research during the past 40 years has shown that rhizosphere microorganisms affect the plant availability of phosphates, through the production of organic acids. Research has shown the fungi genera (including Penicillium sp.) are superior to bacteria in solubilizing various insoluble forms of calcium phosphates (Subba-Rao and Bajpai 1965). Phosphate solubilizing Penicillium sp. have been isolated in native soils in western Canada (Kucey 1983), with P. bilaji being one of the most effective P solubilizers (Kucey 1987; 1988a; Asea et al. 1988).

It is possible to enhance the Penicillium population in the soil and Keyes (1990) reported that application of P. bilaji to soil increases the canola rhizosphere population 23 and 100-fold after 36 and 49 days, respectively, with the population declining for the remainder of the season. Increases in the this fungal population has been shown to improve early season P uptake. In greenhouse experiments, inoculation of wheat with P. bilaji increased P uptake in the plant by 14%, resulting from an 11% increase in the proportion of P derived from native P sources, at 8 weeks after emergence (Asea et al. 1988). As well, the combination of rock phosphate (RP) and P. bilaji increased dry matter production, at 8 weeks after emergence (Asea et al. 1988) and at harvest (Kucey 1987). Similar results were obtained in field experiments on alkaline soils. Rock phosphate plus P. bilaji resulted in P uptake and grain yield equivalent to the same rate of MAP without P. bilaji (Kucey 1987). Increases in yield from P. bilaji inoculation decreased as the rate of P fertilizer increased (Gleddie et al. 1991).

Based on the importance of early season uptake of P by the plant, combined with the potential for PB-50 inoculation to increase the soil microbial populations early in the growing season, the first objective of this research was to determine the time of enhanced P uptake for plants inoculated with PB-50 over a range of P fertilizer rates, and what fraction of P contributed to the increased plant uptake. The second objective of this research was to assess if the enhanced plant P nutrient status from PB-50 would result in dry matter and grain yield increases, under field and growth chamber conditions.

The solubilization and release of inorganic phosphates into soil solution involves both acidification and chelation processes (Sperber 1958a; Asea et al. 1988). In experiments with wheat, it was shown that P. bilaji increased the solubilization of insoluble forms of Cu, Fe and Zn, but only the Zn concentration increased in the plant (Kucey 1988a). Keyes (1990) analyzed for a number of nutrients and concluded that P. bilaji inoculated canola was associated with significantly greater plant Fe and Mn concentrations. To study the availability of Ca, Mg, Fe and Al, the third objective of this research was to determine if the organism affects the concentration of these nutrients in the crop.

With the majority of the research being conducted on alkaline soils (Kucey 1987; 1988a; Asea et al. 1988), it is important to determine the efficiency of PB-50 on a range of pH soils. Since flax responds poorly to P fertilization, and because previous research on PB-50 has been done mostly with wheat, it was appropriate to test the above objectives of PB-50 inoculation on both wheat and flax plants.

II. LITERATURE REVIEW

Most soils in Western Canada are deficient in available P and require the addition of P fertilizer to maximize crop production (Ozanne 1980). The highly water soluble phosphate salts of ammonium are the most common forms of P fertilizers applied to agricultural soils in Western Canada (Engelstad and Terman 1980; Tisdale et al. 1985).

The ability of a crop to utilize fertilizer P additions depends on the fertilizer and the initial reactions that occur in the soil. When added to the soil, the P fertilizer dissolves very rapidly, even at low moisture conditions (Lehr et al. 1959). As the P fertilizer granule dissolves, a saturated solution of P will diffuse from the origin, with the rate determined by an osmotic potential gradient between the fertilizer solution and the soil water (Lehr et al. 1959). This diffusion of P fertilizer and inward movement of water will continue until the concentrated P solution is decreased, either by dilution or by reactions with soil constituents (Lindsay et al. 1962).

The rate and distance of P fertilizer movement in soil is increased with increasing concentration, water solubility, initial soil moisture, time and soil temperature (Sutton 1969) and with decreasing soil carbonate content (Lewis and Racz 1969, Khasawneh et al. 1974; Eghball et al. 1990). Most of the P movement occurs within the first few days of application. By three weeks, the maximum diffusion of about 3 cm, will have occurred. (Lewis and Racz 1969; Hasimoto and Lehr 1973; Khasawneh et al. 1974; Eghball et al. 1990). The ortho- and polyphosphate fertilizers will move at different rates initially but

after three weeks, these various water soluble fertilizers will diffuse similar distances (Khasawneh et al. 1974).

The applied P will readily react with the soil constituents, making it both less mobile and less available to the plant. Up to 90% of the added P fertilizer can be temporarily unavailable, as it is retained in sparingly soluble forms (Stevenson 1986). The added P can be fixed in the soil through chemical reactions such as adsorption and precipitation. It is difficult to separate the two reactions as both have similar bonding structures (Larsen 1967). Under low P concentrations, adsorption predominates, whereas in high P concentrations, such as in the case of a P fertilizer band, precipitation of P with hydrous oxides, alumino-silicates minerals and soil carbonates are more important (Sample et al. 1980). The maximum availability of P fertilizer will persist the longest on neutral to slightly acid (pH=6.5) soils (Sanchez and Uehara 1980; Stevenson 1986).

Phosphorus fixation reactions are very rapid on acid soils. There can be over 90% of P fixed within the first hour of soil contact, in these soils (Sanchez and Uehara 1980). In the acidic soils, Fe and Al concentrations are high, causing the initial precipitated reaction products of a P fertilizer such as MAP to be predominantly two forms of ammonium taranakite [$Al_5(NH_4)_3H_6(PO_4)_8$ and $Fe_3NH_4H_8(PO_4)_6$] (Lindsay et al. 1962). These precipitates are sparingly soluble but are plant available. In addition, the phosphate ions will react with Al and Fe oxides on the clay surface, becoming tightly absorbed and insoluble (Lindsay et al. 1962; Sanchez and Uehara 1980). These initial reaction products such as the taranakites and amorphous Al and Fe phosphates are

chemically precipitated to more stable compounds, such as variscite ($AlPO_4$) and strengite ($FePO_4$) (Das and Datta 1969). The conversion of the Fe-phosphate compounds to a more stable form is faster than for Al compounds (Taylor et al. 1963; Juo and Ellis 1968).

For neutral to alkaline soils, Ca is the predominant cation. The initial, precipitated reaction product from orthophosphate fertilizers (particularly, monoammonium phosphate, MAP), is dicalcium phosphate [(DCP), $CaHPO_4$] (Lindsay et al. 1962; Bell and Black 1970a). If the ratio of Ca to Mg is less than 1.5, dimagnesium phosphate [(DMP), $MgHPO_4$] as well as DCP can be the reaction product (Racz and Soper 1967). Both reaction products are sparingly soluble, however the plant has the ability to absorb P from these compounds. The DCP and DMP can persist in some soils for periods up to and greater than 15 months, indicating the P added to neutral and alkaline soils can remain plant available for a considerable time (Racz and Soper 1970; Strong and Racz 1970).

With time these initial precipitated reaction products will be converted to more stable and less soluble P compounds. The DCP and DMP precipitate to octacalcium phosphate [(OCP), $Ca_8H_2(PO_4)_6$] and or trimagnesium phosphate [(TMP), $Mg_3(PO_4)_2$], respectively (Racz and Soper 1967; Bell and Black 1970b; Strong and Racz 1970). Both OCP and TMP are available to plants and can persist in soils for extremely long periods of time. This accounts for the availability of the P from the fertilizer source to future crops, for over eight years (Bailey et al. 1977; Read et al. 1977; Sander et al. 1990). The rate of conversion from DCP to OCP has been reported to increase with an increase in soil

pH, temperature and carbonate content and decreased with an enrichment of Mg and organic matter (Bell and Black 1970b). In addition, the presence of Fe and Al oxides under both acidic and near neutral pH conditions will slow down the conversion of DCP to OCP (Moreno et al. 1960). Also, OCP can undergo further precipitation reactions with the soil solution to form the very stable, highly insoluble and unavailable P compound, hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] (Lehr and Brown 1958).

Another initial reaction product found with the application of MAP to soils high in Mg will be $MgNH_4PO_4$ (Bell and Black, 1970a). For neutral and slightly acidic soils, $MgNH_4PO_4$ reacts to form $Mg_3(PO_4)_2$ and $Ca(NH_4)_2(HPO_4)_2$ with the latter being converted to DCP. In highly calcareous soils, $MgNH_4PO_4$ is not detectable with time (Bell and Black 1970a).

The ability of a crop to respond to P fertilizer is dependent on the level of plant available P in the soil, which in turn is determined by phosphate fixation reactions in the soil as well as by the crop itself. Due to the limited mobility of P in the soil, the reaction products are restricted to a small fraction of the soil volume (Lewis and Racz 1969). Consequently, P fertilizer placement is critical in obtaining maximum P fertilizer efficiency and plant yield. In general, the banded P is more efficient and has a greater yield potential compared to other fertilizer placements, especially if both the fertilizer and soil P levels are low (Ridley and Tayakepisuthe 1974; Bullen et al. 1983; Fiedler et al. 1989). When comparing broadcast versus banded P application, 2 to 3 times more broadcast P is required to achieve the same grain yields as for seed placed P (Peterson et al.

1981). In terms of fertilizer utilization, 2 to 5 times more P is taken up by wheat from a band as compared to a broadcast application (Alessi and Power 1980; Hanway and Olson 1980; Sander et al. 1990).

Continuous P bands are more effective for increased fertilizer P uptake as compared to discontinuous P bands (Sander and Eghball 1988). When fertilizer particles are too far apart, many root-fertilizer contacts are required to provide adequate fertilizer P uptake, whereas a continuous band allows for root proliferation and P uptake from a single root-fertilizer contact (Eghball and Sander 1987).

Efficiency of banded P fertilizer depends on placement of the band, moisture content of the soil and the ability of the roots to proliferate into the P fertilizer band. Phosphorus fertilizer banded in the seed row can be superior to side-banded P, when the rate applied is moderate to low and the plant available soil P is low. The reverse is true when high rates of P fertilizer are applied to soil high in available soil P (Nyborg and Hennig 1969; Eghball and Sander 1987), because high levels of P in the seed row can cause injury to the root system of seedling plants (Bullen et al. 1983). For example, Soper and Kalra (1969) reported that a pellet of P with a high P concentration inhibited root growth of flax and oat plants.

The ability of the plants rooting system to proliferate into the P fertilizer band helps to explain the utilization, efficiency and yield response to P. Phosphorus adsorption by wheat roots is inhibited under dry soil conditions (Boatwright et al. 1964), so placement below the seed is necessary to increase P uptake and yield (M^{c}Connell et al. 1986). In adequate moisture conditions, the best response to P

fertilizer occurs when the band is placed 2.5 cm below the seed row (Nyborg and Hennig 1969; Bullen et al. 1983) and increasing the distance between the P band and seed row decreases P uptake and yield (Mitchell 1957; Sherell et al. 1965). Plant root hairs aid in ion uptake, in particular immobile ions like P (Barber 1984). The P depletion zone around wheat roots is 1 mm, which is the approximate length of the plants root hairs (Strong and Soper 1974a).

Crops differ in their ability to extend roots into the fertilizer band. Rape (canola) roots show a high degree of proliferation into the fertilizer band. In comparison, wheat and flax will proliferate into the P fertilizer bands, but the quantity of roots is much less (Kalra and Soper 1968; Soper and Kalra 1969; Strong and Soper 1973; 1974a). As the P concentration of the band increases, the quantity of roots in the P rich zone decreases, with the flax roots being more sensitive than the wheat, to P concentration increases. (Strong and Soper 1973).

This difference in sensitivity to P helps to explain why crops are different in their time and rate of P uptake. Early season P uptake has been shown to be important for maximizing yield, and it appears that a limited P supply to the plant later in the growing season has negligible effects on yield (Boatwright and Viets 1966; Sutton et al. 1983). Boatwright and Viets (1966) showed the importance of early season P uptake. They suggest a supply of P for the first five weeks of growth was adequate to produce maximum dry matter and grain yields for wheat, and a four week initial supply achieved maximum root development. If P was withheld from wheat for 1, 2 or 3 weeks, grain yields were reduced by 25, 58 and 81%, respectively (Boatwright and Viets 1966; Claassens

1990).

If cereal seedlings are initially starved of P, and then exposed to high levels, the plants are capable of a faster uptake and can accumulate abnormally high P concentrations, from 1.5 to 3% (Boatwright and Viets 1966; Green et al 1973; Green and Warder 1973). In general, the tissue content of P sufficient plants at similar growth stages will be 0.2 to 0.4% P, on a dry matter basis (Bieleski 1976). The abnormal high P concentration may be caused from a lower dry matter production of the P starved compared to P sufficient plants, indicating a dilution effect.

On average wheat and flax extract fertilizer P from bands (2.5 cm beside and below the seed), 5 and 8 days after seeding, respectively, for soils with a wide range of pH (Beever 1987). Maximum plant P concentration has been reported to occur at 25 and 35 days after emergence for wheat and flax, respectively (Spinks and Barber 1947; Boatwright and Haas 1961; Racz et al. 1965; Kalra and Soper 1968; Alessi and Power 1980).

Wheat plants have a moderate rate of P uptake, occurring up to 60 days whereas flax has slower, but longer duration of P uptake (Racz et al. 1965). In contrast, rape has a rapid uptake of P in its initial stages of growth and continues beyond 60 days (Racz et al. 1965). This assists in explaining why rape has the greatest response, in terms of P uptake and yield, to P fertilizer, for the 3 crops (Racz et al. 1965). The inefficiency of early season P uptake by flax may be caused by too high a P concentration in the fertilizer zone for the roots to enter, thereby delaying fertilizer uptake.

The response of plants to P fertilizer in alkaline soils has been reported to be greatest for rape, followed by wheat and then flax (Racz et al. 1965). Wheat has been reported to use two to three times more fertilizer P than flax, but only up to 35% of the total plant P content has been reported to be derived from the fertilizer fraction (Strong and Soper 1973; Bailey et al. 1977). In addition, if the P fertilizer applied exceeds the recommended rate, the P fertilizer efficiency decreases dramatically and the amount of residual available P increases (Spinks and Barber 1947; Dion et al. 1949; Bailey et al. 1977; Read et al. 1977; Alessi and Power 1980; Sander and Eghball 1988; Sander et al. 1990).

Although flax utilizes minimal fertilizer P, soil P is continuously extracted throughout the growing season (Racz et al 1965; Soper and Kalra 1969). In contrast, wheat utilizes fertilizer P as its main P source early in the growing season and reverts to extracting more soil P for the remainder of the growing season (Spinks and Barber 1947; Racz et al 1965; Barber and Olson 1968; Soper and Kalra 1969).

Due to its reactive nature, P fertilizer quickly forms insoluble compounds, resulting in a small fraction of P existing in the soil solution. Therefore, other transformations must be exploited to assist in solubilizing inorganic P into an available form for plant uptake (Ozanne 1980; Stevenson 1986). Since plants contain a rich population of microorganisms around the roots, it would be unrealistic to assess plant P uptake without considering the microbial activity in the soil (Hayman 1975). However, there are reports which discount the contribution of rhizosphere microorganisms to the plant P nutrient

status. This is based on the fact that any phosphate released by the bacteria will enter the normal adsorption equilibria of inorganic phosphate in the soil and suffer the same transport impedance as other phosphate ions. In addition the quantities of organic acids produced by the plant roots, or its associated microflora, appear to be too small to affect phosphate solubilization (Tinker and Sanders 1975).

The presence of phosphate dissolving microorganisms in soil, was first detected by Sackett et al. (1908). Pure culture conditions were used as the initial criterion for isolation and enumeration of phosphate solubilizing (PS) microorganisms (Gerretsen 1948; Sperber 1958a; Katznelson and Bose 1959; Louw and Webley 1959b). The zone of solubilization (clear zone) around a microbial colony was the visual evaluation of the PS capability of an organism (Sperber 1957).

The rate and amount of P solubilization by these organisms is dependent on the source of inorganic P. Various forms of calcium phosphates can be solubilized by microorganisms, with a greater proportion of DCP being solubilized than TCP, followed by hydroxyapatite. (Louw and Webley 1959b; Katznelson and Bose 1959). A wide variety of soil fungi isolated from nursery seedbeds significantly solubilized both hydroxy- and fluoroapatite (Agnihotri 1970).

The soil microorganisms can also solubilize Fe and Al phosphates, but to a much lesser extent than for the various Ca phosphate compounds (Gerretsen 1948; Duff et al. 1963; Banik and Dey 1981a; 1982). When soluble KH_2PO_4 was added to cultures maintained on TCP, or Fe and Al to cultures maintained on DCP, the solubilizing capability of the isolates decreased (Duff et al. 1963; Chhonkar and

Subba-Rao 1967).

When dealing with soil phosphates, the microbial population examined could not solubilize RP in agar media or variscite, strengite and taranakite in both agar and liquid media (Louw and Webley 1959a; 1959b). In addition, variscite and strengite were resistant to solubilization by the PS bacteria tested (Duff et al. 1963).

Another important consideration is the activity of the entire PS microbial population in the soil (Kucey et al. 1989). In 1948, Gerretsen was the first to report on the important role of rhizosphere microorganisms concerning plant availability and uptake of soil phosphates. Almost all soils contain PS microorganisms, but the actual population and genera vary a great deal depending on soil climate and cropping history (Sperber 1958a; Katznelson and Bose 1959; Katznelson et al. 1962; Chhonkar and Subba-Rao 1967; Agnihotri 1970; Banik and Dey 1982; Kucey 1983; Venkateswarlu et al. 1984; Thomas et al. 1985).

Phosphate solubilizing microorganisms have also been isolated from the surface of seeds, with 40-70% of the bacterial isolates able to solubilize P in agar media (Katznelson et al. 1962). However, soil-borne organisms contribute the majority of microbial colonization on plant roots (Rouatt 1959; Katznelson et al. 1962). The phosphate dissolving microbial population can be 2 to 70 times higher in the rhizosphere as compared to the root free soil (Gerretsen 1948; Sperber 1958a; Rouatt 1959; Greaves and Webley 1965).

The distribution and elevated microbial populations on and around legume roots may be dependent on the total amount of root exudates and time of development for the population (Rouatt 1959; Tinker and Sander

1975). By a predicted amount of substrate available to the rhizosphere organisms, a maximum of 7 x 10^7 bacteria/g of dry roots can be expected, for barley (Barber and Gunn 1974; Tinker and Sanders 1975). Reports for wheat indicate 4 to 10% of the root surface is encased by bacteria, with another 3% by fungal hyphae (Newman and Bowen 1974; Rovira et al. 1974). The root hairs on young tomato plants are free from bacteria, while oat plants support a large number of organisms (Rovira 1956). In both plants the root tips are free from organisms.

There is evidence legume plants support a larger number of PS microorganisms than non-legumes (Sobieszczanski 1961) and each legume variety has different phosphate dissolving microbial populations (Paul and Sundara Rao 1971). Katznelson et al. (1962) report the incidence of PS fungi on the seed and roots of cereal crops is negligible in comparison to clover and perennial grasses. In addition, only 10% of the microbial population showed an incidence of phosphate dissolving ability in the rhizoplane, rhizosphere and root-free soil microorganisms of barley, corn, flax and oat plants. The percentage of the total microbial population dissolving P varies between 26 and 41% for wheat, ryegrass and subterranean clover (Sperber 1958a). This is in agreement with Katznelson and Bose (1959) who found approximately one-third of the bacteria from the wheat rhizoplane dissolved P and these bacteria were more metabolically active than other bacteria from the same soil.

Numerous researchers have isolated PS organisms in the rhizosphere and rhizoplane of various crops (Sperber 1958a; Katznelson and Bose 1959; Louw and Webley 1959a; Rouatt and Katznelson 1961; Katznelson et al. 1962; Raghu and MacRae 1966; Chhonkar and Subba-Rao 1967; Taha et

al. 1969; Moghimi et al. 1978a; 1978b). The most frequently isolated PS organisms are genera of bacteria, including Bacillus (Taha et al. 1969; Banik and Dey 1981a; 1982; Venkateswarlu et al. 1984), Mycobacterium (Sperber 1958a; Louw and Webley 1959b), Norcardia (Sperber 1958b; Louw and Webley 1959b) and Pseudomonas (Rouatt and Katznelson 1961; Venkateswarlu et al. 1984); actinomycetes, including Streptomyces (Taha et al. 1969; Banik and Dey 1981a; 1982); and fungi, including Aspergillus (Sperber 1958b; Katznelson et al. 1962; Chhonkar and Subba-Rao 1967; Banik and Dey 1981a; 1982; Kucey 1983; Venkateswarlu et al. 1984) and Penicillium (Sperber 1958b; Katznelson et al. 1962; Subba-Rao and Bajpai 1965; Chhonkar and Subba-Rao 1967; Banik and Dey 1981a; Kucey 1983; Venkateswarlu et al. 1984).

The majority of isolates which initially solubilize P irreversibly lose this ability after colonies have undergone one or two sub-culturing (Sperber 1957; 1958a; 1958b; Kucey 1983). The root-free microflora (both bacteria and fungi) lose the apatite solubilizing capability more readily than rhizosphere microorganisms (Sperber 1958b). In contrast it has been reported that only bacteria have a tendency to lose PS ability upon sub-culturing, with fungi retaining their ability for over eight sub-culturing and for more than 2 years (Kucey 1983).

In pure cultures, rhizosphere microorganisms are actively dissolving P, three to four days after inoculation (Kucey 1983; Molla et al. 1984) with fungi initiating solubilization before bacteria (Kucey 1983). In addition, fungi compared to bacteria, solubilize more P with solubilization continuing for a longer time (Kucey 1983).

In the rhizosphere, microbial populations are isolated 2 to 3 days

after seed germination, for oat, pea, tomato and wheat plants (Rovira 1956; Rouatt 1959; Brown 1974). Brown (1974) reports after 27 days, microbial populations increased tenfold on wheat seedling roots. Researchers indicate an increase in microbial population up to 21 days, followed by a decline for the remainder of the growing season (Rouatt 1959; Raghu and MacRae 1966; Raj et al. 1981). In contrast, the rhizosphere population continued to increase during the entire growing season for annual crops (Louw and Webley 1959a). For pasture grasses, populations begin decreasing after 10 months, with a continual decline thereafter (Greaves and Webley 1965).

In prairie soils, the PS bacteria and fungi account for 0.5 and 0.1% respectively, of the soil microbial population, with PS bacteria outnumbering fungi 2 to 150 fold (Kucey 1983). This is in agreement with research conducted on Indian soils, where bacterial populations are 3 and 50 fold greater than actinomycetes and fungi, respectively (Banik and Dey 1982; Venkateswarlu et al. 1984).

When determining the efficiency of PS microorganisms for solubilizing calcium phosphates, conflicting reports occur. Banik and Dey (1981a) conclude the overall most efficient phosphate solubilizer was B. firmus followed by a Penicillium sp., an Aspergillus sp. and a Streptomyces sp.. It should be noted that mixed cultures of Bacillus sp. were even more effective than individual strains (Banik and Dey 1981a; Molla et al. 1984). Taha et al. (1969) were in agreement with this, and report bacteria are the predominant P solubilizers for Egyptian soils. In contrast, many researchers indicate the fungi are superior to bacteria, up to ten times greater, in solubilizing various

insoluble forms of calcium phosphate (Subba-Rao and Bajpai 1965; Banik and Dey 1981b; 1982; Kucey 1983; Venkateswarlu et al. 1984). In particular, A. niger (Banik and Dey 1981b; Venkateswarlu et al. 1984) and A. candidus (Banik and Dey 1982) solubilized the greatest amount of P as compared to a Penicillium sp. and various bacteria. Subba-Rao and Bajpai (1965) report a Penicillium sp. released the maximum amount of P as compared to a Aspergillus sp. and various bacteria.

Even with a large microbial population existing in the soil, inoculation with PS organisms on a broad range of soil types and conditions can increase the rhizospheric population (Sperber 1958a; Katznelson et al. 1962; Saber et al. 1977; Banik and Dey 1982; Kucey 1983; Thomas et al. 1985). However, to obtain a potential benefit by inoculating a soil with PS microorganism(s), the particular strain added must be native to the soil, to allow the organism to compete and survive (Rakhno and Ryys 1963; Brown 1974). In addition, the effectiveness of a microbial inoculant is dependent on the number of viable organisms and their ability to multiply once applied to seed, root or soil (Mishustin 1963). Inoculation of soil with phosphobacterin has been a common practice in the Soviet union for a number of years.

Phosphobacterin was introduced in 1947 as a bacterial fertilizer that would be able to provide the necessary P for plant nutrition (Cooper 1959; Mishustin 1963). The commercial product contained 70% kaolinite, impregnated with spores of bacterium, Bacillus megatherium var. phosphaticum (Menkina 1956; Smith et al. 1961). The product was used on a commercial basis, as a substitute for mineral fertilizers for winter wheat and corn, with 1 million hectares being treated in 1962

(Kudzin and Yaroshevich 1962).

There are conflicting reports on the effectiveness of phosphobacterin. Several authors claim the mineralization of organic P to an available plant form is the activity by which B. megatherium improves crop yield (Kudzin and Yaroshevich 1962; Kvaratskheliya 1962; Menkina 1963; Molla et al. 1984). In addition, B. megatherium causes mineralization of nucleic acid-P (Menkina 1963) and myo-inositol phosphate in sand cultures, but did not release the latter compound in soil (Greaves and Webley 1965). Martin (1973) concluded B. megatherium var. phosphaticum was incapable of affecting the mineralization of P in soil. Fungi, including Penicillium and Aspergillus solubilized more $AlPO_4$, and bacteria including Bacillus, Streptomyces and Aspergillus solubilized more $FePO_4$, than phosphobacterin.

For the organism to be beneficial to the plant, proliferation in the root zone or rhizosphere must occur (Gerretsen 1948). Inoculation of B. megatherium on pea plants, increased the microbial population of the rhizosphere (Saber et al. 1977). In contrast, B. megatherium var. phosphaticum populations are not enhanced in the root rhizosphere, but many other microflora are abundant in the rhizosphere and capable of increasing P to the plant (Mishustin 1963; Mishustin and Naumova 1962). The largest population increase from microbial inoculation occurs in partially sterilized soils, possibly due to a reduction in the native P solubilizing organisms (Taha et al. 1969; Kundu and Gaur 1980). However, Gerretsen (1948) reports non-sterilized soils had faster growth, larger plant yields and P uptake than sterilized soil for all P sources, as a result of the increased microbial population.

For Soviet soils, inoculation with phosphobacterin resulted in a yield increase ranging from 0 to 70%, with an average of 10% for a variety of grain crops such as winter and spring wheat and corn (Dorosinskii 1962; Mishustin and Naumova 1962; Samtsevich 1962). Horticultural crops (cabbage, tomatoes and cucumber) showed a more substantial response to phosphobacterin, with yield increases being approximately 35% larger than yields on non-inoculated treatments (Mishustin and Naumova 1962). Lucerne and orchardgrass also responded to the inoculation of B. megatherium var. phosphaticum (Zenkova 1955), but cotton plants showed no positive benefits (Cooper 1959).

The extreme variability reported on the consistent effectiveness of the inoculum can be accounted for by a lack of a definition of an effective response to the bacteria. The greatest yield increases with the addition of phosphobacterin occurred on soils with a neutral or alkaline pH and with a high organic matter content, particularly chernozems (Samoilou and Berezova 1953; Mishustin and Naumova 1962). Response was also obtained on acidic and low organic P soils with the addition of lime, manure, or inorganic P fertilizer to increase pH, organic matter content or nutrient status of the soil, respectively (Cooper 1959; Mishustin and Naumova 1962; Menkina 1963).

Reports indicated that grain yields of superphosphate fertilizers treatments would be two to three times greater than B. megatherium (Kudzin and Yaroshevich 1962; Mishustin and Naumova 1962; Mishustin 1963; Voznyakovskaya 1963). In addition, the combination of organic and/or inorganic fertilizers, plus the bacteria, would result in increased yields, as compared to non-inoculated treatments (Cooper 1959;

Dorosinskii 1962; Kvaratskheliya 1962; Mishustin 1963).

