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Effect of *Penicillium bilaii* on Root Morphology and Architecture of Pea (*Pisum sativum* L.)

by
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EFFECT OF *PENICILLIUM BILAII* ON ROOT MORPHOLOGY AND ARCHITECTURE OF PEA (*PISUM SATIVUM* L.)

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ABSTRACT

Penicillium bilaii (ATCC strain no. 20851) is a rhizospheric fungus sold commercially in western Canada to improve phosphate (P) uptake of various crops. However, the mechanism underlying the stimulation of P uptake is not known. The objective of our research was to determine if the stimulation of P uptake in pea (*Pisum sativum* L.) treated with *P. bilaii* is the result of increased root surface area for P absorption. In addition, we examined the effect of *P. bilaii* and P concentration on root architecture (branching patterns) of pea. Experiments were carried out under controlled environment and field conditions. Under controlled conditions, treatment of peas with *P. bilaii* resulted in increased root and shoot P concentration and accumulation when no P fertilizer was added, or when rock phosphate (17.2 mg P kg^{-1} soil) fertilizer was applied. This stimulation of P uptake was not a result of increased root growth. In fact, it was observed that inoculation with *P. bilaii* resulted in a reduction in root length and had no effect on P concentration, accumulation, or dry matter effect. *Penicillium bilaii* inoculated plants, when no P fertilizer was added, had greater mean root diameters. Under field conditions, when no P fertilizer was applied, *P. bilaii* treatment resulted in a 48% increase in upper root length, and roots were significantly finer in the sample core (an area 15 cm in depth, and 6.5 cm in diameter around the stem base). This increase in root length may have contributed to the 13% increase in shoot P concentration of inoculated plants. However, it is proposed that *P. bilaii* may have increased the availability of soil P in the sampled area, thus resulting in the proliferation of plant roots. Under controlled conditions, using growth pouches, it was found that P concentration in

the nutrient solution had a greater effect on branching patterns of pea than *P. bilaii*. It was found that *P. bilaii* treatment resulted in a reduction in root length, and increased average root diameter at the intermediate P levels. The results of these studies suggest that the stimulation of P uptake is not a result of increased root surface area for phosphate absorption.

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1. Introduction

Phosphorus (P) is an essential nutrient for plant life and is usually second only to nitrogen in terms of limiting plant growth. Phosphorus plays many structural, metabolic, and regulatory roles within the plant (Marschner, 1995).

Prairie soils are often high in total P (Doyle and Cowell, 1993a). Unfortunately, this does not translate into large quantities of plant-available P. In most soils, P in the plant-available form rarely exceeds 0.01% of the total P in soils (Brady, 1990). The availability of P to plants may be limited by solution P precipitating with soil cations as secondary minerals and by adsorption onto mineral surfaces (Barber, 1984).

Plants have various ways in which to adapt to P-limiting soil conditions. Sensitivity analysis of models of phosphate uptake by roots has indicated that plant properties that affect root surface area (eg. root length and diameter) can greatly affect the rate of phosphate uptake by the plant (Silverbush and Barber, 1983). Increased phosphate uptake may result from plants that have a finer and longer root system (Silverbush and Barber, 1983). Soil microorganisms may stimulate, inhibit, or do not affect root growth depending on the type of microbe, plant species, and the environmental conditions (Marschner, 1995). Soil microorganisms may promote plant growth by altering root growth and morphology, or by influencing the influx kinetics of part of or the whole root surface to improve water and mineral nutrient acquisition by the plant (Barber, 1984; Marschner, 1995; Tinker, 1980).

Penicillium bilaii (ATCC strain no. 20851) is a rhizospheric fungus isolated from a southern Alberta soil by Kucey (1983). This fungus has been shown to solubilize

calcium phosphate in an agar medium (Kucey, 1983) and rock phosphate in liquid culture (Asea *et al.*, 1988). *Penicillium bilaii* is thought to solubilize sparingly soluble phosphates by secreting organic acids that acidify the surrounding soil and/or by cation chelation, thereby increasing the availability of soluble phosphate to the plant and thus promoting plant growth (Kucey, 1987, 1988; Asea *et al.*, 1988). This fungus has been shown to increase dry matter production, grain yield and P uptake of wheat, canola, bean, pea and lentil in growth chamber and field experiments (Asea *et al.*, 1988; Chambers, 1992; Gleddie, 1993; Gleddie *et al.*, 1991, 1993; Kucey, 1987, 1988; Kucey and Leggett, 1989). Gleddie *et al.* (1991) observed that increase in yields from *P. bilaii* inoculation decreases as the rate of P fertilizer increases. However, Chambers (1992) in field and growth chamber studies observed increased dry matter production of wheat with *P. bilaii* inoculation, and the increases were greater at the higher rates of added P fertilizer. Keyes (1990) found that dry matter yield of canola was consistently greater with inoculation with *P. bilaii* but P uptake was not promoted. This suggests that *P. bilaii* may promote plant growth by mechanisms other than increased P availability.

The objective of this study was to determine the effects of inoculating pea seed with *P. bilaii* and *Rhizobium leguminosarum* bv *viciae* on assimilate partitioning (P, dry matter), nodulation, and root morphology and architecture. To meet this objective, the following experiments were conducted:

1. Growth chamber experiments using a low-P (Malmo) and a high-P (Hochfeld) soil to investigate: a) if inoculation with *P. bilaii* affects shoot or root growth (dry weight, root length), b) if *P. bilaii* affects P uptake (P concentration and accumulation) of pea,

and c) if *P. bilaii* inoculation has a stimulatory or inhibitory effect on nodulation of pea.

2. Field experiments were conducted on low P and N soils using soil cores to investigate: a) if inoculation with *P. bilaii* affects shoot or root growth (dry weight, root length), b) if *P. bilaii* affects P uptake (P concentration and accumulation) of pea, and c) if *P. bilaii* inoculation has a stimulatory or inhibitory effect on nodulation of pea.

3. A growth chamber experiment was conducted utilizing growth pouches to investigate: a) if *P. bilaii* affects root architecture (branching patterns) of pea, b) if inoculation with *P. bilaii* affects shoot or root growth (dry weight, root length), and c) if *P. bilaii* inoculation has a stimulatory or inhibitory effect on nodulation of pea.

2. Literature Review

2.1 The Roles of Phosphorus in Plant Growth

Phosphorus (P) is an essential nutrient for plant life and is usually second only to nitrogen in terms of limiting plant growth. Phosphorus can be considered essential for all forms of life because of its role in the formation of RNA and DNA. The importance of P to plant nutrition can be appreciated by its many structural, metabolic, and regulatory roles within the plant (Marschner, 1995). Phosphorus nutrition can also influence the process of N_2 fixation at various steps of the symbiotic interaction between legumes and *Rhizobium*.

2.1.1 Structural, Metabolic, and Regulatory Roles

Phosphorus is a component of phospholipid that plays a key structural role in biomembrane synthesis. Phosphorus is involved in energy transformations within the plant through its role in the formation of adenosine triphosphate (ATP). The energy-rich pyrophosphate bonds of ATP supply the energy for vital metabolic processes within the plant. Phosphorus also is a component of reductants such as NADP. Phosphorylation of enzyme proteins modulates their activity and may play a role in signal transduction within the plant (Marschner, 1995). Phosphorus is considered essential for flowering, fruiting, and seed formation (Ozanne, 1980; Tisdale *et al.*, 1993). Increased P content of forages, fruits, and vegetables improves their quality (Tisdale *et* al., 1993).

2.1.2 Roles in Root Growth and Development

Phosphorus is considered an essential nutrient for plant life and can affect the plant root system. Plant root systems are highly variable both within and between plant species

(Marschner, 1995). The growth and development of a root system are influenced by both the soil environment and its shoot.

The ability of the soil to supply an adequate amount of P to the plant root is essential for proper root development. Phosphorus nutrition of the plant can affect the length of primary laterals and the production of secondary laterals but has less effect on the length of the seminal axis (Christie, 1975; Fitter, 1982; Price *et al.*, 1989). Enhanced lateral initiation can occur at sites of high P supply such as those created by phosphate fertilizer bands (Marschner, 1995). It has been suggested that roots growing under high P supply should be more branching and have finer roots (greater length per unit dry weight). This has been suggested because of the observed increase in the length of primary laterals, and their frequency of initiation compared to root growth under low P supply (Christie, 1975; Fitter, 1982; Price *et al.*, 1989).

Plant root morphology (root hairs, root diameter and length) can also be influenced by soil P supply. Formation of root hairs is strongly influenced by phosphate supply and the P content of the plant (Föhse and Jungk, 1983). Under low P supply, root hair density and length may increase depending on the plant species (Bhat and Nye, 1974; Föhse and Jungk, 1983; Marschner, 1995). The increase of root hair length increases the phosphate absorbing surfaces of the root and the effective root radius that can aid in P uptake by the root (Barber, 1984; Föhse *et al.*, 1991). Root hairs and root size are important for the efficiency of P uptake and P nutrition (Itoh and Barber, 1983).

The amount of root growth is important for supplying nutrients to the shoot and can be affected by P supply. Under P deficient conditions, some plant species may

allocate more of their resources to advance root growth at the expense of shoot growth, thus increasing the root to shoot ratios (Atkinson, 1973; Föhse *et al.*, 1988; Lynch *et al.*, 1991). Some plant species do not alter their root to shoot ratios as P supply varies in the soil (Powell, 1974). The response of plant root systems to the level of P supply in the soil environment seems species specific. Schenk and Barber (1979b) found that weight and length of corn (*Zea mays* L.) roots were not affected by added P while Khasawneh and Copeland (1973) found P increased root length of cotton (*Glossypium hirsutum* L.). On the other hand, Powell (1974) working with *Carex coriacea* found that root length decreased as P supply increased and the roots also became thicker. However, Ascenio (1996) found severely P stressed *Desmodium* plants had significantly shorter roots than those of P sufficient plants. Similarly, Paynter (1993) found burr medics (*Medicago polymorpha* L.) produced shorter, thicker roots under P deficiency. Schenk and Barber (1979b) showed that low soil P resulted in a smaller root radius for corn compared with plants grown with the same soil under high P supply. For soybean, Hallmark and Barber (1981) observed a reduction in root radiuses with added P. Severe P stress is likely to reduce overall plant growth. Changes in root morphology and the ability to adapt to low P conditions to improve plant P nutrition seems species specific.

2.1.3 Roles in N_2 Fixation

Phosphorus plays an essential role in the growth and development of plants and is required for the establishment and maintenance of the legume (macro-symbiont) - *Rhizobium* (micro- symbiont) mutualistic symbiosis. The advantage of this intimate relationship to the host plant is that it receives approximately 90% of the nitrogen fixed by

the micro-symbiont (Hansen, 1994). The benefits to the bacteria are that the bacteria are housed in a competition-free environment (nodule) and are supplied with a steady flow of photosynthate from the macro-symbiont.

Phosphorus is essential to the micro-symbiont for life processes (as it is in plants) and is essential for the functioning of nitrogenase, the enzyme complex responsible for the reduction of molecular nitrogen (N_2). Dinitrogen fixation carried out by the micro-symbiont requires a significant amount of energy, which is supplied by the plant. Approximately 16 moles of ATP are required to reduce N_2 to 2 moles NH_3 (Hansen, 1994). Phosphorus is required for the production of ATP and therefore its importance in maintaining N_2 fixation is quite apparent. Legumes that rely on N_2 fixation for their supply of N and are not receiving an adequate supply of P may become N deficient (Marschner, 1995). Sink strength of nodules for P is high. The high sink strength is most likely the result of the high demand of ATP for nitrogenase and because the P concentration of microbial tissue is greater than plant cells (Jakobsen, 1985). Thus, P is maintained at high concentrations within the nodules and is usually greater than the content of roots and shoots on a per unit dry weight basis (Adu-Gyamfi *et al.*, 1989; Graham and Rosas, 1979; Pereira and Bliss, 1987; Robson *et al.*, 1981).

Phosphorus nutrition may influence the process of N_2 fixation at various steps of the symbiotic interaction. The P status of the rhizosphere may affect host plant growth, growth and survival of the bacteria, nodule initiation and development, and nodule function. Phosphorus is essential for optimum growth and development of the host plant. If the host plant is P deficient, this may affect the supply of photosynthate to the nodule,

which fuels the nitrogenase enzyme. Robson *et al* (1981), working with subterranean clover (*Trifolium subterranean* L.) concluded that P increases N_2 fixation by stimulating host plant growth rather than by enhancing rhizobial growth and survival or nodule development and function. This view that P nutrition of the host plant indirectly affects N_2 fixation by its effects on host plant growth is shared by Graham and Rosas (1979) and Jakobsen (1985).

Researchers have observed that P also plays a direct role in rhizobial growth and survival, nodule initiation, and function. Rhizobial growth can be adversely affected by an inadequate supply of P (Beck and Munns, 1984; Gates, 1974). Adequate growth and colonization of the rhizosphere by the bacteria are essential for nodule initiation (Beck and Munns, 1984; Cassman *et al.*, 1981; Gates, 1974; Israel, 1987). Nodule development can be greatly enhanced by P; nodule number and mass have been shown to increase with increased supply of P (Cassman *et al.*, 1981; Dhingra *et al.*, 1988; Gates, 1974; Graham and Rosas, 1979; Pereira and Bliss, 1987). Ultimately, it has been observed that the rate of N_2 fixation can be stimulated by improved P nutrition (Adu-Gyamfi, 1989; Cassman *et al.*, 1981; Dhingra *et al.*, 1988; Graham and Rosas, 1979; Israel, 1987). Further, Sa and Israel (1991) have observed decreased specific-nitrogenase activity in nodules of phosphorus-deficient soybean plants that was associated with decreased energy status (i.e., ATP concentration and energy charge) of host-plant cells of nodules. Israel (1993) observed that decreased nodule function of soybean plants preceded decreased plant growth under P-limiting conditions. Israel (1993) interpreted the effect of altered P supply on symbiotic N_2 fixation to depend on both the indirect effects on host-plant

growth and the direct effects on the metabolic function of nodules.

2.2 Phosphorus in Prairie Soils

Phosphorus is an essential nutrient for plant growth and development. Plant growth can be restricted if the soil supply of plant available P is low and no supplemental fertilizer is added. Although total soil P levels may appear adequate to meet plant requirements, often the P present in the soil is in a form unavailable to plants. Further, when P fertilizer is applied to soils, it can rapidly become unavailable to plants because of P fixation or retention reactions that occur in soil.

2.2.1 Inorganic, Organic, and Solution Phosphorus

Phosphorus may exist in many chemical forms within the soil but can be divided into three general categories: (1) inorganic P compounds, (2) organic P compounds, and (3) soil solution P. Prairie soils are generally high in total P (Doyle and Cowell, 1993a). Unfortunately this does not translate into large quantities of plant-available P (soil solution P). In most soils, P in the plant available forms, rarely exceeds 0.01% of the total P in soils (Brady, 1990).

Soil solution P supplies the plant with soluble P as primary or secondary orthophosphate ions ($H_2PO_4^-$, HPO_4^{2-}) depending on the soil solution pH. The $H_2PO_4^-$ ion is the major form present at pH values less than 7.2, and the HPO_4^{2-} ion at solution pH values above 7.2. Optimum phosphate availability occurs around pH 6.5 (Tisdale *et al.*, 1993). However, since plants can absorb both P forms, the concentration of P in the soil solution is more important to P availability to plants, as is the ability of various P compounds to resupply soil solution P (Tisdale *et al.*, 1993). The soil solution P

concentration is low regardless of whether the soil is acidic, neutral, or alkaline in nature (Barber, 1984). Solution P levels are buffered and replenished through the release of adsorbed P from mineral and clay surfaces (labile P) quite readily; while P from the mineralization of organic P and the dissolution of solid P minerals (non-labile P) will slowly replenish labile P (Barber, 1984; Tisdale *et al.*, 1993). Phosphorus in the soil solution that is not absorbed by plant roots or immobilized by soil microbes can become adsorbed on mineral surfaces or precipitated with soil cations as secondary minerals; further restricting plant availability (Barber, 1984; Tisdale *et al.*, 1993). The P concentration in the soil solution is ultimately controlled by the solubility of inorganic P minerals in soils. Al- and Fe-P minerals are the most common P minerals found in acid soils while Ca-P minerals predominate in neutral, alkaline, and calcareous soils (Barber, 1984; Tisdale *et al.*, 1993).

Organic P compounds generally represent 50% of total P in most soils (Ozanne, 1980). In Canadian soils, the range is between 9 and 54% (Tisdale *et al.*, 1993). Plant and animal residues are the initial source of soil organic P. These residues must be degraded by soil microbes to produce organic compounds that undergo mineralization to release inorganic P. Only 50 to 70% of organic P compounds in soils have been identified (Stewart and McKercher, 1982). Most of these compounds are esters of orthophosphoric acid. The organic P compounds have been classified as inositol phosphates, phospholipid, nucleic acids, nucleotides, and sugar-phosphates. The mineralization of organic compounds is catalysed by the enzyme phosphatase, which is produced by soil microorganisms or is free within the soil (Tisdale *et al.*, 1993).

2.2.2 Phosphorus Fertilizers

Another source of P for plant uptake is phosphate fertilizers. During the first four weeks of crop growth, applied P fertilizer is the primary source of P whereas native soil P is an important source of P later in crop development (Barber and Olson, 1968; Spinks and Barber, 1947). Calcium orthophosphate and ammonium phosphates are the two types of P fertilizers that are currently used in the prairies for crop production (Doyle and Cowell, 1993b). In western Canada, use of monoammonium phosphate ($NH_4H_2PO_4$; 11-55-0) predominates while triple superphosphate {$Ca(H_2PO_4)_2 \cdot H_2O$; 0-45-0} is used to a lesser extent. The fertilizer use efficiency (FUE), the proportion of fertilizer applied which is retained by the crop in a single growing season, for P fertilizer is quite low. The FUE for P fertilizers is less than 25% for band applications and less than 10% for broadcast applications (Tisdale *et al.*, 1993). Low FUE may result from the rapid reactions that occur when water soluble P fertilizers dissolve in moist soil and react with soil constituents, which leads to the formation of less soluble compounds, and with time to highly insoluble forms. These reactions between P fertilizers and soil constituents are referred to as P fixation or retention (Soper and Racz, 1980). Alumina-silicate minerals, soil carbonates, organic matter, and Al and Fe hydrous acids play key roles in P retention (Soper and Racz, 1980). In acid soils, P may be precipitated as iron- or aluminum-phosphates, while in alkaline soils, P may precipitate as calcium- or magnesium-phosphates. Phosphorus may also be chemically bonded to cations at the surfaces of soil constituents (Soper and Racz, 1980). Mechanisms by which soil constituents retain P can be considered special cases of precipitation or adsorption reactions (Sample, Soper and

Racz, 1980).

Because of these retention mechanisms, only a small fraction of the fertilizer remains in the soil solution and available to plants. However, the applied P is not easily lost from the agroecosystem because P has no gaseous phase and is not easily leached (Tisdale *et al.*, 1993). Combined with low FUE of P fertilizers, and the increased use of P fertilizers on the prairies (Doyle and Cowell, 1993b), residual P fertilizer contributes a substantial quantity of P to the total P content of soils.

2.2.3 Plant Available Phosphorus and Plant Absorption

Phosphorus must be in a soluble form ($H_2PO_4^-$, HPO_4^{2-}) to be absorbed by plant roots. The concentration of plant available P in soils is in the order of 0.05 to 1.0 ppm (Ozanne, 1980; Paul and Clark, 1989) and varies widely among soils. However, plants require concentrations between 0.003 and 0.3 ppm depending on crop species and level of production (Tisdale *et al.*, 1993). Therefore, in some situations native soil solution P will not meet crop demand.

The primary mechanism of transport of P from the bulk soil solution to the root is by diffusion (Barber, 1984). Mass flow of P in soil solutions contributes less than 20% of movement to plant roots (Tisdale *et al.*, 1993). As plants absorb P from the surrounding soil solution, a region of phosphate depletion develops adjacently to the root surface (Bhat and Nye, 1973). As a result, a concentration gradient is established in the surrounding soil-water films and P ions diffuse toward the root (Doyle and Cowell, 1993a). Nye and Tinker (1977) concluded that phosphate movement across the depletion zone is diffusion limited and therefore plant uptake is diffusion limited. However, if phosphate

concentrations are high in the soil solution, the rate limiting steps is the uptake of P across the plant root membrane (Nye, 1977).

The cytoplasm of root cells of plants generally contains much higher ionic concentrations than the rhizosphere. To absorb phosphate ions, root cells must surmount a concentration gradient in the order of 10^3 to 10^4 (cytoplasm verse rhizosphere) which is much greater than for most other ions (Bieleski, 1973). The expenditure of metabolic energy for phosphate uptake is considerable (Ozanne, 1980). The exact mechanism of transport of phosphate across the plasmalemma is not known. However, it is believed that phosphate uptake is linked to an ATPase (Barber, 1984). There is evidence supporting an H^+- cotransport mechanism (Ullrich-Eberius *et al.*, 1984) for phosphate uptake and an OH^--antiporter mechanism (Lin, 1979). Experimental evidence of P uptake has been shown to be biphasic (Barber, 1972, Ullrich-Eberius *et al.*, 1984) or multiphasic (Michalík, 1982; Nandi *et al.*, 1987; Nissen, 1974; Nissen, 1996) over a wide range of external solution concentrations (0.001-50 mM P). Whatever the number of experimental phases for P uptake, soil solution concentrations are generally between 1 and 20 μM, which is in the range of the lowest concentration phase (Barber, 1984). Phosphorus uptake by plant root cells from the soil solution will generally follow the Michaelis-Menten equation for ion uptake kinetics (Barber, 1984). The kinetics of P uptake is important for the flux across the root surface and for the movement of P from the bulk soil to the rhizosphere (Jungk, 1996).

Sensitivity analysis of models for phosphate uptake into roots has suggested that phosphate uptake is affected more by changes in soil parameters {initial P concentration in

the soil solution (C_l), buffering power (b), and effective diffusion coefficient (D_e)} than ion uptake properties of the root {maximal influx (I_{max}), concentration in solution where net influx equals 0.5 I_{max} (K_m), concentration in solution where no net influx occurs (C_{min})} (Silverbush and Barber, 1983). However, plant properties that affect root surface area (eg. root length, and diameter) show the greatest sensitivity and therefore can greatly affect the rate of phosphate uptake by the plant (Silverbush and Barber, 1983). Increased phosphate uptake may result from plants that have a finer and longer root system (Silverbush and Barber, 1983). This root system could exploit a greater volume of soil and access more soil solution, which is of great significance in acquiring P because it diffuses slowly in the soil (Nye and Tinker, 1977).

Föhse *et al.* (1988) working with seven different plant species found that P efficiency was related to the uptake efficiency of the plant, which was determined by both root to shoot ratio and the absorption rate per unit of root length (influx). Phosphorus efficiency (external P requirements) may be defined as a percentage of a plant maximum yield produced at a certain level of soil P (Föhse *et al.*, 1988). Species that had low P efficiency had low influx rates and low root to shoot ratios, whereas species of medium to high efficiency had either high influx rates or a high root to shoot ratios. However, the combination of both high influx rate and a high root to shoot ratios was not found in the species evaluated in this study. The P influx rate differed greatly among species under P-deficient soil conditions. Föhse *et al.* (1988) suggested that this indicates that plant species differ in their ability to improve phosphate transport from the soil to the roots and that these differences may be attributed to differences in root morphology (root diameters,

root hairs), mycorrhizal associations, and chemical changes of the root environment.