The phosphobacterin was tested in growth chamber experiments on six chernozem and chernozem like soils from the USA (Smith et al. 1961). Tomatoes and wheat showed little or no yield response to the bacteria and neither an increased plant P concentration or increased fertilizer recovery was observed. In field trials with tomatoes, wheat, oats and sorghum, yield differences with the addition of phosphobacterin were negligible and inconsistent (Smith et al. 1961).

The inconsistent results obtained with the phosphobacterin may be explained through specificity of the strain of B. megatherium. This was demonstrated in an Egyptian soil, where B. megatherium var. phosphaticum (phosphobacterin) had poor solubilizing potential, but a local strain of B. megatherium was the most superior PS microorganism tested (Taha et al. 1969). This would indicate that isolated local strains are more effective than the foreign strains in solubilizing P, due to the organism ability to compete and survive in the soil. The same theory may be applicable to research conducted in the USA, where the plants did not show a beneficial response to the addition of phosphobacterin (Cooper 1959). In addition, the commercial product of phosphobacterin had high levels of contamination and low viability of bacterial spores (Samtsevich 1962).

Inoculation of B. megatherium var. phosphaticum decreased the incidence of viral and bacterial diseases (potato) and fungal diseases of sunflower, millet and winter wheat (Dorosinskii 1962; Mishustin and Naumova 1962; Samtsevich 1962). This may relate to general improvements of plant growth. In summary, it is evident the organism can cause plant

growth responses, but the effectiveness of B. megatherium does not suggest that it is a reliable substitute for organic and/or inorganic fertilizers, but perhaps a beneficial addition.

Many different types of plants have been inoculated with other PS microorganisms, including both bacteria and fungi, in both greenhouse and field experiments. However, the majority of the organisms have not been produced on a commercial scale. In general, the increased microbial population in the rhizosphere of plants contributes an increased level of available P to the plant. Inoculation of wheat seeds with B. polymyxa and Pseudomonas striata has been shown to increase the rhizosphere population (Kundu and Gaur 1980). In addition, red pine seedlings inoculated with a Pseudomonas sp. results in an increased microbial population (Ralston and McBride 1976).

In terms of specific organisms tested, a Bacillus sp. increased soil P availability and plant uptake for rice (Banik and Dey 1982). Raj et al. (1981) labelled tricalcium and superphosphate with ^{32}P and inoculated the soil with B. circulans and determined a reduction in P fixation and an increase in plant dry matter production for both fertilizers, as compared to non-inoculated soil. Datta et al. (1982) reported similar results, as B. firmus plus RP increases plant dry matter production.

Inoculation of red pine seedlings with a Pseudomonas sp. enhanced dry matter and P uptake, when calcium phosphate to the soil (Ralston and McBride 1976). An unidentified bacteria was reported to increase dry matter production of alfalfa (Piccini and Azcon 1987). Another unidentified bacteria, when inoculated on soybeans, enhanced VAM

colonization and in doing so, increased P uptake (Azcon-Aguilar et al. 1986). The inoculation of Ps. striata to lentil seed increased the efficiency of P uptake from RP and increased grain yield (Sharma et al. 1983).

Researchers obtained similar or greater responses with a combination of two or more PS organisms. Wheat seeds inoculated with B. polymyxa and Ps. striata resulted in an increased plant P concentration and uptake, and dry matter production (Kundu and Gaur 1980). Similar results were obtained for lavender plants, when a Pseudomonas sp. and Agrobacterium sp. were added with RP (Azcon et al. 1976). This is in agreement with Banik and Dey (1981c) who used a combination of Bacillus sp., Penicillium sp. and Aspergillus sp. to increase available soil P, plant P concentration and uptake and dry matter for rice. Kundu and Gaur (1984) inoculated Ps. striata and A. awamori on rice and reported similar results.

Research conducted in Western Canada using Penicillium bilaji as an inoculant for wheat, canola and field beans, showed significant increases in plant P uptake, dry matter and grain production in both greenhouse and field conditions (Kucey 1987; 1988a; Asea et al. 1988; Kucey and Leggett 1989). The 14% increase in P uptake by wheat may have been caused by an additional 11% P being derived from the soil fraction, as evidenced through the use of ^{32}P labelled soil (Asea et al. 1988).

It should be noted however, the majority of research conducted on the effectiveness of PS microorganisms on increasing plant growth is tested in greenhouse conditions, where plant rooting volumes are restricted. Consequently, the contribution of soil P towards plant

nutrition is reduced and plant response is greater if P is solubilized by organisms (Kucey et al. 1989).

Organic acid production and or enhanced biochemical processes, are two possible mechanisms by which PS microorganisms increase P availability to the plant. Organic acid production by microorganisms can affect P availability by releasing P through either acidification or chelation of cations (Sperber 1958b; Katznelson and Bose 1959; Molla et al. 1984). The increased production of various growth hormones (Brown 1974; Azcon et al. 1976; Tinker 1980; Kucey 1988a), or phosphatase enzymes (Casida 1959; Cooper 1959; Greaves and Webley 1965; Raghu and MacRae 1966), by microorganisms are the two potential means of increasing P availability through biochemical processes.

There is evidence organic acids accumulate in localized zones in the soil, in sufficient quantities to appreciably increase the availability of phosphates to plants (Stevenson 1967). Organic acids occur in excretion products from plant roots and decomposing leaf litter (Stevenson 1967). As well, rhizosphere organisms can also produce organic acids (Sperber 1958b; Katznelson and Bose 1959; Molla et al. 1984).

The organic acids which have been reported to be produced by rhizosphere microorganisms include: citric (Struthers and Sieling 1950; Sperber 1958b; Banik and Dey 1982); glycollic (Sperber 1958b; Venkateswarlu et al. 1984); lactic (Sperber 1957; Sperber 1958b; Louw and Webley 1959b; Taha et al. 1969; Venkateswarlu et al. 1984); malic (Taha et al. 1969); malonic (Banik and Dey 1981a; 1982); oxalic (Banik and Dey 1981a; 1982); succinic (Sperber 1958b; Banik and Dey 1981a;

Venkateswarlu et al. 1984); tartaric acid (Banik and Dey 1982); and 2-ketogluconic acid (Duff and Webley 1959; Louw and Webley 1959b; Rouatt and Katznelson 1961; Duff et al. 1963; Moghimi et al. 1978b; Banik and Dey 1981b). A more detailed list of the principle organic acids produced by PS microorganisms has been reported by Kucey et al. (1989).

Phosphate release from mineral salts may be due to a drop in pH of the mineral suspension (Taha et al. 1969; Moghimi et al. 1978a; Venkateswarlu et al. 1984). Inorganic P solubilization was found to be directly related to a drop in pH generated by P. bilaji and P. cf. fuscum (Asea et al. 1988). The production of 2-ketogluconic acid acts as a readily available source of hydrogen ions for the dissolution of hydroxyapatite (Moghimi and Tate 1978). In contrast, there is evidence that there is a lack of significant correlation between the ability of the PS isolates to acidify the surrounding media and to solubilize inorganic P (Sperber 1958b; Chhonkar and Subba-Rao 1967; Gaur et al. 1973; Surange 1985). Using ^{32}P analysis, it was observed that larger amounts of P from RP are solubilized by P. bilaji than by 0.1 N HCl at equivalent solution pH levels (Asea et al. 1988), indicating the organism is not strictly relying on the production of acid to dissolve P.

It is suggested the nature of the organic acid produced has a greater significance on the amount of phosphate solubilized than the quantity produced, with the lowering of the media pH being a result of an accumulation of a particular acid (Sperber 1958a; Louw and Webley 1959a; Chhonkar and Subba-Rao 1967).

Tinker and Sanders (1975) calculated the quantity of acid required

to solubilize the levels of P that have been reported, and concluded microorganisms are not capable of producing this amount. The drop in pH associated with organic acid production can be accomplished by root exudates without microorganisms being present (Hedley et al. 1982; Bekele et al. 1983). Hale et al. (1971) and Moghimi et al. (1978a) are in agreement with this theory of the production of exudates by the plant, but add the material produced would not persist long enough in the rhizosphere to affect P solubilization.

The production of organic acids by PS organism and the subsequent chelation of cations in a micro-environment, like the rhizosphere, or in close proximity to phosphatic fertilizers, is the suggested alternative mechanism (Sperber 1958b; Katznelson and Bose 1959; Moghimi et al. 1978a; Tinker 1980). The organic acids chelate with cations such as Ca, Mg, Fe and Al and consequently P is released into solution from these insoluble phosphate compounds (Stevenson 1967; Banik and Dey 1981a; 1981b). In addition, chelation reduces the precipitation of phosphate by Fe and Al (Sanchez and Uehara 1980) and is dependent on the stability of the complexes formed. The stability will increase as the number of hydroxyl groups in organic acids increases (Struthers and Sieling 1950).

Emphasis is placed on 2-ketogluconate when dealing with P solubilization, since it is a strong acid, with good chelating ability, that represents approximately 20% of the rhizosphere products for wheat seedlings (Moghimi et al. 1978b). Duff and Webley (1959) first reported the production of 2-ketogluconic acid by soil microflora and the ability to chelate calcium from various insoluble salts and minerals.

Subsequently, Webley and Duff (1965) reported that there are soils rich in organic matter which contain a large number of microorganisms producing 2-ketogluconic acid, which form divalent-metal chelates that remain stable at pH 3.0. Phosphobacterin has been reported to produce an unidentified organic acid, with characteristics of both 2-ketogluconic and tartaric acid (Banik and Dey 1981a; 1982). It is concluded organisms producing this acid are capable of dissolving certain insoluble phosphate and silicate minerals (Duff et al. 1963). Although no correlation exists between organic acid production by a particular genera and phosphate solubilization, isolates producing 2-ketogluconic acid are efficient P solubilizers (Webley and Duff 1965; Banik and Dey 1981a).

Other organic acids such as lactic, glycollic, citric, oxalic and succinic have been shown to solubilize P, due to the chelation of the Ca ion. (Sperber 1958b; Louw and Webley 1959b; Duff et al. 1963; Banik and Dey 1981a).

The solubilization of minor elements and metals can assist in the explanation of chelation by the organic acids. The 2-ketogluconic acid, isolated from a culture of bacterium, Erwinia sp., extracted more inorganic ions, including Co, Ni, Zn, Fe, Ti and V than ammonium acetate, and was equivalent to EDTA and DTPA in extracting Cu, Mn, Mo, Ni and Zn (Berrow et al. 1982). Similar findings were reported for P. bilaji, which produced an unidentified acid that could solubilize both Cu and Zn as well as the EDTA (Kucey 1988a).

Enhanced biochemical processes, in particular, the production of plant growth hormones, has been proposed by a number of researchers as

the mechanism by which the microbial population increases P availability (Brown 1974; Azcon et al. 1976; Tinker 1980; Kucey 1988a). The common plant hormones produced by a range of isolates in culture filtrate include auxin, gibberellin and cytokinins-like substances (Brown and Walker 1970; Brown 1972; Barea et al. 1976).

The function of auxins is to increase the root growth of a plant and retard abscission in leaves, thereby promoting increased plant growth (Scott 1972; Brown 1974). Gibberellins affect the aerial portion of plants with responses being elongated stems, larger leaf area and enhanced flowering and fruit setting, but yield responses are not necessarily present (Brown 1974). Cytokinins can increase shoot and fruit development and delay leaf senescence, thereby giving rise to a potential yield increase (Salisbury and Ross 1978).

A large number of bacteria have been reported to have the capability of producing hormones. In addition, PS organisms producing several growth-promoting substances, give the most beneficial effects to the plant (Barea et al. 1976). As a consequence of the increased plant size, enhanced P uptake would occur. This would allow PS organisms to act as a secondary role in solubilizing extra P to the plant, especially in P-deficient soils (Barea et al. 1976).

Gibberellin-like substances were identified in cultures of B. subtilis and a Pseudomonas sp. (Katznelson and Cole 1965). Wheat inoculated with a Bacillus sp. had similar growth responses in comparison to the addition of gibberelic acid (Kucey 1988b). The inoculation of B. firmus on rice increased the plants growth response, with the organism demonstrating both PS and auxin producing ability

(Datta et al. 1982). The stimulation of plant growth with the addition of B. megatherium may have been caused by the production of auxins and gibberellin-like substances (Brown 1972; 1974). The magnitude of the plant response would be influenced by the amount and type of hormone produced (Brown 1974). Meyer and Linderman (1986) inoculated a plant growth promoting isolate, Ps. putida, on subterranean clover and found an increased uptake of Fe, Cu, Al, Zn, Co and Ni.

An alternative mechanism through which P may be made more available is by increased production of phosphatase enzymes by microorganisms (Casida 1959; Cooper 1959; Greaves and Webley 1965; Raghu and MacRae 1966). Greaves and Webley (1965) indicated phosphatase activity from the root surface and rhizosphere soil was higher than nonrhizosphere soil. In addition, the enhanced microbial population in this area was capable of attacking organic phosphates, such as phenolphthalein diphosphate, glycerophosphate and sodium phytate. Some members of the genus Aspergillus produce phosphatase, in particular acid phytase, which is able to dephosphorylate inert ferric phytate (Casida 1959). Aspergillus ficuum produces two enzymes, each for a specific pH, that hydrolyze myo-inositol hexaphosphate to various pentaphosphate forms (Irving and Cosgrove 1972). A Pseudomonas sp. can produce phytase and in turn dephosphorylate myo-inositol hexaphosphate to pentaphosphate (Cosgrove 1970).

III. EFFECT OF PB-50 INOCULATION ON EARLY SEASON P UPTAKE, DRY MATTER AND GRAIN PRODUCTION OF WHEAT - FIELD STUDY

3.1 Introduction

Crops grown in western Canada require approximately 10 kg ha^{-1} of P in the form of H_2PO_4 and HPO_4 for the entire growing season. Although the soils of western Canada contain large amounts of P, many soils need to be supplemented with P fertilizer to obtain optimal yields. Plants grown on soils that test less than 10 mg kg^{-1} $NaHCO_3$ extractable P should in theory, respond to the application of P fertilizer (Kamprath and Watson 1980). After dissolution of P fertilizer, the soluble P will readily react with the soil decreasing the solubility and bioavailability of the P fertilizer (Racz and Soper 1970; Strong and Racz 1970). The initial precipitated phosphate compounds are sparingly soluble compounds such as dicalcium phosphate dihydrate, in soils having neutral to high pH (Sample et al. 1980), and iron and aluminum phosphates in low pH soils (Lindsay and Stevenson 1959). Residual benefits of P fertilizer occur as organic acids accumulate in localized zones in the soil in sufficient quantities to appreciably increase the availability of these insoluble phosphates to the plant (Stevenson 1967).

Research over the past 40 years has shown that rhizosphere microorganisms will affect the plant availability of phosphates, probably through the production of organic acids. The most frequently isolated organisms that have the ability to solubilize P are genera of bacteria and fungi, with the fungi being more efficient (Kucey 1983;

Venkateswarlu et al. 1984). Banik and Dey (1982) found that phosphate-solubilizing fungi and bacteria, on average, solubilized considerably more $Ca_3(PO_4)_2$ than $AlPO_4$ and $FePO_4$.

To obtain a beneficial yield increase to the addition of P. bilaji, the crop must first show a response to P fertilizer application (Bullock et al. 1990; Hnatowich et al. 1990; Gleddie et al. 1991). Field experiments on an alkaline soil showed the addition of RP (20 kg P ha^{-1}) plus P. bilaji resulted in P uptake and grain yield to be equivalent to the same rate of MAP, without P. bilaji added (Kucey 1987). However, as the rate of TSP fertilizer increased, the yield response to P. bilaji inoculated treatments decreased (Hnatowich et al. 1990; Gleddie et al. 1991). It would appear at the higher rates of P fertilization, the crop is able to obtain adequate levels of phosphate, for optimum plant growth, without the addition of P. bilaji.

Due to the limited information on the effectiveness of PB-50 inoculation during the early stages of plant growth, in field conditions, the first objective of the research was to determine the time of initiation of P uptake by PB-50 inoculated on wheat. The second objective was to test if PB-50 could increase plant P uptake, over a range of P fertilizer rates, and if this increase would be result in increased dry matter production and grain yield. To help understand the range of soil pH in which the inoculation of PB-50 may be beneficial to plant growth, the final objective was to compare the effect of PB-50 on plant growth in acidic, neutral and alkaline soils.

3.2 Materials and Methods

Six field trials were conducted in 1989 and 1990 at three experimental sites in southern Manitoba. Two experimental sites were located north of Carberry, MB, on a Wellwood (Orthic Black) sandy clay loam and a Stockton (Rego Black) fine sandy loam. The third site was located north of Portage La Prairie, MB, on a Portage (Orthic Black) silty clay loam (Table 3.1). To eliminate any residual carryover of inoculated fungal populations, the experiments in 1990 were located adjacent to the sites of the previous cropping year.

Some characteristics of the soils studied are reported in Table 3.1. All analyzes, except N, were conducted on surface (0-15 cm) samples. The solution pH was determined with a glass electrode (soil:water ratio, 1:1) (McLean 1982). Inorganic C was analyzed by a titrimetric method as described by Bundy and Bremner (1972). Organic C content was assessed using a dichromate oxidation procedure (Yeomans and Bremner 1988). Particle size analysis was determined using the standard pipette method as described by Kilmer and Alexander (1949). Plant available phosphate was extracted using $NaHCO_3$ as described by Olsen et al. (1954) and the P in solution was measured by the acid-molybdate procedure (Murphy and Riley 1962). Nitrogen as nitrate, was determined to a depth of 60 cm, by the hydrazine sulfate reduction procedure (Kampshake et al. 1967). Plant available Cu, Fe, Mn and Zn were assessed using the DTPA method of Lindsay and Norvell (1978).

The experiments were arranged in a split plot completely randomized design (Little and Hills 1978), with five rates of P (0, 5, 10, 20 and 40 kg P_2O_5 ha^{-1}) as the main treatments, and with and without

Table 3.1. Soil characteristics.

Soil	Texture	Ph	Org. C	Inorg. C	N	P	Cu	Fe	Mn	Zn
			--------%	--------	----------mg kg^{-1}----------					
Wellwood-89	SCL	5.7	2.75	0.0	88	5.9	1.2	95	48	2.1
Stockton-89	FSL	6.6	3.11	0.3	44	5.9	1.5	81	46	3.6
Portage-89	SiCL	8.1	3.29	1.1	47	3.8	0.7	9	22	0.5
Wellwood-90	SCL	5.5	2.49	0.0	64	6.7	1.0	98	52	2.0
Stockton-90	FSL	6.4	3.20	0.4	56	5.7	1.6	83	44	3.7
Portage-90	SiCL	8.0	3.64	1.3	74	4.8	0.6	8	20	0.6

PB-50 fungal seed coating as the subtreatments. All sites used wheat (Triticum aestivum cv. Katepwa) as the test crop and treatments were replicated five times.

Preparation of all field sites prior to planting included basal treatments of N, K and S broadcast according to soil test recommendations, using a Valmar air-flow granular applicator. Incorporation, to a depth of 8 cm, using a tandem disc, was performed immediately after application, followed by a cross working with diamond tooth harrows.

Prior to planting, wheat seeds were coated with P. bilaji inoculant, PB-50™ (obtained from PhilomBios Biotechnological Products, Saskatoon, SA). The coating was prepared by suspending 4.0 g (1989) or 2.0 g (1990) of PB-50 in 80 mL of deionized water. The suspension was pipetted over 8.0 kg of wheat seed in a polyethylene bag and shaken to obtain an even distribution of fungal spores on the seed. Non-inoculated wheat (8.0 kg) also received 80 mL of deionized water to maintain uniformity in water uptake by the seeds. Both the treated and untreated seeds were allowed to dry before planting. The PB-50 suspension and samples of the coated seeds were analyzed by PhilomBios, for fungal viability (Table 3.2). In 1990, the wheat seeds were contaminated with fungi common to seeds (Fusarium, Alternaria and Cladosporium sp.), so accurate spore counts could not be obtained. Wheat was seeded using a 6 row (1989) or 8 row (1990) small plot double disc press drill with 15 cm spacing between rows. The crop was sown at a rate of 90 kg ha^{-1} and to a depth of 2.5 cm (1.2 cm into soil moisture). In addition, the outer drill run on either side of the

Table 3.2. Spore counts of PB-50 suspension and treated wheat seeds.

Soil	PB-50 Suspension	Treated Seed
1989	---cfu g^{-1}---	--cfu $seed^{-1}$--
Wellwood	7.2×10^8	2.2×10^5
Stockton	6.0×10^8	1.5×10^5
Portage	7.2×10^8	2.2×10^5
1990		
Wellwood	2.1×10^9	9.4×10^1
Stockton	7.2×10^9	4.8×10^1
Portage	7.2×10^9	5.7×10^1

planter was defined as guard rows and sown with winter wheat. Each plot received 2 passes by the drill. The various rates of P fertilizer, as monoammonium phosphate (MAP), were banded in the seed row. To balance the contribution of N from varying rates of MAP, additional N, as NH_4NO_3, was also placed with the seed. To control volunteer weeds, herbicides were applied at the recommended rates.

Plant samples from one of the drill runs were randomly taken from all plots at 1, 2, 4, and 8 weeks after emergence, and at plant maturity (15 weeks). The sampling area was 0.5 m^2 for week 1, 2 and 4, and 1.0 m^2 for week 8 and maturity. All samples were obtained from the inside 4 (1989) or 6 (1990) rows. Final harvest samples were separated

into seed and straw components in order to determine grain and dry matter yields. The second drill run for each plot was grown to maturity, at which time the plot was trimmed to 10 m to eliminate any edge effect. The entire plot was then straight-cut combined using a Wintersteiger nurseryman small plot combine, and grain yields were determined. The grain yields reported in this section were obtained from the entire plot, but the straw yield is reported from the 1.0 m^2 sample.

The plant samples were air dried for 2 weeks, weighed (for dry matter and grain yields) and ground through a 2-mm sieve using a Thomas-Wiley Laboratory Mill.

Samples of the ground tissue were prepared for P analysis by digesting the plant material using a nitric-perchloric procedure (Isaac and Kerber 1971), followed by the acid-molybdate procedure (Murphy and Riley 1962). The results of the experiment were statistically analyzed using the GLM procedure (Goodnight et al. 1988).

3.3 Results and Discussion

In the field experiments, plant samples were taken at 1, 2 and 4 weeks (early season growth) and at 8 weeks and maturity (late season growth). The importance of P at the early growth stages affects plant nutrition in such a way that, although plants P to maturity, a five week supply of initial P is sufficient for maximum yields (Boatwright and Viets 1966). Considering the importance of P supply early in the growing season, the results for the two time periods of the field experiments will be discussed separately.

3.3.1 Early Season Growth

The concentration of P in the plants, at all site-years, significantly increased with increasing rates of P fertilizer at most of the early season sampling dates (Tables 3.3, 3.4 and 3.5) (Figures 3.1, 3.2 and 3.3). As well, the increase in the P content of the plant generally was associated with significant increases in plant dry matter production (Tables 3.6, 3.7 and 3.8) (Figures 3.4, 3.5 and 3.6). However, at the Wellwood-89 site (Table 3.6) and the Portage-90 site (Table 3.8) increases in the P content of the plant, with increasing rates of fertilizer P added, were associated with statistically significant increases in dry matter production only at the four week sampling date.

The effect of PB-50 on the concentration of P in the plants was inconsistent among field sites and cropping years (Tables 3.3, 3.4 and 3.5) (Figures 3.1, 3.2 and 3.3). However, when there was a response to PB-50 inoculation, it generally was positive. The one exception was at the Wellwood-89 site (4 week sampling date) where the plants inoculated with PB-50 had a significantly lower P content than the non-inoculated plants (Table 3.3). On P deficient soils, the addition of P fertilizer usually increases plant growth and P uptake, but the concentration of P in the plant may remain constant or even decrease (Jarrell and Beverly 1981). Similarly, inoculation with PB-50 may increase plant growth with inconsistent effects on the P content of the plant (Kucey 1987; 1988a; Asea et al. 1988; Keyes 1990).

Even though PB-50 did not consistently cause a significant increase in the P content of the plant, when a significant increase

Table 3.3. Effect of P fertilizer and PB-50 on P concentration in wheat, at various sampling times (Wellwood soil).

P_2O_5 Added (kg ha^{-1})	PB-50	Phosphorus Concentration (mg kg^{-1})					
		Week 1	Week 2	Week 4	Week 8	Grain	Straw
		Crop Year 1989					
0	-	4572	3574	4581	2106	4372	116
	+	4659	3595	4297	2111	4001	108
5	-	4896	3827	4479	2238	4065	168
	+	4661	4048	4420	2176	3971	131
10	-	4911	3895	4757	2131	3945	214
	+	4818	3768	4461	2014	4227	119
20	-	5299	4403	5045	2209	3735	248
	+	5237	4041	4567	2239	4238	126
40	-	6204	4576	4970	1855	3566	218
	+	6485	4557	4787	2202	4008	129
Rate		0.01	0.01	0.02	NS	NS	0.01
PB		NS	NS	0.01	NS	NS	0.02
Rate*PB		NS	NS	NS	NS	0.03	0.05
$LSD_{(P=0.05)}$†		--	--	--	--	430	38
		Crop Year 1990					
0	-	3993	3655	4321	1773	3320	283
	+	3996	3703	4467	2047	3396	302
5	-	4078	3763	4254	1925	3348	283
	+	4295	3769	4745	2058	3452	316
10	-	4926	4399	4239	1945	3405	300
	+	5044	4365	4773	2090	3447	291
20	-	5501	4878	4350	1966	3423	284
	+	5521	4845	4909	2108	3500	298
40	-	6633	5563	4767	1955	3416	286
	+	6504	5481	5083	2114	3478	321
Rate		0.01	0.01	0.02	0.02	NS	NS
PB		NS	NS	0.01	0.01	NS	NS
Rate*PB		NS	NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$		--	--	--	--	--	--

† LSD values apply only to comparison of means for treatments with and without PB-50

Figure 3.1. Effect of P fertilizer and PB-50 on P concentration in wheat, at various sampling times, for the Wellwood soil, in the 1989 (A) and 1990 (B) crop year.