2.3 Plant Adaptations to Phosphorus-Limiting Soil Conditions

Plant species differ widely in P efficiency and their ability to adapt to P-limiting soil conditions (Christie and Moorby, 1975; Föhse *et al.*, 1988; Mclachlan, 1976; Paynter, 1993). Plants may alter morphological and physiological properties of the root system to improve their P status. Root induced changes in the rhizosphere may also aid in improving P availability and uptake by the plant root.

2.3.1 Alteration of Morphological and Physiological Properties of the Root System

Under field conditions, the total volume of soil exploited by the root system may be as important as the ability of plant roots to absorb phosphate at low concentrations (Asher and Loneragan, 1967). The relative importance of total root length versus uptake efficiency depends on the P supply in the soil. Römer *et al.* (1988) found that the P absorbing root surfaces (root length) of wheat is more important in soils of low P availability, whereas in soils of high P availability, uptake efficiency is relatively more important. Under P-limiting conditions, increased P uptake may result from plants that have a finer and longer root system, with root hairs of the same morphology, as this root system would exploit a greater volume of soil, and increase the likelihood of P uptake (Barber, 1980; Jungk, 1996; Marschner, 1995; Silverbush and Barber, 1983). High uptake efficiency of root hairs results from their perpendicular growth into the soil from the root axis and their small radii (Föhse *et al.*, 1991). Root hairs will contribute to increasing the absorbing surfaces of the root and in accessing a larger volume of soil. Autoradiographic evidence is supporting the fact that the root hair zone depletes soil P

(Bhat and Nye, 1974). Itoh and Barber (1983), using the Cushman model (Barber and Cushman, 1981) to predict P uptake, observed that the addition of the contribution of root hairs to P uptake by plant roots resulted in predicted P uptake values similar to the observed values. However, Bole (1973) found no significant relationship between root hairs of wheat and P uptake of wheat lines differing in root hair length. Another potential way a plant could improve its P status, besides morphological adaptations, is to maintain a low P concentration at the root surface. This may aid in P diffusion to the roots because lowering the threshold concentration increases the diffusion gradient (Paynter, 1993).

2.3.2 Root Induced Changes in the Rhizosphere

Plant roots will generally contact less than 1% of the available P in the soil, and this amount will only supply a small fraction of the plant P requirement (Barber, 1980). Thus, plant roots must modify their soil environment to increase P availability and root uptake. Plant roots may alter conditions in the rhizosphere chemically to increase P availability from organic and inorganic P sources. Changes in P availability in the rhizosphere may result from the alteration of rhizosphere pH, and through root exudation of organic compounds (Marschner, 1995; Nye, 1986). Rhizosphere pH may be affected by the following processes: (1) the imbalance of cation and anion uptake and the coinciding releases of protons (H^+) or hydroxyl (OH^-) or bicarbonate (HCO_3^-); or (2) through CO_2 release from root and microbial respiration, which is hydrated to form carbonic acid (H_2CO_3); or (3) the excretion of low molecular weight organic acids by the root, which can act directly to mobilize phosphate by their dissociation to produce H^+ or indirectly by providing the energy for microbial activity; and (4) through microbial

production of acids (Marschner, 1995; Nye, 1986).

Cation-anion uptake imbalances are thought to be the most important source of pH change in the rhizosphere (Nye, 1986). The alteration of rhizosphere pH that results from cation-anion uptake imbalances is most significantly affected by the form of N supply. Ammonium (NH_4^+) uptake is correlated more with a higher rate of H^+ excretion and the reduction of rhizosphere pH, while nitrate (NO_3^-) uptake is correlated more with a higher rate of HCO_3^- net release or H^+ consumption, and thus an increase of rhizosphere pH (Marschner, 1995). Under neutral soil conditions, a reduction in rhizosphere pH may increase P availability while under acid soil conditions, the opposite is true. However, it has been observed that P deficient rape seedlings can decrease rhizosphere pH and increase P uptake by solubilizing P under nitrate nutrition (Grinsted *et al.*, 1982). The decrease in rhizosphere pH was attributed to H^+ excretion although nitrate was the main form of N taken up by the plant (Hedley *et al.*, 1982a).

Legumes which derive their N by symbiotic N_2 fixation rather than utilization of soil nitrate take up proportionally more cations than anions since the uncharged N_2 enters the root instead of NO_3^- (Nye, 1986). Nitrate normally constitutes more than 50% of the total absorbed anions (Nye, 1986). Therefore, the cation-anion imbalance will likely result in the reduction of soil pH. However, the net excretion of H^+ per unit assimilated N is less than that produced by ammonium-fed plants (Raven *et al.*, 1990).

Other soil processes such as CO_2 release from respiration will have more of an acidifying effect on bulk soil pH than rhizosphere pH, as it will diffuse away from the root through air filled pores unless the soil is completely saturated with water (Nye, 1986).

The significance of organic acid secretion by plant roots or microbes on rhizosphere pH is uncertain. No significant quantities of organic acids have been found in the rhizosphere either because the amounts produced by roots or microbes are small or because they are rapidly metabolized by soil microorganisms (Nye, 1986). However, Bar-Yosef (1996) believes that H^+/OH^-, HCO_3^- and citrate exudation at similar rates that occur under field conditions may induce a 2 to a 3-fold increase in P solution concentration in the rhizosphere. The means by which organic acids increase P availability are not confined to lowering rhizosphere pH. Citric and malic acid and phenolics can form relatively stable chelates with Fe^{3+} and Al^{3+} and may increase the solubility of P (Marschner, 1995). In calcareous soils, P availability may be increased by lowering the Ca^{2+} concentration through chelation and formation of sparingly soluble salts such as calcium citrates (Marschner, 1995). Organic acids may also compete with orthophosphate on common absorption sites and may also modify soil surface characteristics, which may increase P availability (Bar-Yosef, 1996).

Plants may also use organic P sources to improve their P situation. Organic P compounds generally represent about 50% of total P in soil (Ozanne, 1980). The hydrolysis of organic P compounds may result from root-borne acid phosphatases to release inorganic P. The release of acid phosphatases by roots has been shown to increase under P deficient conditions (Hedley *et al.*, 1982b; Tadano *et al.*, 1993). However, Hedley *et al.* (1982b) found no significant evidence of the hydrolysis of organic P by P-deficient *Brassica napus* var. Emerald seedlings even though acid phosphatase was released by the roots. The authors concluded that the increased acid phosphatase activity

was a consequence of root growth and low P supply but does little to enhance P uptake during short periods of growth.

2.4 Roles of Soil Microorganisms in Plant Nutrition and Growth Promotion

Plant roots have various ways in which to improve the P status of plants. One way plants can improve their P situation is through the development of a symbiotic relationship with fungi, known as mycorrhizae. Mycorrhizal associations are important for P uptake in many vascular plants. In species which have been investigated, mycorrhizas occur in 79% of monocotyledonous and 83% of dicotyledonous plants and all gymnosperms are mycorrhizal (Wilcox, 1996). Suggested mechanisms by which mycorrhizal fungi increase the uptake of P by plants are: the exploration of a larger volume of soil by the fungal hyphae, faster movement of P into the fungal hyphae, and through solubilization of soil P by the fungi (Bolan, 1991). Other free living soil-borne fungi, and bacteria may also increase the availability of P to plants and may influence plant growth more directly.

Free-living and symbiotic soil bacteria that enhance plant growth are often referred to as plant growth promoting rhizobacteria (PGPR) (Kalpulnik, 1996). Rhizobacteria is used to refer to bacteria which colonize plant roots. However, the exact mechanism (s) of how soil microorganisms enhance plant growth and P nutrition is unclear.

A large proportion of plant net photosynthetic carbon is released into the rhizosphere (the soil adjacent to the roots, which is influenced by the presence of the root) and may support the growth of rhizospheric microorganisms (Marschner, 1995). The population density of soil microbes, especially bacteria, in the rhizosphere is considerably higher than the bulk soil (Tinker, 1980). Rhizosphere microorganisms may promote plant

growth indirectly by influencing the rhizosphere environment, or directly by the production of plant growth regulators (Kalpulnik, 1996). The ways in which rhizosphere microorganisms may promote the uptake of P by plant roots include: alteration of root growth and morphology (in particular root length, and root hair length and density), altering the influx kinetics of part of or the whole root surface, increasing the concentration of P in the soil solution near the root surface, and by facilitating the transport of P to the root surface for absorption (Barber, 1984; Marschner, 1995; Tinker, 1980).

2.4.1 Microorganisms that Increase the Availability of Phosphorus

Most of the early research with soil microorganisms focused on increasing the availability of P in soil for plant root uptake. Most of this work was likely prompted after Gerretsen (1948) showed that the uptake of phosphate by plants growing in sterilized sand containing insoluble P compounds was increased by the addition of unsterilized soil. An explosion of investigations on the distribution of organisms capable of releasing phosphate from organic and inorganic phosphorus sources, isolated from many soil types, rhizospheres, and from seeds, followed. Numerous phosphate-solubilizing (PS) bacteria and fungi were characterized and evaluated in terms of their ability to solubilize organic and inorganic P (Banik and Dey, 1981; Chonkar and Subba-Rao, 1967; Duff *et al.*, 1963; Greaves and Webely, 1965; Katznelson and Bose, 1959; Katznelson et al., 1962; Kucey, 1983; Martin, 1973; Molla *et al.*, 1984; Paul and Sundaro Rao, 1971; Raghu and Macrae, 1966; Ralston and McBride, 1976; Sperber, 1957, 1958a, 1958b; Taha *et al.*, 1969; Thomas *et al.*, 1985; Venkateswarlu *et al.*, 1984). A number of the isolates were shown

to produce organic acids in pure culture and in some cases, a reduction in media pH and an increase in titratable acidity was observed (Banik and Dey, 1981, 1982; Cunningham and Kuiack, 1992; Duff *et al.*, 1963; Sperber, 1958b; Taha *et al.*, 1969; Venkateswarlu *et al.*, 1984). Some researchers have proposed that the major means by which microorganisms solubilize insoluble P compounds is through the production of the organic acids, which could act directly to dissolve P, or act through chelation of the cationic partners of the phosphate ion (Agnihotri, 1970; Banik and Dey, 1981; Duff *et al.*, 1963; Katznelson and Bose, 1959; Sperber, 1958b). Alteration of P concentration by pH change, organic acid production, or through chelation is thought to be unlikely to occur in soil because of the soil buffering capacity (for H^+, phosphate, and cations) and because the supply of cations to be chelated is very large (Tinker, 1984). However, other researchers suggest that the mode of action of microorganisms are not completely related to solubilization of P by organic acid production and that these microorganisms may promote plant growth by other mechanisms (Banik and Dey, 1982; Barea *et al.*, 1975; Brown, 1974; Kundu and Gaur, 1984; Ralston and McBride, 1976; Thomas *et al.*, 1985; Tinker, 1980; Yoshikawa *et al.*, 1993). Some attention was also focused on microorganisms capable of releasing inorganic P from organic P sources (Casida, 1959; Greaves and Webley, 1965; Martin, 1973; Molla *et al.*, 1984). The hydrolysis of organic P by microorganisms is mediated by fungal acid and alkaline phosphatases and bacterial alkaline phosphatases (Marschner, 1995). The most notable organism thought to possess the ability to solubilize organic P compounds was *Bacillus megatherium* var. *phosphaticum*. This organism was used in the Soviet Union as a bacterial fertilizer called Phosphobacterin

(Brown, 1974). Plant growth responses to *B. megatherium* have been observed, but it is unlikely that the promotion of plant growth was a result of mineralization of organic P compounds (Brown, 1974). Martin (1973) utilizing ^{32}P, found that rhizosphere flora, or bacterial isolates shown to possess phytase activity, did not affect the incorporation of ^{32}P into soil grown wheat plants.

2.4.2 Microorganisms that Promote Root Growth

Soil microorganisms may stimulate, inhibit or not affect root growth depending on the type of microbe, plant species, and the environmental conditions (Marschner, 1995). Most of the current research with soil microbes that promote plant growth focuses on rhizobacteria, more specifically, diazotrophic bacteria. These N_2 fixing PGPRs include *Azospirillum*, *Azotobacter*, and *Pseudomonas* species. Rhizobacteria may promote plant growth by altering root growth and morphology or by influencing the influx kinetics of part of or the whole root surface to improve water and mineral nutrient acquisition by the plant (Barber, 1984; Marschner, 1995; Tinker, 1980). Rhizobacteria are thought to promote plant growth either directly or indirectly. These bacteria may affect plant growth directly through the production of plant growth regulating substances, which may enhance root growth or indirectly by suppressing root pathogens, thereby creating a more favourable environment for root growth.

Rhizobacteria have been shown to reduce the disease severity caused by soil borne root pathogens (Reddy *et al.*, 1993; Scher and Baker, 1980; Suslow and Schroth, 1982; Weller, 1988). Because of their ability to reduce plant disease, these organisms are often referred to as bacterial biocontrol agents. These PGPRs may improve plant growth by

suppressing both major and minor plant root pathogens (Weller, 1988). Promotion of plant growth through suppression of disease by rhizobacteria may involve direct or indirect effects on the pathogen. Direct mechanisms include substrate competition and niche exclusion, siderophore release, and production of antibiotics, whereas indirect effects involve alteration of plant defence responses (Weller, 1988). However, rhizobacteria may use more than one mechanism to suppress a pathogen.

Aggressive colonization of the root system by PGPRs may result in the displacement or exclusion of deleterious microbes (Schroth and Hancock, 1982). Competition for nutrients in the rhizosphere (root exudates) likely occurs between PGPRs and deleterious microbes (Weller, 1988). Thus, occupation of nutritionally favourable sites by PGPRs may aid in biocontrol of plant pathogens. Rhizobacteria may also exclude plant pathogens by releasing siderophores. Siderophores are defined as low molecular weight, mostly Fe (III)-specific ligands, that act as scavenging agents for Fe (Neilands and Leong, 1986). Virtually all fungi and most bacteria have been found to form siderophores (Neilands and Leong, 1986). Rhizobacteria may release siderophores to acquire Fe for their growth and may exclude deleterious microbes from the rhizosphere and rhizoplane (root surface) by limiting the supply of Fe, because the pathogens do not produce siderophores in sufficient quantities, or their siderophores have less affinity for Fe than those produced by PGPRs (Schroth and Hancock, 1982).

Production of microbial compounds that have antibiotic activity may be a major component in suppression of plant disease (Bull *et al.*, 1991; Suslow and Schroth, 1982; Weller, 1988). Bull *et al.* (1991) found phenazine-1-carboxylic acid to be the major factor

in disease suppression of take-all of wheat (*Gaeumannomyces graminis* var. *tritici*). Bacterial cyanide production has also been implicated in the suppressive effect of PGPRs (Voisard *et al.*, 1989; Wei *et al.*, 1991).

Antagonism of the plant pathogen is not the only way PGPRs may reduce the incidence of disease. Bacterial enhancement of plant host resistance to phytopathogens is another way in which rhizobacteria may promote plant growth. Protection may result from induction of systemic resistance through mechanisms that include accumulation of antimicrobial compounds such as phytoalexin, and/or the formation of lignin, callose, and hydroxyproline-rich glycoproteins (Kalpulnik, 1996). Seed and root inoculation with PGPRs has been shown to induce systemic resistance, which may act to reduce the severity of the disease (Alstrom, 1991; van Peer *et al.*, 1991; Wei *et al.*, 1991). Antagonism of deleterious soil microbes by Fe-chelating siderophores, antibiotics and hydrogen cyanide produced by PGPRs and competition for favourable sites have been shown to reduce plant disease and thus creating a more favourable environment for root growth.

The production of plant growth regulating compounds by PGPRs may also aid in promoting plant growth. Lifshitz *et al.* (1987) observed inoculation of canola (*Brassica campestris*) seeds with a N_2 fixing strain of *Pseudomonas putida* dramatically increased the root length of seedlings under gnotobiotic conditions suggesting that PGPRs may not promote growth strictly by antagonistic interactions. The effect was not caused by improved N status of the plant. The bacterial effect on root growth consistently promoted an increase in the uptake of ^{32}P. The authors came to two possible explanations for their

results. The stimulation of root elongation could be a result of a hormone-like factor, which then resulted in the increased nutrient and water absorptive capacity of the plant, or the bacteria increased the P uptake capacity of the plant, which then stimulated root elongation. Lin *et al.* (1983) observed increased uptake of NO_3^-, K^+, and $H_2PO_4^-$ in 3 to 4 day and 2 week old corn root segments inoculated with *Azospirillum brasilense*, but no changes in root morphology were observed. The mechanism of increased ion uptake by *A. brasilense* inoculated plants is unknown but is thought to be a result of phytohormone production. Other researchers have observed increased root elongation, root hair length and number as a result of *A. brasilense* inoculation, which was not related to improved N status (Hadas and Okon, 1987; Kalpulnik, *et al.*, 1985). It is believed that these changes in root morphology are the result of phytohormone production, in particular, auxin production (Kapulnik, 1996; Marschner, 1995). Many rhizobacteria are known to produce auxins, gibberellins, and cytokinins in culture media with or without added precursors (Barea *et al.*, 1975; Barea and Brown, 1974; Brown, 1972, 1974; Leinhos and Vacek, 1994). The ability of rhizobacteria to produce these active substances may contribute to their ability to promote plant growth. Rhizosphere microorganisms may possess various mechanisms to promote plant growth and some of them may yet to be discovered.

2.5 *Penicillium bilaii*

Penicillium bilaii (ATCC strain no. 20851) (formerly *P. bilaji)* is a rhizospheric fungus isolated from a southern Alberta soil by Kucey (1983). This fungus was selected for its superior ability to solubilize precipitated calcium phosphate. *Penicillium bilaii*

solubilized P from rock phosphorus (rock P) at rates two to four times greater than other isolates, grew actively on a wide range of nutrient sources, and maintained its ability to solubilize phosphate at temperatures as low as 4^0 C (Gleddie, 1992; Kucey, 1983). Unlike other phosphorus-solubilizing (PS) microorganisms, *P. bilaii* retained its PS ability over repeated subculturing. This fungus has been shown to grow and survive under field conditions (including the ability to survive the winter) and does not move below 10 cm in the soil to any great extent (Keyes, 1990).

The ability of *Penicillium bilaii* to solubilize phosphate was determined by the dissolution of precipitated calcium phosphate in an agar medium (Kucey, 1983) and through solubilization of rock phosphate in liquid culture (Asea *et al.*, 1988). Microorganisms which possess PS ability are identified by their ability to dissolve calcium phosphate and apatite in pure culture (Sperber, 1958a; Katznelson and Bose, 1959). The acidic or chelating metabolites possibly involved in *P. bilaii*'s ability to solubilize phosphate was investigated by Cunningham and Kuiack (1992). These researchers revealed that the major acidic metabolites produced by *P. bilaii* in a sucrose nitrate liquid medium was oxalic and citric acid. Promotion of oxalic acid occurred under carbon-limited conditions, while citric acid was promoted under nitrogen-limited conditions. Other PS microorganisms which produce oxalic and citric acid in pure culture have been reported (Banik and Dey, 1982; Sperber, 1958b). Researchers have attributed the PS capability of microorganisms to the secretion of organic acids that directly dissolve phosphatic materials and/or chelate cationic partners of the phosphate ion (Agnihotri, 1970; Banik and Dey, 1982; Duff *et al.*, 1963; Katznelson and Bose, 1959; Sperber,

1958b). Asea *et al.* (1988) concluded that inorganic P solubilization was directly related to the drop in pH generated by *P. bilaii*. However, the quantity of P present in the media could not be attributed to acidity alone. *Penicillium bilaii* was able to release more P from Idaho rock phosphate than released by 0.1 M HCl added to achieve equivalent media pH levels. Other researchers have reported the lack of correlation between PS microorganisms' ability to acidify the surrounding media with the ability to solubilize inorganic phosphate (Gaur *et al.*, 1973; Surange, 1985). Kucey (1988) showed that inoculation with *P. bilaii* had the same solubilizing effect using a solution containing insoluble copper and zinc compounds as 0.05 M EDTA and at a level greater than 0.1 M HCl at pH equal to 4.

Penicillium bilaii is thought to solubilize precipitated phosphate by secreting organic acids thereby increasing the availability of soluble phosphate to the plant. Organic acids could act as direct proton donors to aid in the solubilization of precipitated phosphate and/or act as chelating agents binding cationic partners (eg. Ca^{2+}) thereby shifting the soil equilibrium toward the solubilization of phosphate, if these organic acids were produced *in situ* (Kucey, 1988; Kucey *et al.*, 1989). No reports have identified *in situ* production of organic acids or antibiotics by *P. bilaii*. It is the action of the organic acids that are believed to result in increased phosphorus uptake, dry matter production, and grain yields of various crop plants.

Crop responses to *P. bilaii* inoculation are generally greater on soils with low available P and in treatments without added phosphatic fertilizer (Asea *et al.*, 1988; Gleddie *et al.*, 1991; Kucey, 1987, 1988; Kucey and Leggett, 1989). This would be

expected if increased P availability is the primary mechanism of response to *P. bilaii* inoculation, since if P is otherwise available to the plant, the plant would be less dependent on solubilized P and the net effect of inoculation would be less (Gleddie, 1992). However, Chambers (1992) in field and growth chamber studies, observed increased dry matter production of wheat with *P. bilaii* inoculation, and the increases were generally greater at the higher rates of added P fertilizer. This increase in dry matter production was not associated with increased P content of the plant suggesting that *P. bilaii* may promote plant growth by other mechanisms. If *P. bilaii* was solubilizing unavailable forms of P in the rhizosphere, thereby increasing the P concentration in the soil solution, it would be expected that the response to inoculation with *P. bilaii* would be similar to that obtained from added P fertilizer. At higher rates of P fertilizer, one would expect that the effects of *P. bilaii* on plant growth would be diminished or equal to those produced by P fertilizer alone. Gleddie (1992) also observed increased dry matter production and P uptake of pea plants under P-responsive field conditions at 2 weeks after emergence at all levels of added P fertilizer, and concluded that *P. bilaii* may affect plant growth by other mechanisms besides solubilizing P. Work by Keyes (1990) also suggests that *P. bilaii* may promote plant growth by mechanisms other than increased P availability. Keyes found that dry matter yield of canola grown on an acidic chernozemic soil was consistently greater with inoculation of *P. bilaii* but P uptake was not promoted. Inoculation with this fungus did result in greater Fe and Mn concentrations in canola.

2.5.1 Crop Plant Responses to Inoculation with *Penicillium bilaii*

2.5.1.1 Wheat

Several researchers have investigated the effect of *P. bilaii* inoculation on plant P uptake, dry matter production, and grain yield. In-furrow application of bran inoculated with *P. bilaii* and seed treatment has been shown to increase dry matter production and P uptake of wheat (*Triticum aestivum* L.) in controlled environment and field experiments (Chambers, 1992; Gleddie *et al.*, 1991; Kucey, 1987, 1988). Under field conditions, treatments consisting of rock phosphate (rock P) plus *P. bilaii* resulted in wheat yields and P uptake equivalent to increases due to the addition of monoammonium phosphate added at an equivalent rate of P (Kucey, 1987, 1988). Kucey (1987) found that *P. bilaii* alone or in combination with straw also affected grain and straw dry weights. *Penicillium bilaii* alone affected P content of wheat, however when added in combination with rock P there was no effect. Gleddie *et al.* (1991) found that seed inoculation with *P. bilaii* increased grain yields compared with uninoculated P fertilizer control treatments in over more than 55 trials established in western Canada in 1988-1990. As P fertilizer rates approached 30 kg P_2O_5 ha^{-1}, yield increases from inoculation with *P. bilaii* generally decreased. Grain yields of inoculated plants at 10 and 20 kg P_2O_5 ha^{-1} were equivalent to those of the uninoculated control plants at 20 and 30 kg P_2O_5 ha^{-1} respectively. Goos *et al.* (1994) observed increased grain yields of 66 kg ha^{-1} averaged over four sites. However, early crop growth was not enhanced nor was P uptake increased.

In a field study conducted by Chambers (1992), plant samples were taken throughout the growing season to observe how *P. bilaii* affected plant P uptake. At all sites, in one year of the study, *P. bilaii* inoculation resulted in increased dry matter production in early season growth (1, 2, and 4 weeks after emergence) of wheat. This

increase in dry matter production was not associated with increased P content of the plant, suggesting that the fungus may have additional effects to increasing the availability of P to the plant. Further, the increased dry matter production was greater at the higher rates of P fertilizer. However, at 2 sites, inoculation with *P. bilaii* resulted in increased P content of the plant at the eight week (after emergence) sample period and the grain. Increased dry matter production at 8 weeks, at all site years, was observed with *P. bilaii* inoculation and the increases were generally greater at the higher rates of added P fertilizer. These results are in contrast to Kucey (1987) and Gleddie *et al.* (1991) who reported the best response to *P. bilaii* inoculation occurred at the lower rates of P fertilization. Yield responses decreased as the rate of P fertilizer increased. A combined site analysis showed an overall increase in grain yields across all fertilizer rates of 172 kg ha^{-1} or 5.9% greater than non-inoculated treatments.

In a controlled environment study utilizing ^{32}P, Chambers (1992), found that inoculation of wheat with *P. bilaii* generally did not affect the concentration of P in the plants. At one sampling date (1 week after emergence) on one soil type, *P. bilaii* increased the P concentration in the plants as a result of an increased proportion of P derived from the soil. Inoculation with *P. bilaii* resulted in increased dry matter production at most of the sampling dates for all rates of P fertilizer added on both soil types. Inoculation of wheat with *P. bilaii* did not affect the P concentration in the grain and did not affect the contribution of fertilizer or soil P to the total amount of P in the grain. The grain yields averaged for both soils were increased by 9%. In a greenhouse experiment, Asea *et al.* (1988) reported *P. bilaii* inoculation resulted in a 14% increase in

total plant P uptake (early heading stages). This was attributed to the increase of the proportion (11%) of P derived from native P sources even in the presence of added rock P. Inoculation also resulted in increased plant dry matter yields of 16%. Kucey (1987, 1988) using controlled conditions observed increased dry matter production and P uptake of inoculated plants both with and without added rock P fertilizer. Inoculation with *P. bilaii* also increased the $NaHCO_3$-extractable P in potted soils and the incidence of P-solubilizing fungi in the rhizosphere (Kucey, 1988). *Penicillium bilaii* plus rock P at 45 μg of P g^{-1} of soil resulted in plant dry matter production and P uptake by wheat that was not significantly different from increases resulting from the addition of 15 μg of P g^{-1} of soil as triple superphosphate (TSP) (Kucey, 1987).

2.5.1.2 Canola

Kucey and Leggett (1989) conducted greenhouse and field experiments using in-furrow application of bran inoculated with *P. bilaii*. Inoculation with this fungus resulted in increased canola (*Brassica napus* L.) plant yield and P uptake. Addition of P at a rate of 20 mg P kg^{-1} soil as Florida rock P, plus inoculation with *P. bilaii*, resulted in P uptake by canola nearly equivalent to that resulting from the addition of monoammonium phosphate (MAP) alone at the same rate, under controlled conditions. Inoculation however did not affect straw or pod dry matter production. Canola P concentrations were increased with addition of *P. bilaii* in the unfertilized control, and in combination with rock P (20 mg P kg^{-1} soil), and MAP (10 mg P kg^{-1} soil), but was not affected by the addition of P fertilizer alone. In the field study, inoculation with *P. bilaii* at both sites increased canola seed yields with and without the addition of P fertilizer. Seed P content

at one site was increased by *P. bilaii* inoculation in the unfertilized treatments, while at the other site, inoculation increased the oil content of the canola seeds in treatments receiving 6.1 kg P ha^{-1} as MAP, and in the rock P treatment (12.2 kg P ha^{-1}). The greatest effects observed in both the greenhouse and at one field site as a result of *P. bilaii* inoculation, were when no P fertilizer was added. The authors suggested that this would be expected since the favoured theory of how *P. bilaii* increases plant yields is by increasing P availability to the plant by accessing sources of P, which would generally be unavailable to the plant. In field trials established in western Canada, Gleddie *et al.* (1993) observed increased vegetative growth, P uptake, and grain yields of canola with in-furrow granular or seed inoculation with *P. bilaii*. These increases were apparent with or without application of P fertilizer. Increased P uptake was largely a result of the greater dry matter production of inoculated plants. Inoculation with *P. bilaii* at 10 kg P_2O_5 ha^{-1} resulted in equivalent P uptake and grain yield compared with an uninoculated treatment at 20 kg P_2O_5 ha^{-1}. Keyes (1990) under field conditions observed that dry matter and seed yields were consistently greater with *P. bilaii* inoculation but P uptake was not affected. Inoculation however did result in increased Fe and Mn concentrations.

2.5.1.3 Flax

Chambers (1992) in a growth chamber study, utilizing ^{32}P, found *P. bilaii* inoculation resulted in a greater contribution of soil-P to the total amount of P in flax (*Linum usitatissimum* L.) the early season growth (1, 2, and 4 weeks after emergence). However, no effect on plant dry matter production was observed with *P. bilaii* inoculation. Inoculation with *P. bilaii*

increased P concentration in the grain over the non-inoculated flax. The increase in P concentration in the grain was attributed to the greater contribution from soil P. However, the increased P concentration in the grain as a result of inoculation did not translate into increased grain yields.

2.5.1.4 Legumes

In a greenhouse experiment, Kucey (1987) observed inoculation with *P. bilaii* along with rock P at 45 μg P g^{-1} of soil resulted in field bean (*Phasseolus vulgaris* L.) plant dry matter production and P uptake that was not significantly different from the addition of 15 μg P g^{-1} of soil as triple superphosphate (TSP). Rock phosphate addition alone did not affect plant growth. The addition of *P. bilaii* alone did not affect dry matter production or P content of field beans.

Inoculation with *P. bilaii* has been shown to increase vegetative growth, P and N uptake of pea (*Pisum sativum* L.) under controlled environment conditions, and to increase vegetative growth, P and N uptake and grain yields of pea and lentil under P responsive field conditions (Gleddie, 1993). Increases in P uptake occurred both with and without P fertilizer addition. Increased root to shoot ratios of inoculated pea plants was observed under controlled conditions. Gleddie (1993) reported *P. bilaii* with no additional P fertilizer resulted in equivalent P uptake in pea plants receiving 10 mg P kg^{-1} soil in the controlled environment study and 10 kg P_2O_5 ha^{-1} in the P responsive field trials. *Penicillium bilaii* inoculation resulted in increased nodulation and N uptake of pea in the controlled environment study and increased N uptake of pea and lentil in the field study. Grain yields of pea or lentil inoculated with *Rhizobium leguminosarum* bv. *viciae* was

increased or not affected by the inoculation with *P. bilaii*. These results are not in agreement with those of Downey and van Kessel (1990). These researchers observed in a controlled environment study a decrease in pea dry matter production and total N accumulation as a result of the inoculation with *P. bilaii* in combination with *R. leguminosarum* bv. *viciae* (no additional P fertilizer). However, *P. bilaii* inoculation alone increased dry matter production 22% over the control. The reduction in N_2 fixation as a result of the addition of *P. bilaii* was proposed to be a result of the production of organic acids by the fungus, which would adversely affect nodulation. The *P. bilaii* plus *R. leguminosarum* bv. *viciae* treatment in this study had a lower dry matter production, but the %NDFA was not different from the *Rhizobium* only treatment. The addition of P fertilizer to this treatment, *P. bilaii* in combination with *R. leguminosarum* bv. *viciae*, produced similar results to the *R. leguminosarum* bv. *viciae* plus P fertilizer treatment. Therefore it is questionable that *P. bilaii* adversely affected the *Rhizobium*-legume symbiosis.

2.6 Summary

Phosphorus plays many vital roles within the plant and is essential for the *Rhizobium*-legume symbiosis. Although prairie soils are generally high in total P, the amount of plant available P is often limited (Brady, 1990; Doyle and Cowell, 1993a). Therefore, plants have developed various mechanisms to overcome P-limiting soil conditions. Mycorrhizae and other soil fungi and bacteria appear to play an important role in the P nutrition of plants and may influence plant growth more directly. *Penicillium bilaii* is a rhizospheric fungus thought to solubilize precipitated phosphate by secreting

organic acids thereby increasing the availability of soluble phosphate to the plant and thus promoting plant growth. *Penicillium bilaii*'s abilities to promote plant growth, P uptake, and dry matter production have been demonstrated. However, researchers have observed that *P. bilaii* promotes plant growth without increasing phosphorus uptake and that the effect of the fungus was more pronounced at all and/or higher rates of P fertilization (Chambers, 1992; Gleddie, 1992; Goos *et al.*, 1994; Keyes, 1990). This suggests that *P. bilaii* may promote plant growth via other mechanisms.

3. Effect of *Penicillium bilaii* on Root Morphology of Pea (*Pisum sativum* L.) - Controlled environment study

3.1 Introduction

Although prairie soils are often high in total phosphorus (P), only limited quantities are available to plants (Doyle and Cowell, 1993a). The soil solution supplies P in a soluble form ($H_2PO_4^-$, HPO_4^{2-}) for plant root uptake. However, the availability of soil solution P may be limited as P may precipitate with soil cations as secondary minerals or be adsorbed onto mineral surfaces (Barber, 1984).

Plants have various ways in which to adapt to P-limiting soil conditions. Sensitivity analysis of models for phosphate uptake into plant roots has indicated that plant properties that affect root surface area (eg. root length and diameter), can greatly affect the rate of phosphate uptake by the plant (Silverbush and Barber, 1983). Increased phosphate uptake may result from plants that have a finer and longer root system. Soil microorganisms may promote plant growth by altering root growth and morphology or by influencing the influx kinetics of part of or the whole root surface to improve water and mineral nutrient acquisition by the plant (Barber, 1984; Marschner, 1995; Tinker, 1980).

Penicillium bilaii (ATCC strain no. 20851) is a rhizospheric fungus reported to increase dry matter production, grain yield, and P uptake of wheat, canola, bean, pea, and lentil in growth chamber and field experiments (Asea *et al.*, 1988; Chambers, 1992; Gleddie, 1993; Gleddie *et al.*, 1991, 1993; Kucey, 1987, 1988; Kucey and Leggett, 1989). The mechanism (s) underlying the stimulation of plant growth and P-uptake is not known.

The fungus has been shown to solubilize calcium phosphate in an agar medium (Kucey, 1983), and rock phosphate in liquid culture (Asea *et al.*, 1988). In potted soils, *P. bilaii* inoculation increased the $NaHCO_3$-extractable P and the incidence of P-solubilizing fungi in the rhizosphere (Kucey, 1988). The major acidic metabolites produced by *P. bilaii* are oxalic and citric acid (Cunningham and Kuiack, 1992). This indirect evidence suggests that *P. bilaii* may increase the availability of phosphate to the plant by releasing organic acids, which may act to acidify localised areas of the rhizosphere and/or act as a chelator of cationic partners of the phosphate anion (Kucey, 1988). However, alternative evidence suggests *P. bilaii* may stimulate plant growth by other mechanisms (Chambers, 1992; Downey and van Kessel, 1990; Gleddie, 1992; Keyes, 1990).

The objective of this study was to determine if the stimulation of P uptake in pea (*Pisum sativum* L.) treated with *P. bilaii* is the result of a generalized stimulation of plant root growth. This stimulation of root growth would result in a greater root surface area to access soil phosphorus. We also determined the effect of this fungus on assimilate partitioning (P, dry matter) and nodulation of pea.

3.2 Materials and Methods

3.2.1 Soil and Pot Preparation

Two growth room experiments were carried out on pea (*Pisum sativum* L. cv. Express), one using a Hochfeld (high in available P) and another using a Malmo (low in available P) soil. These experiments had a completely randomized design with factorial (2 X 3) treatment structure. The experiments had two factors, *P. bilaii* and fertilizer type, with two levels (inoculated, non-inoculated) and three levels of P (no fertilizer, 17.2 mg P

or 40 mg P_2O_5 kg^{-1} soil as triple superphosphate, and 17.2 mg P or 40 mg P_2O_5 kg^{-1} soil equivalent of Idaho rock phosphate) respectively, resulting in six treatments with six replications.

Surface soil (0-15 cm) was collected near Carmen MB (49° 28' N, 98° W) (N 0.5-23-T6-R5-W) for the Hochfeld soil and near Ellerslie AB (53° 22' N, 113° 28' W) (NE-24-T51-R25-W4) for the Malmo soil. The characteristics of the soils are presented in Table 3.1. All analyses of the soils were carried out by NorWest Labs (Winnipeg, MB). Surface soil (0-15 cm) was used for analysis of the Malmo soil, while soil core samples (0-30 cm, 30-60 cm) were used for the Hochfeld soil. Soil texture was determined by the hand-texturing method (by feel and rolling). Organic matter (%) was determined by loss on ignition. Soil pH was determined using a 1:2 soil water paste and pH meter (Kalra, 1995). Soil electrical conductivity (E.C.) was determined using a saturated paste and conductivity meter. Soil nitrogen (NO_3-N) was determined using a $CaCl_2$ extract and analysis for nitrate and nitrite by automated colorimetry (Manual Soil Sampling, 1978). Soil phosphorus (P) was determined by ammonium acetate/acetic fluoride extract and analysis for phosphate by automated molybdate colorimetry (Ashworth and Mrazek, 1995). Soil potassium (K) was determined by ammonium acetate/acetic fluoride extract and analysis by flame photometry (Ashworth and Mrazek, 1995). Soil sulphate (SO_4-S) was determined using a $CaCl_2$ extract followed by analysis for sulphate by methyl thymol blue automated colorimetry (APHA, 1992).

Table 3.1. Characteristics of two soils collected from Carman MB (Hochfeld) and Ellerslie AB (Malmo).

Soil name	Soil group (Chernozemic)	Surface texture*	Field capacity	Organic matter	pH	E.C.	NO_3-N	P	K	SO_4-S
			----------%----------			-mS cm^{-1}	----------mg kg^{-1} ----------			
Hochfeld	Orthic black	FSL	20	3.3	5.6	0.6	41	36	250	10
Malmo	Black	CL	33	9.6	6.4	0.2	7	5	74	3

*FSL = fine sandy loam
*CL = clay loam

The soil was collected, air dried, and sieved (2 mm) and stored at 4^0 C until it was used. The soil and fertilizer were mixed in a custom-made mixer (similar to a cement mixer) to obtain an even distribution of fertilizer. Soil that did not receive any fertilizer was also mixed. Rock phosphorus (rock-P) and triple superphosphate (TSP) were analysed for P (P_2O_5) content using sulfur wet digestion (Kingston and Jassie, 1988) and were determined at 14.37% P (33.42% P_2O_5) and 20.87% P (48.53% P_2O_5) respectively. The fertilizers were ground to a consistent powder form using a mortar and pestle. Fertilizers were added at a rate to obtain 17.2 mg P kg^{-1} soil (40 mg P_2O_5 kg^{-1} soil). This was achieved by adding 0.1197 g rock-P fertilizer kg^{-1} soil and 0.0824 g TSP fertilizer kg^{-1} soil and mixing for 5 m.

Two (Hochfeld) or 1.95 (Malmo) kg of soil was placed into 2 L pots constructed from polyvinyl chloride pipe (10 cm diameter, 26 cm length). The Malmo soil was deficient in K and S (Table 3.1) so 10 ml of a solution containing 0.28 M of K and 0.14 M S was added to each pot. Pots were watered, randomly placed into the growth chamber, and the soil was given a week to equilibrate.

Gravimetric field capacity was determined for both soils. The procedure for the determination of gravimetric field capacity of the soils is described briefly as follows: the sieved (2 mm) soil was placed into beakers at the same consistency used for the pots. Distilled water was slowly poured onto the surface of the soil, to prevent the water from running down the sides of the beaker, until the upper portion of soil was saturated with water. The wetting front was marked and the beaker was sealed with parafilm to prevent water loss by evaporation. After 48 hours, the top 2 cm of soil was removed, and a

sample was then taken between the surface and the wetting front. The sample was weighed and then oven-dried for 48 hours. The amount of water held by the sample was determined by the difference between the moist and dried sample. Field capacity (%) was determined by dividing the weight of water by the oven-dried sample weight and multiplying by 100 to give a percentage. Field capacity was determined to be 20% for the Hochfeld soil and 33% for the Malmo soil.

During the period that the soil was given to equilibrate (a week prior to seeding), the pots were watered by weight every second day to maintain gravimetric field capacity (Hochfeld soil) or 70% field capacity (Malmo soil).

3.2.2 Seed Preparation and Planting

All pots were watered prior to seeding. Pea (*Pisum sativum* L. cv. Express) seeds were exposed to 0.5% hypochlorite solution for 5 m and rinsed thoroughly with distilled water. In the experiment using the Malmo soil, two weeks prior to seeding the soil was inoculated with 2 ml pot^{-1} of yeast mannitol broth containing a minimum of 3×10^9 colony forming units (CFU) ml^{-1} of *Rhizobium leguminosarum* bv *viceae* (Hup^- strain 128A1, Liphatech, Milwaukee) and treatments including *Penicillium bilaii* (ATCC strain no. 20851) received 2 ml pot^{-1} of a diluted inoculum, supplied by Philom Bios Inc., Saskatoon SK, containing a minimum of 6.4×10^4 CFU ml^{-1}. Three seeds per pot were placed into holes dug to 1.25 cm from the soil surface. All seeds were inoculated with 1 ml of a yeast mannitol broth containing a minimum of 8.4×10^8 CFU ml^{-1} of the same *Rhizobium* strain mentioned previously, as determined by dilution plating. Seeds which received the *P. bilaii* treatment were inoculated outside the chamber with 1 ml of a diluted inoculum,

containing a minimum of 2.9 x 10^4 CFU ml^{-1}, as determined by dilution plating. Seeds not receiving the *P. bilaii* treatment had 1 ml of distilled water placed on the seed after *Rhizobium* inoculation. Nine days after planting, pots were thinned to 1 plant per pot.

In the experiment using the Hochfeld soil, two seeds per pot were placed into holes dug to 2.5 cm from the soil surface. All seeds were inoculated with 1 ml of a yeast mannitol broth containing a minimum of 3 x 10^9 CFU ml^{-1} of the same *Rhizobium* strain used for the experiment using the Malmo soil, as determined by dilution plating. Seeds which received the *P. bilaii* treatment were inoculated outside the chamber with 1 ml of a diluted inoculum, containing a minimum of 1.6 x 10^3 CFU ml^{-1}, as determined by dilution plating. Nine days after planting, pots were thinned to 1 plant per pot.

The plants in both experiments were grown in a controlled environment cabinet (model GRV36-Econaire, Winnipeg, MB) under a 16/8 hr, 20/16° C day/night regime and exposed to an average photon flux density of 610 ± 60 µmol m^{-2} s^{-1} for the Hochfeld soil experiment, and 620 ± 60 µmol m^{-2} s^{-1} for the Malmo soil experiment, provided by a combination of cool white VHO and Grolux fluorescent lamps (Sylvania, Inc., Drummonville, PQ) at a ratio of 4:1, respectively.

The soils were watered to maintain gravimetric field capacity (Hochfeld soil) or 70% field capacity (Malmo soil) by adding adequate amounts of distilled water to the surface daily (Hochfeld soil), or every second day (Malmo soil), for the duration of the experiment (see section 3.2.1).

3.2.3 Sampling

Plants were harvested at 14, 21, and 28 days after planting. The soil was removed by soaking the pots in containers filled with tap water and gently shaking the pot to remove the soil and roots. The soil adhering to the roots was removed by placing the roots and soil onto a sieve (1.2 or 1.4 mm) and gently washing. Fresh root volume was determined by water displacement using a graduated cylinder. Shoots were removed at the soil surface and were frozen at -20° C, then freeze dried, weighed, and finally ground using a coffee grinder.

Root lengths were determined using IMAGEX, a digital analysis system, developed by L. Lamari, Dept. of Plant Science, Winnipeg, MB. This image analysis system reduces (skeletonizes) images to one pixel thick images, then "draws" a line through the length of each root piece. The system was calibrated using pieces of paper of known dimensions of (i.e., 1 pixel = "x" mm). Plant root segments were floated in water in a glass tray and care was taken to prevent root pieces from overlapping or touching. A video camera was used to capture the image of the roots. The picture was digitized, and skeletonized, and root lengths were determined.

All root material from the first harvest was used in root length determination. Roots from the second harvest were split down the main axis and approximately half was used for P analysis. The other half of the root system was used for root length determination. A ratio for root length to dry weight (DW) was used from the analysed portion of the roots in order to determine the total root lengths for the plants (i.e., root length:DW of analysed plants x DW of the total root system). Roots from the third

harvest were split down the main axis, and approximately half of the root system was cut into 3 cm segments, mixed, and a subsample (approximately half) was used for root length determination. The remaining root material was used for P analysis. Total root length was determined by the method described from harvest two. Roots from the second and third harvest used for root length determination were stained with methylene blue (0.1% w/v distilled water). Roots were placed into the dye solution for 30 s and then rinsed with distilled water for 30 seconds. All root material was frozen at -20^0 C, and then later thawed to remove and count the nodules. The roots were then refrozen, freeze dried, and weighed. The unstained roots that were used for P analysis were ground using a coffee grinder.

Specific root length (SRL) was calculated as the ratio of total root length to dry weight of the root system. It was used to describe mean root diameters of the plant root systems.

3.2.4 P Analysis

The concentration of P in shoot and root samples from the Hochfeld soil experiment were prepared for P analysis by digesting the plant material using a nitric-perchloric acid procedure (Isaac and Kerber, 1971), followed by the acid molybdate procedure (Murphy and Riley, 1962) for colour development and colorimetric assay. For the Malmo soil experiment, P concentration in the plant tissue was determined by procedures described by the Department of Fisheries and Oceans special publication no. 25 (1974), and is briefly described as follows: the P in the sample was oxidized using a combustion technique (muffled at 500^0 C), then hydrolysed using hydrochloric acid,

followed by the acid-molybdate procedure for colour development and colorimetric assay. Total P accumulation was determined by multiplying the dry weight of the root or shoot by the P concentration of the sample of known weight.