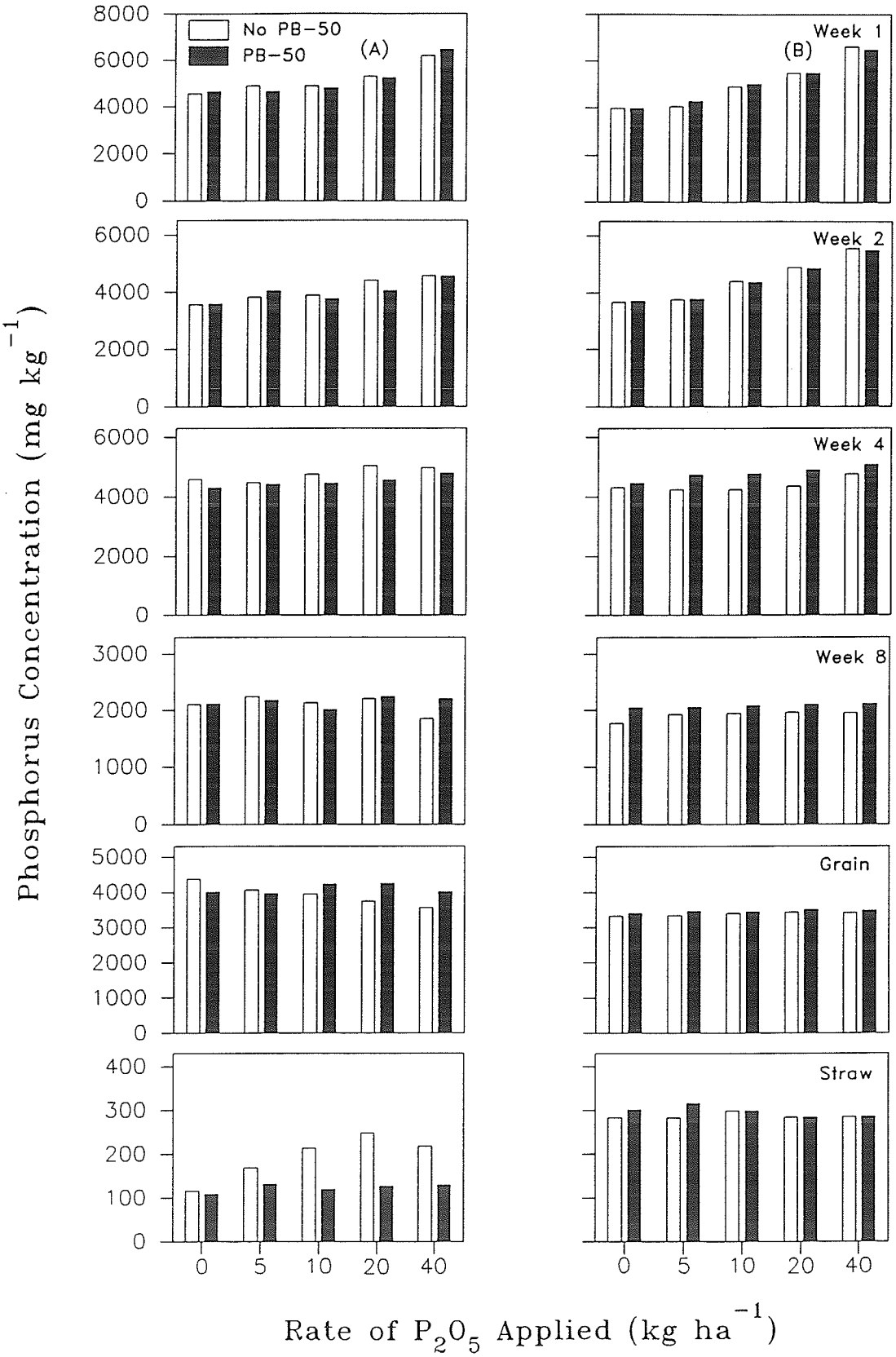

No PB-50
PB-50
(A)
(B)
Week 1
Week 2
Week 4
Week 8
Grain
Straw
Phosphorus Concentration (mg kg^{-1})
Rate of P_2O_5 Applied (kg ha^{-1})
0
5
10
20
40

Table 3.4. Effect of P fertilizer and PB-50 on P concentration in wheat, at various sampling times (Stockton soil).

P_2O_5 Added (kg ha^{-1})	PB-50	Phosphorus Concentration (mg kg^{-1})					
		Week 1	Week 2	Week 4	Week 8	Grain	Straw
		Crop Year 1989					
0	-	3763	2390	4050	1829	3291	163
	+	3799	2420	3916	2121	3926	248
5	-	3625	2616	3956	1952	3628	179
	+	3702	2557	4086	1887	4119	241
10	-	4122	3142	4193	2034	3288	260
	+	4024	2647	4067	2111	4079	252
20	-	4169	3364	4360	2009	3313	232
	+	3897	3095	4427	1923	3768	251
40	-	4651	3999	4274	1881	3080	170
	+	4508	3946	4584	2013	3869	268
Rate		0.01	0.01	0.01	NS	NS	NS
PB		NS	NS	NS	0.05	0.01	NS
Rate*PB		NS	NS	NS	0.02	NS	0.01
$LSD_{(P=0.05)}$†		--	--	--	216	--	50
		Crop Year 1990					
0	-	2569	2962	4030	1815	3081	216
	+	2593	3002	4278	1850	3395	239
5	-	2766	3126	4086	1900	3119	208
	+	2709	3388	4179	1951	3462	255
10	-	3007	3255	4004	1932	3174	216
	+	3264	3508	4253	1966	3513	246
20	-	4040	3849	4272	1904	3171	245
	+	4051	3706	4303	2005	3569	282
40	-	4170	4345	4173	1872	3131	259
	+	4862	4552	4527	2154	3570	280
Rate		0.01	0.01	NS	0.02	NS	0.05
PB		0.01	NS	0.01	0.04	0.01	0.01
Rate*PB		0.01	NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$		396	--	--	--	--	--

† LSD values apply only to comparison of means for treatments with and without PB-50

Figure 3.2. Effect of P fertilizer and PB-50 on P concentration in wheat, at various sampling times, for the Stockton soil, in the 1989 (A) and 1990 (B) crop year.

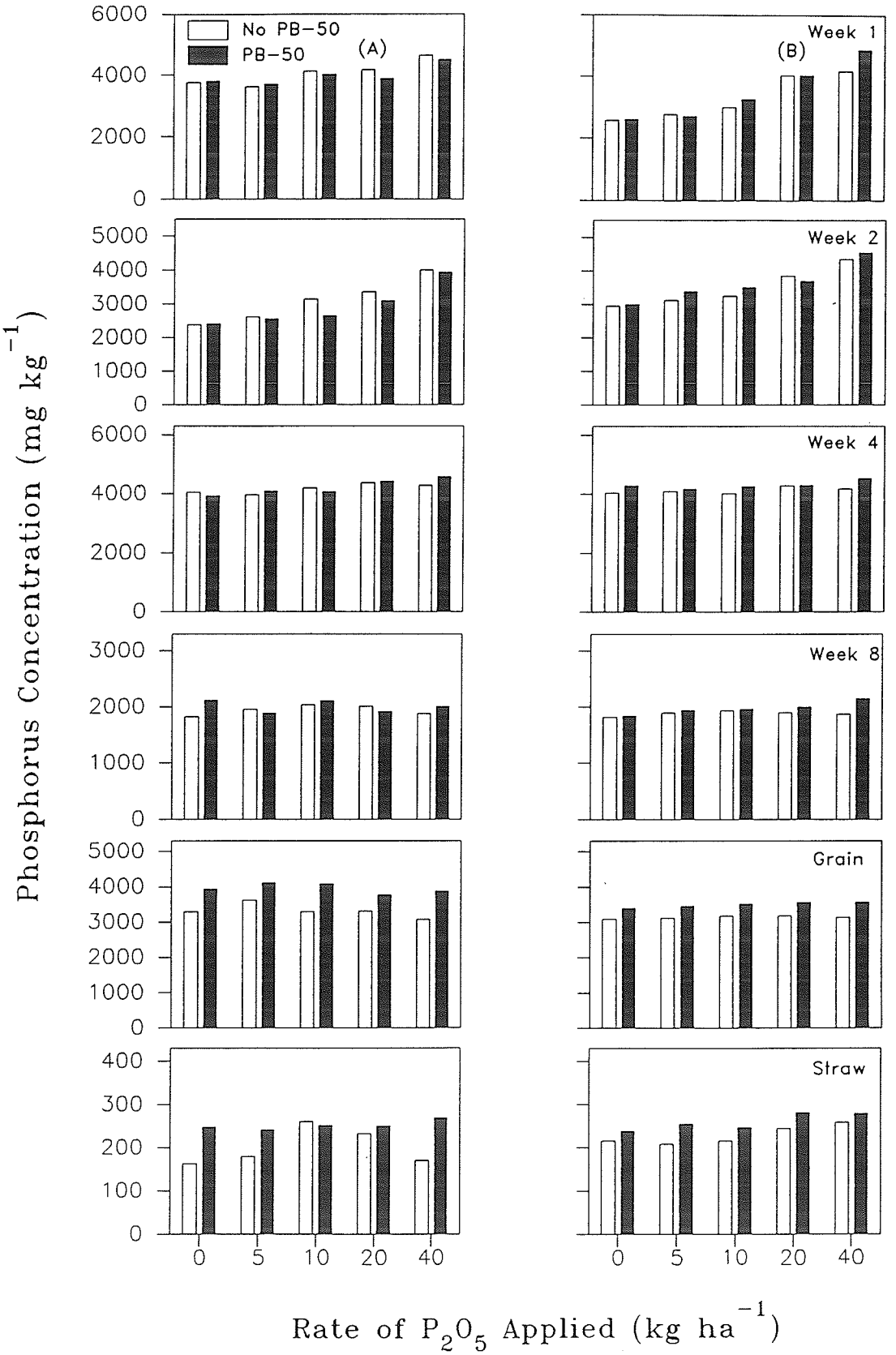

No PB-50
PB-50
(A)
(B)
Week 1
Week 2
Week 4
Week 8
Grain
Straw
Phosphorus Concentration (mg kg^{-1})
Rate of P_2O_5 Applied (kg ha^{-1})

Table 3.5. Effect of P fertilizer and PB-50 on P concentration in wheat, at various sampling times (Portage soil).

P_2O_5 Added (kg ha^{-1})	PB-50	Phosphorus Concentration (mg kg^{-1})					
		Week 1	Week 2	Week 4	Week 8	Grain	Straw
		Crop Year 1989					
0	-	4410	4104	3624	1978	3898	137
	+	5047	4447	3811	1966	3690	278
5	-	4384	4156	3827	1971	3487	130
	+	4254	4276	3690	2091	3759	249
10	-	3866	4326	3606	1975	3486	148
	+	4471	4686	3805	1793	4022	267
20	-	4929	4848	4146	1972	3586	148
	+	4923	5027	3906	2170	4010	225
40	-	5588	5217	4107	2117	3321	138
	+	5713	5342	4290	2078	4030	232
Rate		0.01	0.01	0.03	NS	NS	NS
PB		0.03	NS	NS	NS	0.01	0.01
Rate*PB		NS	NS	NS	NS	0.02	NS
$LSD_{(P=0.05)\dagger}$		--	--	--	--	156	--
		Crop Year 1990					
0	-	2404	3722	3477	1740	3225	212
	+	2905	4164	3838	1893	3555	232
5	-	2845	4182	3653	1760	3324	218
	+	2966	4406	4019	2041	3636	243
10	-	2897	4096	3601	1839	3379	226
	+	3118	4447	3915	2113	3633	261
20	-	3754	4505	3805	1884	3510	242
	+	3982	4641	4091	2171	3645	266
40	-	4345	4501	4029	1865	3540	236
	+	4462	4596	4062	2169	3659	329
Rate		0.01	0.01	0.05	NS	NS	0.03
PB		NS	0.02	0.01	0.01	0.01	0.01
Rate*PB		NS	NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$		--	--	--	--	--	--

† LSD values apply only to comparison of means for treatments with and without PB-50

Figure 3.3. Effect of P fertilizer and PB-50 on P concentration in wheat, at various sampling times, for the Portage soil, in the 1989 (A) and 1990 (B) crop year.

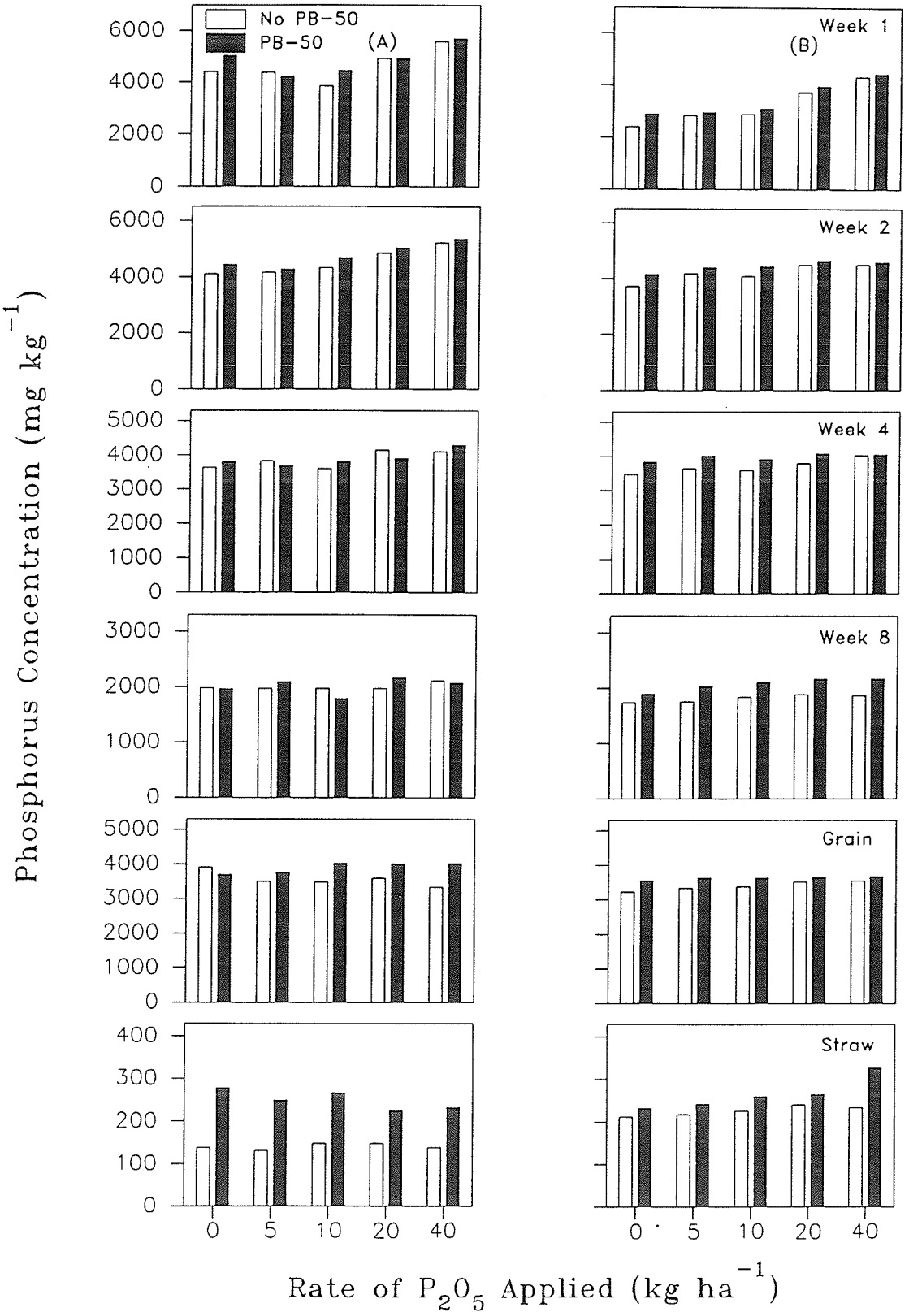

No PB-50
PB-50
(A)
(B)
Week 1
Week 2
Week 4
Week 8
Grain
Straw
Phosphorus Concentration (mg kg^{-1})
Rate of P_2O_5 Applied (kg ha^{-1})
0
5
10
20
40

Table 3.6. Effect of P fertilizer and PB-50 on dry matter and grain production in wheat, at various sampling times (Wellwood soil).

P_2O_5 Added (kg ha^{-1})	PB-50	Dry Matter (g m^{-2})					
		Week 1	Week 2	Week 4	Week 8	Grain	Straw
		Crop Year 1989					
0	-	2.02	5.59	41.8	398	234	270
	+	2.26	7.77	44.6	488	253	287
5	-	1.96	5.87	35.7	448	238	283
	+	2.58	7.42	44.0	547	269	318
10	-	1.88	5.84	44.4	433	183	246
	+	2.71	7.36	59.2	437	222	252
20	-	1.81	6.23	39.7	437	190	222
	+	2.74	7.96	62.9	569	267	308
40	-	2.15	7.29	53.0	497	240	301
	+	2.81	7.68	56.6	550	262	304
Rate		NS	NS	0.02	NS	NS	NS
PB		0.01	0.01	0.01	0.03	0.02	0.03
Rate*PB		NS	NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$†		--	--	--	--	--	--
		Crop Year 1990					
0	-	5.75	12.1	67.6	642	394	514
	+	6.53	12.5	80.7	781	395	527
5	-	6.68	12.5	69.0	656	392	516
	+	7.13	13.8	81.8	860	394	525
10	-	6.63	13.4	74.9	654	407	543
	+	8.50	18.8	95.6	939	425	531
20	-	6.95	14.4	77.9	790	415	507
	+	9.88	20.4	108	1088	418	552
40	-	8.16	18.9	89.3	754	400	443
	+	8.47	20.7	110	1124	401	542
Rate		0.03	0.01	0.05	0.01	NS	NS
PB		0.01	0.01	0.01	0.01	NS	NS
Rate*PB		NS	NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$		--	--	--	--	--	--

† LSD values apply only to comparison of means for treatments with and without PB-50

Figure 3.4. Effect of P fertilizer and PB-50 on dry matter and grain production in wheat, at various sampling times, for the Wellwood soil, in the 1989 (A) and 1990 (B) crop year.

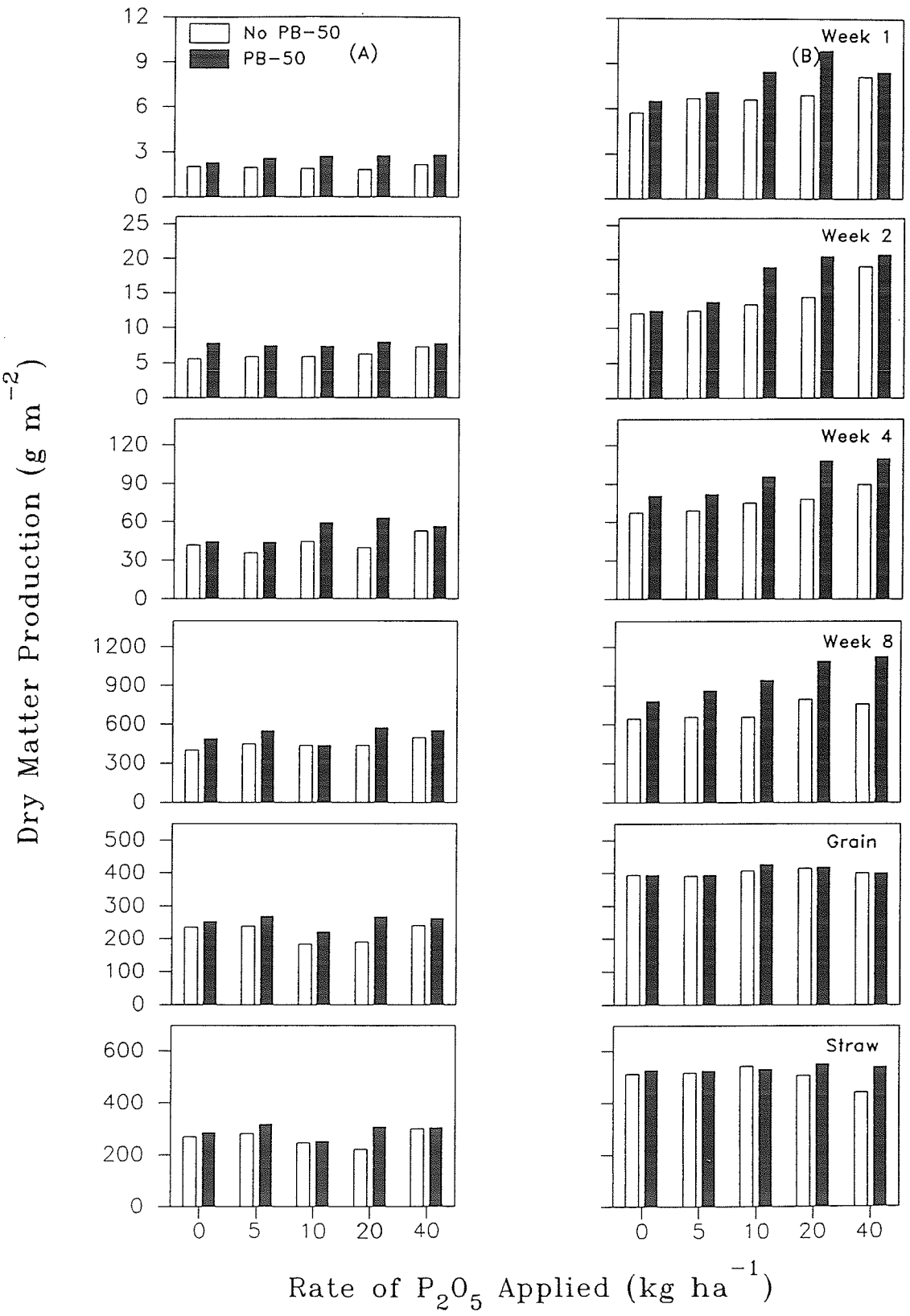

No PB-50
PB-50
(A)
(B)
Week 1
Week 2
Week 4
Week 8
Grain
Straw
Dry Matter Production (g m^{-2})
Rate of P_2O_5 Applied (kg ha^{-1})
0
5
10
20
40

Table 3.7. Effect of P fertilizer and PB-50 on dry matter and grain production in wheat, at various sampling times (Stockton soil).

P_2O_5 Added (kg ha^{-1})	PB-50	Dry Matter (g m^{-2})					
		Week 1	Week 2	Week 4	Week 8	Grain	Straw
		Crop Year 1989					
0	-	1.58	5.31	18.5	416	278	319
	+	2.19	5.57	27.7	430	280	332
5	-	1.79	5.11	26.7	399	228	259
	+	2.58	6.70	30.7	419	266	324
10	-	1.90	5.13	26.3	410	235	345
	+	2.13	6.29	36.7	503	242	357
20	-	2.04	5.78	32.8	395	293	360
	+	2.26	6.67	41.5	482	296	366
40	-	1.92	5.89	36.0	446	262	353
	+	2.70	7.03	48.7	603	280	386
Rate		0.05	0.01	0.01	NS	NS	NS
PB		0.01	0.01	0.01	0.02	NS	0.05
Rate*PB		NS	NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$†		--	--	--	--	--	--
		Crop Year 1990					
0	-	5.16	10.2	56.4	510	267	371
	+	5.01	10.3	58.2	709	282	399
5	-	5.51	11.3	60.3	611	308	419
	+	5.72	11.3	60.0	889	308	432
10	-	5.75	11.3	64.3	660	313	431
	+	6.97	15.3	67.6	866	314	432
20	-	5.94	13.4	71.1	686	311	423
	+	6.92	15.8	95.3	892	316	429
40	-	7.29	14.5	82.9	758	322	435
	+	8.19	15.7	98.3	910	320	427
Rate		0.02	0.01	0.01	0.01	0.05	NS
PB		NS	NS	0.03	0.01	NS	NS
Rate*PB		NS	NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$		--	--	--	--	--	--

† LSD values apply only to comparison of means for treatments with and without PB-50

Figure 3.5. Effect of P fertilizer and PB-50 on dry matter and grain production in wheat, at various sampling times, for the Stockton soil, in the 1989 (A) and 1990 (B) crop year.

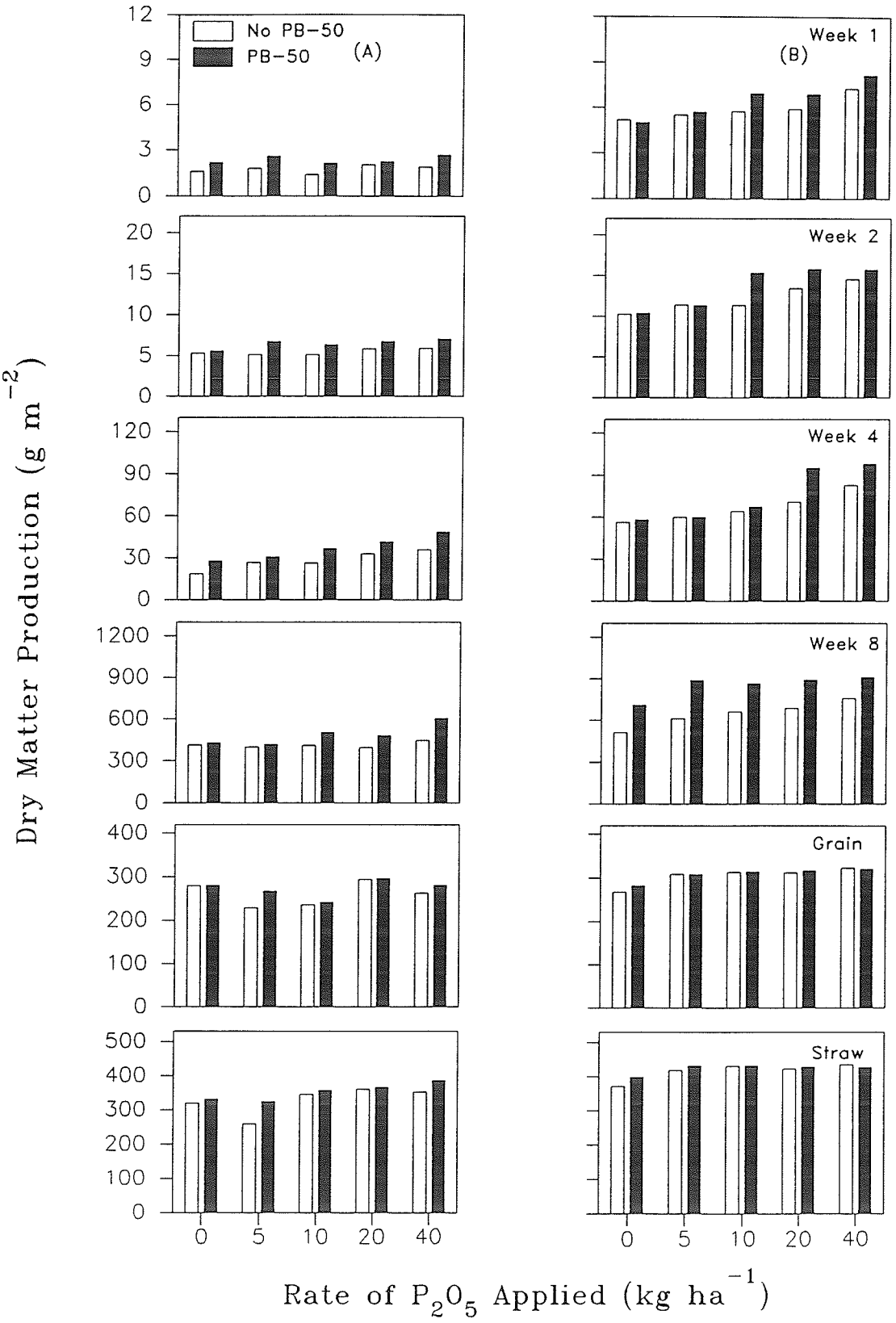
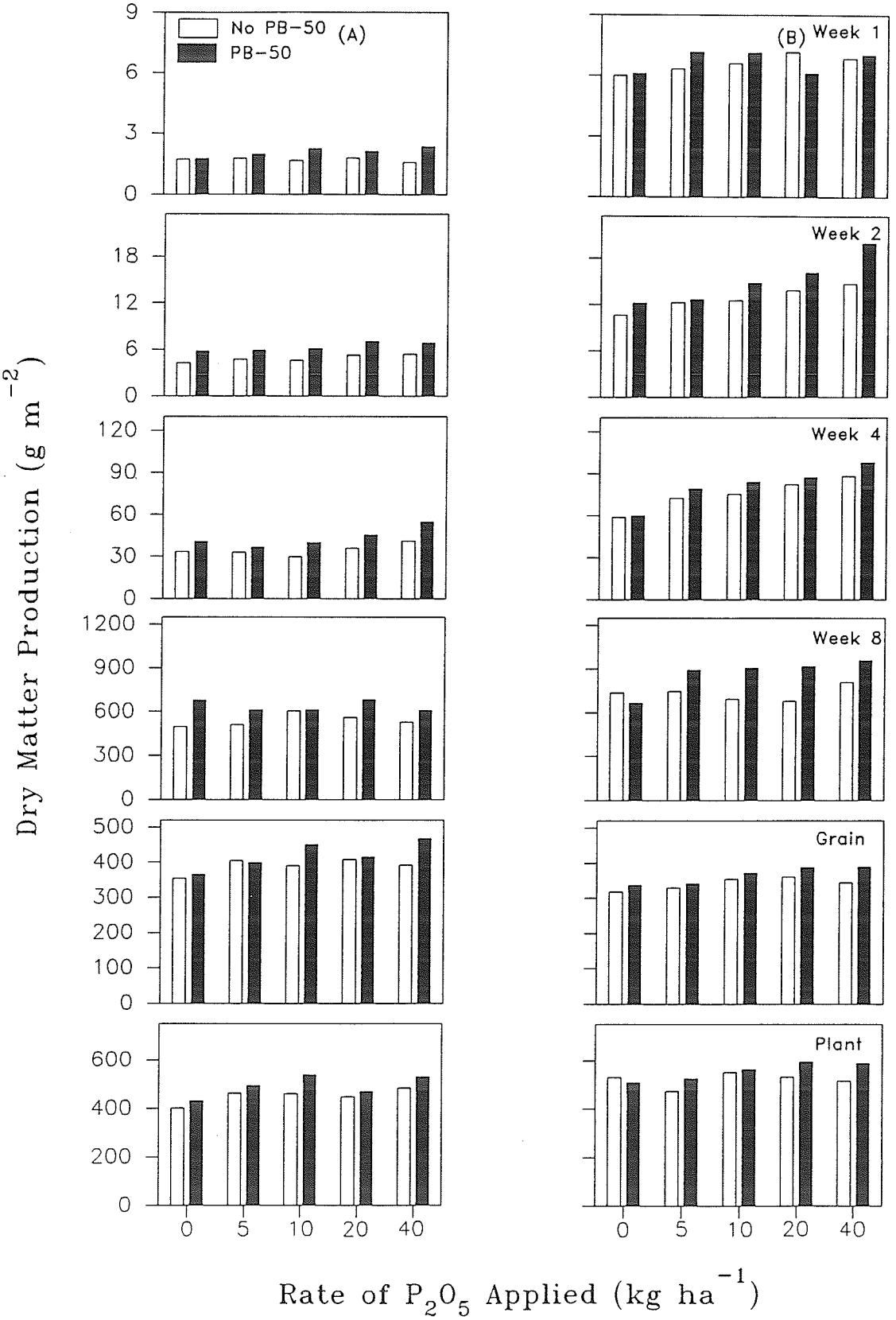

No PB-50
PB-50
(A)
(B)
Week 1
Week 2
Week 4
Week 8
Grain
Straw
Dry Matter Production (g m^{-2})
Rate of P_2O_5 Applied (kg ha^{-1})
0
5
10
20
40

Table 3.8. Effect of P fertilizer and PB-50 on dry matter and grain production in wheat, at various sampling times (Portage soil).