Data was analysed using the General Linear Model procedure of the Statistical Analysis System package (SAS Institute Inc., 1986) and single degree of freedom contrasts were made. Treatment means were separated using the Fisher protected least significant difference test (LSD) at $\alpha = 0.05$ level, after the analysis of variances indicated significant differences at the same level.

3.3 Results

The growth chamber experiments were conducted to investigate the effect of *Penicillium bilaii* and phosphorus fertilizer on the stimulation of root growth, and the resulting P concentration and accumulation of pea over time. The sampling dates were 14, 21, and 28 days after planting (DAP). Since the effects of *P. bilaii* and P fertilizer may be influenced by time, each sampling date of the parameters investigated will be discussed separately.

3.3.1 Root Morphology

The results on root morphology have been summarized on Tables 3.2 and 3.3 which presents significance levels for main effects (*P. bilaii* inoculation, P fertilizer) and interactions (*P. bilaii* x P fertilizer) for all harvest dates. Details of treatment effects for each harvest date are presented below.

Table 3.2. Significance of root responses [root volume, root length, and specific root length (SRL)] of pea in the Hochfeld soil to three P fertilizer treatments (no added P, rock phosphate, triple superphosphate), inoculation with *Penicillium bilaii* (Pb), to interactions between P fertilizer treatments and Pb inoculation, to Pb inoculation within each P fertilizer treatment.

	14 DAP			21 DAP			28 DAP		
	Volume	Length	SRL	Volume	Length	SRL	Volume	Length	SRL
Main effects									
P fertilizer	-	-	*	-	-	-	*	-	-
P. bilaii	-	-	-	-	-	-	-	-	-
Interaction									
P x Pb	-	-	-	-	-	-	-	-	-
Contrasts									
Pb @ 0P[1]	-	-	-	-	-	-	-	-	-
Pb @ RP[2]	-	-	-	-	-	-	-	-	-
Pb @ TSP[3]	-	-	-	-	-	-	-	-	-

* $P \leq 0.05$. ** $P \leq 0.01$. *** $P \leq 0.001$. - = not significant.

[1]0P = no added P fertilizer. [2]RP = Idaho rock phosphate at 17.2 mg P kg^{-1} soil. [3]TSP = Triple superphosphate at 17.2 mg P kg^{-1} soil.

Table 3.3. Significance of root responses [root volume, root length, and specific root length (SRL)] of pea in the Malmo soil to three P fertilizer treatments (no added P, rock phosphate, triple superphosphate), inoculation with *Penicillium bilaii* (Pb), to interactions between P fertilizer treatments and Pb inoculation, to Pb inoculation within each P fertilizer treatment.

	14 DAP			21 DAP			28 DAP		
	Volume	Length	SRL	Volume	Length	SRL	Volume	Length	SRL
Main effects									
P fertilizer	-	-	-	-	**	**	**	*	-
P. bilaii	-	-	-	-	*	-	-	-	-
Interaction									
P x Pb	-	-	-	-	-	-	-	-	*
Contrasts									
Pb @ 0P[1]	-	-	-	-	-	-	-	-	**
Pb @ RP[2]	-	-	-	-	-	-	-	-	-
Pb @ TSP[3]	-	-	-	-	-	-	-	-	-

* $P \leq 0.05$. ** $P \leq 0.01$. *** $P \leq 0.001$. - = not significant.

[1]0P = no added P fertilizer. [2]RP = Idaho rock phosphate at 17.2 mg P kg^{-1} soil. [3]TSP = triple superphosphate at 17.2 mg kg^{-1} soil.

3.3.1.1 14 DAP

Penicillium bilaii inoculation failed to show any significant effect ($P \leq 0.05$) on pea root length, specific root length (SRL) or root volume in either soil type at 14 DAP (Tables 3.2, 3.3; Figures 3.1, 3.2). Similarly, no response to P fertilizer was observed in the Malmo soil, but the main effect of P fertilizer for SRL was significant ($P \leq 0.05$) for the Hochfeld soil (Tables 3.2, 3.3). Plants that did not receive any fertilizer had significantly finer roots (i.e., a higher SRL) than plants that received triple superphosphate (TSP) or rock phosphate (rock-P) fertilizer in the Hochfeld soil (Table 3.4; Figure 3.1). The main effect of P fertilizer was significant at $P = 0.08$ for root length for the Hochfeld soil only, and plants in the treatment with no added P had the greatest root length (Table 3.2; Figure 3.1).

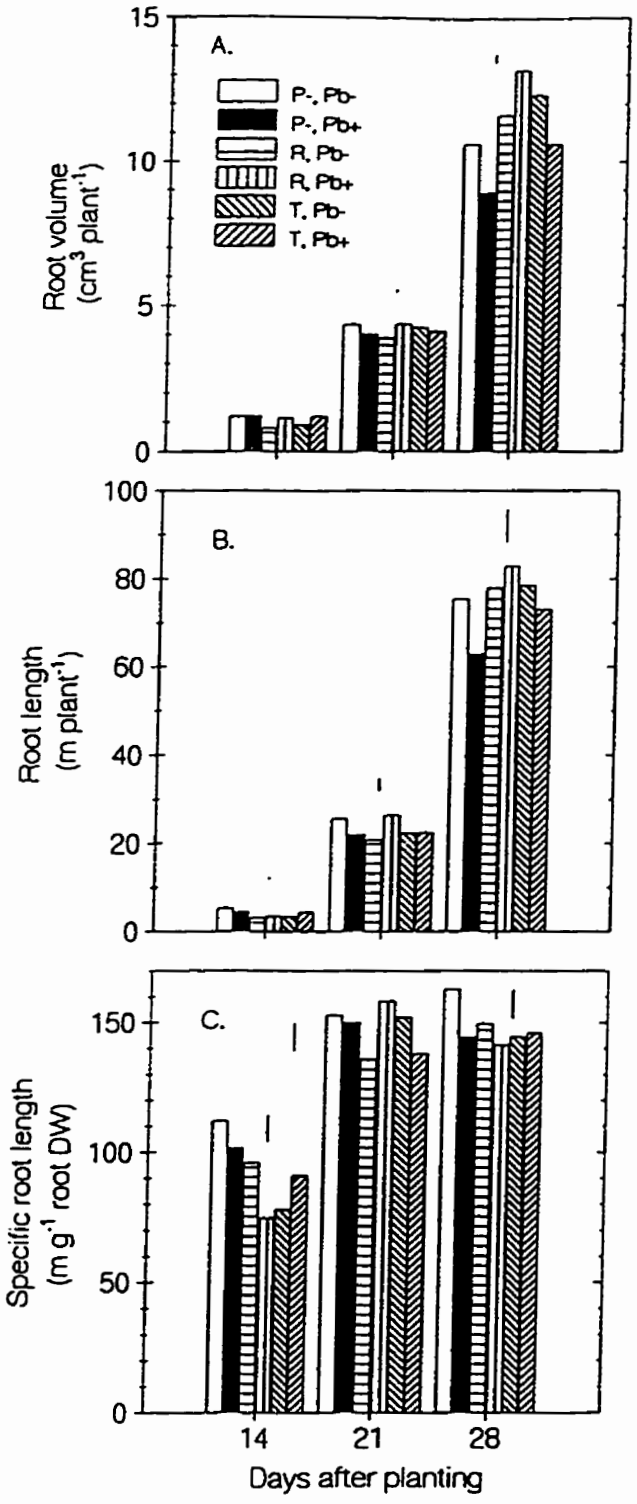

Figure 3.1. The effect of inoculation with (Pb+) or without (Pb-) *Penicillium bilaii* and P sources (0 P fertilizer added, P-; 17.2 mg P of Idaho rock P kg^{-1} soil, R; 17.2 mg P of triple superphosphate kg^{-1} soil, T) on root volume (A), root length (B), and specific root length (c) of pea in the Hochfeld soil. Bar represents the mean standard error of treatments within dates.

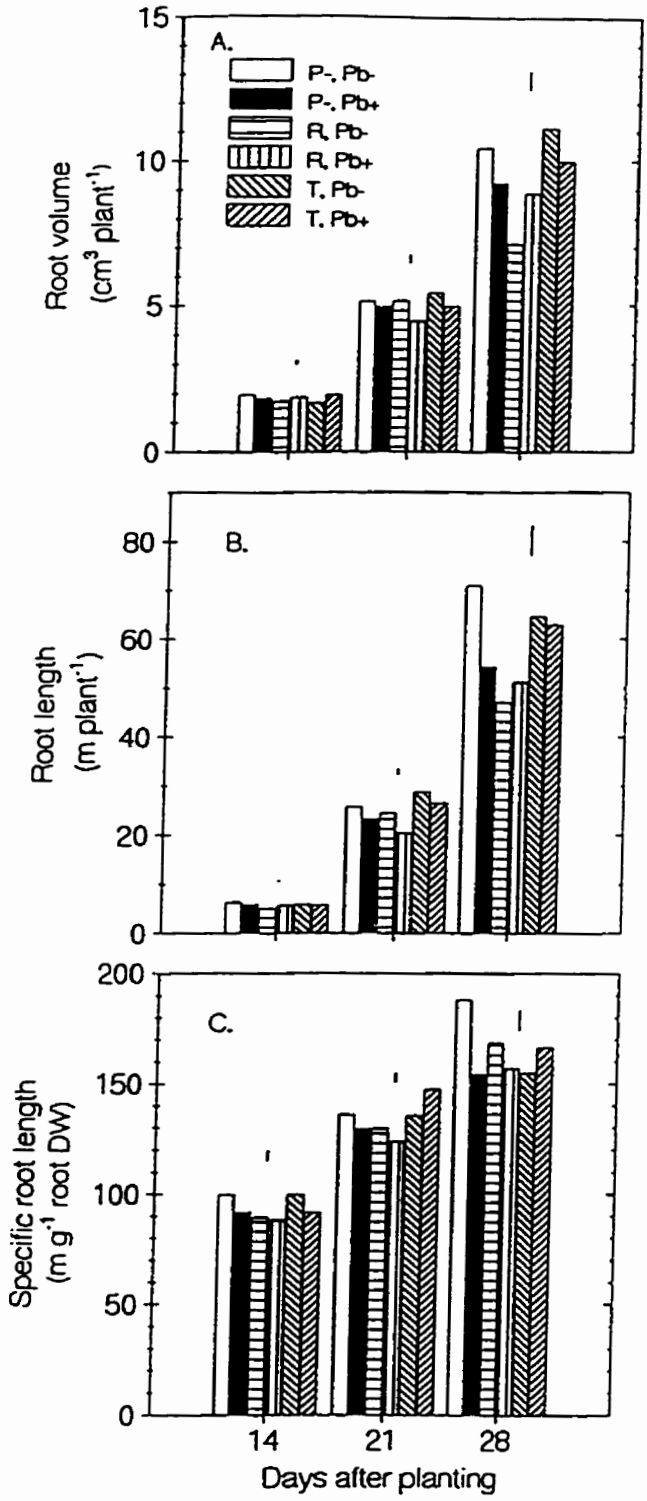

Figure 3.2. The effect of inoculation with (Pb+) or without (Pb-) *Penicillium bilaii* and P sources (0 P fertilizer added, P-; 17.2 mg P of Idaho rock P kg^{-1} soil, R; 17.2 mg P of triple superphosphate kg^{-1} soil, T) on root volume (A), root length (B), and specific root length (c) of pea in the Malmo soil. Bar represents the mean standard error of treatments within dates.

Table 3.4. Effect of P fertilizer on specific root length (SRL) of pea in the Hochfeld soil, 14 days after planting.

P fertilizer	SRL (m g^{-1} DW root)
No added P	106.86
TSP[2]	84.52
Rock-P[1]	84.21
LSD ($P \leq 0.05$)	20.02

[1]Rock-P = Idaho rock phosphate at 17.2 mg P kg^{-1} soil.
[2]TSP = triple superphosphate at 17.2 mg P kg^{-1} soil.

3.3.1.2 21 DAP

At 21 DAP, no detectable treatment effects on root morphology of pea for the Hochfeld soil were observed (Table 3.2; Figure 3.1). For the Malmo soil, the effects of P fertilizer and *P. bilaii* inoculation were significant ($P \leq 0.01$, $P \leq 0.05$ respectively) for root length (Table 3.3; Figure 3.2). The effect of P fertilizer was also significant ($P \leq 0.01$) for specific root length (Table 3.3). Triple superphosphate fertilizer significantly increased root length 23% and SRL 12%, over the rock-P fertilizer level in the Malmo soil (Table 3.5). Over all P fertilizer levels, *P. bilaii* inoculation reduced root length 12% in the Malmo soil (Table 3.6). No detectable treatment effects on root volume were observed in the Malmo soil (Table 3.3; Figure 3.2).

3.3.1.3 28 DAP

At 28 DAP, no detectable treatment effects on pea root length or SRL for the Hochfeld soil were observed (Table 3.2; Figure 3.1). Root volume was significantly ($P \leq 0.05$) affected by P fertility in the Hochfeld soil (Table 3.2; Figure 3.1). Overall, the addition of P fertilizer (TSP or rock-P) significantly increased root volume compared with the no added P fertilizer level (Table 3.7).

For the Malmo soil, inoculating with *P. bilaii* did not effect pea root length. However, inoculation at the no added P level was significant at $P = 0.06$ for reducing root length (Table 3.3; Figure 3.2). The main effects were not significant for SRL, but the contrast statements revealed that *P. bilaii* inoculation at the no added P fertilizer level significantly ($P \leq 0.01$) reduced SRL by 22% in the Malmo soil (Table 3.3; Figure 3.2).

Table 3.5. Effect of P fertilizer on root length and specific root length (SRL) of pea in Malmo soil, 21 days after planting.

P fertilizer	Length (m)	SRL (m g $^{-1}$ DW root)
TSP[2]	27.62	141.66
No added P	24.60	132.76
Rock-P[1]	22.44	126.70
LSD (P≤ 0.05)	3.26	8.99

[1]Rock-P = Idaho rock phosphate at 17.2 mg P kg^{-1} soil.
[2]TSP = triple superphosphate at 17.2 mg P kg^{-1} soil.

Table 3.6. Effect of *Penicillium bilaii* (with, +; without, -) inoculation on root length of pea in Malmo soil, 21 days after planting.

P. bilaii	Length (m)
-	26.33
+	23.45
LSD (P≤ 0.05)	2.66

Table 3.7. Effect of P fertilizer on root volume of pea in Hochfeld soil, 28 days after planting.

P fertilizer	Volume (cm^3)
Rock-P[1]	12.31
TSP[2]	11.64
No added P	9.78
LSD (P≤ 0.05)	1.83

[1]Rock-P = Idaho rock phosphate at 17.2 mg P kg^{-1} soil.
[2]TSP = triple superphosphate at 17.2 mg P kg^{-1} soil.

Table 3.8. Effect of P fertilizer on root length and volume of pea in Malmo soil, 28 days after planting.

P fertilizer	Length (m)	Root volume (cm^3)
TSP[2]	63.73	10.49
No added P	63.50	9.89
Rock-P[1]	49.21	7.99
LSD (P≤ 0.05)	12.27	1.51

[1]Rock-P = Idaho rock phosphate at 17.2 mg P kg^{-1} soil.
[2]TSP = triple superphosphate at 17.2 mg P kg^{-1} soil.

Phosphorus fertilizer had a significant effect on root length ($P \leq 0.05$) and volume ($P \leq 0.01$) (Table 3.3). Triple superphosphate fertilizer increased root length 29% and volume 31% over the rock-P fertilizer level (Table 3.8).

3.3.2 Phosphorus Concentration and Accumulation

The results on shoot and root P concentration and accumulation have been summarized on Tables 3.9 and 3.10 which presents significance levels for main effects (*P. bilaii* inoculation, P fertilizer) and interactions (*P. bilaii* x P fertilizer) for all harvest dates. Details of treatment effects for each harvest date are presented below.

3.3.2.1 14 DAP

Penicillium bilaii inoculation did not affect shoot P concentration or accumulation (total P) of pea in Hochfeld soil at 14 DAP (Table 3.9; Figure 3.3). Phosphorus fertilizer was significant for shoot P concentration ($P \leq 0.05$) and accumulation in the Hochfeld soil ($P \leq 0.01$) (Table 3.9). Triple superphosphate fertilizer increased shoot P concentration 19% over the rock-P fertilizer level, and TSP and the no added P fertilizer level accumulated more P than the rock-P fertilizer level (Table 3.11). *Penicillium bilaii* inoculation at the TSP fertilizer level was significant at $P = 0.08$ for increasing shoot P accumulation in the Hochfeld soil (Figure 3.3).

In the Hochfeld soil, the interaction effect between P treatment and *P. bilaii* was significant ($P \leq 0.05$) for root P concentration and accumulation (Table 3.9). *Penicillium bilaii* inoculation at the no additional P level resulted in a significant ($P \leq 0.01$) decrease of 40% in root P concentration and a significant ($P \leq 0.05$) decrease of 45% in root P accumulation (Figure 3.3).

Table 3.9. Significance of shoot and root P concentration (conc.) and accumulation (total P) responses of pea in the Hochfeld soil to three P fertilizer treatments (no added P, rock phosphate, triple superphosphate), inoculation with *Penicillium bilaii* (Pb), to interactions between P fertilizer treatments and Pb inoculation, to Pb inoculation within each P fertilizer treatment.

	14 DAP				21 DAP				28 DAP			
	Shoot P Conc.	Root P Conc.	Shoot P Total	Root P Total	Shoot P Conc.	Root P Conc.	Shoot P Total	Root P Total	Shoot P Conc.	Root P Conc.	Shoot P Total	Root P Total
Main effects												
P fertilizer	*	-	**	-	***	-	**	-	-	*	**	-
P. bilaii	-	*	-	-	-	-	-	-	-	-	-	-
Interaction												
P x Pb	-	*	-	*	**	-	-	-	-	-	-	-
Contrasts												
Pb @ 0P[1]	-	**	-	*	-	-	-	-	-	-	-	-
Pb @ RP[2]	-	-	-	-	*	-	-	-	-	-	-	-
Pb @ TSP[3]	-	-	-	-	**	-	-	-	-	-	-	-

* $P \leq 0.05$. ** $P \leq 0.01$. *** $P \leq 0.001$. - = not significant.

[1]0P = no added P fertilizer. [2]RP = Idaho rock phosphate at 17.2 mg P kg^{-1} soil. [3]TSP = triple superphosphate at 17.2 mg kg^{-1} soil.

Table 3.10. Significance of shoot and root P concentration (conc.) and accumulation (total P) responses of pea in the Malmo soil to three P fertilizer treatments (no added P, rock phosphate, triple superphosphate), inoculation with *Penicillium bilaii* (Pb), to interactions between P fertilizer treatments and Pb inoculation, to Pb inoculation within each P fertilizer treatment.

	14 DAP				21 DAP				28 DAP			
	Shoot P Conc.	Root P Conc.	Shoot P Total	Root P Total	Shoot P Conc.	Root P Conc.	Shoot P Total	Root P Total	Shoot P Conc.	Root P Conc.	Shoot P Total	Root P Total
Main effects												
P fertilizer	*	-	-	-	***	-	-	-	-	**	-	**
P. bilaii	-	-	-	-	-	-	-	**	-	-	-	-
Interaction												
P x Pb	*	-	*	-	-	-	-	-	-	**	-	*
Contrasts												
Pb @ 0P[1]	*	-	**	-	-	-	-	-	-	-	-	-
Pb @ RP[2]	-	*	-	-	-	-	-	*	*	**	*	*
Pb @ TSP[3]	-	-	-	-	-	-	-	*	-	-	-	-

* $P \leq 0.05$. ** $P \leq 0.01$. *** $P \leq 0.001$. - = not significant.

[1]0P = no added P fertilizer. [2]RP = Idaho rock phosphate at 17.2 mg P kg^{-1} soil. [3]TSP = triple superphosphate at 17.2 mg kg^{-1} soil.

Table 3.11. Effect of P fertilizer on shoot P concentration and accumulation of pea in Hochfeld soil, 14 days after planting.

P fertilizer	Shoot P concentration (mg P g^{-1} DW)	Shoot P accumulation (mg)
TSP[2]	4.634	0.793
No added P	4.310	0.719
Rock-P[1]	3.880	0.604
LSD ($P \leq 0.05$)	0.594	0.109

[1]Rock-P = Idaho rock phosphate at 17.2 mg P kg^{-1} soil.
[2]TSP = triple superphosphate at 17.2 mg P kg^{-1} soil.

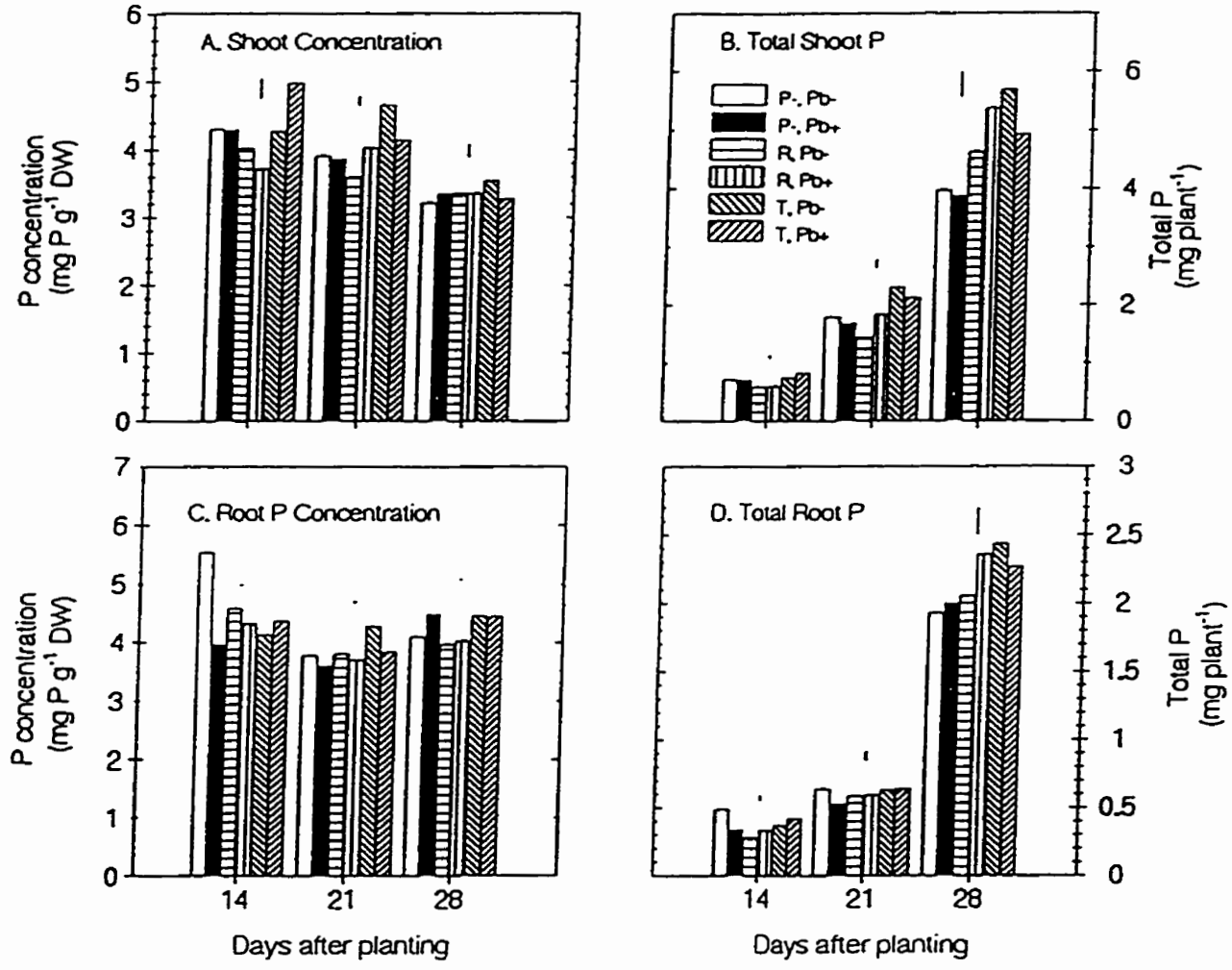

Figure 3.3. The effect of inoculation with (Pb+) or without (Pb-) *Penicillium bilaii* and P sources (0 P fertilizer added, P-; 17.2 mg P of Idaho rock P kg^{-1} soil, R; 17.2 mg P of triple superphosphate kg^{-1} soil, T) on shoot P concentration (A), shoot P accumulation (B), root P concentration (C), and root P accumulation (D), of pea in the Hochfeld soil. Bar represents the mean standard error of treatments within dates.

For the Malmo soil, the interaction effect between P treatment and *P. bilaii* was significant ($P \leq 0.05$) for shoot P concentration, and *P. bilaii* inoculation was significant ($P \leq 0.01$) at the no added P level resulting in a 29% increase in shoot P concentration and a 45% increase in shoot P accumulation (Table 3.10; Figure 3.4). The only significant ($P \leq 0.05$) effect of *P. bilaii* inoculation on root P concentration in the Malmo soil occurred at the rock-P fertilizer level, in which inoculation resulted in a reduction of root P concentration by 20% (Table 3.10; Figure 3.4).

3.3.2.2 21 DAP

For the Hochfeld soil, the interaction effect between P treatment and *P. bilaii* was significant ($P \leq 0.01$) for shoot P concentration, and contrast statements indicated a significant effect of *P. bilaii* inoculation at the rock-P ($P \leq 0.05$) and TSP ($P \leq 0.01$) fertilizer levels at 21 DAP (Table 3.9). *Penicillium bilaii* inoculation at the rock-P fertilizer level increased shoot P concentration by 12% whereas inoculation resulted in a 12% decrease at the TSP fertilizer level in the Hochfeld soil (Figure 3.3).

The main effect of P fertilizer was significant for shoot P accumulation ($P \leq 0.01$) in the Hochfeld soil (Table 3.9). The TSP fertilizer level resulted in plants with significantly increased shoot P accumulation over the no added P and rock-P fertilizer levels (Table 3.12; Figure 3.3).

For the Malmo soil, *P. bilaii* inoculation did not affect shoot P concentration or accumulation (Table 3.10; Figure 3.4). The main effect of P fertilizer was highly significant for shoot P concentration ($P \leq 0.001$) (Table 3.10). The TSP fertilizer level resulted in plants with significantly increased shoot P concentration over the no added P

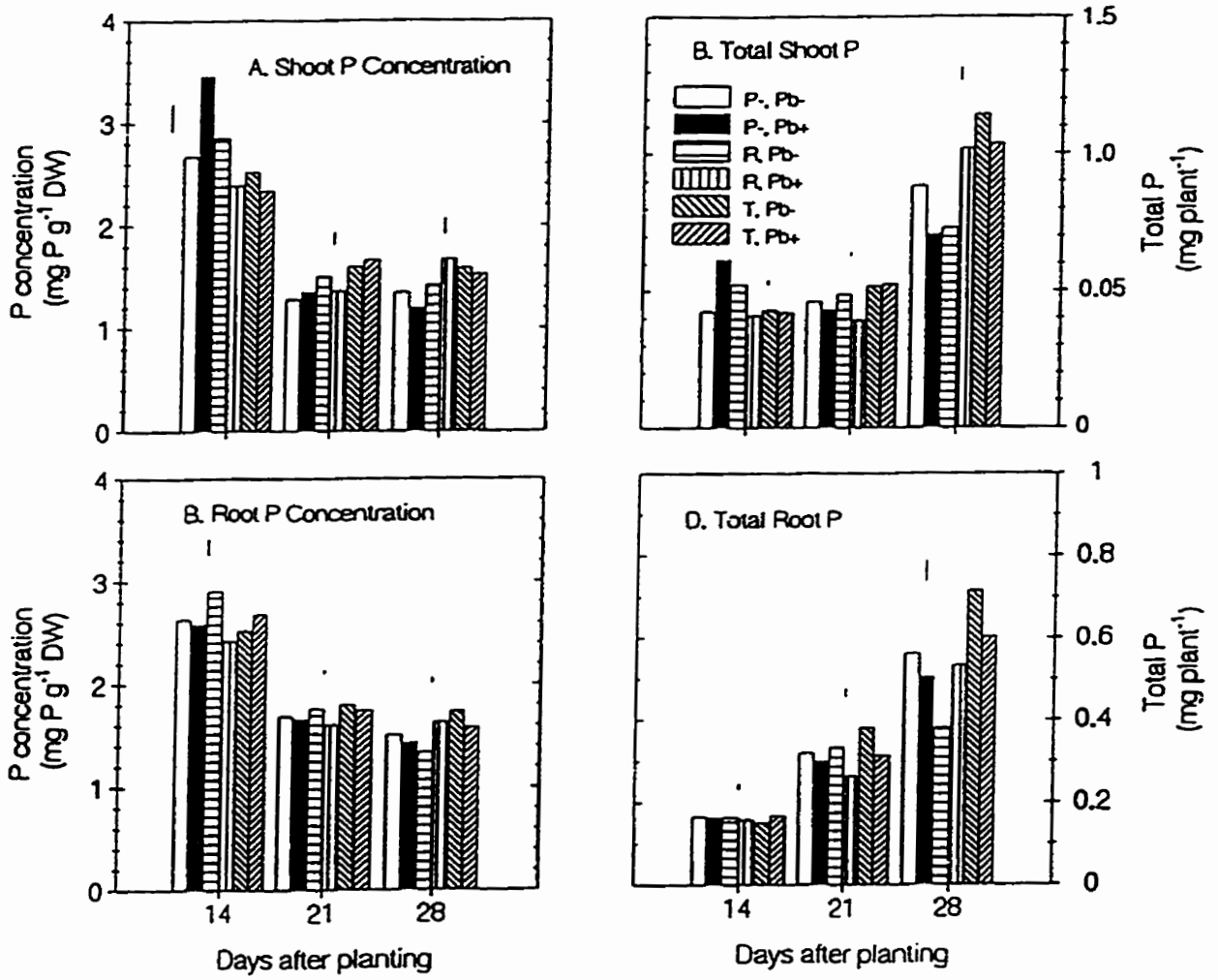

Figure 3.4. The effect of inoculation with (Pb+) or without (Pb-) *Penicillium bilaii* and P sources (0 P fertilizer added, P-; 17.2 mg P of Idaho rock P kg^{-1} soil, R; 17.2 mg P of triple superphosphate kg^{-1} soil, T) on shoot P concentration (A), shoot P accumulation (B), root P concentration (C), and root P accumulation (D), of pea in the Malmo soil. Bar represents the mean standard error of treatments within dates.

Table 3.12. Effect of P fertilizer on shoot P accumulation of pea in the Hochfeld soil, 21 days after planting.

P fertilizer	Shoot P accumulation (mg)
TSP[2]	2.226
No added P	1.754
Rock-P[1]	1.624
LSD (P≤ 0.05)	0.323

[1]Rock-P = Idaho rock phosphate at 17.2 mg P kg^{-1} soil.
[2]TSP = triple superphosphate at 17.2 mg P kg^{-1} soil.

Table 3.13. Effect of P fertilizer on shoot P concentration of pea in the Malmo soil, 21 days after planting.

P fertilizer	Shoot P concentration (mg P g^{-1} DW)
TSP[2]	1.639
Rock-P[1]	1.444
No added P	1.322
LSD (P≤ 0.05)	0.131

[1]Rock-P = Idaho rock phosphate at 17.2 mg P kg^{-1} soil.
[2]TSP = triple superphosphate at 17.2 mg P kg^{-1} soil.

Table 3.14. Effect of *Penicillium bilaii* (with, +; without, -) inoculation on root P accumulation of pea in the Malmo soil, 21 days after planting.

P. bilaii	Root P accumulation (mg)
-	0.345
+	0.293
LSD (P≤ 0.05)	0.038

and rock-P fertilizer levels in the Malmo soil (Table 3.13; Figure 3.3).

There were no detectable treatment effects on root P concentration in the Malmo soil at 21 DAP (Table 3.10; Figure 3.4). A contrast statement for *P. bilaii* inoculation at the rock-P fertilizer level was significant at P = 0.06 for reducing root P concentration (Table 3.10). *Penicillium bilaii* inoculation in the Malmo soil had a significant effect ($P \leq 0.01$) on root P accumulation, which resulted in a 18% decrease in P accumulation over all P fertilizer levels (Table 3.14). Contrast statements indicated a significant effect ($P \leq 0.05$) of *P. bilaii* inoculation at the rock-P and TSP fertilizer levels in the Malmo soil (Table 3.10). *Penicillium bilaii* inoculation resulted in a 26% and 22% decrease in root P accumulation at the rock-P and TSP fertilizer levels respectively (Figure 3.4).

3.3.2.3 28 DAP

No detectable treatment effects on shoot P concentration of pea at 28 DAP in the Hochfeld soil were observed (Table 3.9; Figure 3.3). The main effect of P fertilizer in the Hochfeld soil was significant ($P \leq 0.01$) for shoot P accumulation (Table 3.9). Adding P fertilizer (TSP or rock-P) significantly increased shoot P accumulation over the no added P fertilizer level (Table 3.15). This is likely a result of the significant ($P \leq 0.01$) increase in shoot dry matter production for the TSP and rock-P fertilizer level (Figure 3.5).

Penicillium bilaii inoculation did not affect root P concentration or accumulation in the Hochfeld soil (Table 3.9; Figure 3.3). The main effect of P fertilizer had a significant effect ($P \leq 0.05$) on root P concentration but not P accumulation (Table 3.9).The TSP fertilizer level increased root P concentration 11% over the rock-P fertilizer level (Table 3.16).

Table 3.15. Effect of P fertilizer on shoot P accumulation of pea in Hochfeld soil, 28 days after planting.

P fertilizer	Shoot P accumulation (mg)
TSP[2]	5.391
Rock-P[1]	4.977
No added P	3.935
LSD (P≤ 0.05)	0.769

[1]Rock-P = Idaho rock phosphate at 17.2 mg P kg^{-1} soil.
[2]TSP = triple superphosphate at 17.2 mg P kg^{-1} soil.

Table 3.16. Effect of P fertilizer on root P concentration of pea in Hochfeld soil, 28 days after planting.

P fertilizer	Root P accumulation (mg)
TSP[2]	4.46
No added P	4.30
Rock-P[1]	4.00
LSD (P≤ 0.05)	0.36

[1]Rock-P = Idaho rock phosphate at 17.2 mg P kg^{-1} soil.
[2]TSP = triple superphosphate at 17.2 mg P kg^{-1} soil.

For the Malmo soil, the only significant response on shoot P concentration and accumulation resulted from *P. bilaii* inoculation at the rock-P fertilizer level (Table 3.10; Figure 3.4). *Penicillium bilaii* inoculation resulted in a 42% increase in shoot P concentration and a 63% increase in P accumulation (Figure 3.4).

The only significant effect of *P. bilaii* inoculation on root P concentration and accumulation in the Malmo soil occurred at the rock-P fertilizer level (Table 3.10; Figure 3.4). *Penicillium bilaii* inoculation significantly ($P \leq 0.01$) increased root P concentration 22%, and significantly ($P \leq 0.05$) increased P accumulation 39% at the rock-P fertilizer level (Figure 3.4).

3.3.3 Dry Weight Responses

The results on dry weight responses and root:shoot ratios have been summarized on Tables 3.17 and 3.18 which presents significance levels for main effects (*P. bilaii* inoculation, P fertilizer) and interactions (*P. bilaii* x P fertilizer) for all harvest dates. Details of treatment effects for each harvest date are presented below.

3.3.3.1 14 DAP

At 14 DAP, no detectable treatment effects on dry weights (root, shoot, plant) or root:shoot ratios for both the Hochfeld and Malmo soils were observed (Tables 3.17, 3.18; Figures 3.5, 3.6, 3.7, 3.8).

Table 3.17. Significance of dry weight and root:shoot ratio responses of pea in the Hochfeld soil to three P fertilizer treatments (no added P, rock phosphate, triple superphosphate), inoculation with *Penicillium bilaii* (Pb), to interactions between P fertilizer treatments and Pb inoculation, to Pb inoculation within each P fertilizer treatment.

	14 DAP					21 DAP					28 DAP				
	Root	Shoot	Nodule	Plant	RSR	Root	Shoot	Nodule	Plant	RSR	Root	Shoot	Nodule	Plant	RSR
Main effects															
P fertilizer	-	-	-	-	-	-	-	-	-	**	*	**	*	**	*
P. bilaii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Interaction															
P x Pb	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Contrasts															
Pb @ 0P[1]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pb @ RP[2]	-	-	-	-	-	-	-	**	-	-	-	-	-	-	-
Pb @ TSP[3]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* $P \leq 0.05$. ** $P \leq 0.01$. *** $P \leq 0.001$. - = not significant.

[1]0P = no added P fertilizer. [2]RP = Idaho rock phosphate at 17.2 mg P kg^{-1} soil. [3]TSP = triple superphosphate at 17.2 mg kg^{-1} soil.

Table 3.18. Significance of dry weight and root:shoot ratio responses of pea in the Malmo soil to three P fertilizer treatments (no added P, rock phosphate, triple superphosphate), inoculation with *Penicillium bilaii* (Pb), to interactions between P fertilizer treatments and Pb inoculation, to Pb inoculation within each P fertilizer treatment.

	14 DAP					21 DAP					28 DAP				
	Root	Shoot	Nodule	Plant	RSR	Root	Shoot	Nodule	Plant	RSR	Root	Shoot	Nodule	Plant	RSR
Main effects															
P fertilizer	-	-	*	-	-	-	-	**	-	**	**	**	-	**	-
P. bilaii	-	-	-	-	-	**	-	**	*	-	-	-	-	-	-
Interaction															
P x Pb	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-
Contrasts															
Pb @ 0P[1]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pb @ RP[2]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pb @ TSP[3]	-	-	-	-	-	**	-	*	-	-	-	-	**	-	-

* $P \leq 0.05$. ** $P \leq 0.01$. *** $P \leq 0.001$. - = not significant.

[1]0P = no added P fertilizer. [2]RP = Idaho rock phosphate at 17.2 mg P kg^{-1} soil. [3]TSP = triple superphosphate at 17.2 mg kg^{-1} soil.

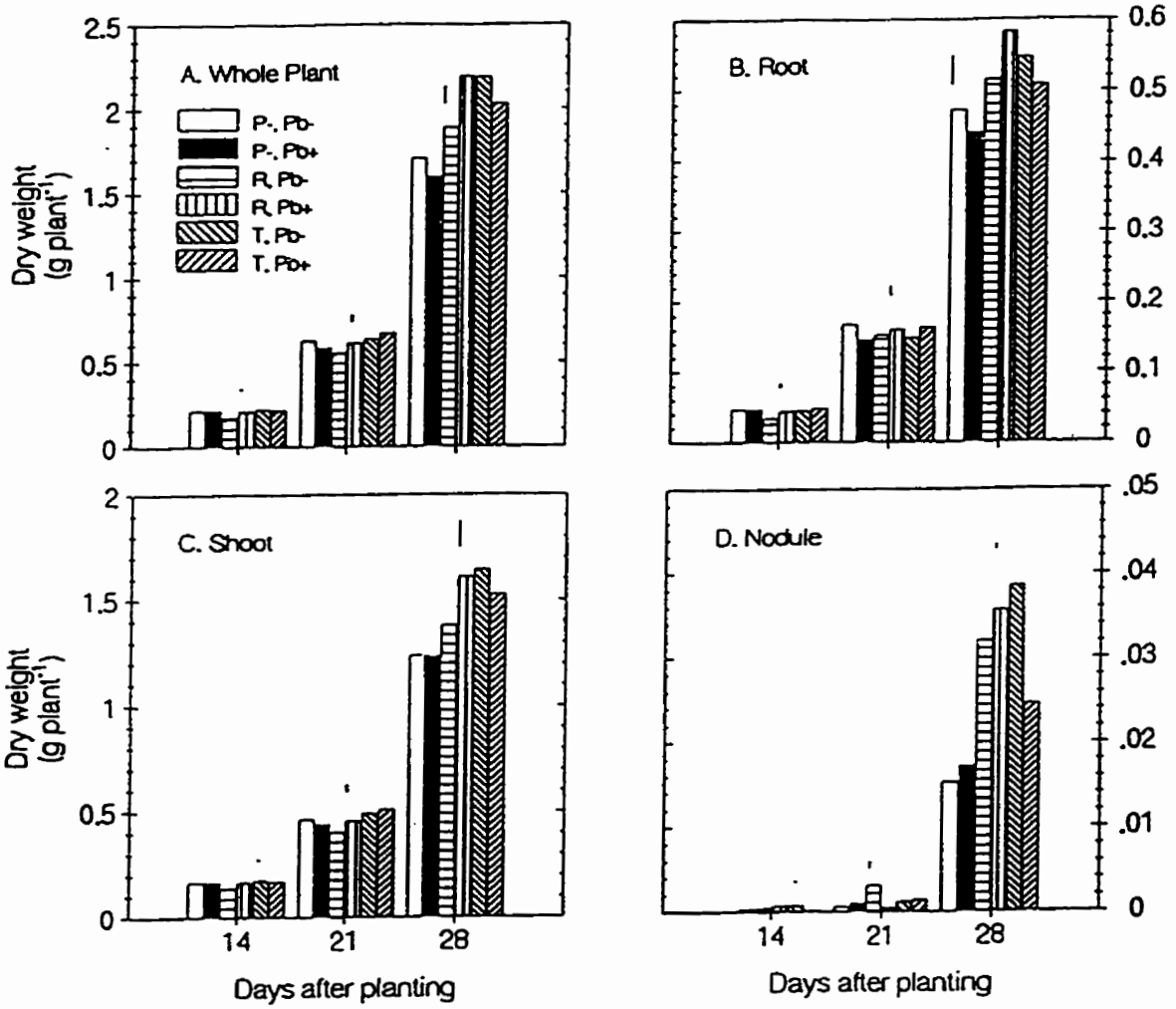

Figure 3.5. The effect of inoculation with (Pb+) or without (Pb-) *Penicillium bilaii* and P sources (0 P fertilizer added, P-; 17.2 mg P of Idaho rock P kg^{-1} soil, R; 17.2 mg P of triple superphosphate kg^{-1} soil, T) on whole plant (A), root (B), shoot (C), and nodule (D) dry weight of pea in the Hochfeld soil. Bar represents the mean standard error of treatments within dates.

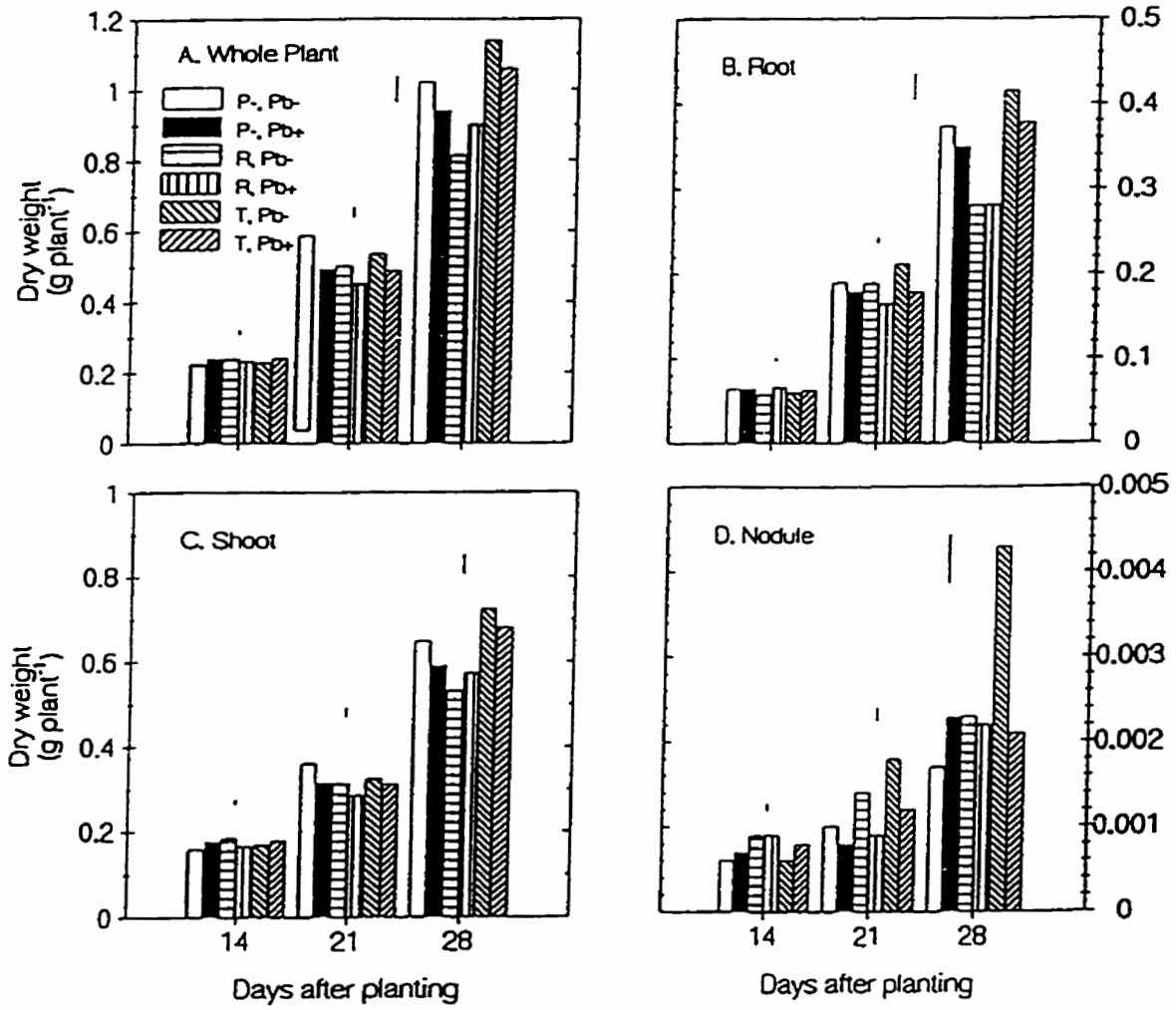

Figure 3.6. The effect of inoculation with (Pb+) or without (Pb-) *Penicillium bilaii* and P sources (0 P fertilizer added, P-; 17.2 mg P of Idaho rock P kg^{-1} soil, R; 17.2 mg P of triple superphosphate kg^{-1} soil, T) on whole plant (A), root (B), shoot (C), and nodule (D) dry weight of pea in the Malmo soil. Bar represents the mean standard error of treatments within dates.