P_2O_5 Added (kg ha^{-1})	PB-50	Dry Matter (g m^{-2})					
		Week 1	Week 2	Week 4	Week 8	Grain	Straw
		Crop Year 1989					
0	-	1.71	4.23	33.4	495	354	401
	+	1.74	5.74	40.8	679	366	430
5	-	1.76	4.71	33.3	511	405	462
	+	1.96	5.90	36.6	611	399	495
10	-	1.66	4.59	29.6	606	390	460
	+	2.24	6.12	40.1	612	450	539
20	-	1.78	5.23	36.1	560	408	448
	+	2.13	6.99	45.5	682	415	468
40	-	1.60	5.37	41.0	529	392	484
	+	2.37	6.81	54.8	608	467	529
Rate		NS	0.01	0.02	NS	NS	NS
PB		0.01	0.01	0.01	0.05	NS	NS
Rate*PB		0.05	NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$†		0.40	--	--	--	--	--
		Crop Year 1990					
0	-	6.01	10.6	59.1	734	318	530
	+	6.11	12.2	60.0	666	337	508
5	-	6.33	12.2	72.6	745	329	473
	+	7.17	12.6	79.2	894	341	526
10	-	6.60	12.5	75.3	693	355	550
	+	7.15	14.8	84.1	908	372	564
20	-	7.17	13.8	82.4	678	360	532
	+	6.13	16.1	87.3	918	388	595
40	-	6.88	14.6	88.1	808	344	516
	+	7.04	20.0	97.9	958	388	588
Rate		NS	NS	0.01	NS	NS	NS
PB		NS	0.02	NS	0.01	NS	NS
Rate*PB		NS	NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$		--	--	--	--	--	--

† LSD values apply only to comparison of means for treatments with and without PB-50

Figure 3.6. Effect of P fertilizer and PB-50 on dry matter and grain production in wheat, at various sampling times, for the Portage soil, in the 1989 (A) and 1990 (B) crop year.


No PB-50
PB-50
(A)
(B)
Week 1
Week 2
Week 4
Week 8
Grain
Plant
Dry Matter Production (g m^{-2})
Rate of P_2O_5 Applied (kg ha^{-1})
0
5
10
20
40

occurred it generally was associated with a significant increase in dry matter production (Tables 3.3, 3.4, 3.5, 3.6, 3.7 and 3.8). In addition, for both cropping seasons, at the Portage site (Table 3.8) (Figure 3.3), inoculation with PB-50 resulted in increased P content of the plants and increased dry matter production, at the control treatment (0 kg P_2O_5 ha^{-1}). However, these increases were not always significant.

At all sites in 1989 and at all sampling dates, inoculation with PB-50 resulted in significant increases in plant dry matter production (Tables 3.6, 3.7 and 3.8). For the same treatments in 1990, only plants at the Wellwood site had significant increases in dry matter production (Table 3.6). These increases in dry matter production with PB-50 inoculation generally were not associated with increased P content of the plant, suggesting that the organisms have additional effects to increasing the availability of P to the plant. The organism may affect the availability of other nutrients, in particular Cu, Mn, Fe and Zn.

Although not an objective of this study, additional plant analyses were conducted to determine the micronutrient (Cu, Zn and Mn) status of plants from the Wellwood site, in both site-years. The results showed inconsistent effects of the PB-50 inoculation on the micronutrient status of the plants (Appendix, Tables A.1, A.2 and A.3). Other research conducted with wheat has shown that inoculation with P. bilaji increases the solubilization of insoluble forms of Cu, Fe and Zn, but only enhanced the uptake of Zn (Kucey 1988a). Doyle et al. (1991) found that inoculation of wheat with P. bilaji increased Cu uptake, as compared to unfertilized treatments. They also reported similar results of increased Zn content of navy beans from P. bilaji inoculation (Doyle

et al. 1991). Significantly higher concentrations of Fe and Mn have been found in canola plants inoculated with P. bilaji (Keyes 1990).

The increased dry matter production, as a result of PB-50 inoculation, generally was greater at the higher rates of P fertilization. The levels of microbial populations on the root rhizosphere were not enumerated in this experiment, but viable spore counts on the treated seed were at levels high enough to ensure an adequate population in the rhizosphere (Table 3.2). Also, the population density of P. bilaji has been found to be enhanced as the rate of P fertilization increases (Keyes 1990). This may explain the significantly increased dry matter production, with PB-50 inoculation, particularly at the higher rates of fertilization.

The early season response to PB-50 is conceivable as P. bilaji populations have been shown to increase 23 and 100 fold, 36 and 49 days after inoculation, respectively (Keyes 1990). As well, P. bilaji has been shown to solubilize RP as early as four days after inoculation (Asea et al. 1988).

3.3.2 Late Season Growth

Although significant increases in the P concentration of the plant were observed with increasing rates of P fertilizer added, during early season growth, this trend did not continue during the late season growth (Tables 3.3, 3.4 and 3.5) (Figures 3.1, 3.2 and 3.3). The level of P in the plants remained relatively constant across all rates of fertilizer added. This is not surprising since a good discrimination in P content between P-stressed and P-sufficient plants is usually only apparent

during the first three to four weeks after emergence (Tomasiewicz and Racz 1991). If cereal seedlings are initially deprived of P, and then exposed to high levels, elevated concentrations of P in the plant can accumulate (Boatwright and Viets 1966; Green et al. 1973; Green and Warder 1973). In addition, Tomasiewicz and Racz (1991) showed that P content of tissues may be above the critical level during the later stages of growth, even though plants were P deficient earlier in the season.

Tomasiewicz and Racz (1991) found that the critical level of P in the plant at three weeks after emergence and at heading, was 4000 mg kg^{-1} and 2400 mg kg^{-1}, respectively. In the experiment reported here, the P content of the plant tissue at four and eight weeks after emergence was approximately 4100 mg kg^{-1} and 2100 mg kg^{-1}, respectively, indicating the plants were marginally sufficient.

Increasing rates of fertilizer P added also had a minimal effect on the dry matter production, at the later sampling dates (Tables 3.6, 3.7 and 3.8) (Figures 3.4, 3.5 and 3.6). The limited response to P fertilizer (dry matter production and grain yield) was not expected as the soils at all sites tested low in $NaHCO_3$-ext P (Table 3.1). This lack of response to P fertilizer makes it difficult to study the effects of PB-50, because to obtain a beneficial yield increase from the inoculation with P. bilaji, the crop must first show a response to P fertilizer application (Bullock et al. 1990; Hnatowich et al. 1990; Gleddie et al. 1991).

In field experiments conducted on P. bilaji inoculated wheat, it was found that the plants generally did not show a P fertilizer response

at maturity (Bullock et al. 1990; Hnatowich et al. 1990; Androsoff et al. 1991; Doyle et al. 1991; Gleddie et al. 1991). For example, PB-50 experiments were conducted at 27 different sites, with only plants at one location responding to P fertilizer additions (Androsoff et al. 1991). As a result, in these experiments, grain yields were not statistically increased with the addition of P. bilaji. In contrast, where plants responded to P fertilizer additions, there were significant yield increases with the inoculation of P. bilaji (Kucey 1987; 1988a; Hnatowich et al. 1990; Gleddie et al. 1991).

As the crops matured, differences between inoculated and non-inoculated plants were observed, particularly at the Stockton and Portage sites (Tables 3.3, 3.4 and 3.5) (Figures 3.1, 3.2 and 3.3). At both sites, inoculation with PB-50 generally resulted in significant increases in the P content of the plant (eight-week sampling period) and always significantly increased the P content of the grain (Tables 3.4 and 3.5). These results are in contrast to previous research on P. bilaji which showed that the P content of inoculated plants remained constant (Kucey 1987; Androsoff et al. 1991; Doyle et al. 1991) or decreased (Kucey 1988a), as compared to non-inoculated treatments, at the recommended rate of P fertilizer. However, Kucey (1987; 1988a) did not find an increased P content in plants inoculated with P. bilaji, when no fertilizer P was applied.

At heading (eight-week sampling date), plants at all site-years had a significant increase in dry matter production with the PB-50 treatment (Tables 3.6, 3.7 and 3.8). However these increases were not P fertilizer dependent, as the increases generally were greater at the

higher rates of added P fertilizer (Figures 3.3, 3.4 and 3.5). In contrast, Kucey (1987), Hnatowich et al. (1990) and Gleddie et al. (1991) reported that the best response to inoculation with P. bilaji occurred at the lower rates of fertilization, with the yield response of the treated plants decreasing as the rate of P fertilizer application increased. They suggested that at the higher rates of fertilization, the crop was able to obtain the P necessary for optimum growth, without the addition of P. bilaji.

By maturity, the inoculation with PB-50 had no effect on grain production even though, for most sites, significant increases in plant dry matter production had been observed over most sampling dates (Tables 3.6, 3.7 and 3.8). Only at the Wellwood-89 site did the plants have a significantly higher grain yield with the PB-50 treatment (Table 3.6). Also at this site, the P content of the grain was not affected by inoculation with PB-50 (Table 3.3). Similar results were observed by Kucey (1988a) and Keyes (1990) who found increased yields with the P. bilaji treatments even though the P content of the plants were reduced.

This lack of significant increase in grain yield with the PB-50 inoculation, suggests that any additional P taken up by the inoculated plant later in the growing season could not contribute to an increased grain yield. This was probably because that plants contained sufficient P and any additional P added was in excess of the plants requirements.

It is interesting to compare the grain yields obtained for the two field seasons. Yields for all sites and treatments averaged 3021 kg ha^{-1} and 3545 kg ha^{-1} in 1989 and 1990, respectively. Lower yields in 1989 were due to less rainfall 1989 than in 1990, at the Wellwood and

Stockton sites. Also, wind erosion at the Wellwood site in 1989 damaged seedlings and an infestation of grasshoppers at the same site reduced plant stand and grain yield. In contrast, at the Portage site optimal moisture conditions existed in 1989 and excess moisture reduced yields at the same site in 1990.

The grain yield, averaged across all sites in 1989 was 2887 kg ha^{-1} for the non-inoculated treatments and 3156 kg ha^{-1} for the inoculated treatments. The difference between the two treatments, averaged across all sites, was less in 1990, 3490 kg ha^{-1} for non-inoculated treatments and 3599 kg ha^{-1} for the inoculated treatments. The grain yields averaged across all site-years, for the non-inoculated and inoculated treatments, at increasing rates of P fertilizer additions, are reported in Table 3.9. The grain yield increases ranged from 69 to 263 kg ha^{-1}, with an average statistically significant increase across all fertilizer rates of 172 kg ha^{-1} (5.9% greater than the non-inoculated treatments). In field experiments where the plants showed a response to P fertilizer, Gleddie *et al*. (1991) reported yield increases, with the addition of *P. bilaji*, of up to 65 kg ha^{-1}, with an average increase of 30 kg ha^{-1}. In the field experiment reported here, the greatest increases in dry matter production and grain yield, with the PB-50 treatment, occurred at the higher rates of fertilizer P additions, similar to what was observed at the earlier sampling dates.

The beneficial effects of PB-50, in terms of increased P concentration in the plant and increased dry matter production, were similar for the three soils studied, selected for their difference in pH

Table 3.9. The overall effect of P fertilizer and PB-50 on the grain yield of wheat (6 site years).

P_2O_5 Added (kg ha^{-1})	Grain Yield (kg ha^{-1})	
	No PB-50 Added	PB-50 Added
0	3119	3188
5	3192	3294
10	3111	3374
20	3274	3484
40	3275	3492
Rate	NS	
PB	0.01	
Rate*PB	NS	
$LSD_{(P=0.05)}$†	--	

† LSD values apply only to comparison of means for treatments with and without PB-50

(Tables 3.3, 3.4, 3.5, 3.6, 3.7 and 3.8). Keyes (1990) studied the effects of P. bilaji inoculation on crop growth in an acidic soil and found results similar to those obtained by researchers who studied the effects of P. bilaji inoculation on crop growth in alkaline soils (Kucey 1983; 1987; 1988a; Asea et al. 1988). From the results presented here, it appeared that the responsiveness of the plant to P fertilization, the environmental conditions and the crop grown, were more important in determining the effect of PB-50 on crop growth, than was reaction of the soil.

3.4 Summary

Field trials were conducted in 1989 and 1990 at three locations in Manitoba, to determine the influence of PB-50 on early season uptake of P, dry matter and grain yield of wheat, over a range of P fertilizer rates. Three soils were studied to compare the effectiveness of PB-50 on different pH soils.

For all site years, the addition of P fertilizer enhanced the P content in the plant and dry matter production of wheat at the early growth stages. However these increases were not associated with a significant yield increase at final harvest, with the exception of the wheat at the Stockton-90 site.

The inoculation of PB-50 initiated an inconsistent, but enhanced uptake of P by wheat, early in the growing season. However, for the majority of the 1989 sites, the increased early season uptake of P by PB-50 inoculated plants was represented by an enhanced dry matter production, rather than an enhanced plant P concentration. In contrast,

in 1990, enhanced dry matter production as well as an increased plant P concentrations were evident early in the growing season. In addition, response of the wheat to the PB-50 inoculation was not always followed by a similar response trend to P fertilization.

By maturity, the benefits of PB-50 had dissipated, as only the Wellwood-89 site reported a significant grain yield increase in response to inoculation with PB-50. A combined site analysis indicated an overall significant grain yield response from PB-50 inoculation. In addition, comparison of the field years indicate that the 1989 wheat crop had a larger grain yield response to PB-50 than in 1990, even though the 1990 crop demonstrated an increased response in terms of plant P concentration, from PB-50 inoculation. For the majority of the experimental sites, as the rate of P fertilization increased, the responses to PB-50 increased. In addition, more significant responses to PB-50, in terms of P concentration of wheat, occurred at the later sampling times, and were associated with increased dry matter production. The results are inconclusive, but the trends of an increased dry matter production when wheat was inoculated with PB-50, may have been due to the organism affecting the uptake of additional nutrients.

When comparing the different pH soils, it appeared that the benefits of PB-50 were not limited because of the pH of the soil. Instead, PB-50 inoculation was limited to the reponse of the plants to P fertilizer.

IV. INFLUENCE OF PB-50 INOCULATION ON THE AVAILABILITY OF SOIL AND FERTILIZER P FOR WHEAT AND FLAX, AND THE IMPLICATIONS ON CROP GROWTH - GROWTH CHAMBER STUDY

4.1 Introduction

The recovery of fertilizer P in the plant for the year of fertilizer P application varies with the amount added, soil characteristics, time of application and the crop species (Wild 1988). In terms of crop species, wheat utilizes up to 35% of the fertilizer P applied, whereas flax extracts only one-third to one-half as much P fertilizer (Strong and Soper 1973; Bailey et al. 1977). It has been found for wheat that the principal source of P uptake during the first four weeks after seeding is from the applied fertilizer, whereas native soil P provides the main source later in plant development (Barber and Olson 1968; Spinks and Barber 1947). In contrast, flax utilizes negligible amounts of fertilizer P, as soil P continuously supplies the plant throughout the growing season (Racz et al. 1965; Soper and Kalra 1969).

Due to the limited information on the effects of PB-50 at the early plant growth stages, the objective of this research was to determine the effectiveness of PB-50 for increasing early season uptake of P in wheat and flax, and for enhancing dry matter production and grain yield. Fertilizer containing labelled ^{32}P was applied to assist in determining the influence of PB-50 on crop availability of soil and fertilizer P at various sampling times during the growing season. The final objective was to determine the effectiveness of PB-50 on soils of

different pH, by comparing an acidic and a neutral soil.

4.2 Materials and Methods

Wheat (Triticum aestivum cv. Katepwa) and flax (Linum usitatissimum cv. Norlin) were grown in a growth chamber experiment using a surface (0 - 15 cm) sample of a Wellwood and a Willowcrest soil. The characteristics of these soils are presented in Table 4.1. The solution pH was determined with a glass electrode (soil:water ratio, 1:1) on <2 mm air-dry soil (McLean 1982). Moisture content for field capacity was determined by a gravimetric procedure (Viehmeyer and Hendrickson 1949). The cation exchange capacity was assessed by saturation with sodium and displacement by Mg (Polemio and Rhoades 1977). Inorganic C was analyzed by a titrimetric method (Bundy and Bremner 1972) and organic C content was assessed using a dichromate oxidation procedure (Yeomans and Bremner 1988). Particle size analysis was determined using the standard pipette method as described by Kilmer and Alexander (1949). Plant available phosphate was extracted using $NaHCO_3$ as described by Olsen et al. (1954) and the P in solution was measured by the acid-molybdate procedure (Murphy and Riley 1962). Plant available Cu, Fe, Mn and Zn were assessed using the DTPA method of Lindsay and Norvell (1978).

Both wheat and flax seeds were coated with P. bilaji inoculant, PB-50. The coating was prepared by suspending 0.5 g of PB-50 in 20 mL of deionized water. The suspension was pipetted over 500 g of wheat or flax seed in a polyethylene bag and shaken to obtain an even distribution of fungal spores on the seed. Non-inoculated wheat and

Table 4.1. Soil characteristics.

Soil	Ph	CEC	Field Capacity	Org. C	Inorg. C	Texture			P	Cu	Fe	Mn	Zn
						Sand	Silt	Clay					
		cmol kg^{-1}	----------%						---------mg kg^{-1}---------				
Wellwood	5.3	44.0	23.4	2.63	0.0	56	20	24	8.8	1.2	9.7	48.0	2.1
Willowcrest	7.4	20.6	21.2	1.81	0.4	87	3	10	5.3	0.1	7.0	8.2	0.4

flax seeds (500 g) also received 20 mL of deionized water to maintain uniformity in water uptake by the seeds. Both the treated and untreated seeds were allowed to dry before planting. The PB-50 suspension and samples of the coated seeds were analyzed by PhilomBios for fungal viability (Table 4.2).

Table 4.2. Spore counts of PB-50 suspension and treated wheat and flax seeds.

Crop	PB-50 Suspension	Treated Seed
	---cfu g^{-1}---	--cfu $seed^{-1}$--
Wheat	1.9×10^9	5.1×10^3
Flax #1	7.2×10^9	6.0×10^4
Flax #2	7.1×10^9	4.3×10^3

Prior to use, the soil was sieved (<2-mm) in the field moist condition and stored at 4 C. Samples of soil (2500 g, oven-dry basis) were evenly treated with 20 mL of deionized water containing a basal nutrient solution of 100 mg N kg^{-1} soil as NH_4NO_3 and 400 mg K kg^{-1} soil and 80 mg S kg^{-1} soil as K_2SO_4, and placed in 3 L pots. Five rates of ^{32}P-labelled P fertilizer in solution, 0.0, 2.5, 5.0, 10.0 and 20.0 mg P kg^{-1} soil as $NH_4H_2PO_4$, labelled with 0, 370, 740, 1480 and 2960 kBq ^{32}P, respectively, and two PB-50 seed treatments (inoculated and non-inoculated) were applied. For wheat and flax, an additional 100 mg N kg^{-1} as NH_4NO_3 was applied at 3 and 6 weeks after emergence.

To apply the ^{32}P-labelled P fertilizer, 10 mL of solution was placed in a 8-cm band in the centre of the pot, 3 cm below the soil surface. Twelve wheat or 20 flax seeds were placed in 2 rows, 1.5 cm from the soil surface and 2 cm on either side of the ^{32}P-labelled P fertilizer band. All treatments were replicated 4 times, and pots were arranged in a completely randomized design (Little and Hills 1978).

The pots were placed in a growth chamber set at a 16 h day (23 C), 8 h night (17 C) cycle. The soils were moistened and maintained at the gravimetric field capacity (Table 3.1) by adding adequate distilled water to the soil surface daily, for the duration of the experiment.

For wheat, plant samples were randomly taken from all pots at 1, 2 and 4 weeks after emergence, and at plant maturity (15 weeks). Six plants were harvested at one and two weeks and 4 plants were collected at four weeks and maturity. After the sampling at one week, pots were thinned to 14 plants.

The flax experiment was conducted two times to ensure that adequate plant material was collected to perform plant nutrient analysis. For the first experiment, plant samples were randomly taken from all pots at 1 week (20 plants) and 2 weeks (16 plants) after emergence. The experiment was conducted a second time in the same manner. For both experiments, the pots were thinned to 16 plants, 1 week after emergence. For the second experiment, plants were randomly sampled at 4 weeks (6 plants) and 8 weeks (4 plants) and at plant maturity (6 plants) (21 weeks). Both wheat and flax plants grown to maturity were separated into seed and straw components in order to determine grain and dry matter yields.

The plant samples were oven dried (65 C) for 1 week, weighed (for dry matter and grain yields) and ground through a 2-mm sieve using a Thomas-Wiley Laboratory Mill.

Samples of the ground tissue were prepared for ^{31}P and ^{32}P analysis by digesting the material using a nitric-perchloric procedure (Isaac and Kerber 1971). The samples were analyzed for ^{31}P by the acid-molybdate procedure described by Murphy and Riley (1962) and ^{32}P radiation was analyzed by liquid scintillation counting using Beckman Ready Solv CP Cocktail and a Beckman model 7500 counter (Wang et al. 1975).

The fraction of P in the plant derived from the fertilizer (FP) was calculated using isotopic dilution. For all plant samples, counts were corrected for background and radioactive decay, whereby:

$$FP = \frac{\text{specific activity of } ^{32}P \text{ in plant}}{\text{specific activity standard (SAS)}} \times \text{dilution factor} \quad (1)$$

The SAS was determined from the formula:

$$SAS = \frac{\text{specific activity of the standard } ^{32}P \text{ sample}}{\text{concentration of the standard sample}} \quad (2)$$

Phosphorus derived from the soil, was the difference between the total P (^{31}P) in the plant and the FP (^{32}P).

The results of the experiment were statistically analyzed using the GLM procedure (Goodnight et al. 1988).

4.3 Results and Discussion

As discussed in Section 3.3, the importance of P supply early in the growing season must be considered. Therefore, the results for early

season growth (1, 2 and 4 week sampling dates) and late season growth (8 week [flax only] and maturity sampling dates), for the growth chamber experiments will be discussed separately. In addition, two crops, wheat and flax, were grown and results for each also will be discussed separately.

4.3.1 Wheat: Early Season Growth

The concentration of P in the wheat plants, on both soils, significantly increased with increasing rates of P fertilizer added (Table 4.3 and Figure 4.1). In addition, on both soils, the level of labelled fertilizer P in the plant significantly increased, whereas the level of soil P in the plant generally decreased or was unaffected by increasing rates of P fertilizer added (Table 4.3 and Figure 4.1). It is interesting to note that the contribution of soil P to the total amount of P in the plant was almost always greater than the contribution by the P fertilizer. However, the overall contribution of soil P may be overestimated as the P in the seed can also make a contribution to the total concentration of P in the plant (Bolland and Baker 1988).

The increase in the P content of the plant was associated with a significant increase in plant dry matter production, with increasing rates of P fertilizer added (Table 4.4 and Figure 4.2).

The inoculation of wheat with PB-50 generally did not significantly affect the concentration of P in the plant (Table 4.3 and Figure 4.1). Only PB-50 treated plants grown in the Willowcrest soil, at the one week sampling date, had a significant increase in the concentration of P in the plant, as a result of an increased proportion

Table 4.3. The contribution of fertilizer and soil P as affected by P fertilizer and PB-50 in wheat, at various sampling times.

P Added (kg ha^{-1})	PB-50	Phosphorus Concentration (mg kg^{-1})					
		Week 1			Week 2		
		Total	Fert	Soil	Total	Fert	Soil
		Wellwood Soil					
0	-	4728	0	4728	3057	0	3057
	+	4960	0	4960	3232	0	3232
2.5	-	5233	423	4810	2921	409	2512
	+	5243	535	4708	3495	370	3125
5	-	5487	625	4862	3627	787	2840
	+	5115	818	4298	3611	822	2788
10	-	5732	1437	4295	4194	1473	2720
	+	6156	1624	4531	4404	884	3520
20	-	6237	2801	3436	4621	2436	2185
	+	6570	2948	3622	5271	1560	3711
Rate		0.01	0.01	0.01	0.01	0.01	NS
PB		NS	NS	NS	NS	0.01	NS
Rate*PB		NS	NS	NS	NS	0.05	NS
$LSD_{(P=0.05)}$†		--	--	--	--	511	--
		Willowcrest Soil					
0	-	2188	0	2188	1274	0	1274
	+	2369	0	2369	1356	0	1356
2.5	-	2697	583	2114	1550	484	1066
	+	2916	637	2279	1603	473	1130
5	-	3327	1033	2294	1553	748	805
	+	3423	1061	2362	1564	685	879
10	-	3818	1808	2010	2202	1435	767
	+	3901	1539	2362	2134	1455	679
20	-	3868	2954	914	3248	2568	680
	+	4814	2399	2415	3067	2019	1047
Rate		0.01	0.01	0.01	0.01	0.01	0.01
PB		0.01	0.03	0.01	NS	NS	NS
Rate*PB		NS	NS	0.01	NS	NS	NS
$LSD_{(P=0.05)}$		--	--	365	--	--	--

† LSD values apply only to comparison of means for treatments with and without PB-50

Week 4			Grain			Straw		
Total	Fert	Soil	Total	Fert	Soil	Total	Fert	Soil
1955	0	1955	3889	0	3889	179	ND	ND
1997	0	1997	3725	0	3725	156	ND	ND
1961	163	1798	3742	119	3623	150	ND	ND
2180	160	2021	3571	103	3468	143	ND	ND
2588	332	2256	3990	243	3746	140	ND	ND
1968	312	1656	3733	221	3512	151	ND	ND
2371	681	1690	4256	469	3787	165	ND	ND
2708	652	2056	4038	461	3577	173	ND	ND
2908	1457	1450	4631	895	3736	214	ND	ND
3090	1381	1710	4990	964	4026	216	ND	ND
0.01	0.01	NS	0.01	0.01	NS	0.01	ND	ND
NS	NS	NS	NS	NS	NS	NS	ND	ND
0.05	NS	NS	NS	NS	NS	NS	ND	ND
474	--	--	--	--	--	--	--	--
1335	0	1335	2850	0	2850	184	ND	ND
1352	0	1352	2626	0	2626	357	ND	ND
1298	317	981	2955	343	2612	177	ND	ND
1204	335	869	3126	323	2803	200	ND	ND
2317	660	1657	2860	669	2191	164	ND	ND
2916	597	2319	3426	715	2712	204	ND	ND
3546	1130	2415	3361	1271	2089	270	ND	ND
3985	1221	2763	3143	1141	2002	195	ND	ND
3566	1480	2084	4038	2273	1765	381	ND	ND
3989	1597	2392	4227	2167	2060	422	ND	ND
0.01	0.01	0.01	0.01	0.01	0.05	0.01	ND	ND
NS	NS	NS	NS	NS	NS	0.03	ND	ND
NS	NS	NS	NS	NS	NS	0.01	ND	ND
--	--	--	--	--	--	75	--	--

Figure 4.1. The contribution of fertilizer and soil P as affected by P fertilizer and PB-50 in wheat, at various sampling times, for the Wellwood (A) and Willowcrest (B) soils.