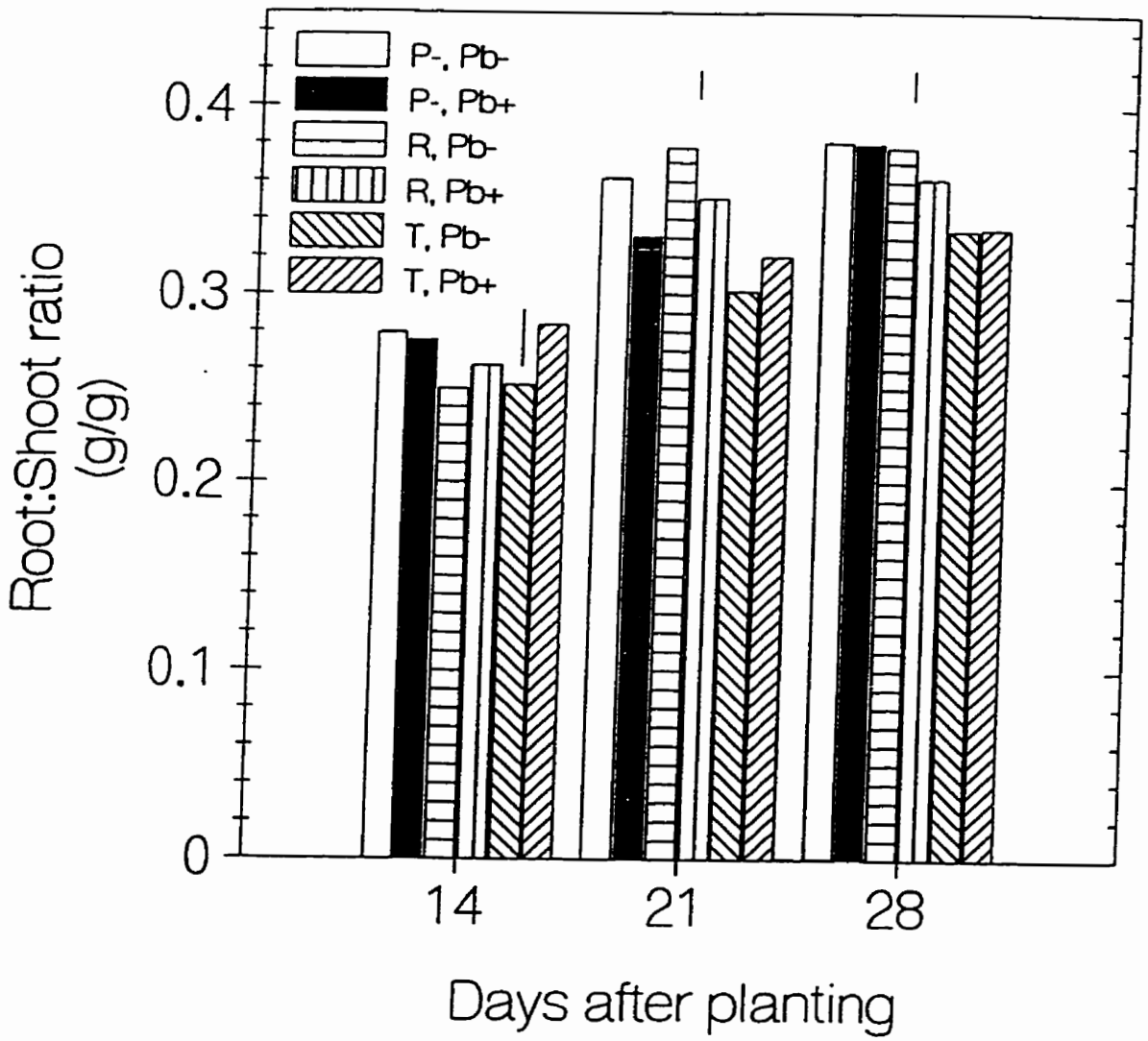

Figure 3.7. The effect of inoculation with (Pb+) or without (Pb-) *Penicillium bilaii* and P sources (0 P fertilizer added, P-; 17.2 mg P of Idaho rock P kg^{-1} soil, R; 17.2 mg P of triple superphosphate kg^{-1} soil, T) on root:shoot ratio of pea in the Hochfeld soil. Bar represents the mean standard error of treatments within dates.

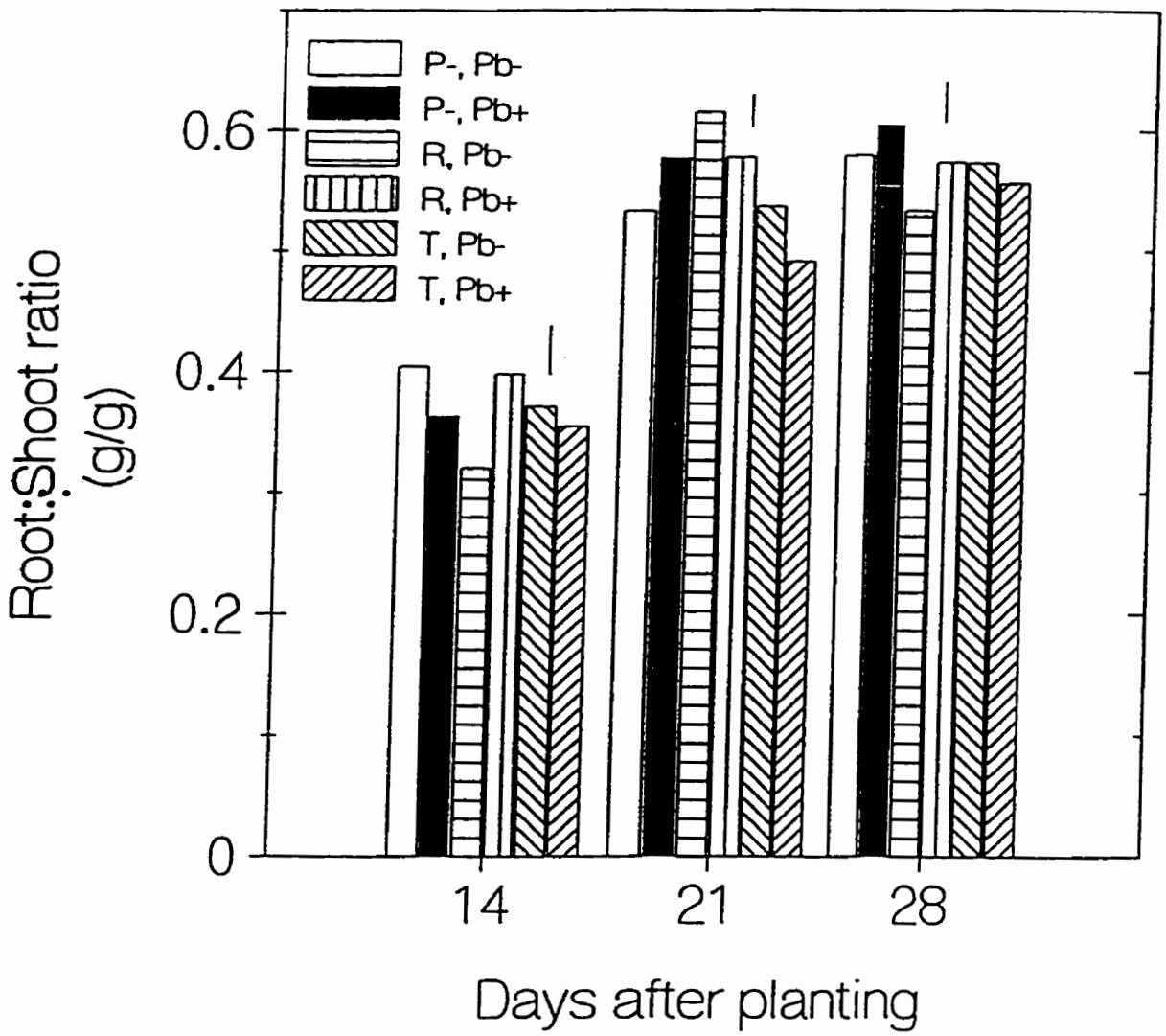

Figure 3.8. The effect of inoculation with (Pb+) or without (Pb-) *Penicillium bilaii* and P sources (0 P fertilizer added, P-; 17.2 mg P of Idaho rock P kg^{-1} soil, R; 17.2 mg P of triple superphosphate kg^{-1} soil, T) on root:shoot ratio of pea in the Malmo soil. Bar represents the mean standard error of treatments within dates.

3.3.3.2 21 DAP

For the Hochfeld soil, no detectable treatment effects on dry weights (root, shoot, plant) of pea were observed at 21 DAP (Table 3.17; Figure 3.5). However, P fertilizer had a significant effect ($P \leq 0.01$) on root:shoot ratios, but *P. bilaii* inoculation had no effect on this parameter (Table 3.17; Figure 3.7). The rock-P and no added P fertilizer level produced significantly greater root:shoot ratios than the TSP fertilizer level (Table 3.19).

For the Malmo soil, there were no dry matter responses to P fertilizer, but *P. bilaii* inoculation had a significant effect on root dry weight ($P \leq 0.01$) and plant dry weight ($P \leq 0.05$) (Table 3.18). Over all levels of P fertilizer, *P. bilaii* inoculation reduced root dry weight 13% and plant dry weight 11% over non-inoculated plants (Table 3.20; Figure 3.6). At the TSP fertilizer level, *P. bilaii* inoculation significantly ($P \leq 0.01$) reduced root dry weight by 18% (Figure 3.6). Root:shoot ratio was significantly ($P \leq 0.01$) affected by P fertilizer in the Malmo soil (Table 3.18). The rock-P fertilizer level significantly increased root:shoot ratio 16% over the TSP fertilizer level (Table 3.21).

3.3.3.2 28 DAP

For the Hochfeld soil at 28 DAP, *P. bilaii* inoculation did not affect dry matter production or root:shoot ratios of pea (Table 3.17; Figures 3.5, 3.7). However, P fertilizer had a significant effect on root ($P \leq 0.05$), shoot ($P \leq 0.01$), and plant ($P \leq 0.01$) dry weights and root:shoot ratios ($P \leq 0.05$) (Table 3.17; Figures 3.5, 3.7).

Table 3.19. Effect of P fertilizer on root:shoot ratio of pea in the Hochfeld soil, 21 days after planting.

P fertilizer	Root:shoot ratio (g/g)
Rock-P[1]	0.365
No added P	0.347
TSP[2]	0.310
LSD (P≤ 0.05)	0.033

[1]Rock-P = Idaho rock phosphate at 17.2 mg P kg^{-1} soil.
[2]TSP = triple superphosphate at 17.2 mg P kg^{-1} soil.

Table 3.20. Effect of *Penicillium bilaii* (with, +; without, -) inoculation on root and plant dry weight of pea in the Malmo soil, 21 days after planting.

P. bilaii	Root dry weight (g)	Plant dry weight (g)
-	0.1969	0.5301
+	0.1745	0.4793
LSD (P≤ 0.05)	0.0152	0.0505

Table 3.21. Effect of P fertilizer on root:shoot ratio of pea in the Malmo soil, 21 days after planting.

P fertilizer	Root:shoot ratio (g/g)
Rock-P[1]	0.5965
No added P	0.5553
TSP[2]	0.5139
LSD (P≤ 0.05)	0.0559

[1]Rock-P = Idaho rock phosphate at 17.2 mg P kg^{-1} soil.
[2]TSP = triple superphosphate at 17.2 mg P kg^{-1} soil.

Rock-P fertilizer significantly increased root dry weight 19% over the no added P fertilizer level (Table 3.22). The addition of P fertilizer (TSP or rock), significantly increased shoot and plant dry matter production in the Hochfeld soil (Table 3.22). The TSP fertilizer level produced an average increase of 29 and 28% over the no added P level for shoot and plant dry weight, respectively, while the rock-P fertilizer level produced an average increase of 20 and 23%, respectively (Table 3.22). Root:shoot ratios were significantly greater for the no added P and rock-P fertilizer levels than for the TSP fertilizer level in the Hochfeld soil (Table 3.23).

Similarly, *P. bilaii* inoculation did not affect dry matter production or root:shoot ratios of pea on the Malmo soil (Table 3.18; Figures 3.6, 3.8). However, P fertilizer had a significant effect on root ($P \leq 0.01$), shoot ($P \leq 0.01$), and plant ($P \leq 0.01$) dry weight in the Malmo soil (Table 3.18). The TSP and no added P fertilizer levels produced significantly greater root dry matter (40 and 29%, respectively) than the rock-P fertilizer level (Table 3.24). For shoot and plant dry matter, TSP fertilizer significantly increased production (27 and 28%, respectively) over rock-P fertilizer (Table 3.24). Phosphorus fertilizer did not affect root:shoot ratios in the Malmo soil (Table 3.18; Figure 3.8).

3.3.4 Nodulation Responses

The results on nodule number, and specific nodulation have been summarized on Tables 3.25 and 3.26 and nodule dry weights on Tables 3.17 and 3.18 which presents significance levels for main effects (*P. bilaii* inoculation, P fertilizer) and interactions (*P. bilaii* x P fertilizer) for all harvest dates. Details of treatment effects for each harvest date are presented below.

Table 3.22. Effect of P fertilizer on root, shoot, and plant dry weight of pea in the Hochfeld soil, 28 days after planting.

P fertilizer	Root (g)	Shoot (g)	Plant (g)
TSP[2]	0.5308	1.597	2.127
Rock-P[1]	0.5455	1.485	2.031
No added P	0.4564	1.234	1.655
LSD (P≤ 0.05)	0.0783	0.236	0.299

[1]Rock-P = Idaho rock phosphate at 17.2 mg P kg^{-1} soil.
[2]TSP = triple superphosphate at 17.2 mg P kg^{-1} soil.

Table 3.23. Effect of P fertilizer on root:shoot ratio of pea in the Hochfeld soil, 28 days after planting.

P fertilizer	Root:shoot ratio (g/g)
No added P	0.380
Rock-P[1]	0.370
TSP[2]	0.335
LSD (P≤ 0.05)	0.032

[1]Rock-P = Idaho rock phosphate at 17.2 mg P kg^{-1} soil.
[2]TSP = triple superphosphate at 17.2 mg P kg^{-1} soil.

Table 3.24. Effect of P fertilizer on root, shoot, and plant dry weight of pea in the Malmo soil, 28 days after planting.

P fertilizer	Root (g)	Shoot (g)	Plant (g)
TSP[2]	0.3953	0.7009	1.0962
No added P	0.3626	0.6228	0.9855
Rock-P[1]	0.2816	0.5537	0.8577
LSD (P≤ 0.05)	0.0713	0.098	0.1433

[1]Rock-P = Idaho rock phosphate at 17.2 mg P kg^{-1} soil.
[2]TSP = triple superphosphate at 17.2 mg P kg^{-1} soil.

Table 3.25. Significance of nodulation response of pea in the Hochfeld soil to three P treatments (no added P, rock phosphate, triple superphosphate), inoculation with *Penicillium bilaii* (Pb), to interactions between P fertilizer treatments and Pb inoculation, to Pb inoculation within each P fertilizer treatment.

	14 DAP		21 DAP		28 DAP	
	Nodule no.	Specific Nodulation	Nodule no.	Specific Nodulation	Nodule no.	Specific Nodulation
Main effects						
P fertilizer	-	-	-	-	*	-
P. bilaii	-	-	-	-	-	-
Interaction						
P x Pb	-	-	-	-	-	-
Contrasts						
Pb @ 0P[1]	-	-	-	-	-	-
Pb @ RP[2]	-	-	-	*	-	-
Pb @ TSP[3]	-	-	-	-	-	-

* P ≤ 0.05. ** P ≤ 0.01. *** P ≤ 0.001. - = not significant.

[1]0P = no added P fertilizer. [2]RP = Idaho rock phosphate at 17.2 mg P kg^{-1} soil. [3]TSP = triple superphosphate at 17.2 mg P kg^{-1} soil.

Table 3.26. Significance of nodulation response of pea in the Malmo soil to three P treatments (no added P, rock phosphate, triple superphosphate), inoculation with *Penicillium bilaii* (Pb), to interactions between P fertilizer treatments and Pb inoculation, to Pb inoculation within each P fertilizer treatment.

	14 DAP		21 DAP		28 DAP	
	Nodule no.	Specific Nodulation	Nodule no.	Specific Nodulation	Nodule no.	Specific Nodulation
Main effects						
P fertilizer	-	**	*	*	-	-
P. bilaii	-	-	**	*	-	-
Interaction						
P x Pb	-	-	-	-	-	-
Contrasts						
Pb @ 0P[1]	-	-	-	-	-	-
Pb @ RP[2]	-	-	**	*	-	-
Pb @ TSP[3]	-	-	-	-	-	-

* $P \leq 0.05$. ** $P \leq 0.01$. *** $P \leq 0.001$. - = not significant.

[1]0P = no added P fertilizer. [2]RP = Idaho rock phosphate at 17.2 mg P kg^{-1} soil. [3]TSP = triple superphosphate at 17.2 mg P kg^{-1} soil.

3.3.4.1 14 DAP

For the Hochfeld soil at 14 DAP, no detectable treatment effects on nodule number, dry weight, or specific nodulation of pea was observed (Tables 3.17, 3.25; Figures 3.5, 3.9).

For the Malmo soil, there were no detectable treatment effects on nodule number (Table 3.26; Figure 3.10). However, the main effect of P fertilizer was significant in the Malmo soil for nodule dry weight ($P \leq 0.05$) and specific nodulation ($P \leq 0.01$) (Tables 3.18, 3.26). Rock-P fertilizer significantly increased nodule dry weight 50% over the no added P fertilizer level and 28% over the TSP fertilizer level (Table 3.27). In addition, rock-P fertilizer significantly increased specific nodulation 40% over the no added P level (Table 3.27).

3.3.4.2 21 DAP

For the Hochfeld soil at 21 DAP, the only significant nodulation response occurred for *P. bilaii* inoculation at the rock-P fertilizer level (Tables 3.17, 3.25). Inoculation resulted in a significant reduction in nodule dry weight ($P \leq 0.01$) and specific nodulation ($P \leq 0.05$) by 7- and 3-fold respectively (Figures 3.5, 3.9) at the rock-P fertilizer level.

For the Malmo soil, both *P. bilaii* inoculation and P fertilizer affected nodule number, dry weight, and specific nodulation of pea (Tables 3.18, 3.26, 3.28, 3.29; Figures 3.6, 3.10). Over all P fertilizer levels, *P. bilaii* inoculation on average, reduced nodule number by 38%, nodule dry weight by 56% and specific nodulation 22% compared with non-inoculated plants in the Malmo soil (Table 3.28). Further to this, contrast statements

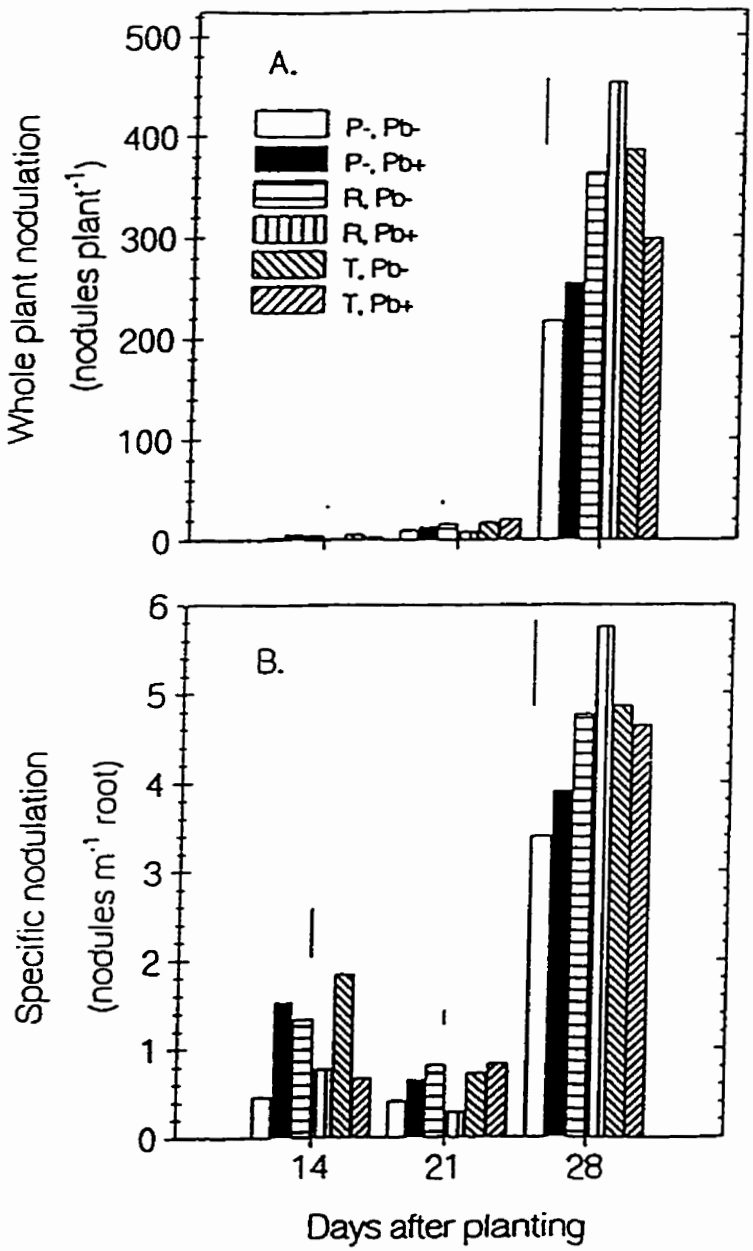

Figure 3.9. The effect of inoculation with (Pb+) or without (Pb-) *Penicillium bilaii* and P sources (0 P fertilizer added, P-; 17.2 mg P of Idaho rock P kg^{-1} soil, R; 17.2 mg P of triple superphosphate kg^{-1} soil, T) on whole plant nodulation (A), and specific nodulation (B) of pea in the Hochfeld soil. Bar represents the mean standard error of treatments within dates.

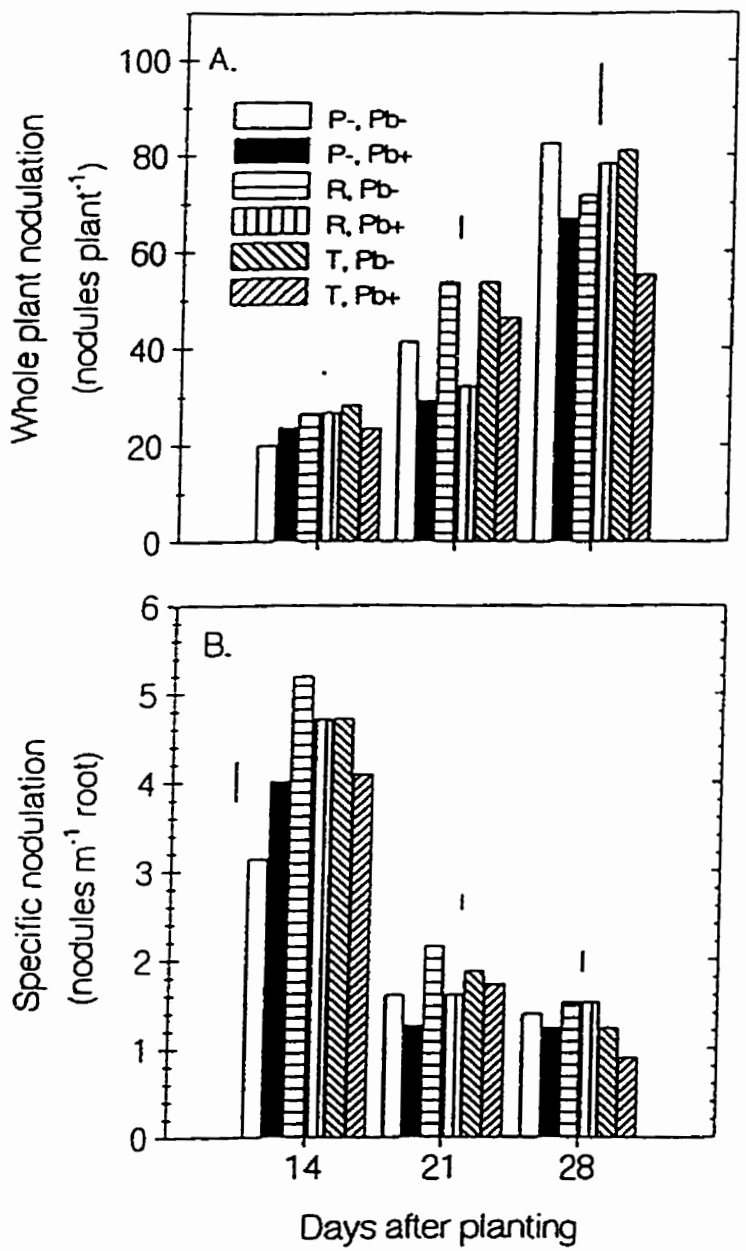

Figure 3.10. The effect of inoculation with (Pb+) or without (Pb-) *Penicillium bilaii* and P sources (0 P fertilizer added, P-; 17.2 mg P of Idaho rock P kg^{-1} soil, R; 17.2 mg P of triple superphosphate kg^{-1} soil, T) on whole plant nodulation (A), and specific nodulation (B) of pea in the Malmo soil. Bar represents the mean standard error of treatments within dates.

Table 3.27. Effect of P fertilizer on nodule dry weight and specific nodulation of pea in the Malmo soil, 14 days after planting.

P fertilizer	Nodule dry weight (g)	Specific nodulation (no. m^{-1} root)
Rock-P[1]	0.0009	4.95
TSP[2]	0.0007	4.38
No added P	0.0006	3.54
LSD ($P \leq 0.05$)	0.0002	0.94

[1]Rock-P = Idaho rock phosphate at 17.2 mg P kg^{-1} soil.
[2]TSP = triple superphosphate at 17.2 mg P kg^{-1} soil.

indicated *P. bilaii* inoculation at the rock-P fertilizer level significantly ($P \leq 0.01$) reduced nodule number 66% and specific nodulation ($P \leq 0.05$) 34% compared with the non-inoculated plants (Table 3.26; Figure 3.10). *P. bilaii* inoculation at the TSP fertilizer level significantly ($P \leq 0.05$) reduced nodule dry weight 50% compared with the non-inoculated plants (Table 3.18; Figure 3.10). *P. bilaii* inoculation at the rock-P fertilizer level was significant at $P = 0.07$ for reducing nodule dry weight (Table 3.18).

The main effect of P fertilizer had a significant effect on nodule number ($P \leq 0.05$), dry weight ($P \leq 0.01$), and specific nodulation ($P \leq 0.05$) of pea in the Malmo soil (Tables 3.18, 3.26; Figures 3.6, 3.10). Triple superphosphate fertilizer significantly increased nodule number 41%, and dry weight 67% over the no added P fertilizer level (Table 3.29). Rock-P fertilizer significantly ($P \leq 0.05$) increased specific nodulation 32% over the no added P fertilizer level in the Malmo soil (Table 3.29).

3.3.4.3 28 DAP

At 28 DAP, nodule number, dry weight, and specific nodulation of pea was not affected by *P. bilaii* inoculation on the Hochfeld soil (Tables 3.17, 3.25; Figures 3.5, 3.9). Phosphorus fertilizer in the Hochfeld soil had a significant ($P \leq 0.05$) effect on nodule number and dry weight (Tables 3.17, 3.25). The rock-P fertilizer level produced significantly more (72%) nodules than the no added P fertilizer level (Table 3.30). Rock-P fertilizer increased nodule dry weight 108% and TSP fertilizer 103% over the no added P fertilizer level in the Hochfeld soil (Table 3.30).

For the Malmo soil, the only significant response occurred for *P. bilaii* inoculation at the TSP fertilizer level (Tables 3.18, 3.26; Figures 3.6, 3.10). Overall, *P. bilaii*

Table 3.28. Effect of *Penicillium bilaii* (with, +; without, -) inoculation on nodule number, nodule dry weight, and specific nodulation of pea in the Malmo soil, 21 days after planting.

P. bilaii	Nodule number	Nodule dry weight (g)	Specific nodulation (no. m^{-1} root)
-	49.61	0.0014	1.89
+	36.00	0.0009	1.54
LSD (P≤ 0.05)	8.63	0.0003	0.32

Table 3.29. Effect of P fertilizer on nodule number, nodule dry weight, and specific nodulation of pea in the Malmo soil, 21 days after planting.

P fertilizer	Nodule number	Nodule dry weight (g)	Specific nodulation (no. m^{-1} root)
TSP[2]	50.00	0.0015	1.43
No added P	43.08	0.0011	1.81
Rock-P[1]	35.33	0.0009	1.90
LSD (P≤ 0.05)	10.57	0.0004	0.39

[1]Rock-P = Idaho rock phosphate at 17.2 mg P kg^{-1} soil.
[2]TSP = triple superphosphate at 17.2 mg P kg^{-1} soil.

Table 3.30. Effect of P fertilizer on nodule number, and nodule dry weight of pea in the Hochfeld soil, 28 days after planting.

P fertilizer	Nodule number	Nodule dry weight (g)
Rock-P[1]	404.55	0.0337
TSP[2]	351.30	0.0330
No added P	235.20	0.0162
LSD ($P \leq 0.05$)	126.36	0.0154

[1]Rock-P = Idaho rock phosphate at 17.2 mg P kg^{-1} soil.
[2]TSP = triple superphosphate at 17.2 mg P kg^{-1} soil.

inoculation significantly ($P \leq 0.01$) reduced nodule dry weight 105% compared with the non-inoculated plants (Figure 3.10).

3.4. Discussion

These experiments were conducted to assess the stimulatory effect of the rhizospheric fungus *Penicillium bilaii* on pea root morphology and phosphate absorption. The favoured hypothesis of how *P. bilaii* promotes plant growth is that the fungus is thought to solubilize sparingly soluble phosphates by secreting organic acids, which acidify the surrounding soil and/or by cation chelation, thereby increasing the availability of soluble phosphate to the plant (Asea *et al.*, 1988; Kucey, 1987, 1988). The three occurrences where inoculation with *P. bilaii* increased P concentration or accumulation in pea tissue, there was no observed stimulation of plant root length (see below). The observed increases in P concentration and accumulation associated with *P. bilaii* inoculation occurred when there was no addition of P fertilizer, or when rock phosphate fertilizer was added. Other researchers have observed increased P uptake as a result of *P. bilaii* inoculation, which occurred both with and without added P fertilizers (Asea *et al.*, 1988; Gleddie, 1992; Kucey, 1988; Kucey and Leggett, 1989). At 14 days after planting (DAP) on the Malmo soil (low P), *P. bilaii* inoculation when no P fertilizer was added, increased shoot P concentration 29% and shoot P accumulation 45%, but there was no resultant increase in dry matter yields (Tables 3.3, 3.10, 3.18; Figures 3.2, 3.4, 3.6). At 28 DAP on the Malmo soil, *P. bilaii* inoculation and rock phosphate fertilizer increased both root and shoot P concentration (22, and 42% respectively) and accumulation (39, and 63% respectively) but no increase in dry matter yields was observed (Tables 3.3, 3.10,

3.18; Figures 3.2, 3.4, 3.6). At 21 DAP on the Hochfeld (high P) soil, *P. bilaii* inoculation and rock phosphate fertilizer increased shoot P concentration 12%, but no effect on dry matter nor increase in P accumulation was observed (Tables 3.2, 3.9, 3.17; Figures 3.1, 3.3, 3.5). Other researchers have demonstrated that inoculation with *P. bilaii* resulted increased dry matter production (Downey and van Kessel, 1990) and increased P uptake and dry matter yield of pea shoots and roots (Gleddie, 1992).

As the Malmo soil was P deficient (Table 3.1), it was expected that any increase in P availability from the application of P fertilizer would have resulted in increased dry matter production, provided that other factors were not limiting growth. Triple superphosphate (TSP) fertilizer at 21 DAP increased shoot P concentration over rock phosphate and no added P treatments, but since there was no dry matter response, accumulation was not increased (Tables 3.10, 3.13, 3.18; Figures 3.4, 3.6). The Hochfeld soil had sufficient soil P levels (Table 3.1) and responses to P fertilizer were unexpected. Shoot P accumulation and shoot and plant dry matter yields responded to the addition of P fertilizer (Tables 3.9, 3.15, 3.17, 3.22; Figures 3.3, 3.5). Allaway (1971) has concluded that the effect of adding a nutrient as fertilizer may range from no increase in the concentration of the element in the plant although marked yield increases may be obtained, to significant increases in the level of the element in plant tissue without change in yields. Paynter (1993) has described the two components of a plant response to applied P to be: first, the ability of the plant to take up P, and second, its ability to utilise this absorbed P within the roots and shoots. At 21 DAP, the plants on the Malmo soil were able to take up P as there was an increase in the shoot P concentration when TSP fertilizer was added.

However, the plants were unable to utilise this P for dry matter production. By the third harvest, plants at the TSP fertilizer level were not significantly different from those that received no P fertilizer in terms of P concentration and accumulation and dry matter production.

The reasons for the lack of dry weight response may be threefold. First, if we look at the P concentration values in the plant tissues for the Malmo soil compared with the Hochfeld soil (Figures 3.3, 3.4), there is a dramatic difference between the soils. The P concentration in the plant can reflect the adequacy of P for plant growth (Hanway and Olson, 1980). Generally for leaf samples of grain crops (wheat, oat, corn, soybean) at anthesis or full bloom, if the percentage of P (expressed on an oven-dry basis) exceeds 0.25% (2.5 mg g^{-1}), the P concentration of the plant is considered sufficient. If the P concentration is less than 0.2% (2 mg g^{-1}), the plant is considered low in P, and if less than 0.15% (1.5 mg g^{-1}), the plant is considered to be very P deficient (Hanway and Olson, 1980). Although these ranges are for more mature leaf tissue, the P concentration of young tissue is usually greater than mature tissue, and therefore these ranges are applicable for this discussion (see below). For the Malmo soil, the P concentrations of the shoots at 14 DAP are in the sufficient range (Figure 3.4). This is likely a result of the supply of P from cotyledon P reserves. At 21 and 28 DAP, the P concentration of the shoot tissue is in the low to deficient range (Figure 3.4). The effects of P deficiency are reflected in the reduction in dry weights of the roots, shoots, and nodules of plants and the increased root:shoot ratios on the Malmo soil compared with the Hochfeld soil (Figures 3.5, 3.6, 3.7, 3.8). The Hochfeld soil was sufficient in P (Table 3.1) and the shoot P

concentrations were in the sufficient range at all dates (Figure 3.3). Researchers have found that biomass gain per unit leaf area is low in P-stressed plants because each unit of leaf area has a greater respiratory burden of heterotrophic tissue to sustain. As a result, a greater proportion of fixed carbon is lost to respiration in P deficient plants (Lynch *et al.*, 1991). Lynch *et al.* (1991) determined that the proportionally greater biomass partitioned to heterotrophic tissue (i.e., roots) could entirely account for the reduced growth of P-deficient plants. If a greater rate of P was applied, or if the P fertilizer was added to the pots to be positionally available (i.e., mimic a side band application), a greater response to P in the Malmo soil may have been observed. Since an increase in the P concentration of shoots at 21 DAP was observed with the addition of triple superphosphate fertilizer in the Malmo soil (Table 3.13), other factors may have been limiting growth.

The second factor that may have contributed to the lack of dry weight response may be related to N deficiency. The Malmo soil was rated as P and N deficient (Table 3.1) and nodulation response was poor (Figures 3.6 and 3.10) compared with the Hochfeld soil, which had optimum P levels and nodulation response was good (Figures 3.5 and 3.9). Researchers have observed that P plays direct roles in rhizobial growth and survival, nodule initiation, and function (Beck and Munns, 1984; Cassman *et al.*, 1981; Gates, 1974; Israel, 1987). Legumes that rely on N_2 fixation for their supply of N, and are not receiving an adequate supply of P, may become N deficient (Marschner, 1995).

The third factor that may have contributed to the lack of dry weight response on the Malmo soil is the irradiance in the growth chamber (approximately 615 $\mu mol\ m^{-2}\ s^{-1}$). Given that the irradiance in the field can be up to three times the irradiance of our growth

chambers, photosynthesis may have been a limiting factor, and masking a P fertilizer response on the low-P Malmo soil.

One observed effect of *P. bilaii* on root morphology was that inoculation resulted in a reduction of root length. At 21 DAP on the Malmo soil, inoculation resulted in a 12% reduction of root length over all P fertilizer levels (Table 3.6). This reduction in root length did not result in a change in the P concentration of roots or shoots, or shoot P accumulation, but resulted in less P accumulation by the roots as significantly less root dry matter was produced compared with non-inoculated plants (Figures 3.2, 3.4, 3.6). There was also a reduction of plant (whole) dry matter yield of 11% as a result of *P. bilaii* inoculation (Table 3.20). However, by 28 DAP, this reduction in root length did not affect P concentration or accumulation, nor dry matter production (Figures 3.4, 3.6). This suggests that *P. bilaii* inoculated plants require less root surface area to produce shoot dry matter and possibly for P absorption as less root length of inoculated plants did not negatively affect P concentration or accumulation, nor dry matter production.

Another observed effect of *P. bilaii* inoculation on root morphology, in particular mean root diameter, occurred on the Malmo soil at 28 DAP at the no additional P fertilizer level. Inoculation resulted in a 22% reduction in SRL (i.e., a decrease in root length per unit root dry weight), which translates into plant roots that are thicker (Figure 3.2). Root length was reduced by 20% at P=0.06. This alteration of root morphology did not affect pea tissue P concentration or accumulation. Interestingly, at 14 DAP, shoot P concentration and accumulation were significantly greater for the *P. bilaii* inoculated plants at the no additional P fertilizer level (Table 3.10, Figure 3.4). At 21 DAP, there

was a reduction in root length plus root and plant dry weight, but no subsequent effect on shoot P concentration, accumulation, or dry weight occurred (Tables 3.3, 3.10, 3.18; Figures 3.2, 3.4, 3.6). This may also suggest that *P. bilaii* inoculated plants may not require as large of a root surface area for P absorption as non-inoculated plants. The observed increase in shoot P concentration and accumulation at 14 DAP may have lead to the subsequent reduction in root length (21 DAP) and increase in SRL at 28 DAP, as plants with sufficient levels of P may actually have shorter roots with greater diameter compared with P-deficient plants (Asher and Loneragan, 1967; Christie and Moorby, 1975; Ozanne *et al.*, 1969; Powell, 1974).

Generally, plants will increase the root surface area to improve the P supply to the shoot (Atkinson, 1973; Fitter and Hay, 1987; Powell, 1974; Römer *et al.*, 1988; Schenk and Barber, 1979 a, b). Plant roots are known to proliferate in P-rich regions of soil (Drew *et al.*, 1973). Although root proliferation is stimulated in a P-rich zone, the addition of P may lead to a reduction in the actual amount of roots per unit weight of top (Christie and Moorby, 1975; Ozanne *et al.*, 1969). Total root length and dry weight may be reduced when the levels of P in the soil are increased (Asher and Loneragan, 1967; Powell, 1974). Mean root radius (and thus the diameter) is generally smaller under low P conditions suggesting that roots of P deficient plants are finer (Fitter, 1985; Fitter and Hay, 1987; Powell, 1974; Schenk and Barber, 1979b). Some plants will reduce root radius instead of root length under shortage of assimilates during P deficiency as a mechanism for increasing the root surface per unit of root weight to improve the P supply to the shoot (Schenk and Barber, 1979b). The non-inoculated plants at the no additional

P levels were significantly finer than *P. bilaii* inoculated plants on the Malmo soil (Table 3.3; Figure 3.2). In addition, pea plants in this study appeared to increase the root:shoot ratio under P deficiency (Malmo vs. Hochfeld soil) (Figures 3.7, 3.8). At low P supply, the proportion of root mass produced increased. This mechanism is an adaptation of plants to improve P uptake when P is limiting. This phenomenon has been observed by many researchers for many species (Atkinson, 1973; Föhse *et al.*, 1988; Lynch et al., 1991).

The rate limiting steps in the uptake of phosphate by plants from soil are: a) the diffusion of phosphate ions in the soil solution to the plant roots; b) concentration of phosphate at the root surface; and c) the release of phosphate ions from soil particles (Bolan, 1991). Since the observed increases in P concentration and accumulation by *P. bilaii* inoculated plants were not preceded or accompanied by a greater root surface area for P absorption, this fungus may be involved in increasing the availability of P to the plant. One possible explanation of the observed alteration of root morphology of *P. bilaii* inoculated plants is that the fungus is increasing the availability of P to the plant roots. Thus, no increased investment in root production was required, but was necessary for non-inoculated plants. *Penicillium bilaii* has shown P-solubilizing ability (Asea *et al.*, 1988; Kucey, 1983, 1988) an produces oxalic and citric acid in pure culture (Cunningham and Kuiack, 1992). Production of organic compounds such as citrate can release phosphate from soil particles (Bolan, 1991; Bar-Yosef, 1996). Previous studies using ^{32}P indicated that plants inoculated with *P. bilaii* are able to utilize soil P sources that are unavailable to control plants (Chambers, 1992) as well as rock phosphate (Asea *et al.*,

1988). This is consistent with the results of this study, which demonstrate increased P concentration and accumulation by *P. bilaii* inoculated plants without the addition of P fertilizer as well as with rock phosphate. The lack of response to *P. bilaii* by inoculated plants on the Hochfeld soil (high P availability) supports the favoured mechanism of growth promotion by *P. bilaii*. However, other researchers have observed increased dry matter production with inoculation with *P. bilaii* that was not associated with increased P content of the plants (Chambers, 1992; Downey and van Kessel, 1990; Gleddie, 1992; Keyes, 1990). This evidence suggests that *P. bilaii* may stimulate plant growth by other mechanisms, possibly in addition to increasing the availability of P to plants. Keyes (1990) has suggested that *P. bilaii* may promote plant growth through passive biocontrol or growth-promoting effects. Because this system is so complex, involving a plant, a fungus, and the soil environment, one should not limit the effect of a living component (i.e., *P. bilaii*) to a single mechanism as can be done with the application of P fertilizer.

Another objective of this study was to determine if *P. bilaii* inoculation affected nodulation of peas. Negative effects of *P. bilaii* inoculation on nodulation (nodule no., specific nodulation, dry weight) were observed on both soil types. Nevertheless, by 28 DAP, the only negative effect observed was for nodule dry weight on the Malmo soil at the TSP fertilizer level (Tables 3.17, 3.18, 3.25, 3.26; Figures 3.5, 3.6, 3.9, 3.10). At 21 DAP on the Malmo soil, the reduction in nodulation response was likely a result of the reduction of root dry matter and thus, fewer sites for *Rhizobium* infection (Table 3.18, Figure 3.6).

The coefficient of variation (CV) for nodulation parameters was quite high. For

the Hochfeld soil, the CVs ranged from 37-124% and the CVs for the Malmo soil were appreciably lower ranging from 25-46%. Generally, the lowest CV values were obtained at 28 DAP. These high values for the CV might have been a result of the inherent variability of nodulation in soils. Nevertheless, it may have prevented detecting true significant differences with the number of replicates used in this study.

In situ acetylene reduction assay was attempted to measure nitrogenase activity, but could not be used as there were problems with administering the acetylene to the roots. The N concentration and accumulation of the pea tissue were not measured in this study and could have given a better indication of the N status of the plant.

Gleddie (1992) reported inoculation with *P. bilaii* resulted in increased nodulation of pea roots by *Rhizobium leguminosarum* in a growth room study, and promoted increased N uptake by pea in growth chamber and P responsive field trials. However, Downey and van Kessel (1990) found that inoculating with *P. bilaii* decreased total N accumulation by pea plants in a growth chamber experiment. They proposed that the production of P-solubilizing organic acids by the fungus may have reduced the rhizosphere pH to a degree that would inhibit *Rhizobium* function.

In conclusion, it appears that *P. bilaii* does not stimulate root growth (i.e., root length) to increase root surface area for phosphate absorption or to promote plant growth. Inoculation with this fungus may affect the morphological properties of the root system (root length and diameter) by improving the availability of P to the plant, and thus, the resulting P status of the plant. Changes in P availability, and the resulting plant uptake may result in the alteration of the morphological properties of the root system, as P status

is known to affect root morphology. No effects on dry matter were observed, but this may have been a result of other factors limiting growth besides P. In this study, the effects of *P. bilaii* on nodulation response of pea are inconclusive. However, if root length was reduced by *P. bilaii*, through improved P status of the plant, a smaller number of infection sites would have been expected.

4.0 Effect of *Penicillium bilaii* on root morphology of pea (*Pisum sativum* L.) - Field study

4.1 Introduction

In western Canada, field crops generally take up about 10 kg ha^{-1} yr^{-1} of phosphorus (P) in the form $H_2PO_4^-$ or HPO_4^{2-} from the soil solution (Keyes, 1990). Although the soils on which these crops are grown are often high in total P, only a very small portion (0.01%) is available to the plants (Brady, 1990; Doyle and Cowell, 1993a). To increase the availability of P to plants, phosphate fertilizers can be added to the soil. However, when water soluble P fertilizers dissolve in moist soil they can react with soil constituents which leads to the formation of less soluble compounds, and with time, to highly insoluble forms. The reactions between P fertilizers and soil constituents are referred to as P fixation and retention (Soper and Racz, 1980). Usually the surface 15-20 cm of soil is higher in P than the subsoil because P is transported to the surface by plant growth over years of soil development and from P fertilizer applications (Barber, 1980; Ozanne, 1980). Subsoil P levels are lower because P does not leach and it is difficult to incorporate P fertilizers mechanically.