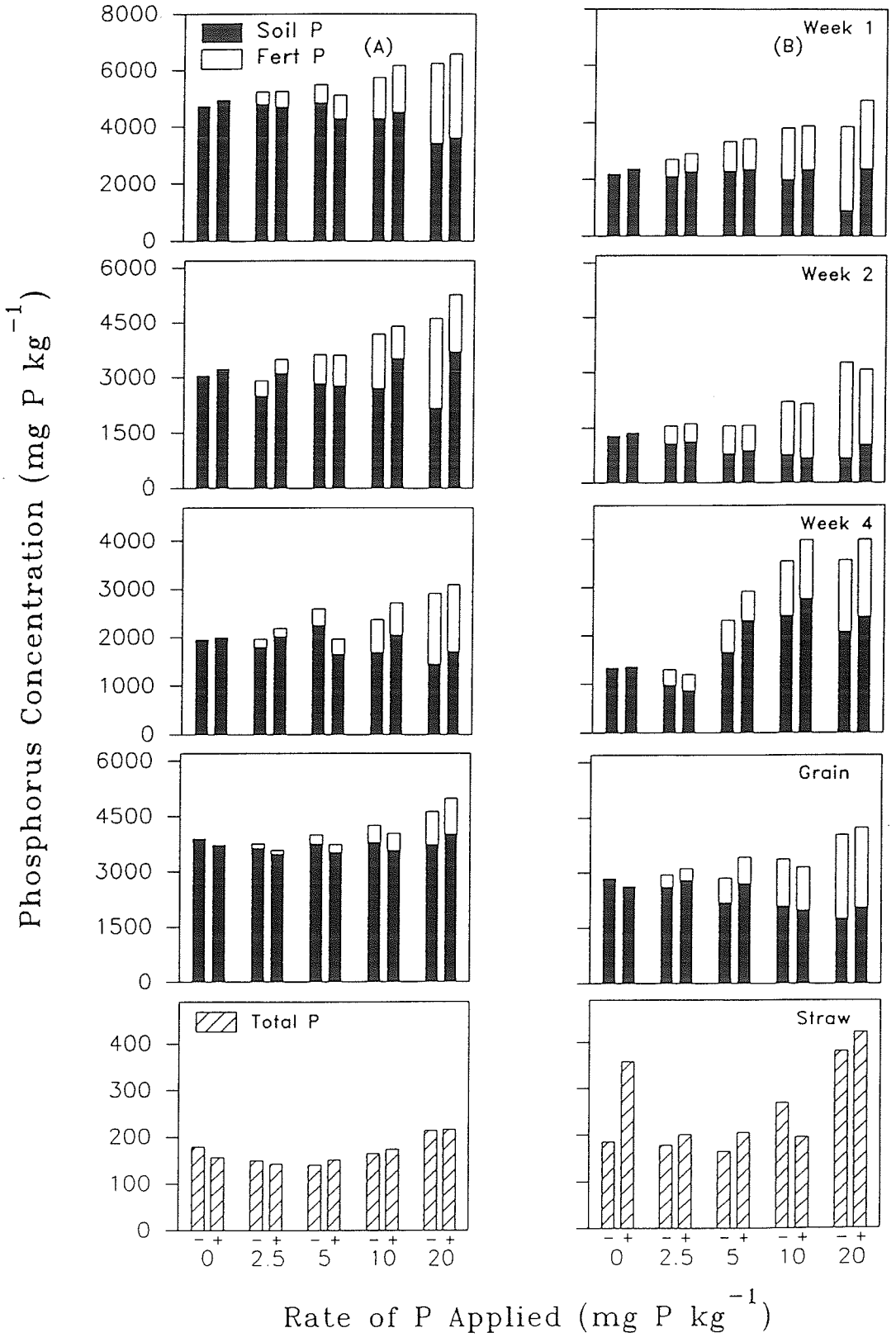
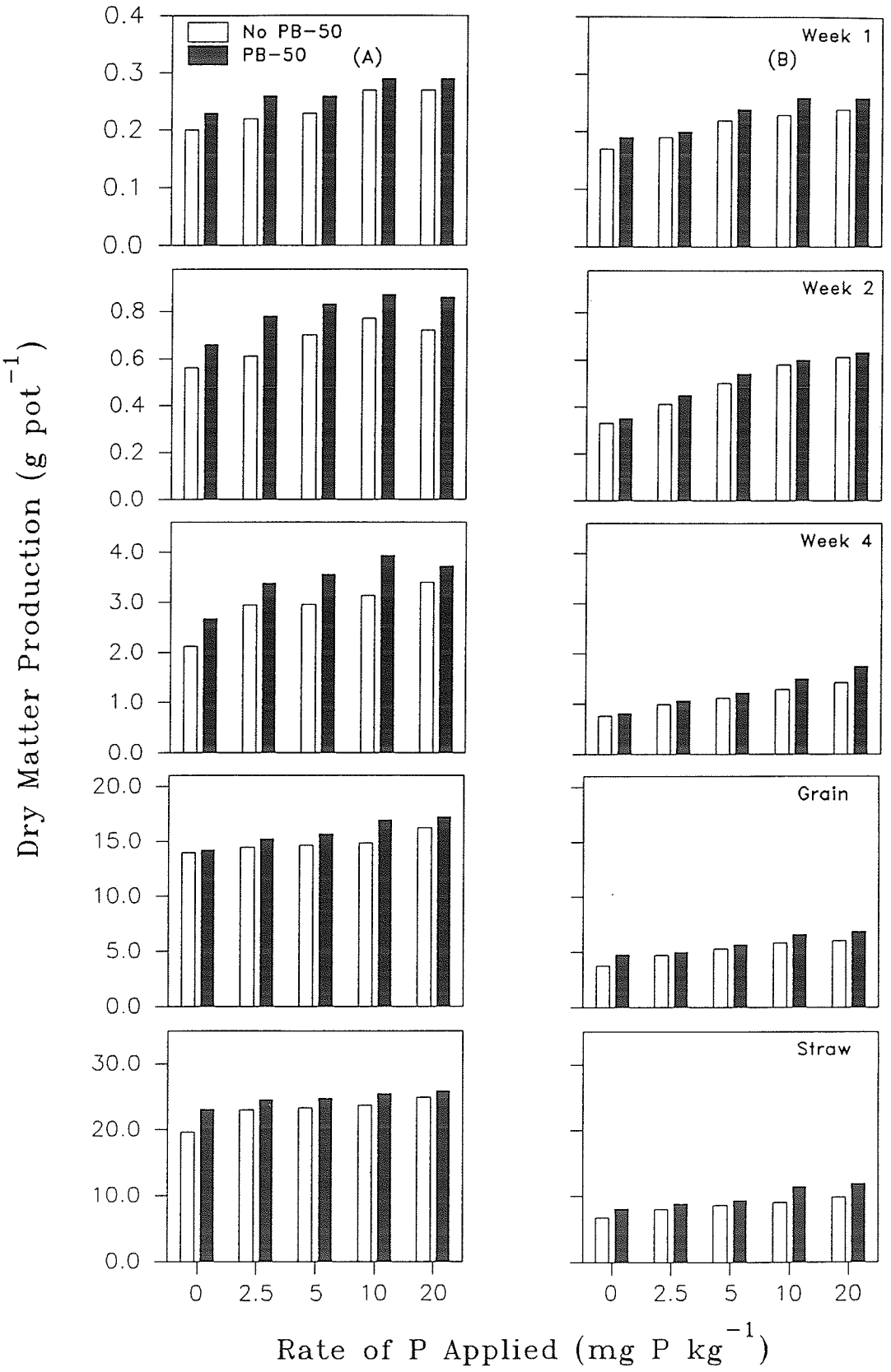

Soil P
Fert P
(A)
(B)
Week 1
Week 2
Week 4
Grain
Straw
Total P
8000
6000
4000
2000
0
6000
4500
3000
1500
0
4000
3000
2000
1000
0
6000
4500
3000
1500
0
400
300
200
100
0
0
2.5
5
10
20
Phosphorus Concentration (mg P kg^{-1})
Rate of P Applied (mg P kg^{-1})

Table 4.4. Effect of P fertilizer and PB-50 on dry matter and grain production in wheat, at various sampling times.

P Added (kg ha^{-1})	PB-50	Dry Matter (g pot^{-1})				
		Week 1	Week 2	Week 4	Grain	Straw
		Wellwood Soil				
0	-	0.20	0.56	2.12	14.0	19.6
	+	0.23	0.66	2.67	14.2	23.1
2.5	-	0.22	0.61	2.94	14.5	23.0
	+	0.26	0.78	3.38	15.2	24.5
5	-	0.23	0.70	2.95	14.6	23.3
	+	0.26	0.83	3.56	15.6	24.8
10	-	0.27	0.77	3.14	14.8	23.7
	+	0.29	0.87	3.94	16.9	25.5
20	-	0.27	0.72	3.40	16.2	24.9
	+	0.29	0.86	3.73	17.2	25.9
Rate		0.01	0.01	0.01	0.01	0.01
PB		0.03	0.01	0.01	0.01	0.01
Rate*PB		NS	NS	NS	0.02	NS
$LSD_{(P=0.05)}$†		--	--	--	0.4	--
		Willowcrest Soil				
0	-	0.17	0.33	0.75	3.77	6.69
	+	0.19	0.35	0.81	4.79	8.04
2.5	-	0.19	0.41	0.99	4.74	8.00
	+	0.20	0.45	1.06	5.04	8.87
5	-	0.22	0.50	1.11	5.31	8.61
	+	0.24	0.54	1.22	5.70	9.39
10	-	0.23	0.58	1.28	5.84	9.04
	+	0.26	0.60	1.49	6.61	11.4
20	-	0.24	0.61	1.42	6.02	9.83
	+	0.26	0.63	1.74	6.89	11.9
Rate		0.01	0.01	0.01	0.01	0.01
PB		0.05	NS	0.02	0.01	0.01
Rate*PB		NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$		--	--	--	--	--

† LSD values apply only to comparison of means for treatments with and without PB-50

Figure 4.2. Effect of P fertilizer and PB-50 on dry matter and grain production in wheat, at various sampling times, for the Wellwood (A) and Willowcrest (B) soils.


No PB-50
PB-50
(A)
(B)
Week 1
Week 2
Week 4
Grain
Straw
Dry Matter Production (g pot^{-1})
Rate of P Applied (mg P kg^{-1})
0.4
0.3
0.2
0.1
0.0
0.8
0.6
0.4
0.2
0.0
4.0
3.0
2.0
1.0
0.0
20.0
15.0
10.0
5.0
0.0
30.0
20.0
10.0
0.0
0
2.5
5
10
20

of P being derived from the soil. Inoculation with PB-50 also had little significant effect on the concentration of labelled fertilizer P in the plant (Table 4.3). With the limited mobility of P in the soil, plant roots must proliferate into the fertilizer band to obtain P. In addition, the quantity of roots in the P band decreases as the concentration of the P fertilizer increases (Strong and Soper 1973). If the quantity of roots and hence the population of inoculated fungi associated with the root rhizosphere is reduced, this may explain why inoculation with PB-50 did not enhance the uptake of labelled P by the plants. Another possible explanation may be that there is isotopic exchange between the fertilizer P and the soil P, resulting in a net tie-up of ^{32}P (Asea et al. 1988).

It should be noted, that an increased contribution of soil P by PB-50 treated plants, was associated with non-significant increases in the total amount of P in the plant, at the higher rates of P fertilization on both soils (Figure 4.1).

Inoculation with PB-50 did result in significant increases in plant dry matter production, at most early season sampling dates, for all rates of P fertilizer added, on both soils (Table 4.4 and Figure 4.2). It is noteworthy that at the higher rates of P fertilization, the non-significant increases in P content, for PB-50 treated plants, was associated with greater significant increases in dry matter production (Figure 4.2). As was observed in the field experiments, the increases in dry matter production with PB-50 inoculation in the growth chamber experiment, suggest that the organisms have additional effects to increasing the availability of P to the plant.

4.3.2 Wheat: Late Season Growth

On both soils, the concentration of P in the grain and in the straw, significantly increased with increasing rates of fertilizer P added, continuing the trend observed at the early season sampling dates (Table 4.3 and Figure 4.1). With increasing rates of P fertilizer, the contribution to the P content of the grain from the labelled fertilizer P significantly increased whereas the contribution to the P content of the grain from the soil P was significantly decreased (Willowcrest soil) or was unaffected (Wellwood soil) (Table 4.3). As was observed at the early sampling dates, the contribution of soil P to the total amount of P in the plant was almost always greater than the contribution by the P fertilizer. Because of low levels of radiation in the straw samples, it was not possible to determine the contribution of labelled fertilizer P to the total amount of P in the plant.

The significant increases in the P content of the plants was related to significant increases in grain and straw production (Table 4.4 and Figure 4.2). This result was expected as both soils tested low in $NaHCO_3$-ext P (Table 4.1).

Inoculation of wheat with PB-50 did not significantly affect the P concentration in the grain and had no significant effect on the contribution of fertilizer or soil P to the total amount of P in the grain (Table 4.3 and Figure 4.1).

Even though plants were not sampled at eight weeks after emergence, Chambers (1989) reported that PB-50 treated wheat, at heading, had significant increases in the soil P content, corresponding to an increased total amount of P in the plant. In addition, the

increases were greater at the higher rates of P fertilizer added. Asea et al. (1988) are in agreement, as an 11% increase in P being derived from native sources was associated with a 14% increase in total P uptake for P. bilaji treated wheat. These increases in P uptake for P. bilaji inoculated plants, resulted in significant increases in dry matter production (Asea et al. 1988; Kucey 1988a; Chambers 1989).

To obtain a beneficial yield increase from the inoculation with P. bilaji, the crop must first show a response to P fertilizer application (Bullock et al. 1990; Hnatowich et al. 1990; Gleddie et al. 1991), which occurred with both soils studied. As a result, the inoculation with PB-50 was related to a significant increase in the grain production, for both soils (Table 4.4 and Figure 4.2). The grain yield, averaged for both soils, for the non-inoculated treatments was 9.9 g pot^{-1} and 10.8 g pot^{-1} for the inoculated treatments (an 9% increase). Kucey (1987) reported a significant increase in grain production with the inoculation of P. bilaji.

4.3.3 Flax: Early Season Growth

On both soils, there generally was a significant increase in the P concentration of the flax plant with increasing rates of P fertilizer added (Table 4.5 and Figure 4.3). As with the wheat in the previous experiment, the contribution of labelled P fertilizer to the total amount of P in the plant was significantly increased with increasing rates of P fertilizer, whereas the contribution of soil P was either decreased or unaffected by the same treatments (Table 4.5). Also, as was noted in the previous experiment, although the contribution of P

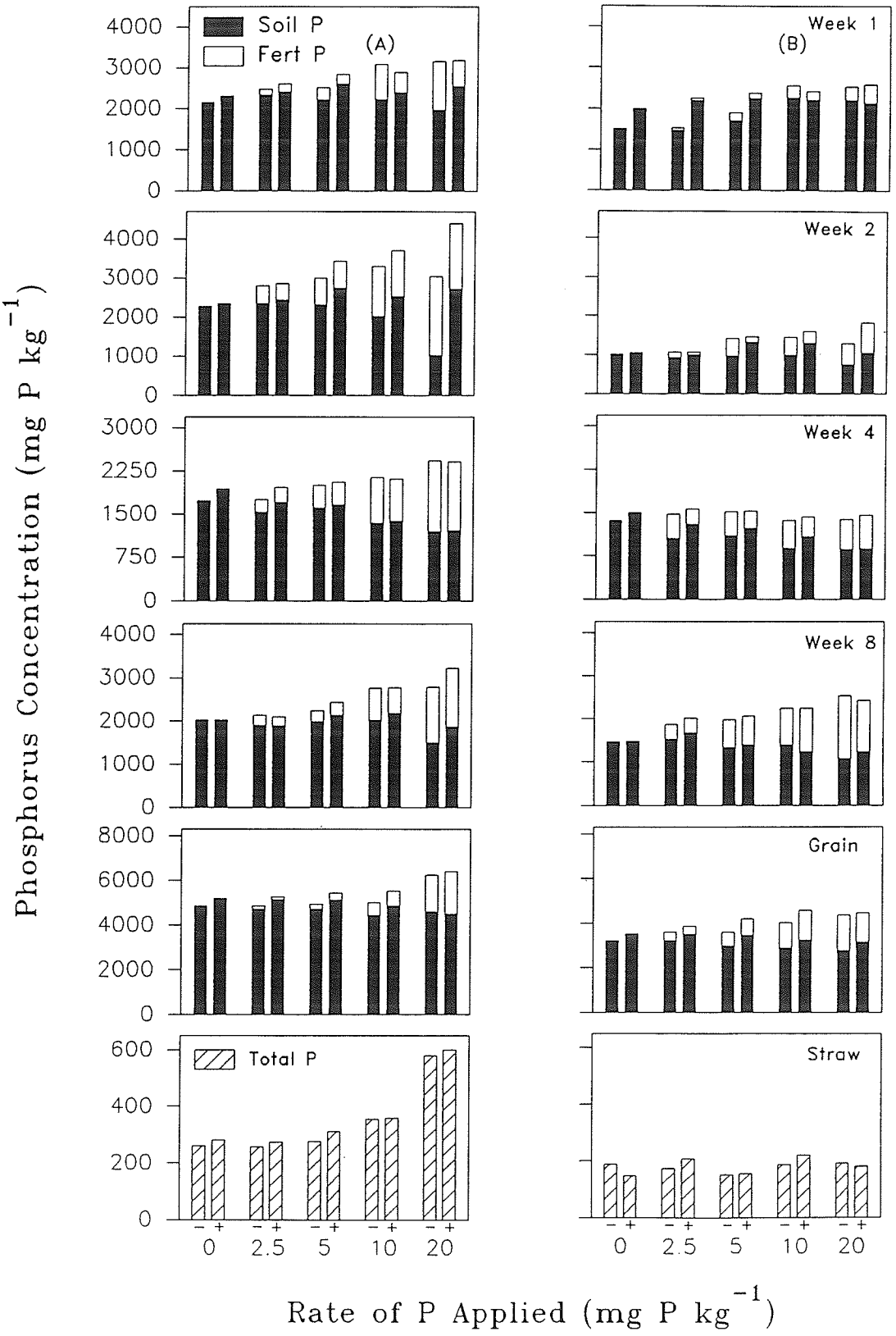
Table 4.5. The contribution of fertilizer and soil P as affected by P fertilizer and PB-50 in flax, at various sampling times.

P_2O_5 Added (kg ha^{-1})	PB-50	Phosphorus Concentration (mg kg^{-1})							
		Week 1			Week 2			Week 4	
		Total	Fert	Soil	Total	Fert	Soil	Total	Fert
		Wellwood Soil							
0	-	2159	0	2159	2277	0	2277	1740	0
	+	2306	0	2306	2338	0	2338	1937	0
5	-	2475	136	2339	2797	459	2338	1760	223
	+	2605	191	2415	2853	419	2434	1967	253
10	-	2518	305	2213	3000	676	2323	1998	393
	+	2839	231	2608	3437	682	2754	2057	391
20	-	3083	849	2233	3297	1279	2017	2140	792
	+	2892	498	2395	3708	1178	2530	2114	727
40	-	3166	1189	1976	3042	2017	1024	2431	1233
	+	3180	630	2550	4402	1683	2719	2410	1194
Rate		0.01	0.01	NS	0.01	0.01	0.05	0.01	0.01
PB		NS	0.01	0.01	0.02	NS	0.01	NS	NS
Rate*PB		NS	0.01	NS	NS	NS	0.01	NS	NS
$LSD_{(P=0.05)}$†		--	226	--	--	--	576	--	--
		Willowcrest Soil							
0	-	1507	0	1507	1011	0	1011	1362	0
	+	1998	0	1998	1043	0	1043	1495	0
5	-	1530	58	1471	1059	133	926	1472	420
	+	2256	46	2210	1056	68	988	1564	267
10	-	1904	190	1714	1413	444	968	1512	413
	+	2388	129	2258	1447	129	1317	1526	302
20	-	2562	292	2270	1450	455	982	1359	483
	+	2422	203	2219	1589	297	1291	1427	350
40	-	2537	326	2211	1283	544	738	1380	521
	+	2594	450	2143	1820	789	1031	1448	581
Rate		0.01	0.01	NS	0.01	0.01	0.01	NS	0.01
PB		0.03	NS	0.01	0.01	NS	0.01	NS	0.05
Rate*PB		NS	NS	NS	0.01	0.05	NS	NS	NS
$LSD_{(P=0.05)}$		--	--	--	199	234	--	--	--

† LSD values apply only to comparison of means for treatments with and without PB-50

	Week 8			Grain			Straw		
Soil	Total	Fert	Soil	Total	Fert	Soil	Total	Fert	Soil
1740	2023	0	2023	4849	0	4849	259	ND	ND
1937	2021	0	2021	5203	0	5203	279	ND	ND
1536	2134	234	1900	4859	142	4716	255	ND	ND
1713	2098	213	1885	5260	120	5139	273	ND	ND
1605	2236	245	1990	4940	221	4719	274	ND	ND
1666	2434	304	2130	5441	322	5118	310	ND	ND
1347	2764	746	2018	5015	575	4440	355	ND	ND
1387	2776	600	2177	5523	659	4863	358	ND	ND
1198	2789	1285	1504	6239	1632	4606	579	ND	ND
1215	3221	1356	1865	6399	1893	4506	600	ND	ND
0.01	0.01	0.01	0.01	0.01	0.01	NS	0.01	ND	ND
0.04	NS	NS	0.05	0.01	NS	0.04	NS	ND	ND
NS	NS	NS	NS	NS	NS	NS	NS	ND	ND
--	--	--	--	--	--	--	--	--	--
1362	1468	0.0	1468	3225	0.0	3225	187	ND	ND
1495	1486	0.0	1486	3538	0.0	3538	148	ND	ND
1052	1872	342	1530	3598	364	3234	173	ND	ND
1297	2011	336	1675	3869	355	3513	207	ND	ND
1099	1986	648	1338	3598	603	2994	150	ND	ND
1224	2060	668	1392	4198	724	3473	155	ND	ND
875	2248	852	1396	4032	1132	2899	186	ND	ND
1076	2243	1011	1232	4583	1343	3240	219	ND	ND
858	2544	1461	1083	4358	1589	2769	191	ND	ND
868	2429	1186	1243	4460	1317	3143	180	ND	ND
0.01	0.01	0.01	0.01	0.01	0.01	0.05	NS	ND	ND
0.02	NS	NS	NS	0.01	NS	0.01	NS	ND	ND
NS	NS	NS	NS	NS	NS	NS	NS	ND	ND
--	--	--	--	--	--	--	--	--	--

Figure 4.3. The contribution of fertilizer and soil P as affected by P fertilizer and PB-50 in flax, at various sampling times, for the Wellwood (A) and Willowcrest (B) soils.


Soil P
Fert P
(A)
(B)
Week 1
Week 2
Week 4
Week 8
Grain
Straw
Total P
Phosphorus Concentration (mg P kg^{-1})
Rate of P Applied (mg P kg^{-1})
0
2.5
5
10
20

fertilizer to the total amount of P in the plant was significantly increased with increasing rates of P fertilizer, a greater amount of the total P in the plant was derived from soil sources of P. For the flax plants this result was expected, since flax has been shown to absorb only 1% of P fertilizer applied, within the first 20 days of growth (Kalra and Soper 1968). Overall, flax utilizes only a small portion of added P fertilizer (10% of that applied, by maturity) (Bailey et al. 1977), whereas soil P is absorbed continually throughout the growing season (Racz et al. 1965; Soper and Kalra 1969).

The increased P concentration in the plant with increasing rates of P fertilizer, was associated with a significant increase in plant dry matter production at the 2 and 4 week sampling date, on both soils (Table 4.6 and Figure 4.4).

The effect of PB-50 on the concentration of P in the plants was inconsistent for the two soils at the early sampling dates. However, when a response was observed, it was positive (Table 4.5 and Figure 4.3). The contribution of labelled P fertilizer to the total amount of P in the plant was generally not affected by the PB-50 treatment. Only the contribution of soil P to the total amount of P in the plant was influenced by PB-50 inoculation, with significantly higher amounts of P in the plant with this treatment (Table 4.5).

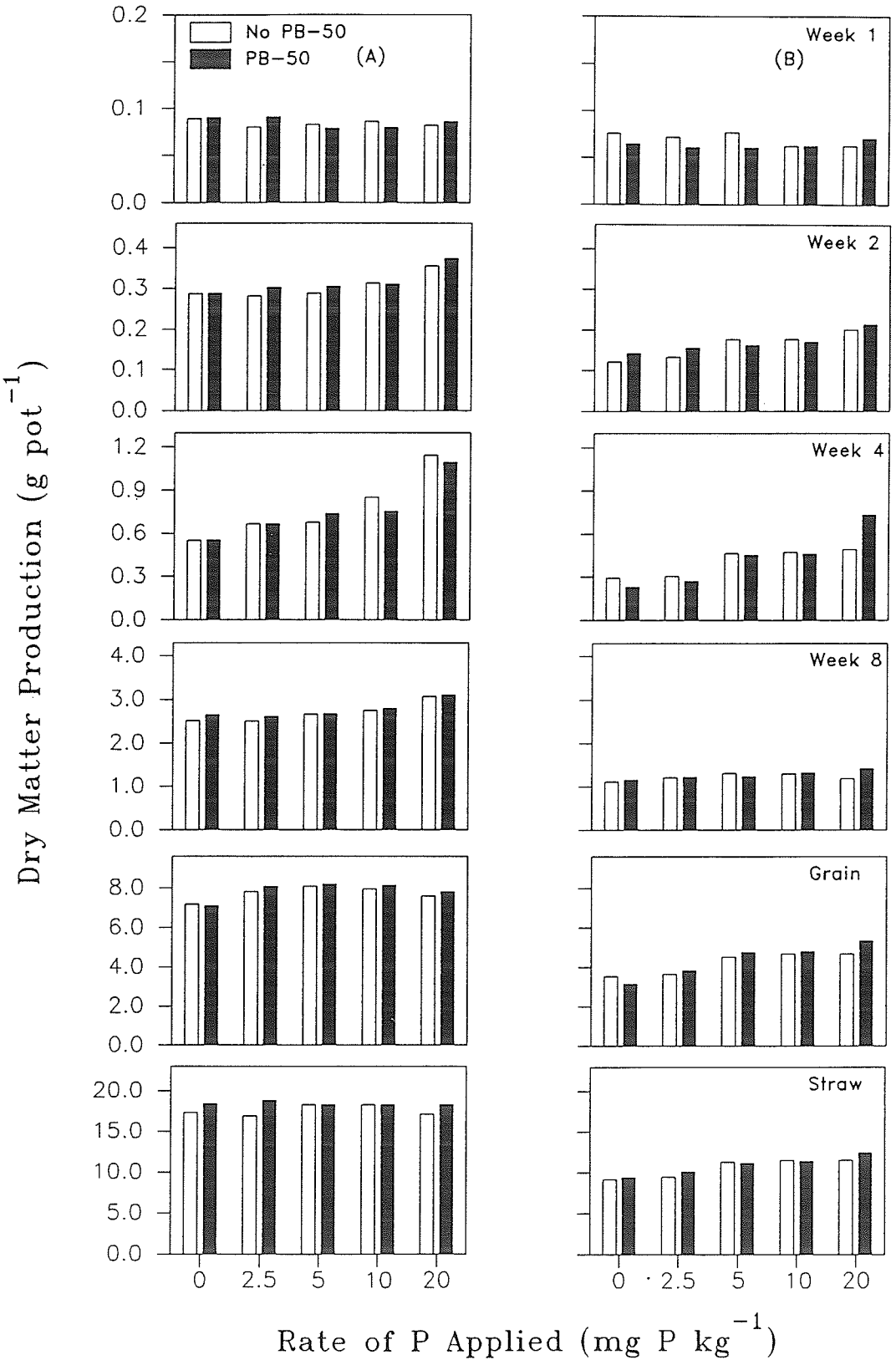
Although inoculation with PB-50 significantly increased the contribution of soil P to the total amount of P in the plant, this treatment had no effect on the plant dry matter production at the early sampling dates (Table 4.6 and Figure 4.4).

Table 4.6. Effect of P fertilizer and PB-50 on dry matter and grain production in flax, at various sampling times.

P Added (kg ha^{-1})	PB-50	Dry Matter (g pot^{-1})					
		Week 1	Week 2	Week 4	Week 8	Grain	Straw
		Wellwood Soil					
0	-	0.09	0.29	0.55	2.51	7.18	17.3
	+	0.09	0.29	0.56	2.64	7.09	18.4
2.5	-	0.08	0.28	0.67	2.50	7.81	16.9
	+	0.09	0.30	0.67	2.61	8.10	18.8
5	-	0.08	0.29	0.68	2.66	8.09	18.3
	+	0.08	0.31	0.74	2.68	8.19	18.3
10	-	0.09	0.31	0.85	2.75	7.94	18.3
	+	0.08	0.31	0.75	2.80	8.15	18.3
20	-	0.08	0.35	1.14	3.06	7.59	17.1
	+	0.09	0.37	0.09	3.11	7.82	18.3
Rate		NS	0.04	0.01	0.01	0.05	NS
PB		NS	NS	NS	NS	NS	0.04
Rate*PB		NS	NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$†		--	--	--	--	--	--
		Willowcrest Soil					
0	-	0.08	0.12	0.29	1.11	3.52	9.13
	+	0.06	0.14	0.23	1.17	3.14	9.38
2.5	-	0.07	0.13	0.31	1.21	3.64	9.50
	+	0.06	0.16	0.27	1.23	3.83	10.1
5	-	0.08	0.18	0.46	1.31	4.52	11.3
	+	0.06	0.16	0.46	1.24	4.76	11.2
10	-	0.06	0.18	0.47	1.29	4.69	11.5
	+	0.06	0.17	0.46	1.32	4.80	11.4
20	-	0.06	0.20	0.49	1.19	4.68	11.5
	+	0.07	0.21	0.73	1.42	5.33	12.4
Rate		NS	0.02	0.01	NS	0.01	0.01
PB		NS	NS	NS	NS	NS	NS
Rate*PB		NS	NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$		--	--	--	--	--	--

† LSD values apply only to comparison of means for treatments with and without PB-50

Figure 4.4. Effect of P fertilizer and PB-50 on dry matter and grain production in flax, at various sampling times, for the Wellwood (A) and Willowcrest (B) soils.


No PB-50
PB-50
(A)
(B)
Week 1
Week 2
Week 4
Week 8
Grain
Straw
Dry Matter Production (g pot^{-1})
Rate of P Applied (mg P kg^{-1})
0.2
0.1
0.0
0.4
0.3
0.2
0.1
0.0
1.2
0.9
0.6
0.3
0.0
4.0
3.0
2.0
1.0
0.0
8.0
6.0
4.0
2.0
0.0
20.0
15.0
10.0
5.0
0.0
0
2.5
5
10
20

4.3.4 Flax: Late Season Growth

As was observed at the early season sampling dates, increases in the rates of P fertilizer added resulted in significant increases in the concentration of P in the plant and in the grain (Table 4.5 and Figure 4.3). Generally this was a result of a significantly increasing proportion of labelled fertilizer P in the plant with a decreasing amount of soil P contributing to the total amount of P in the plant (Table 4.5). As was observed at all sampling dates for both wheat and flax, the amount of soil P in the plant was greater than the amount of labelled fertilizer P, for all rates of fertilizer P added (Table 4.5). As in the previous experiment with wheat, low levels of radiation in the flax straw samples made it impossible to determine the contribution of labelled fertilizer P to the total amount of P in the plant.

Increasing rates of P fertilization generally increased the dry matter production at the late season sampling dates (Table 4.6 and Figure 4.4). The significant increases in the P concentration in the grain, with increasing rates of P fertilization (Table 4.5), resulted in significantly increased grain yields (Table 4.6). This result was anticipated as both soils tested low in $NaHCO_3$-ext P (Table 4.1).

The PB-50 treated flax had a significantly higher P concentration in the grain, on both soils, as compared to the untreated flax (Table 4.5 and Figure 4.3). This increase in P concentration with the PB-50 treatment, was the result of a significantly greater contribution of P being derived from the soil (Table 4.5). However, even with this increased amount of P, the grain yield for the PB-50 treatment was not significantly different from the yield of the non-inoculated plants

(Table 4.6 and Figure 4.4).

There were marked differences in the two soil studied. On the Wellwood soil, both the wheat and flax plants had higher P concentrations in the plant and greater dry matter and grain production, as compared to the plants grown on the Willowcrest soil (Tables 4.3, 4.4, 4.5 and 4.6). Soil analyses indicated that the nutrient status of the Willowcrest soil was much lower than the Wellwood soil (Table 4.1). Because all the soils received basal applications of N, K and S, it is possible that the reduced growth on the Willowcrest soil was due to micronutrient (Cu and Zn) deficiencies. Even though these nutrient deficiencies may have been present on the Willowcrest soil, the two soils demonstrated similar responses to the inoculation of PB-50, for both the wheat and flax experiments.

It is difficult to compare the responses of wheat and flax to the inoculation for PB-50, because of the differences in P uptake by the two crops. It has been reported that wheat plants utilize fertilizer P early in the growing season, whereas the soil P fraction contributes more P at heading and maturity (Spinks and Barber 1947; Racz et al. 1965; Barber and Olson 1968; Soper and Kalra 1969). Flax, as mentioned previously, utilizes soil P throughout the growing season, with much less dependence on fertilizer P (Racz et al. 1965; Soper and Kalra 1969).

4.4 Summary

Wheat and flax were grown on a Wellwood and Willowcrest soil, in the growth chamber, to determine the influence of PB-50 on crop yield

and utilization of fertilizer and soil P at various stages of crop growth.

Phosphorus content and yield of both wheat and flax was increased by fertilization with P, on both soils, at most sampling times. Wheat and flax inoculated with PB-50 increased early season uptake of P, immediately after emergence (1 week), and this continued to maturity. These increases in total plant P content from PB-50 inoculation resulted from an increased contribution from the soil P fraction. The enhanced plant P nutrient status was associated with increases in dry matter production and grain yield for the PB-50 treated wheat. When combining the two soils, an overall grain yield increase of 9% occurred from the PB-50 treatment. For both soils, a trend existed for an increased plant P concentration and dry matter yield at the higher rates of P fertilization, with the addition of PB-50.

The Wellwood and Willowcrest soils responded similarly to the addition of PB-50. However, as the soil fertility status of the two soils were significantly different, it was not possible to draw any conclusions concerning the effectiveness of PB-50 in an acidic versus an alkaline soil.

V. EFFECT OF PB-50 INOCULATION ON Ca, Mg, Fe AND Al CONCENTRATION OF WHEAT AND FLAX - FIELD AND GROWTH CHAMBER STUDY

5.1 Introduction

Over the past 40 years, research has shown that rhizosphere microorganisms can affect the availability of phosphates, likely through the production of organic acids. The solubilization of inorganic phosphates can be attributed to processes involving both acidification and chelation (Sperber 1958a; Molla and Chowdhury 1984; Asea et al. 1988). However, there has been limited correlation between the ability of the phosphate solubilizing organisms to reduce the media pH and solubilize inorganic P (Gaur et al. 1973; Surange 1985). In contrast, organic acids produced by the soil microflora have been reported to chelate with cations such as Ca, Mg, Fe and Al, thus releasing P into solution from these insoluble phosphate compounds.

Phosphorus fertilizer applied to the soil can precipitate rapidly to form sparingly soluble dicalcium phosphates in neutral to alkaline soils and iron and aluminum phosphate (ammonium taranakites) in soils testing low in pH (Lindsay et al. 1962). There is evidence that organic acid production may accumulate in localized zones in the soil, to assist in the solubilization of these precipitated phosphates. The research on P . bilaji has been conducted on neutral pH soils. It is known that neutral soils have Ca and Mg phosphates as the main source of available P (Lindsay and Stephenson 1959), whereas in low pH soils, Fe and Al phosphates are predominant (Sample et al. 1980). Banik and Dey (1982) reported that phosphate solubilizing bacteria and fungi (including

Penicillium species) are more effective in rendering P to the plant from $Ca_3(PO_4)_2$ than from $AlPO_4$ and $FePO_4$. Therefore, the efficacy of P. bilaji under acidic soil conditions is unclear.

Since organic acids have the potential to chelate Ca, Mg, Al and Fe cations, the first objective was to analyze all plant samples taken in both the growth chamber and field experiments for these nutrients, to determine if P fertilizer and PB-50 inoculation affected their concentration in the plant. The second objective was to compare the concentrations of these nutrients in the plant, for the various pH soils, throughout the growing season. This is of importance since chelation by organic acids is highly pH sensitive (Norval 1972), and that acidification may affect phosphate solubilization by P. bilaji (Asea et al. 1988).

5.2 Materials and Methods

The soils studied in the 1989 and 1990 field trials (Section 3) and the two growth chamber experiments (Section 4) were combined to generate a range of soils necessary to aide in explaining the influence of pH on the effectiveness of PB-50.

Some characteristics of the soils used in the two studies are reported in Table 5.1. All analyses were conducted on surface (0-15 cm) samples. The solution pH was determined with a glass electrode (soil:water ratio, 1:1) (McLean 1982). Inorganic C was analyzed by a titrimetric method (Bundy and Bremner 1972) and organic C content was assessed using a dichromate oxidation procedure (Yeomans and Bremner 1988). Particle size analysis was determined using the standard pipette

Table 5.1. Soil characteristics.

Soil	Texture	pH	Org. C.	Inorg. C	Ca	Mg	P	Cu	Fe	Mn	Zn	Al
Growth Chamber			-----%-----		----------mg kg^{-1}----------							
Wellwood	SCL	5.3	2.63	0.0	1800	400	8.8	1.2	97	48	2.1	11.0
Willowcrest	FSL	7.4	1.81	0.4	3000	220	5.3	0.1	7	8	0.4	6.0
1989												
Wellwood	SCL	5.7	2.75	0.0	1800	400	5.9	1.2	95	48	2.1	11.0
Stockton	FSL	6.6	3.11	0.3	2440	400	5.9	1.5	81	46	3.6	16.5
Portage	SiCL	8.1	3.29	1.1	4400	1840	3.8	0.7	9	22	0.5	5.0
1990												
Wellwood	SCL	5.5	2.49	0.0	1800	400	6.7	1.0	98	52	2.0	17.0
Stockton	FSL	6.4	3.20	0.3	2440	400	5.7	1.6	83	44	3.7	16.5
Portage	SiCL	8.0	3.64	1.1	4400	1040	4.8	0.6	8	20	0.6	5.0

method as described by Kilmer and Alexander (1949). The exchangeable cations of Ca and Mg were analyzed by an ammonium acetate procedure (Chapman 1965). Plant available phosphate was extracted using $NaHCO_3$ as described by Olsen et al. (1954) and the P in solution was measured by the acid-molybdate procedure (Murphy and Riley 1962). Plant available Cu, Fe, Mn and Zn were assessed using the DTPA method of Lindsay and Norvell (1978). Exchangeable Al was extracted by using a KCl solution as described by Webber et al. (1974). The experimental designs, preparation and maintenance of the field and growth chamber experiments are explained in detail in section 3 and 4, respectively. Plant samples were randomly taken for all experiments and treatments at 1, 2, 4, and 8 (excluding wheat grown in the growth chamber) weeks after emergence, and at plant maturity. The plant samples were oven dried for one week (growth chamber), or air dried for 2 weeks (field experiments), and ground through a 2-mm sieve using a Thomas-Wiley Laboratory Mill.

Samples of the ground tissue were prepared for Ca, Mg, Fe and Al analysis by digesting the plant material using a nitric-perchloric procedure (Isaac and Kerber 1971). The digested samples were diluted to the appropriate concentrations and analyzed for each element using an atomic absorption spectrophotometer. The results of the experiment were statistically analyzed using the GLM procedure (Goodnight et al. 1988).

5.3 Results and Discussion

The soils used in the field and growth chamber experiments ranged

in pH from 5.3 to 8.0 (Table 5.1). It has been reported that for soils in western Canada, Ca and Mg phosphates are the predominant forms of extractable P in neutral and alkaline soils, whereas Al and Fe phosphates constitute a greater proportion of extractable P in low pH soils (Racz and Soper 1967; Alexander and Robertson 1968). However, analyses showed that Ca and Mg were predominant cations in all the soils studied, even those low in pH. This is expected, since the underlying parent material for the both the acidic (Wellwood) and neutral (Stockton) soils was formed from lacustrine deposits, that are slightly alkaline and calcareous (Manitoba Soil Survey Report 1957).

5.3.1 Field Experiments: Ca and Mg

For all field sites in 1989, the treatments of increasing rates of P fertilizer added did not affect the concentration of Ca and Mg in the plants, at almost all sampling dates (Tables 5.2, 5.3, 5.4, 5.5, 5.6 and 5.7). The one exception was at the Wellwood-89 site where there was a significant decrease in the concentration of Mg in the plant, with increasing rates of P fertilizer added (Table 5.5). In 1990, the effects of adding increasing rates of P fertilizer on the Ca and Mg content of the plants were inconsistent among field sites (Tables 5.2, 5.3, 5.4, 5.5, 5.6 and 5.7). Greenwood and Hallsworth (1960) reported that the application of P, even at high levels, had no direct affect on Ca uptake by the plant. In contrast, Bar-Yosef (1971) showed that a higher concentration of P in the soil solution could increase Ca flux into the roots. As well, Keyes (1990) found that the addition of P fertilizer increased the concentration of Ca and Mg in barley and the Mg

Table 5.2. Effect of P fertilizer and PB-50 on Ca concentration in wheat, at various sampling times (Wellwood soil).

P_2O_5 Added (kg ha^{-1})	PB-50	Calcium Concentration (mg kg^{-1})					
		Week 1	Week 2	Week 4	Week 8	Grain	Straw
		Site Year 1989					
0	-	4310	5620	5210	3590	370	3110
	+	4560	6090	5950	3430	500	2500
5	-	4410	5890	6230	3470	330	3120
	+	4700	5780	5680	3250	530	2730
10	-	4590	6000	5190	3350	520	2910
	+	4650	6540	5660	3300	520	2660
20	-	3910	5960	5620	3190	610	2800
	+	5790	5670	5480	3120	400	2490
40	-	4350	6190	5550	3450	520	2900
	+	5390	6400	6030	3360	430	3070
Rate		NS	NS	NS	NS	NS	NS
PB		0.01	NS	NS	NS	NS	NS
Rate*PB		0.01	NS	NS	NS	0.01	NS
$LSD_{(P=0.05)}$†		760	--	--	--	140	--
		Site Year 1990					
0	-	2400	4200	2660	2600	470	2590
	+	3310	3680	3090	2790	410	2690
5	-	2410	5560	2500	2210	360	2380
	+	3540	3710	3430	2380	420	2450
10	-	2430	6170	3320	2420	320	2930
	+	3600	4150	3540	2070	390	3570
20	-	2470	5880	3300	1880	290	2900
	+	3400	4380	3590	2280	430	2990
40	-	2350	5950	3300	2520	440	2820
	+	3220	3930	3480	2280	310	3640
Rate		NS	0.01	0.02	NS	NS	0.01
PB		0.01	0.01	0.01	NS	NS	0.01
Rate*PB		NS	NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$		--	--	--	--	--	--

† LSD values apply only to comparison of means for treatments with and without PB-50

Table 5.3. Effect of P fertilizer and PB-50 on Ca concentration in wheat, at various sampling times (Stockton soil).

P_2O_5 Added (kg ha^{-1})	PB-50	Calcium Concentration (mg kg^{-1})					
		Week 1	Week 2	Week 4	Week 8	Grain	Straw
		Site Year 1989					
0	-	3500	4860	4420	3200	480	2530
	+	2960	5190	4500	3000	420	2470
5	-	3700	4610	4360	3030	430	2410
	+	3040	6720	4550	2920	410	2440
10	-	3740	4660	4870	2880	430	2470
	+	3180	5480	4470	2890	430	2520
20	-	3160	4600	4760	3140	340	2370
	+	3600	5620	4500	3220	420	2350
40	-	3300	4980	4330	2840	480	2440
	+	3820	5630	4640	2990	510	2380
Rate		NS	NS	NS	NS	NS	NS
PB		NS	0.01	NS	NS	NS	NS
Rate*PB		0.01	0.04	NS	NS	NS	NS
$LSD_{(P=0.05)}$†		490	790	--	--	--	--
		Site Year 1990					
0	-	2700	4140	3070	1970	490	2600
	+	2510	4140	2730	2350	350	2550
5	-	2780	4290	2670	2530	320	2410
	+	2350	4440	2970	2190	320	2840
10	-	3060	4480	2690	1890	240	2570
	+	2520	4230	3220	2050	460	2830
20	-	2950	4380	3210	1970	490	2830
	+	2600	4370	3450	2120	370	3210
40	-	2020	4470	2920	2350	650	2690
	+	2390	4620	3850	2240	590	3120
Rate		0.01	NS	0.01	NS	0.01	0.01
PB		0.05	NS	0.01	NS	NS	0.01
Rate*PB		NS	NS	0.01	NS	NS	0.05
$LSD_{(P=0.05)}$		--	--	170	--	--	120

† LSD values apply only to comparison of means for treatments with and without PB-50

Table 5.4. Effect of P fertilizer and PB-50 on Ca concentration in wheat, at various sampling times (Portage soil).

P_2O_5 Added (kg ha^{-1})	PB-50	Calcium Concentration (mg kg^{-1})					
		Week 1	Week 2	Week 4	Week 8	Grain	Straw
		Site Year 1989					
0	-	3900	6520	4120	1600	220	2010
	+	3940	7390	4380	1370	230	1820
5	-	4000	7050	3830	1560	230	1500
	+	4040	6550	4270	1380	260	2090
10	-	3500	7390	4310	1820	310	1720
	+	3800	6600	3970	1260	240	1870
20	-	4520	7790	4550	1480	240	1790
	+	4800	7190	4310	1160	190	1650
40	-	3920	6830	4220	1590	230	1380
	+	4440	6940	3970	1310	210	1690
Rate		NS	NS	NS	NS	NS	NS
PB		NS	NS	NS	0.01	NS	NS
Rate*PB		NS	NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$†		--	--	--	--	--	--
		Site Year 1990					
0	-	3140	3830	2780	1530	470	1670
	+	3270	4410	2570	1360	400	1480
5	-	2950	3570	2450	1730	460	1220
	+	2940	4220	2560	1440	270	1500
10	-	3260	3420	2380	1440	350	1260
	+	2560	3680	2790	1580	300	1660
20	-	3080	3600	2320	1210	280	1430
	+	3020	3640	3180	1600	300	1920
40	-	2750	3400	2250	1680	480	1380
	+	3290	4120	2900	1260	440	1980
Rate		NS	0.01	NS	NS	0.01	0.01
PB		NS	0.01	0.01	NS	NS	0.01
Rate*PB		NS	NS	0.02	NS	NS	0.01
$LSD_{(P=0.05)}$		--	--	190	--	--	120

† LSD values apply only to comparison of means for treatments with and without PB-50

Table 5.5. Effect of P fertilizer and PB-50 on Mg concentration in wheat, at various sampling times (Wellwood soil).

P_2O_5 Added (kg ha^{-1})	PB-50	Magnesium Concentration (mg kg^{-1})					
		Week 1	Week 2	Week 4	Week 8	Grain	Straw
		Site Year 1989					
0	-	2590	2480	3100	2380	1670	1290
	+	2710	2650	2920	2260	1590	870
5	-	2670	2550	3570	2240	1660	1200
	+	2710	2550	2850	2200	1530	840
10	-	2830	2600	3140	2220	1610	830
	+	2710	2700	2860	2220	1640	850
20	-	2630	2500	3330	2150	1500	880
	+	3170	2560	2880	2180	1680	900
40	-	2510	2560	3120	2310	1410	830
	+	3110	2750	3090	2180	1620	1010
Rate		NS	NS	NS	NS	0.02	NS
PB		0.01	NS	0.01	NS	NS	0.05
Rate*PB		0.01	NS	NS	NS	0.01	NS
$LSD_{(P=0.05)}$†		340	--	--	--	110	--
		Site Year 1990					
0	-	2700	2780	2810	1620	1370	720
	+	2740	3030	3100	1970	1320	800
5	-	2910	2870	2540	1660	1270	710
	+	3080	3020	3050	1710	1330	780
10	-	2940	3210	2670	1840	1220	740
	+	2910	2980	3190	1800	1330	790
20	-	2760	3140	3250	1550	1260	720
	+	2970	3230	3150	1740	1320	860
40	-	2730	2860	3370	1740	1330	780
	+	3000	3050	3250	1640	1370	890
Rate		NS	NS	0.01	NS	NS	NS
PB		NS	NS	0.02	NS	NS	0.01
Rate*PB		NS	NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$		--	--	--	--	--	--

† LSD values apply only to comparison of means for treatments with and without PB-50

Table 5.6. Effect of P fertilizer and PB-50 on Mg concentration in wheat, at various sampling times (Stockton soil).

P_2O_5 Added (kg ha^{-1})	PB-50	Magnesium Concentration (mg kg^{-1})					
		Week 1	Week 2	Week 4	Week 8	Grain	Straw
		Site Year 1989					
0	-	1840	1960	1900	1710	1450	770
	+	1860	2060	1920	1600	1560	620
5	-	1880	1970	1910	1600	1490	640
	+	1820	2140	1980	1520	1620	640
10	-	1770	1960	2030	1640	1540	690
	+	1840	2100	1920	1580	1660	700
20	-	1870	2000	2050	1730	1550	700
	+	1920	2340	2010	1560	1430	690
40	-	2030	2120	1970	1590	1660	690
	+	1820	2090	2040	1560	1430	720
Rate		NS	NS	NS	NS	NS	NS
PB		NS	0.02	NS	0.01	NS	NS
Rate*PB		NS	NS	NS	NS	0.01	NS
$LSD_{(P=0.05)}$†		--	--	--	--	140	--
		Site Year 1990					
0	-	2050	2920	2620	1300	1210	690
	+	2130	3170	2480	1400	1270	650
5	-	2140	2910	2530	1580	1320	560
	+	2030	3350	2430	1140	1210	700
10	-	2080	2910	2430	1270	1320	580
	+	2020	3020	2950	1160	1310	670
20	-	2320	3260	2800	1270	1190	710
	+	2230	3330	2910	1240	1340	750
40	-	1960	3140	2330	1410	1240	670
	+	2260	3710	2990	1360	1300	780
Rate		NS	0.01	0.01	NS	NS	0.02
PB		NS	0.01	0.01	0.04	NS	0.01
Rate*PB		NS	NS	0.01	0.02	0.02	NS
$LSD_{(P=0.05)}$		--	--	250	220	100	--

† LSD values apply only to comparison of means for treatments with and without PB-50

Table 5.7. Effect of P fertilizer and PB-50 on Mg concentration in wheat, at various sampling times (Portage soil).

P_2O_5 Added (kg ha^{-1})	PB-50	Magnesium Concentration (mg kg^{-1})					
		Week 1	Week 2	Week 4	Week 8	Grain	Straw
		Site Year 1989					
0	-	3370	4590	2820	2470	1530	1220
	+	3650	5130	3120	2680	1590	1470
5	-	3270	4280	2620	2310	1570	1130
	+	3430	5220	2970	2650	1480	1210
10	-	3370	4610	2910	2420	1580	1080
	+	3230	4480	2940	2170	1570	1360
20	-	3730	4630	3150	2530	1600	1380
	+	3430	4260	2820	2130	1550	1380
40	-	3330	4230	2850	2590	1690	1100
	+	3870	4730	3220	2390	1520	1300
Rate		NS	NS	NS	NS	NS	NS
PB		NS	NS	NS	NS	0.02	0.02
Rate*PB		NS	NS	NS	NS	0.03	NS
$LSD_{(P=0.05)}$†		--	--	--	--	120	--
		Site Year 1990					
0	-	2720	3590	2590	1910	1150	370
	+	2340	4210	2610	1760	1190	380
5	-	2630	3480	2880	1940	1140	490
	+	2410	4160	2570	1740	1190	420
10	-	2780	3370	3060	1940	1160	460
	+	2380	4270	2470	1870	1280	440
20	-	2330	3520	2610	1830	1140	480
	+	2470	4080	3010	1930	1220	510
40	-	2420	3640	2900	2030	1150	420
	+	2900	4030	2720	1670	1210	550
Rate		NS	NS	NS	NS	NS	0.02
PB		NS	0.01	NS	NS	0.02	NS
Rate*PB		NS	NS	0.01	NS	NS	0.02
$LSD_{(P=0.05)}$		--	--	340	--	--	90

† LSD values apply only to comparison of means for treatments with and without PB-50

content in canola.

The effect of PB-50 on the concentration of Ca and Mg in wheat was inconsistent among field sites and cropping years. Both significant increases and decreases in the concentration of Ca and Mg in the plants at various sampling dates were obtained (Tables 5.2, 5.3, 5.4, 5.5, 5.6 and 5.7) (Figures 5.1 and 5.2). However, the reasons for these increases or decreases was not evident.

The Ca content of the wheat was greatest 2 weeks after emergence, then decreased until the later season sampling dates. Plants grown at the Portage site had a lower concentration of Ca, at the later sampling dates, than plants grown at the Wellwood and Stockton sites (Figure 5.1), even though the soil at the Portage-89 site contained the highest amount of measurable soil Ca (Table 5.1). This may have been a dilution effect, as wheat grown at the Portage-89 site had a higher grain yield than wheat grown at the Stockton-89 and Wellwood-89 sites (Tables 3.6, 3.7 and 3.8). A concentration of Ca in wheat, at heading, of between 0.2 and 0.5% is considered sufficient (Jones et al. 1991). Therefore, the tissue samples collected from all field sites, for both cropping years, were sufficient in Ca (Table 5.2, 5.3 and 5.4).

The level of Mg in the plants followed the same trend as the level of Ca, however, the differences were not as dramatic between early and late season sampling dates (Figure 5.2). Absorption of Ca by the plant occurs mainly via the root tip, whereas Mg absorption occurs along the entire root surface. When the rate of growth of above ground material rapidly increases at about 4 weeks after emergence, the rate of root growth decreases. This would help to explain why the Mg content of the

Figure 5.1. Effect of PB-50 and site year on the Ca concentration of wheat, for the Wellwood, Stockton and Portage soils.

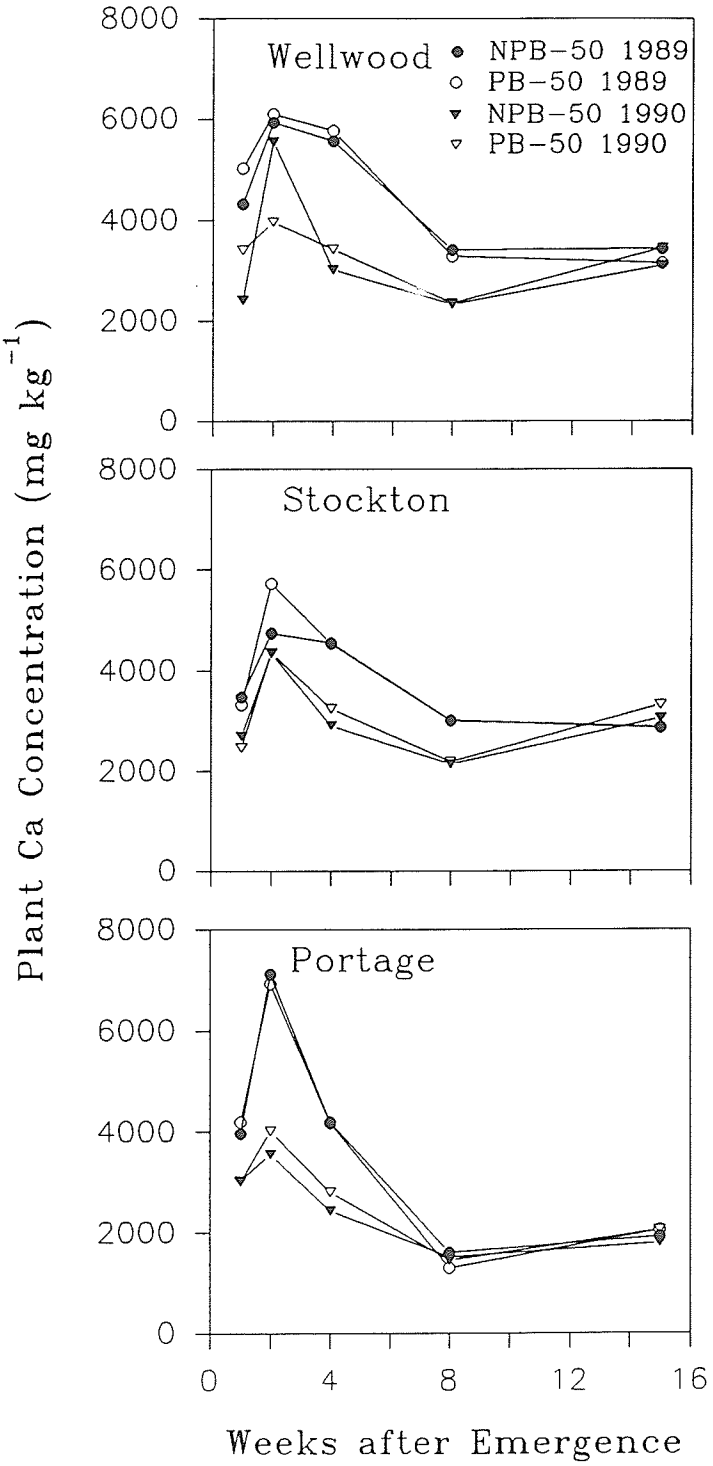
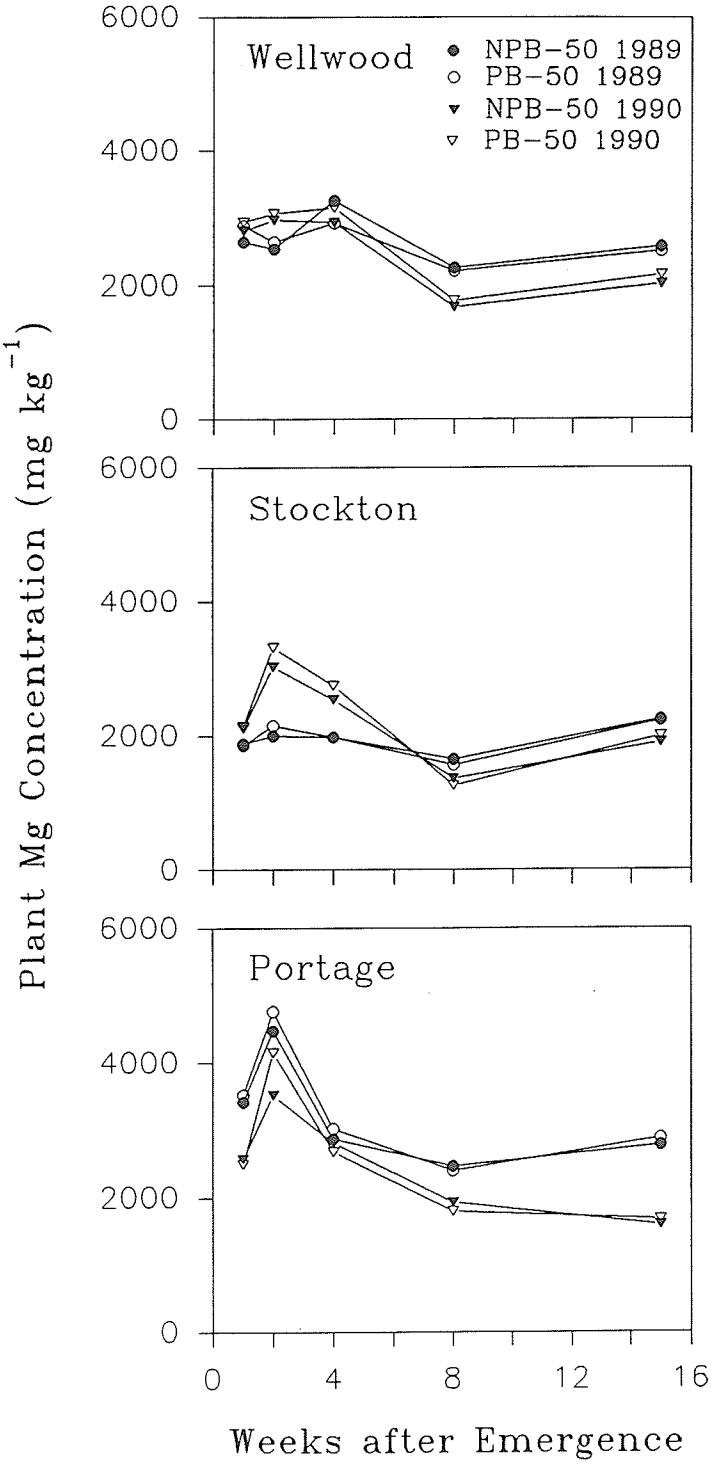


Figure 5.2. Effect of PB-50 and site year on the Mg concentration of wheat, for the Wellwood, Stockton and Portage soils.



plant remained more constant throughout the growing season than did the Ca content (Marschner 1986).

Magnesium uptake can be depressed by the presence of other cations, including Ca in alkaline soils and H in acid soils and this may result in Mg deficiencies in the plant (Marschner 1986). A concentration of Mg in wheat, at heading, of between 0.15 and 0.5% is considered sufficient (Jones et al. 1991). Therefore, it appears that tissue samples collected at all sites were marginally sufficient in Mg (Tables 5.5, 5.6 and 5.7).

5.3.2 Field Experiment: Al and Fe

Increasing rates of P fertilizer added had little or no effect on the concentration of Al and Fe in the plant at all field sites, for both cropping years (Tables 5.8, 5.9, 5.10, 5.11, 5.12 and 5.13). This may have resulted from the strong affinity of Al and Fe to precipitate with high concentrations of phosphates (Adams 1980), combined with a lower solubility of these precipitates as compared to Ca phosphates (Beever 1987). Also, Keyes (1990) reported that the addition of P fertilizer did not affect the uptake of Fe by the plant.

The effect of the PB-50 treatment on the concentration of Al and Fe in the plant was inconsistent among the field sites, for both cropping years (Tables 5.8, 5.9, 5.10, 5.11, 5.12 and 5.13). The responses that did occur also were inconsistent, with both positive and negative effects being observed. It has been reported that inoculation with P. bilaji has no effect on the concentration of Fe in wheat (Kucey 1988a) and barley (Keyes 1990). However, inoculation of canola with P.

Table 5.8. Effect of P fertilizer and PB-50 on Al concentration in wheat, at various sampling times (Wellwood soil).

P_2O_5 Added (kg ha^{-1})	PB-50	Aluminum Concentration (mg kg^{-1})					
		Week 1	Week 2	Week 4	Week 8	Grain	Straw
		Site Year 1989					
0	-	467	243	944	135	70	415
	+	357	303	902	124	78	352
5	-	377	372	862	149	68	337
	+	404	330	843	136	71	320
10	-	417	289	791	163	74	389
	+	389	293	916	154	71	351
20	-	398	241	863	133	75	406
	+	477	244	993	149	68	394
40	-	451	276	799	138	65	377
	+	515	282	961	131	71	358
Rate		NS	0.03	NS	0.05	NS	NS
PB		NS	NS	0.01	NS	NS	NS
Rate*PB		NS	NS	0.02	NS	NS	NS
$LSD_{(P=0.05)}$†		--	--	135	--	--	--
		Site Year 1990					
0	-	452	730	1023	117	29	130
	+	436	762	1379	110	32	132
5	-	535	679	1451	114	38	111
	+	500	829	1383	154	35	126
10	-	431	716	1369	209	35	118
	+	481	775	1201	126	41	140
20	-	617	786	1023	133	37	113
	+	565	864	1123	141	38	146
40	-	613	703	976	129	37	121
	+	560	779	1269	142	31	178
Rate		NS	NS	NS	NS	NS	NS
PB		NS	NS	NS	NS	NS	0.02
Rate*PB		NS	NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$		--	--	--	--	--	--

† LSD values apply only to comparison of means for treatments with and without PB-50

Table 5.9. Effect of P fertilizer and PB-50 on Al concentration in wheat, at various sampling times (Stockton soil).

P_2O_5 Added (kg ha^{-1})	PB-50	Aluminum Concentration (mg kg^{-1})					
		Week 1	Week 2	Week 4	Week 8	Grain	Straw
		Site Year 1989					
0	-	367	295	400	112	77	545
	+	329	384	455	105	62	505
5	-	317	248	430	111	72	467
	+	358	361	504	108	61	550
10	-	333	246	437	109	74	483
	+	339	419	472	106	59	504
20	-	333	234	486	113	69	454
	+	322	483	502	99	73	477
40	-	314	274	507	94	70	465
	+	327	332	481	118	73	471
Rate		NS	NS	NS	NS	NS	NS
PB		NS	0.01	NS	NS	0.01	NS
Rate*PB		0.02	NS	NS	0.01	0.01	NS
$LSD_{(P=0.05)}$†		43	--	--	15	8	--
		Site Year 1990					
0	-	409	719	863	133	18	142
	+	464	690	788	89	17	152
5	-	416	837	879	128	18	147
	+	360	874	683	83	25	189
10	-	300	789	688	154	17	131
	+	323	810	934	76	13	186
20	-	343	858	837	145	23	157
	+	342	787	873	82	15	198
40	-	256	812	669	79	15	169
	+	351	909	723	109	27	195
Rate		NS	NS	NS	NS	NS	NS
PB		NS	NS	NS	0.01	NS	0.01
Rate*PB		NS	NS	0.05	0.04	NS	NS
$LSD_{(P=0.05)}$		--	--	192	54	--	--

† LSD values apply only to comparison of means for treatments with and without PB-50

Table 5.10. Effect of P fertilizer and PB-50 on Al concentration in wheat, at various sampling times (Portage soil).

P_2O_5 Added (kg ha^{-1})	PB-50	Aluminum Concentration (mg kg^{-1})					
		Week 1	Week 2	Week 4	Week 8	Grain	Straw
		Site Year 1989					
0	-	485	636	665	92	64	237
	+	466	677	782	93	68	231
5	-	497	625	667	86	58	189
	+	495	714	665	96	63	198
10	-	449	555	547	90	68	183
	+	509	629	665	77	68	196
20	-	523	693	635	85	64	202
	+	504	674	650	91	71	174
40	-	489	354	550	89	61	157
	+	488	774	719	86	69	210
Rate		NS	NS	NS	NS	NS	NS
PB		NS	0.01	0.02	NS	0.01	NS
Rate*PB		NS	0.01	NS	NS	NS	NS
$LSD_{(P=0.05)}$†		--	141	--	--	--	--
		Site Year 1990					
0	-	369	641	569	125	74	269
	+	541	672	655	115	68	228
5	-	552	619	595	125	35	257
	+	216	794	522	117	34	225
10	-	467	721	718	143	30	231
	+	553	726	640	126	33	235
20	-	578	740	616	127	36	256
	+	344	778	838	127	39	232
40	-	440	594	595	136	41	206
	+	353	636	541	120	41	232
Rate		NS	NS	NS	NS	0.04	NS
PB		NS	NS	NS	0.01	NS	NS
Rate*PB		0.05	NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$		228	--	--	--	--	--

† LSD values apply only to comparison of means for treatments with and without PB-50

Table 5.11. Effect of P fertilizer and PB-50 on Fe concentration in wheat, at various sampling times (Wellwood soil).

P_2O_5 Added (kg ha^{-1})	PB-50	Iron Concentration (mg kg^{-1})					
		Week 1	Week 2	Week 4	Week 8	Grain	Straw
		Site Year 1989					
0	-	289	233	354	138	33	199
	+	284	296	341	130	43	134
5	-	258	330	323	155	36	163
	+	340	311	331	142	44	127
10	-	299	276	305	152	41	175
	+	286	271	366	163	42	151
20	-	331	236	367	125	48	209
	+	295	246	389	156	40	173
40	-	411	256	321	140	45	188
	+	329	297	391	135	41	162
Rate		NS	NS	NS	NS	NS	NS
PB		NS	NS	NS	NS	NS	0.02
Rate*PB		NS	NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$†		--	--	--	--	--	--
		Site Year 1990					
0	-	420	577	1188	68	36	70
	+	407	606	1020	87	36	69
5	-	592	625	1171	74	49	60
	+	422	640	1280	67	32	65
10	-	452	578	1370	86	37	69
	+	413	631	1353	71	38	73
20	-	800	615	1324	72	35	62
	+	415	674	1177	68	41	82
40	-	604	580	1241	79	40	67
	+	454	604	1401	72	40	97
Rate		0.05	NS	NS	NS	NS	NS
PB		0.04	NS	NS	NS	NS	0.01
Rate*PB		NS	NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$		--	--	--	--	--	--

† LSD values apply only to comparison of means for treatments with and without PB-50

Table 5.12. Effect of P fertilizer and PB-50 on Fe concentration in wheat, at various sampling times (Stockton soil).

P_2O_5 Added (kg ha^{-1})	PB-50	Iron Concentration (mg kg^{-1})					
		Week 1	Week 2	Week 4	Week 8	Grain	Straw
		Site Year 1989					
0	-	233	215	247	96	29	222
	+	181	316	271	92	33	190
5	-	204	295	355	85	26	205
	+	213	285	290	90	31	202
10	-	226	230	274	92	26	218
	+	238	325	259	94	31	183
20	-	189	220	287	92	26	184
	+	227	354	309	88	37	157
40	-	213	259	286	77	24	184
	+	235	263	300	99	34	173
Rate		NS	NS	NS	NS	NS	NS
PB		NS	0.02	NS	NS	0.01	NS
Rate*PB		NS	NS	NS	NS	NS	NS
$LSD_{(P=0.05)\dagger}$		--	--	--	--	--	--
		Site Year 1990					
0	-	389	568	670	64	39	85
	+	473	476	626	83	39	87
5	-	372	715	623	72	38	81
	+	436	659	580	86	41	111
10	-	298	624	505	60	37	73
	+	335	682	799	60	38	91
20	-	342	599	648	61	39	89
	+	332	696	680	85	38	98
40	-	288	574	468	60	37	94
	+	363	694	508	84	49	111
Rate		0.01	NS	0.05	NS	NS	NS
PB		NS	NS	NS	0.01	0.05	0.01
Rate*PB		NS	NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$		--	--	--	--	--	--

† LSD values apply only to comparison of means for treatments with and without PB-50

Table 5.13. Effect of P fertilizer and PB-50 on Fe concentration in wheat, at various sampling times (Portage soil).

P_2O_5 Added (kg ha^{-1})	PB-50	Iron Concentration (mg kg^{-1})					
		Week 1	Week 2	Week 4	Week 8	Grain	Straw
		Site Year 1989					
0	-	357	476	261	74	18	94
	+	346	331	295	64	15	115
5	-	360	470	279	63	28	80
	+	378	355	244	65	22	96
10	-	308	465	266	69	32	89
	+	403	475	250	53	20	99
20	-	393	510	226	62	27	75
	+	409	535	254	66	22	83
40	-	397	348	207	62	27	83
	+	402	562	294	62	18	97
Rate		NS	0.02	NS	NS	0.01	0.01
PB		NS	NS	NS	NS	0.01	0.05
Rate*PB		NS	0.01	0.02	NS	0.02	NS
$LSD_{(P=0.05)}$†		--	104	68	--	5	--
		Site Year 1990					
0	-	451	644	720	61	39	93
	+	503	570	867	67	31	82
5	-	409	631	722	77	31	93
	+	551	636	701	72	46	77
10	-	597	612	633	64	26	77
	+	538	644	789	73	30	81
20	-	504	609	704	86	30	78
	+	477	498	783	86	29	82
40	-	448	530	754	88	40	75
	+	483	668	726	78	29	83
Rate		NS	NS	NS	0.01	NS	NS
PB		NS	NS	NS	NS	NS	NS
Rate*PB		NS	NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$		--	--	--	--	--	--

† LSD values apply only to comparison of means for treatments with and without PB-50

bilaji did cause an increase in the Fe concentration of the plant (Keyes 1990).

The wheat tissue samples collected from the Wellwood site (lower pH) had a maximum Al and Fe concentration at the 4 week sampling date, whereas the samples from the Stockton and Portage sites generally peaked at the 2 week sampling date (Figures 5.3 and 5.4). In addition, at the early season sampling dates, tissue samples from the Wellwood site generally contained a higher concentration of Al and Fe, as compared to samples collected from the Stockton and Portage sites (Tables 5.8, 5.9, 5.10, 5.11, 5.12 and 5.13). However, by maturity, the Al and Fe concentrations of the plant tissues were relatively uniform for all field sites in both cropping years (Figures 5.3 and 5.4).

Aluminum is not considered an essential nutrient to the plant, but rather is a trace element and at high concentrations can have a toxic effect on the plant (Asher 1991). Establishing standards for toxic concentrations of Al in plant tissue has been difficult. The general effect of excess Al is to reduce the P content of the plant (Jones et al. 1991). Because a reduced level of P in the plants was not observed in the field experiments (Tables 3.3, 3.4 and 3.5), particularly at the Wellwood site where the plants contained the highest concentration of Al (Table 5.8), it was assumed that the Al content of the plant tissues analyzed was not toxic.

A concentration of Fe in wheat, at heading, of between 25 and 100 mg kg^{-1} is considered sufficient (Jones et al. 1991). Therefore, it appears that the Fe content of the wheat plants, collected from all sites, was sufficient (Tables 5.11, 5.12 and 5.13).

Figure 5.3. Effect of PB-50 and site year on the Al concentration of wheat, for the Wellwood, Stockton and Portage soils.

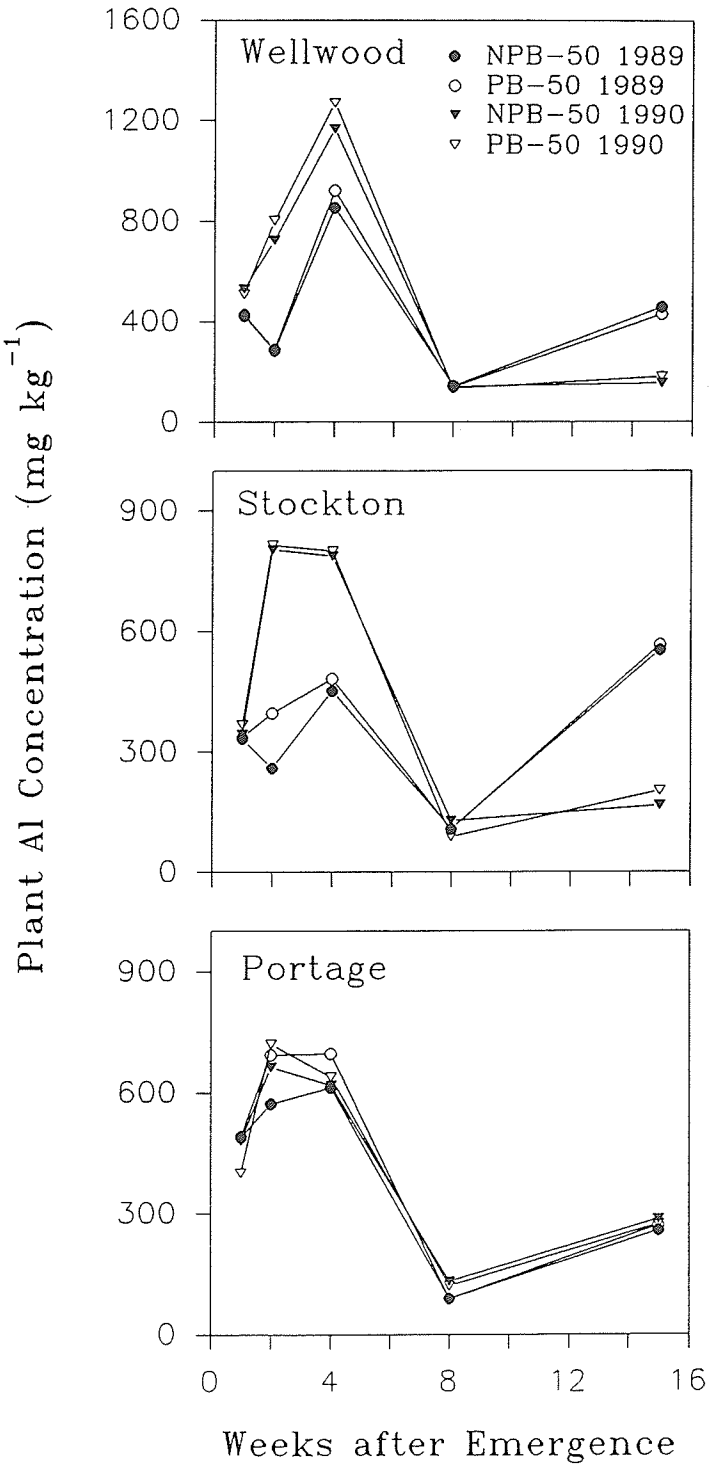
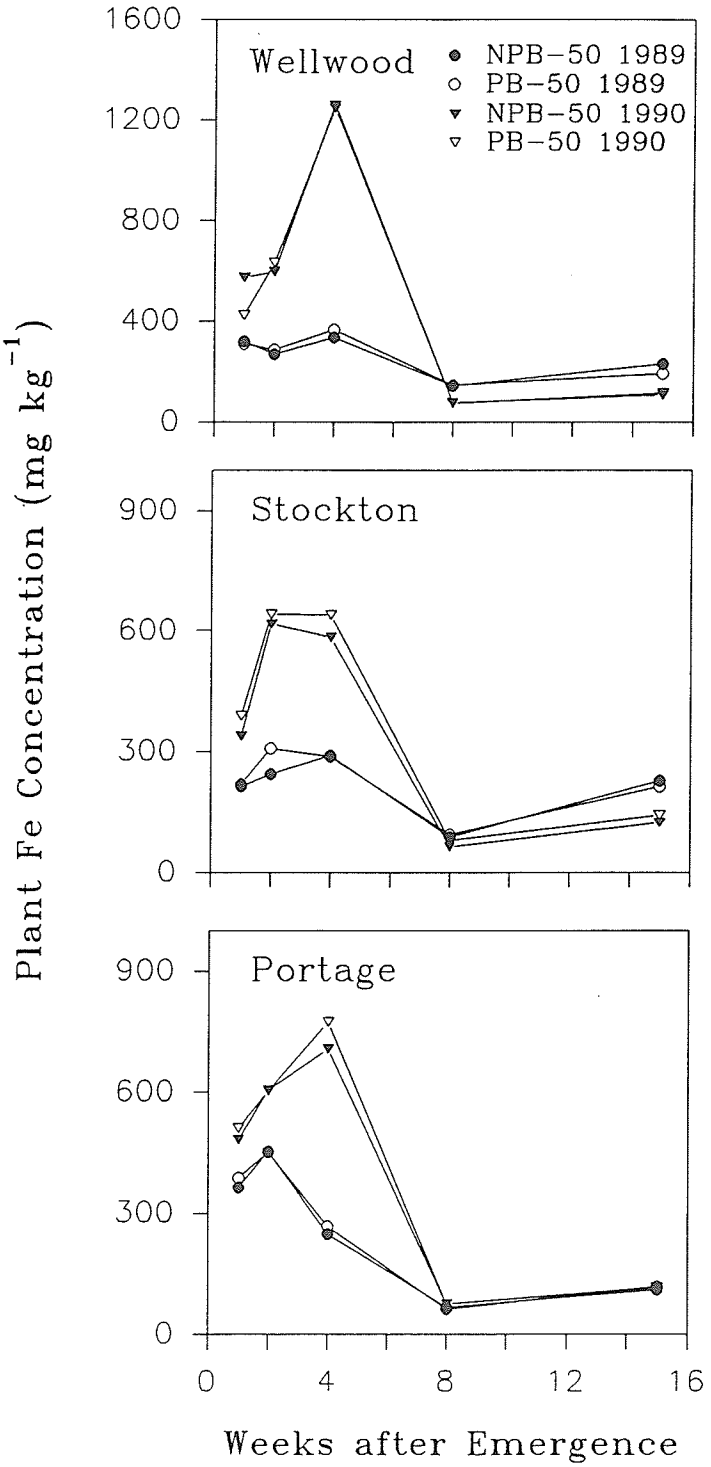


Figure 5.4. Effect of PB-50 and site year on the Fe concentration of wheat, for the Wellwood, Stockton and Portage soils.



5.3.3 Growth Chamber Experiments: Wheat

For the wheat grown on the two soils in the growth chamber, increasing the rates of P fertilizer added had inconsistent effects on the concentration of Ca, Mg, Al and Fe in the plant tissue (Tables 5.14, 5.15, 5.16 and 5.17). Although significant effects were observed, the trends were inconsistent, with significant increases and decreases in the level of the nutrients, at all sampling dates.

The inoculation of wheat with PB-50 also caused inconsistent results, again with both significant increases and decreases in the levels of the nutrients, at all sampling dates (Tables 5.14, 5.15, 5.16 and 5.17) (Figure 5.5).

The inconsistent results from PB-50 inoculation, on the concentration of Al and Fe in the plant tissue, may be the result of contamination of the tissue samples. Studies have shown that for accurate Al and Fe analysis, it is necessary to decontaminate the tissue from dust and soil accumulation (Jones 1991), and this was not done for the tissue samples collected in these experiments. As well, samples were ground using a Wiley mill tissue grinder and these mills contain both Al and Fe components, thereby contributing to the tissue nutrient level (Jones 1991).

The concentration of Ca in the wheat plants grown in the Willowcrest soil was greater than in plants grown on the Wellwood soil, at the early sampling dates, whereas the Mg concentration of the plant seemed to be unaffected by the soil type (Tables 5.14 and 5.15) (Figure 5.5). The concentration of Al and Fe in the plants grown on the

Table 5.14. Effect of P fertilizer and PB-50 on Ca concentration in wheat, at various sampling times.

P Added (kg ha^{-1})	PB-50	Calcium Concentration (mg g^{-1})				
		Week 1	Week 2	Week 4	Grain	Straw
		Wellwood Soil				
0	-	7.1	5.2	5.0	0.3	6.1
	+	6.8	4.9	5.0	0.3	5.0
2.5	-	6.6	7.1	5.2	0.5	6.4
	+	7.0	5.8	5.8	0.3	5.9
5	-	7.6	4.9	3.4	0.5	5.4
	+	6.3	5.7	4.5	0.6	6.4
10	-	5.9	4.6	5.4	0.6	5.0
	+	6.0	5.8	4.4	0.7	6.0
20	-	6.2	4.1	5.2	0.5	5.6
	+	6.2	4.4	4.9	0.6	5.3
Rate		NS	0.01	0.01	0.01	NS
PB		NS	NS	NS	NS	NS
Rate*PB		NS	NS	0.01	NS	NS
$LSD_{(P=0.05)}$†		--	--	0.8	--	--
		Willowcrest Soil				
0	-	6.5	8.2	8.2	0.1	5.3
	+	6.2	7.8	7.0	0.1	5.4
2.5	-	7.6	7.8	6.8	0.4	5.5
	+	6.9	8.4	7.4	0.1	5.7
5	-	6.3	8.5	5.7	0.4	5.1
	+	6.4	8.3	8.1	0.4	5.0
10	-	7.0	6.6	6.6	0.6	5.9
	+	6.8	8.2	6.8	0.5	4.6
20	-	7.5	5.9	6.2	0.2	4.6
	+	7.0	5.7	6.3	0.4	4.7
Rate		NS	0.01	0.04	0.01	0.02
PB		NS	NS	NS	0.02	NS
Rate*PB		NS	NS	0.01	0.01	0.05
$LSD_{(P=0.05)}$		--	--	1.2	0.1	0.8

† LSD values apply only to comparison of means for treatments with and without PB-50

Table 5.15. Effect of P fertilizer and PB-50 on Mg concentration in wheat, at various sampling times.

P Added (kg ha^{-1})	PB-50	Magnesium Concentration (mg g^{-1})				
		Week 1	Week 2	Week 4	Grain	Straw
		Wellwood Soil				
0	-	2.0	1.8	2.2	1.5	1.9
	+	2.2	1.7	1.9	1.5	1.3
2.5	-	2.1	2.3	2.1	1.5	1.4
	+	2.2	2.1	2.2	1.8	1.4
5	-	2.2	1.9	1.7	1.6	1.3
	+	2.0	2.0	2.0	1.9	1.4
10	-	2.0	1.7	2.3	1.0	1.2
	+	1.9	1.6	2.0	1.5	1.3
20	-	1.8	1.6	2.1	1.4	1.2
	+	1.9	2.3	2.1	1.3	1.1
Rate		NS	0.01	NS	0.01	0.01
PB		NS	NS	NS	0.01	NS
Rate*PB		NS	0.01	NS	0.01	0.01
$LSD_{(P=0.05)}$†		--	0.4	--	0.1	0.2
		Willowcrest Soil				
0	-	1.9	1.8	2.5	1.2	1.3
	+	1.8	1.7	2.3	1.2	1.3
2.5	-	2.1	1.6	2.1	1.2	1.8
	+	1.8	1.8	2.3	1.0	1.8
5	-	1.7	1.7	2.0	1.1	1.6
	+	1.7	1.7	2.5	1.2	1.7
10	-	1.8	1.8	2.4	1.2	1.6
	+	1.7	1.7	2.4	1.1	1.4
20	-	2.1	1.7	2.4	1.6	1.5
	+	1.8	1.7	2.4	1.1	1.4
Rate		0.02	NS	NS	0.01	0.01
PB		0.04	NS	NS	0.01	NS
Rate*PB		NS	NS	NS	0.01	NS
$LSD_{(P=0.05)}$		--	--	--	0.1	--

† LSD values apply only to comparison of means for treatments with and without PB-50

Table 5.16. Effect of P fertilizer and PB-50 on Al concentration in wheat, at various sampling times.

P Added (kg ha^{-1})	PB-50	Aluminum Concentration (mg kg^{-1})				
		Week 1	Week 2	Week 4	Grain	Straw
		Wellwood Soil				
0	-	79	62	93	1	23
	+	49	51	74	2	53
2.5	-	33	47	57	5	23
	+	65	66	86	7	26
5	-	26	80	43	7	33
	+	10	34	97	5	34
10	-	40	46	56	7	41
	+	42	29	36	12	58
20	-	88	58	66	20	70
	+	82	37	51	31	51
Rate		0.01	NS	0.01	0.01	0.01
PB		NS	NS	NS	NS	0.01
Rate*PB		NS	NS	0.01	NS	0.01
$LSD_{(P=0.05)}$†		--	--	21	--	25
		Willowcrest Soil				
0	-	55	61	44	10	63
	+	53	44	52	19	52
2.5	-	59	36	40	17	37
	+	59	26	44	11	28
5	-	36	29	13	6	16
	+	57	33	26	11	25
10	-	27	44	15	22	28
	+	20	46	22	10	23
20	-	35	35	6	20	40
	+	37	35	12	11	28
Rate		0.01	0.01	0.01	NS	0.01
PB		NS	NS	0.01	NS	NS
Rate*PB		NS	0.05	NS	0.01	NS
$LSD_{(P=0.05)}$		--	11	--	10	--

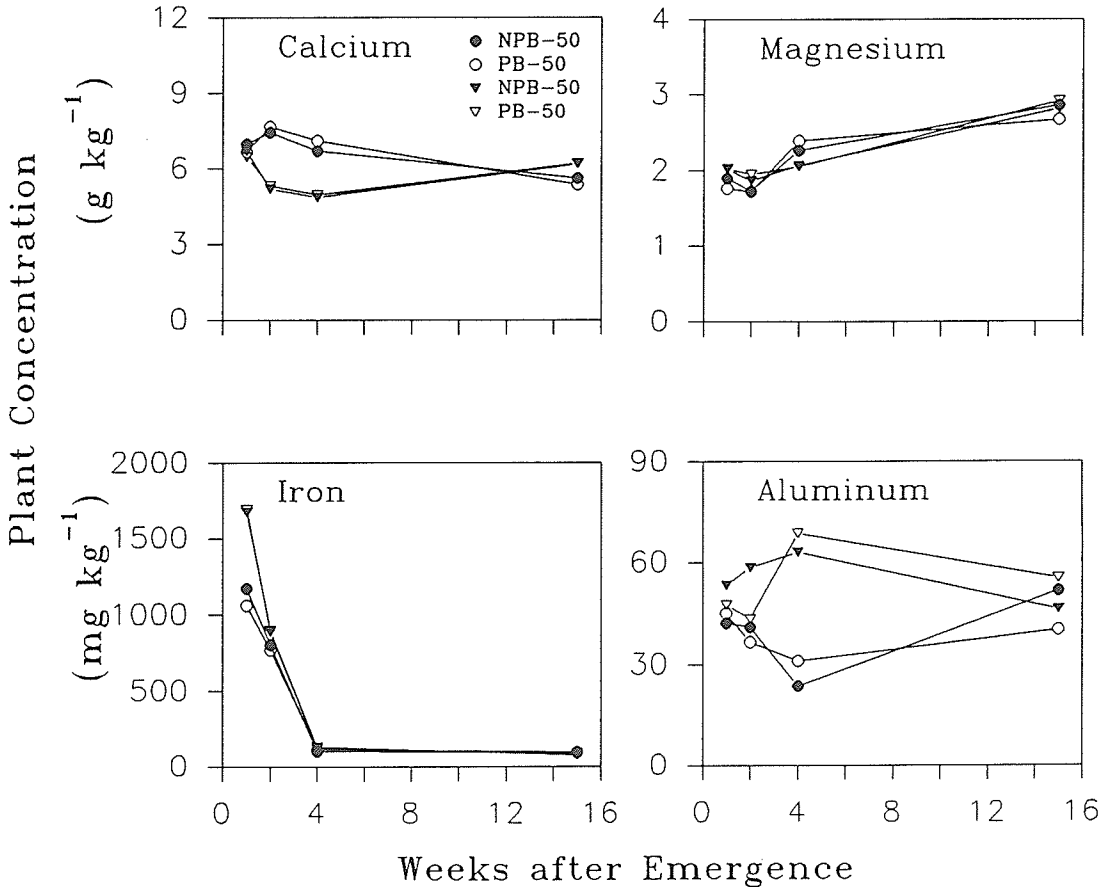
† LSD values apply only to comparison of means for treatments with and without PB-50

Table 5.17. Effect of P fertilizer and PB-50 on Fe concentration in wheat, at various sampling times.

P Added (kg ha^{-1})	PB-50	Iron Concentration (mg kg^{-1})				
		Week 1	Week 2	Week 4	Grain	Straw
		Wellwood Soil				
0	-	1726	1104	138	31	38
	+	1675	984	127	39	54
2.5	-	1721	914	141	36	29
	+	1958	1189	117	42	39
5	-	1741	1154	125	42	33
	+	1737	966	115	39	48
10	-	1804	584	113	47	34
	+	1648	651	107	49	43
20	-	1375	731	130	41	30
	+	1435	656	127	49	34
Rate		0.01	0.01	0.01	0.01	0.01
PB		NS	NS	0.01	0.01	0.01
Rate*PB		NS	0.01	NS	NS	NS
$LSD_{(P=0.05)}$†		--	160	--	--	--
		Willowcrest Soil				
0	-	1129	744	96	51	41
	+	1184	689	112	57	43
2.5	-	1011	697	95	48	50
	+	997	663	96	54	44
5	-	1025	647	93	51	39
	+	1019	732	135	58	42
10	-	961	931	101	51	42
	+	1052	765	112	51	37
20	-	1734	1009	101	38	48
	+	1058	1001	104	53	46
Rate		0.01	0.01	NS	0.03	0.01
PB		0.03	NS	0.01	0.01	NS
Rate*PB		0.01	NS	NS	NS	NS
$LSD_{(P=0.05)}$		226	--	--	--	--

† LSD values apply only to comparison of means for treatments with and without PB-50

Figure 5.5. Effect of PB-50 on the Ca, Mg, Al and Fe concentration of wheat, for the Wellwood (▲-▲) and Willowcrest (●-●) soils.



Wellwood soil were higher than those plants grown on the Willowcrest soil, also at the early sampling dates (Tables 5.16 and 5.17) (Figure 5.5). However, by maturity, plants grown on the two soils had similar concentrations of each of the nutrients studied (Figure 5.5).

Based on the sufficiency nutrient levels, as discussed in the results for the field experiments, all plant samples analyzed were sufficient in Ca, Mg and Fe, and did not contain toxic levels of Al (Table 5.14, 5.15, 5.16 and 5.17). In addition, wheat grown on the Wellwood soil, in the growth chamber, had a substantially lower concentration of Al than did wheat grown in the same soil in the field experiments (Tables 5.8 and 5.16) (Figures 5.3 and 5.5).

5.3.4 Growth Chamber Experiments: Flax

As was observed in the growth chamber experiment with wheat, increasing the rates of P fertilizer added or the inoculation of PB-50, did not consistently affect the concentration of Ca, Mg, Al and Fe in the flax plant tissue (Tables 5.18, 5.19, 5.20 and 5.21) (Figure 5.6).

The concentration of Ca in the flax plants grown on the Willowcrest soil was greater than in plants grown on the Wellwood soil, at the early sampling dates, whereas the opposite trend existed for the Mg concentration of the plants at the same sampling times (Tables 5.18 and 5.19) (Figure 5.6). The concentration of Al and Fe in the plants grown on the Wellwood soil and Willowcrest soil were similar (Tables 5.20 and 5.21) (Figure 5.6). However, by maturity, plants grown on the two soils had similar concentration of each of the nutrients studied (Figure 5.6).

Table 5.18. Effect of P fertilizer and PB-50 on Ca concentration in flax, at various sampling times.

P Added (kg ha^{-1})	PB-50	Calcium Concentration (mg g^{-1})					
		Week 1	Week 2	Week 4	Week 8	Grain	Straw
		Wellwood Soil					
0	-	7.2	11.0	10.4	9.4	1.9	4.3
	+	8.3	10.8	11.5	9.3	1.8	4.1
2.5	-	7.9	11.3	9.9	9.7	1.8	4.3
	+	8.8	11.2	9.8	10.7	1.9	4.4
5	-	8.8	11.8	10.3	8.0	1.9	4.1
	+	10.5	11.3	11.3	8.8	1.7	4.5
10	-	8.0	8.5	8.3	8.7	1.8	4.7
	+	8.6	10.3	9.5	8.9	1.8	4.9
20	-	7.2	8.2	7.9	7.7	1.8	4.7
	+	8.5	10.6	8.9	9.8	1.9	5.2
Rate		NS	NS	0.01	0.04	NS	0.03
PB		NS	NS	0.01	0.03	NS	NS
Rate*PB		NS	NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$†		--	--	--	--	--	--
		Willowcrest Soil					
0	-	8.8	14.4	23.5	13.4	1.8	5.2
	+	8.4	10.8	30.8	13.9	1.9	5.4
2.5	-	8.5	13.7	25.2	12.0	1.7	4.8
	+	8.5	10.7	33.0	13.4	1.7	5.0
5	-	8.1	12.2	19.3	14.4	1.6	3.9
	+	8.9	13.7	24.9	13.5	1.8	4.2
10	-	10.2	12.5	21.8	14.1	1.7	4.2
	+	10.1	13.5	22.3	14.6	1.6	4.6
20	-	9.5	10.2	23.8	14.2	1.9	4.1
	+	8.2	13.1	20.4	14.3	1.8	4.3
Rate		0.01	NS	0.01	NS	NS	0.01
PB		NS	NS	0.01	NS	NS	NS
Rate*PB		0.02	0.01	0.01	NS	NS	NS
$LSD_{(P=0.05)}$		0.7	2.8	2.8	--	--	--

† LSD values apply only to comparison of means for treatments with and without PB-50

Table 5.19. Effect of P fertilizer and PB-50 on Mg concentration in flax, at various sampling times.

P Added (kg ha^{-1})	PB-50	Magnesium Concentration (mg g^{-1})					
		Week 1	Week 2	Week 4	Week 8	Grain	Straw
		Wellwood Soil					
0	-	4.4	5.2	4.9	5.4	3.8	1.1
	+	4.8	5.0	5.2	5.5	3.2	0.9
2.5	-	5.0	5.9	4.6	5.2	3.4	1.1
	+	5.2	5.2	4.8	5.7	3.9	1.1
5	-	5.2	5.6	4.6	4.7	3.1	1.1
	+	6.3	5.7	4.9	5.1	2.8	1.2
10	-	5.0	4.5	4.3	5.4	2.6	1.1
	+	5.1	5.3	4.6	5.3	2.7	1.2
20	-	4.5	4.7	4.3	5.1	3.8	1.4
	+	5.2	5.4	4.7	6.0	3.8	1.4
Rate		NS	NS	0.01	0.04	0.01	0.01
PB		NS	NS	0.01	0.01	NS	NS
Rate*PB		NS	NS	NS	NS	0.03	NS
$LSD_{(P=0.05)}$†		--	--	--	--	0.5	--
		Willowcrest Soil					
0	-	3.8	4.5	3.9	4.9	3.2	1.0
	+	4.1	3.9	5.2	4.9	3.1	1.0
2.5	-	3.8	4.7	4.6	5.0	3.4	0.9
	+	4.2	3.9	5.0	5.7	3.2	1.0
5	-	4.4	5.3	4.3	5.6	3.1	0.8
	+	4.4	4.7	4.6	5.4	3.2	0.8
10	-	5.1	4.6	4.4	5.8	3.1	0.9
	+	4.6	4.7	3.8	5.7	3.0	1.0
20	-	4.8	3.8	4.7	5.6	2.8	0.9
	+	4.4	5.4	4.1	6.1	3.1	0.9
Rate		0.01	NS	0.01	0.01	0.01	NS
PB		NS	NS	NS	NS	NS	NS
Rate*PB		0.05	0.03	0.01	NS	0.02	NS
$LSD_{(P=0.05)}$		0.5	1.0	0.5	--	0.2	--

† LSD values apply only to comparison of means for treatments with and without PB-50

Table 5.20. Effect of P fertilizer and PB-50 on Al concentration in flax, at various sampling times.

P Added (kg ha^{-1})	PB-50	Aluminum Concentration (mg kg^{-1})					
		Week 1	Week 2	Week 4	Week 8	Grain	Straw
		Wellwood Soil					
0	-	284	156	53	26	29	24
	+	230	148	76	31	37	23
2.5	-	283	167	59	35	26	18
	+	230	125	58	37	33	20
5	-	307	127	68	46	27	17
	+	270	234	74	30	32	27
10	-	238	170	47	39	18	22
	+	250	277	61	34	20	27
20	-	289	166	33	34	18	21
	+	313	243	50	35	21	23
Rate		NS	NS	0.03	NS	0.01	NS
PB		NS	NS	0.05	NS	0.04	0.01
Rate*PB		NS	NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$†		--	--	--	--	--	--
		Willowcrest Soil					
0	-	191	136	56	23	53	19
	+	232	168	93	23	16	20
2.5	-	155	154	70	31	23	18
	+	274	203	84	33	32	24
5	-	190	183	45	30	24	18
	+	293	192	76	26	24	23
10	-	220	194	58	27	46	28
	+	233	205	52	25	31	28
20	-	376	203	59	39	48	20
	+	281	171	39	35	23	21
Rate		0.01	NS	0.01	0.01	0.01	0.04
PB		NS	NS	0.04	NS	0.01	NS
Rate*PB		0.04	NS	0.01	NS	0.01	NS
$LSD_{(P=0.05)}$		93	--	22	--	11	--

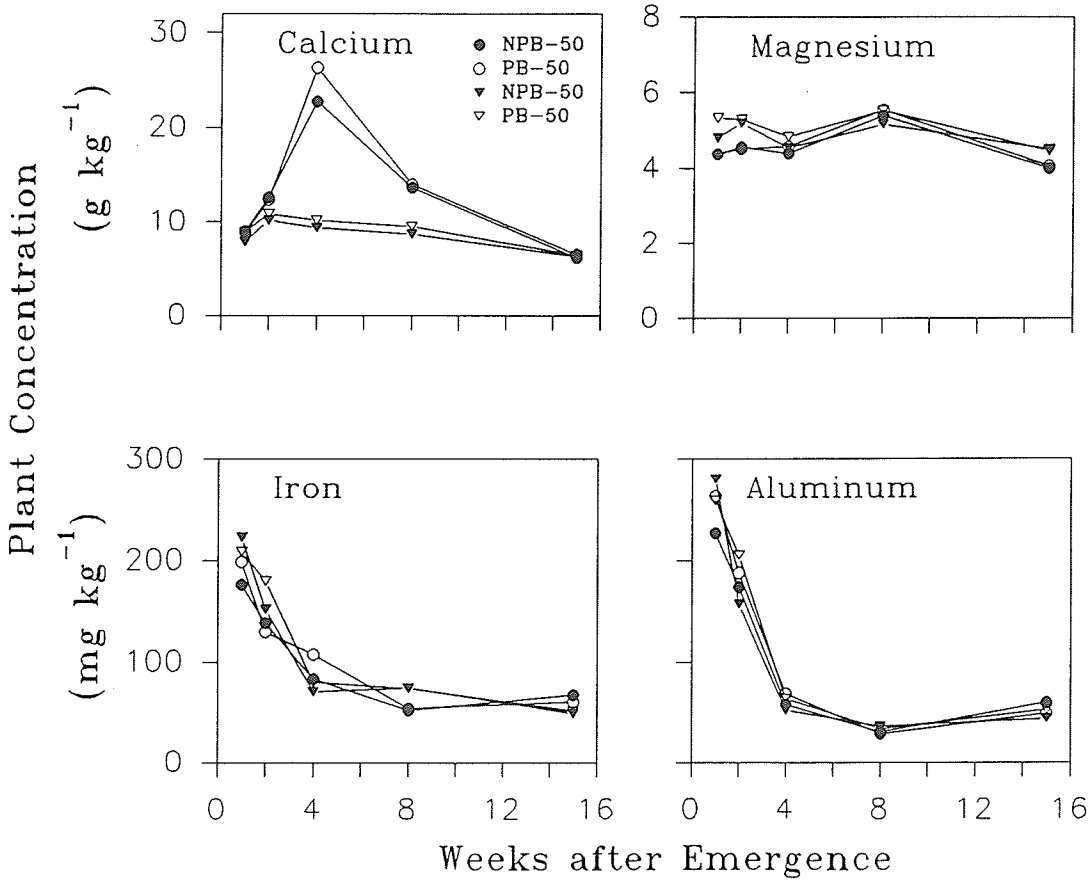
† LSD values apply only to comparison of means for treatments with and without PB-50

Table 5.21. Effect of P fertilizer and PB-50 on Fe concentration in flax, at various sampling times.

P Added (kg ha^{-1})	PB-50	Iron Concentration (mg kg^{-1})					
		Week 1	Week 2	Week 4	Week 8	Grain	Straw
		Wellwood Soil					
0	-	277	144	73	61	34	12
	+	144	130	96	62	36	12
2.5	-	201	160	71	72	31	11
	+	163	116	73	67	33	12
5	-	231	144	84	77	38	12
	+	195	205	91	69	37	16
10	-	198	159	65	82	32	12
	+	193	226	72	79	36	13
20	-	207	153	60	80	48	14
	+	349	223	69	92	46	16
Rate		NS	NS	NS	0.01	0.01	NS
PB		NS	NS	NS	NS	NS	NS
Rate*PB		NS	NS	NS	NS	NS	NS
$LSD_{(P=0.05)}$†		--	--	--	--	--	--
		Willowcrest Soil					
0	-	206	84	60	43	76	6
	+	290	87	122	42	32	7
2.5	-	127	123	72	54	58	8
	+	183	124	158	69	58	10
5	-	140	167	77	49	58	7
	+	166	123	91	49	58	10
10	-	191	170	101	50	67	7
	+	142	158	78	52	56	8
20	-	216	147	106	62	43	6
	+	211	156	89	58	55	8
Rate		NS	0.01	NS	0.01	NS	NS
PB		NS	NS	0.01	NS	0.01	NS
Rate*PB		NS	NS	0.01	NS	0.01	NS
$LSD_{(P=0.05)}$		--	--	39	--	12	--

† LSD values apply only to comparison of means for treatments with and without PB-50

Figure 5.6. Effect of PB-50 on the Ca, Mg, Al and Fe concentration of flax, for the Wellwood (▲-▲) and Willowcrest (●-●) soils.



The flax plants grown in the growth chamber all contained higher levels of Ca, Mg and Al than did the wheat plants, also grown in the growth chamber (Tables 5.14, 5.15, 5.16, 5.17, 5.18, 5.19, 5.20 and 5.21). Based on the sufficiency values for nutrients in leaf tissue [Ca, 0.3 to 1.0%; Mg, 0.25%; and Fe, 50-75 mg kg^{-1} (Jones et al. 1991)] all flax plants samples analyzed, were sufficient in Ca, Mg and Fe (Tables 5.18, 5.19, 5.20 and 5.21). Also, because a reduced level of P in the plants was not observed in the growth chamber experiment (Table 4.5), it was assumed that the Al content of the plant tissues analyzed was not toxic.

5.4 Summary

The plant samples obtained in the field and growth chamber experiments were analyzed for Ca, Mg, Fe and Al to assess the influence of P fertilizer and PB-50 on plant concentration of these nutrients. In addition, the plant nutrient concentrations were compared for the different pH soil studied.

Phosphorus fertilizer can be an integral part of the plant nutrition through its interactions with other nutrients. However in both the field and growth chamber experiments conducted, by varying the rate of P fertilization, the Ca, Mg, Al and Fe concentrations of wheat and flax were not significantly affected, for all sampling times.

The inoculation of PB-50, on both wheat and flax, regardless of soil pH, generated inconsistent results in terms of Ca, Mg, Al and Fe concentrations throughout the growing season, in the field and growth chamber studies. As a result, no beneficial effect could be attributed

to the inoculation of the crops with PB-50.

As a result of the soil characteristics, rather than PB-50 inoculation, variations in the plants nutrient content did exist between the different pH soils. In general, as the soil pH increased, Al and Fe content of the plants decreased, and Ca and Mg concentration increased.

VI. SUMMARY

The studies reported in this manuscript showed that inoculation of wheat and flax with PB-50 can increase P concentration in the plant early in the growing season. This enhanced P concentration in the plant was correlated to a significant increase in the uptake of available P from the soil P fraction.

The increased P nutrient status of the plant, as a result of PB-50 inoculation, continued through the growing season and in most of the experiments wheat had significant increases in dry matter production and overall grain yield. Based on these findings, it appears that the application of PB-50 has the potential to increase crop production of wheat.

In the field experiments, increases in plant P concentrations with PB-50 inoculation also occurred at the later sampling times, and the greater increases in P concentration of the plant and dry matter production with inoculation occurred at the higher rates of P fertilization. In addition, the flax (growth chamber experiment) and the wheat (1990 field experiment) that had significant increases in P concentration of the plant at maturity with PB-50 inoculation, did not have increased grain yields. This suggests that the organism is affecting the uptake of other soil nutrients. Additional studies should be conducted to further elucidate the effects of PB-50 inoculation on crop nutrient uptake. As well, additional research on flax and other crops should be conducted to define the potential of PB-50 inoculation on these crops.

The experiment was conducted on soils of varying pH, however it was evident that the pH of the soil did not influence the response of the crop to the PB-50 inoculation. Instead, the crop type, chemical interaction of the soil with P fertilizer, the soil fertility and the environmental conditions were more critical factors when assessing the possibility of a crop response to inoculation with PB-50.

Based on the analysis of various nutrients that are subject to chelation, it did not appear as if the organism affected the availability of these nutrients to the plant. However, our results were inconclusive and further research is required to determine if chelation is the mechanism by which PB-50 solubilizes P. Research should be conducted to isolate the form of organic acid(s) produced by P. bilaji and to determine how these acids affect plant nutrient availability.

APPENDIX

Table A.1. Effect of P fertilizer and PB-50 on Cu concentration in wheat, at various sampling times (Wellwood soil).

P_2O_5 Added (kg ha^{-1})	PB-50	Copper Concentration (mg kg^{-1})		
		Week 1	Week 2	Week 4
		Crop Year 1989		
0	-	18.5	4.4	5.8
	+	11.5	7.2	6.4
5	-	17.1	4.2	4.9
	+	17.5	7.0	4.8
10	-	22.8	6.9	5.4
	+	18.3	7.5	5.1
20	-	17.1	6.9	5.3
	+	14.8	6.6	5.8
40	-	19.8	6.7	2.9
	+	15.8	4.8	5.3
		Crop Year 1990		
0	-	9.9	6.8	5.7
	+	12.4	5.1	6.1
5	-	14.7	5.7	5.5
	+	19.7	3.1	8.2
10	-	9.0	4.6	5.8
	+	7.2	4.9	7.0
20	-	10.1	4.6	5.9
	+	4.2	5.0	6.0
40	-	6.8	5.0	6.1
	+	3.9	6.3	3.8

Table A.2. Effect of P fertilizer and PB-50 on Zn concentration in wheat, at various sampling times (Wellwood soil).

P_2O_5 Added (kg ha^{-1})	PB-50	Zinc Concentration (mg kg^{-1})		
		Week 1	Week 2	Week 4
		Crop Year 1989		
0	-	40	36	36
	+	86	41	35
5	-	37	38	34
	+	44	40	36
10	-	37	44	40
	+	50	42	38
20	-	39	42	40
	+	41	42	35
40	-	68	43	17
	+	43	41	38
		Crop Year 1990		
0	-	33	41	45
	+	36	39	39
5	-	35	38	40
	+	36	37	48
10	-	37	39	42
	+	39	37	47
20	-	38	38	41
	+	36	43	43
40	-	40	37	43
	+	37	43	36

Table A.3. Effect of P fertilizer and PB-50 on Mn concentration in wheat, at various sampling times (Wellwood soil).

P_2O_5 Added (kg ha^{-1})	PB-50	Manganese Concentration (mg kg^{-1})		
		Week 1	Week 2	Week 4
		Crop Year 1989		
0	-	76	81	94
	+	78	88	98
5	-	73	91	90
	+	81	88	99
10	-	72	91	72
	+	73	85	95
20	-	68	90	92
	+	71	70	84
40	-	84	96	80
	+	81	100	98
		Crop Year 1990		
0	-	72	103	120
	+	66	113	110
5	-	83	111	116
	+	75	110	130
10	-	75	112	132
	+	79	116	134
20	-	81	108	119
	+	73	119	124
40	-	83	116	131
	+	83	127	111

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