Plants have various ways in which to adapt to P-limiting soil conditions. Sensitivity analysis of models for phosphate uptake by plant roots has indicated that plant properties that affect root surface area (eg. root length and diameter), can greatly affect the rate of phosphate uptake by the plant (Silverbush and Barber, 1983). Increased phosphate uptake may result from plants that have a finer and longer root system. Soil

microorganisms may promote plant growth by altering root growth and morphology or by influencing the influx kinetics of part of or the whole root surface to improve water and mineral nutrient acquisition by the plant (Barber, 1984; Marschner, 1995; Tinker, 1980).

Penicillium bilaii (ATCC strain no. 20851) is a rhizospheric fungus reported to increase dry matter production, grain yield, and P uptake of wheat, canola, bean, pea, and lentil in growth chamber and field experiments alone, or in combination with rock phosphate or monoammonium phosphate (MAP) (Asea *et al.*, 1988; Chambers, 1992; Gleddie, 1993; Gleddie *et al.*, 1991, 1993; Kucey, 1987, 1988; Kucey and Leggett, 1989). The mechanism (s) underlying the stimulation of plant growth and P-uptake is not known. The fungus has been shown to solubilize calcium phosphate in an agar medium (Kucey, 1983), rock phosphate in liquid culture (Asea *et al.*, 1988), and in potted soils, *P. bilaii* inoculation increased the $NaHCO_3$-extractable P and the incidence of P-solubilizing fungi in the rhizosphere (Kucey, 1988). The major acidic metabolites produced by *P. bilaii* are oxalic and citric acid (Cunningham and Kuiack, 1992). This indirect evidence that suggests *P. bilaii* may increase the availability of phosphate to the plant by releasing organic acids, which may act to acidify localised areas of the rhizosphere and/or act as a chelator of cationic partners of the phosphate anion (Kucey, 1988). However, alternative evidence suggests that *P. bilaii* stimulates plant growth by other mechanisms (Chambers, 1992; Downey and van Kessel, 1990; Gleddie, 1992; Keyes, 1990).

One possible mechanism of how *P. bilaii* may promote plant growth is by altering root growth and morphology thereby increasing the root surface area for nutrient absorption. In research related to the present study (Chapter 3), *P. bilaii* under controlled

conditions was associated with higher shoot and root tissue P concentration and accumulation, but this increase was not a result of an increase in root surface area for P absorption. In fact, inoculation with this fungus was associated with a reduction of total root length and specific root length (i.e., increased average root diameters) while maintaining the P concentration of the tissue to that of the control plants. This reduction in root length in early growth did not affect the subsequent P status or dry weight of the inoculated pea plants.

The objective of this study was to determine, under field conditions, if the stimulation of P-uptake in peas (*Pisum sativum* L.) treated with *P. bilaii* is the result of a generalized stimulation of plant root growth. This stimulation of root growth would result in a greater root surface area to access soil phosphorus. We also determined the effect of this fungus on above ground biomass and nodulation of crown roots of pea.

4.2 Materials and Methods

4.2.1 Site Preparation and Seeding

Field trials were established at two locations in 1996; Ellerslie AB (49^0 28' N, 98^0 W) (NE-24-T51-R25-W4) and Outlook SK (51° 30' N, 107°, 03' W) (NW-24-T27-R7-W3). These locations were selected because of their low levels of soil P and N (Table 4.1). Wheat was planted at both sites in each of the two previous years. Trials were arranged in a randomized complete block design with factorial (2 x 3) treatment structure. The two factors were *P. bilaii* with two levels (inoculated, non-inoculated) and level of P fertilization with three levels (0, 6.4, 19.3 kg P ha^{-1}) (0, 15, 45 kg P_2O_5 ha^{-1}) resulting in six treatments with five replications.

Soil analysis for the Ellerslie and Outlook sites are summarized in Table 4.1. All analysis of the soil was carried out by Plains Innovative Laboratory Services, Saskatoon SK, using soil cores (30 cm). Soil texture was determined by the hand-texturing method (by feel and rolling). Soil pH was determined by using a 1:2 soil water paste and pH meter (Kalra, 1995). Soil electrical conductivity (EC) was determined using a 1:2 soil water paste and conductivity meter. Soil nitrogen (NO_3-N) was determined using a $CaCl_2$ extract and analysed for nitrate and nitrite by automated colorimetry (Manual Soil Sampling, 1978). Soil phosphorus (P) and potassium (K) was determined using a modified Kelowna extraction (Qian *et al.*, 1994). Soil sulphur (SO_4-S) was determined using a $CaCl_2$ extract and analysed for sulphate by methyl thymol blue automated colorimetry (APHA, 1992).

Prior to seeding, ethalfluralin (Edge DC, 1.15 kg a.i. ha^{-1}) was applied at each field site for weed control and was incorporated in the same direction as plots with a 1.5 m wide rototiller, twice. Trials were hand weeded as necessary to remove weeds not eliminated by the ethalfluralin, and at the Ellerslie site, imazethapyr (Pursuit, 51 g a.i. ha^{-1}) along with a surfactant (Agsurf, 250 ml ha^{-1}), were applied 32 days after planting for post-emergent weed control.

Table 4.1. Characteristics of the soils at the Ellerslie AB, and Outlook SK field sites.

Site	Soil climatic zone	Surface texture*	pH	E.C.	NO_3-N	P	K	SO_4-S
				$mS\ cm^{-1}$	---------- $mg\ kg^{-1}$ ----------			
Ellerslie AB	Moist black central	CL	7.4	0.1	4.2	3.3	118	6.7
Outlook SK	Dark brown	CL	8.5	0.1	2.5	1.7	163	4.7

*CL = clay loam

Trials were seeded by Philom Bios Inc. (Saskatoon, SK) using a small-plot double disc seed drill custom made to minimize contamination between seed inoculant treatments. Pea (*Pisum sativum* L. cv. Majoret) seeds were planted at a depth of 3.1 cm at a rate of 231 kg ha^{-1}. The sites were seeded May 3, 1996 (Outlook) and May 10, 1996 (Ellerslie). All seeds were inoculated with *Rhizobium leguminosarum* bv *viceae* (strain NRG 457) at a rate of 2.0 x 10^5 colony forming units (CFU) $seed^{-1}$. Those seeds receiving *P. bilaii* seed treatment were inoculated at a rate of 1.1 x 10^5 CFU g^{-1} seed.

Individual plots were 10 m in length by 1.5 m in width. Each plot consisted of six treatment rows 15 cm apart, bordered by two guard rows of triazine tolerant canola to eliminate edge effects. Each trial was bordered by untreated plots of pea, also to eliminate edge effects.

Monoammonium phosphate (12-51-0) was banded 5 cm below and 2.5 cm beside the seed at a rate of 0, 6.4, 19.3 kg P ha^{-1} (0, 15, 45 kg P_2O_5 ha^{-1}). Potassium sulphate (0-0-22-22) and zinc sulphate (liquid- 7% Zn, 4% S) were applied according to soil test recommendations.

4.2.2 Sampling

The Outlook and Ellerslie sites were sampled at 41 and 36 days after planting with the plants having an average of 9 and 7-8 nodes, respectively. Two cores per plot were taken, and an average of the two was used for analysis. The sample core was 15 cm in length and 6.5 cm in diameter. The corer was placed directly over the plant with the shoot being in the centre. No weeds or plants were within 7.5 cm (in all directions) of the sample plant. The shoots were removed and later frozen at -20° C, freeze dried, weighed,

taken, and an average of the two was used for analysis. The sample core was 15 cm in length and 6.5 cm in diameter. The corer was placed directly over the plant with the shoot being in the centre. No weeds or plants were within 7.5 cm (in all directions) of the sample plant. The shoots were removed and later frozen at -20° C, freeze dried, weighed, and ground using a coffee grinder. The soil cores were frozen at -20° C and later processed. The soil was removed from the roots by soaking the core in tap water and gently shaking the root core. The soil adhering to the roots was removed by placing the roots and soil onto a sieve (1.2 or 1.4 mm) and gently washing. The roots were then stained with methylene blue (0.1% w/v distilled water) by placing the roots into the dye solution for 30 s, followed by rinsing with distilled water for 30 s. Root lengths were determined using IMAGEX, a digital analysis system, developed by L. Lamari, Dept. of Plant Science, Winnipeg, MB as described previously in Chapter 3. After analysis the roots were frozen at -20° C and later thawed to remove and count the nodules. The samples were then refrozen, freeze dried, and weighed. Phosphorus concentration of the shoot tissue was determined by the same method used in the growth chamber experiments for the Ellerslie soil.

One meter row samples of above ground biomass were removed from each plot, and the number of plants, and dry weights were determined.

Data was analysed using the General Linear Model procedure of the Statistical Analysis System package (SAS Institute Inc., 1986) and single degree of freedom contrasts were made. Treatment means were separated using the Fisher protected least significant difference test (LSD) at $\alpha = 0.05$ level, after the analysis of variances indicated

significant differences at the same level.

4.3 Results

The field experiments were conducted to investigate the effect of *Penicillium bilaii* and phosphorus (P) fertilizer on the stimulation of crown root growth and the resulting concentration and accumulation of P in the shoot tissue. There was only one sampling period at each site, and the results from these sites will be discussed together.

4.3.1 Root Morphology

The results on root morphology have been summarized on Table 4.2 which presents significance levels for main effects (*P. bilaii* inoculation, P fertilizer) and interactions (*P. bilaii* x P fertilizer) for both the Ellerslie and Outlook sites. Details of treatment effects are presented below.

There were no detectable treatment effects on root crown morphology of pea at the Outlook site (Table 4.2; Figure 4.1). However, at the Ellerslie site *P. bilaii* inoculation at the no additional P fertilizer level had a significant effect ($P \leq 0.05$) on root morphology of pea (Table 4.2; Figure 4.2). Inoculation with this fungus resulted in a 48% increase in root crown length and a 21% increase in specific root crown length when no P fertilizer was added. In addition, this treatment produced the greatest root crown length and specific root crown length over all other treatments (Figure 4.2).

Table 4.2. Significance of root crown responses [root length and specific root length (SRL)] of pea grown with and without *Penicillium bilaii* (Pb) and at one of three levels of P fertility (no added P, 6.4 and 19.3 kg P ha^{-1}) at the Ellerslie and Outlook sites.

	Ellerslie		Outlook	
	Root crown† length	Root crown† SRL	Root crown† length	Root crown† SRL
Main effects				
Block	-	-	-	-
P level	*	-	-	-
P. bilaii	**	-	-	-
Interaction				
P level x Pb	*	-	-	-
Contrasts				
Pb @ 0 P	***	*	-	-
Pb @ 6.4 P	-	-	-	-
Pb @ 19.3 P	-	-	-	-

* $P \leq 0.05$. ** $P \leq 0.01$. *** $P \leq 0.001$. - = not significant.

† root recovered from a core 15 cm in depth, and 6.5 cm in diameter taken at the base of the plants.

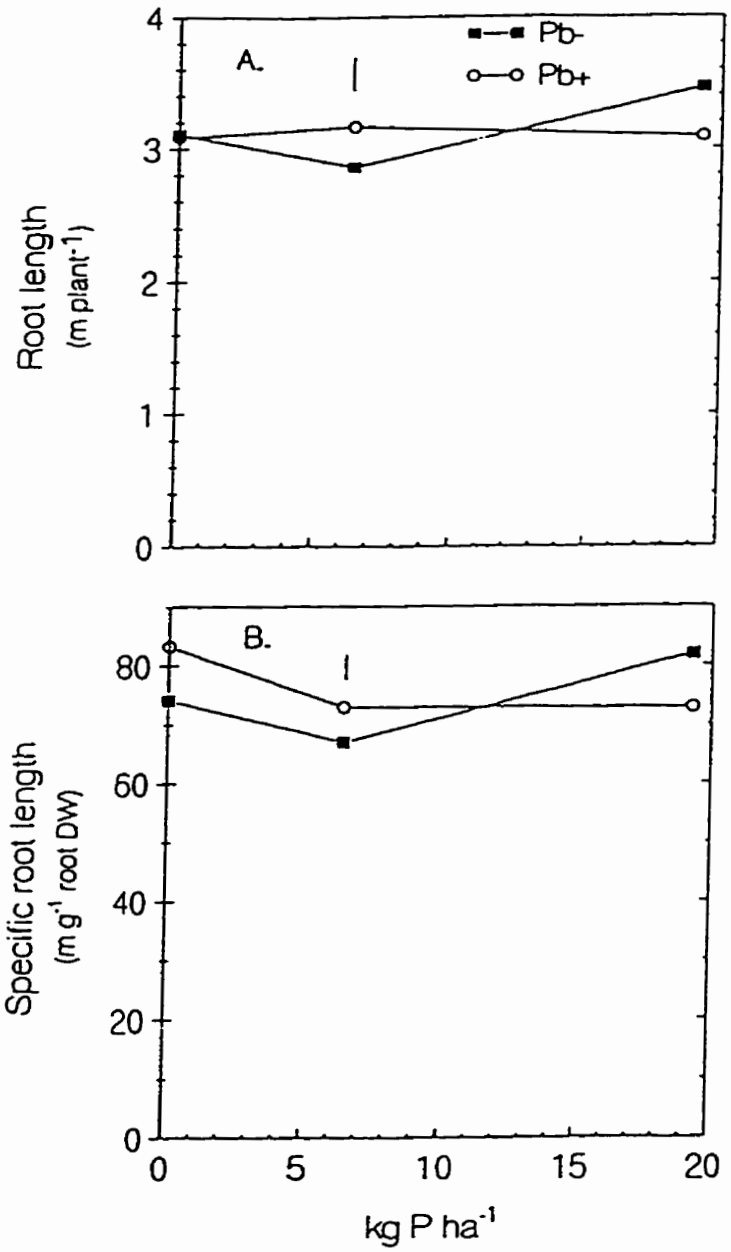

Figure 4.1. The effect of inoculation with (Pb+) or without (Pb-) *Penicillium bilaii* and P fertilizer level (0 P fertilizer added, 6.4 kg P ha^{-1}, and 19.3 kg P ha^{-1}) on root length (A), and specific root length (B) of pea taken from a core 15 cm in depth, and 6.5 cm in diameter, from the base of the plants at the Outlook site. Bar represents the mean standard error of treatments.

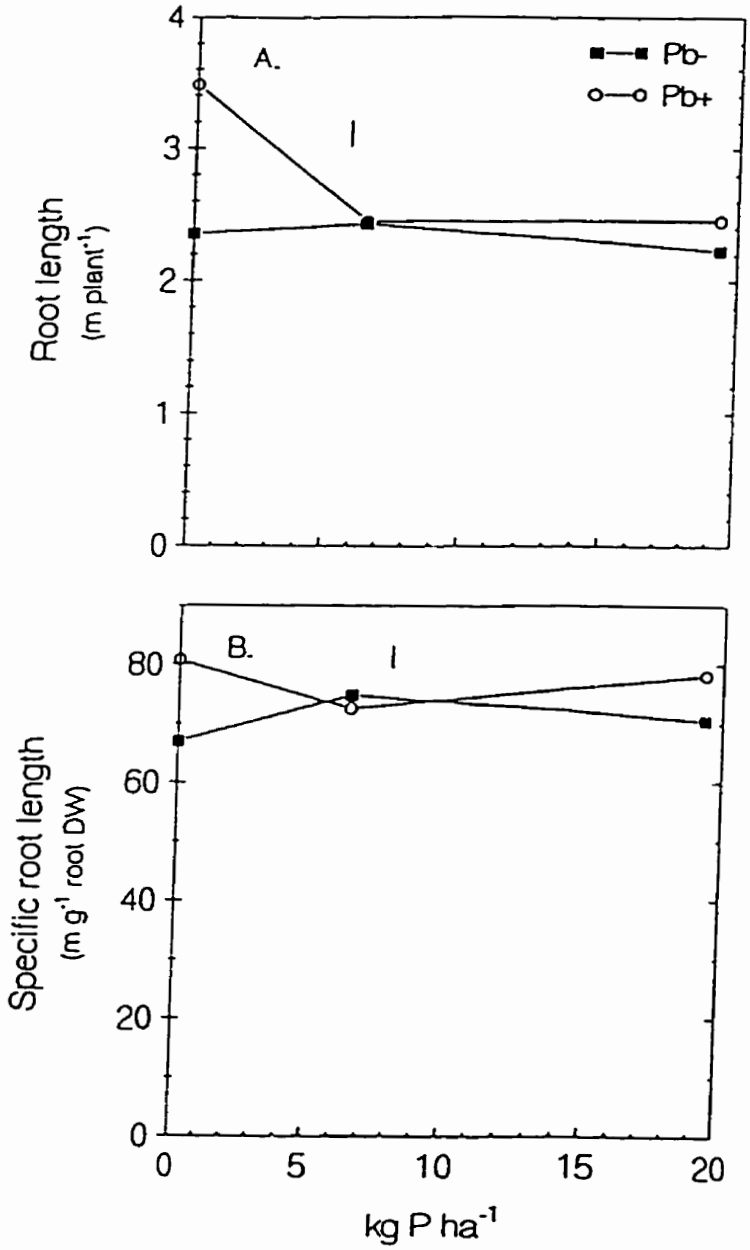

Figure 4.2. The effect of inoculation with (Pb+) or without (Pb-) *Penicillium bilaii* and P fertilizer level (0 P fertilizer added, 6.4 kg P ha^{-1}, and 19.3 kg P ha^{-1}) on root length (A), and specific root length (B) of pea taken from a core 15 cm in depth, and 6.5 cm in diameter, from the base of the plants at the Ellerslie site. Bar represents the mean standard error of treatments.

4.3.2 Shoot P Concentration and Accumulation

The results of shoot P concentration and accumulation have been summarized on Table 4.3 which presents significance levels for main effects (*P. bilaii* inoculation, P fertilizer) and interactions (*P. bilaii* x P fertilizer) for both the Ellerslie and Outlook sites. Details of treatment effects are presented below.

At both sites, the main effect of P fertilizer level was highly significant ($P \leq 0.01$) for shoot P concentration and accumulation (Table 4.3; Figures 4.3, 4.4). At the Ellerslie site, each fertilizer level produced significantly ($P \leq 0.05$) different results for shoot P concentration (Tables 4.3, 4.4; Figure 4.3). As the P fertilizer level increased, shoot P concentration increased. Further, the addition of P fertilizer (as 6.4 or 19.3 kg P ha^{-1}) resulted in significantly ($P \leq 0.05$) greater shoot P accumulation (27% and 42%, respectively) compared with the no added P fertilizer level (Tables 4.3, 4.4; Figure 4.3). At the Outlook site, addition of P fertilizer (as 6.4 or 19.3 kg P ha^{-1}) significantly ($P \leq 0.05$) increased shoot P concentration (25% and 32%, respectively) over the no added P fertilizer level (Tables 4.3, 4.5; Figure 4.4). Each fertilizer level produced significantly ($P \leq 0.05$) different results for shoot P accumulation. The addition of P fertilizer (as 6.4 or 19.3 kg P ha^{-1}) resulted in significantly ($P \leq 0.05$) greater shoot P accumulation (31% and 58%, respectively) compared with the no added P fertilizer level (Tables 4.3, 4.5; Figure 4.4).

At the Ellerslie site, the main effect of *P. bilaii* inoculation was significant at $P = 0.0505$ for increasing shoot P concentration. At the no added P fertilizer level, inoculation significantly ($P \leq 0.01$) increased shoot P concentration by 13% (Table 4.3; Figure 4.3).

Table 4.3. Significance of shoot P concentration (conc.) and accumulation (total) responses of pea grown with and without *Penicillium bilaii* (Pb) and at one of three levels of P fertility (no added P, 6.4 and 19.3 kg P ha^{-1}) at the Ellerslie and Outlook sites.

	Ellerslie		Outlook	
	Shoot P Conc.	Shoot P Total	Shoot P Conc.	Shoot P Total
Main effects				
Block	-	-	*	*
P level	***	**	***	***
P. bilaii	-	-	-	-
Interaction				
P level x Pb	-	-	-	-
Contrasts				
Pb @ 0P	**	-	-	-
Pb @ 6.4 P	-	-	-	-
Pb @ 19.3 P	-	-	-	-

* $P \leq 0.05$. ** $P \leq 0.01$. *** $P \leq 0.001$. - = not significant.

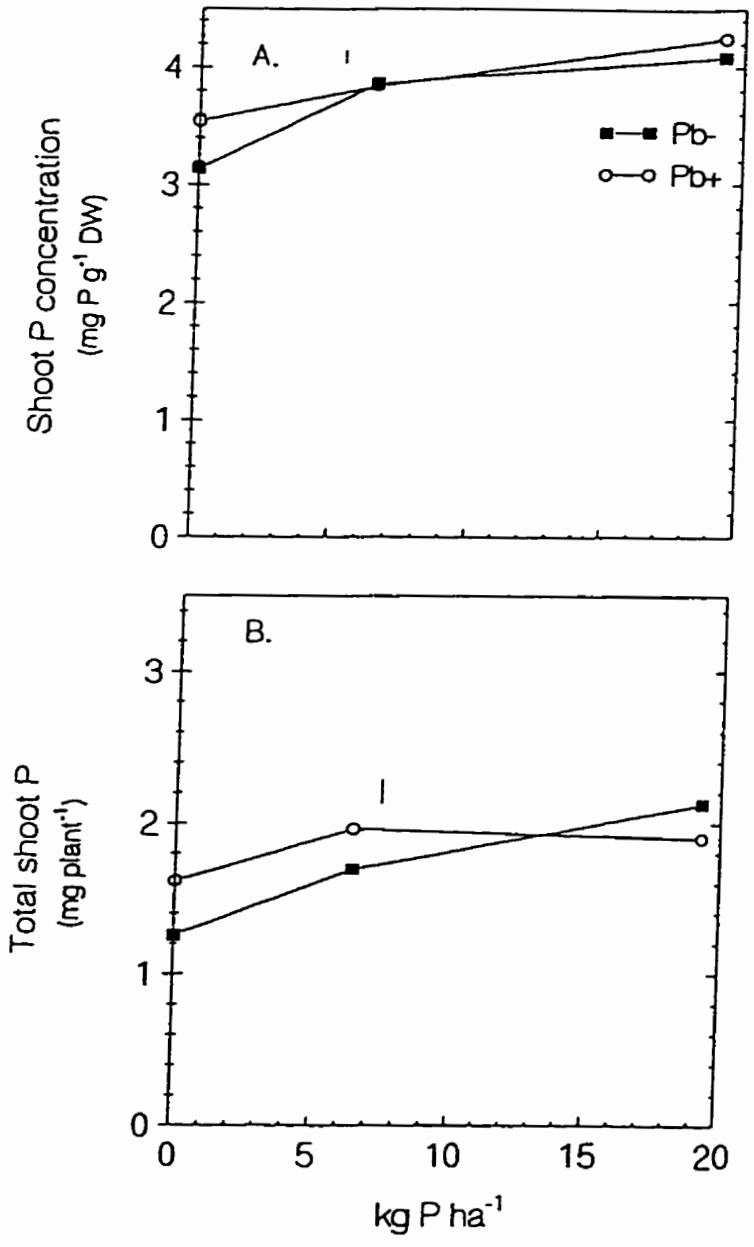

Figure 4.3. The effect of inoculation with (Pb+) or without (Pb-) *Penicillium bilaii* and P fertilizer level (0 P fertilizer added, 6.4 kg P ha^{-1}, and 19.3 kg P ha^{-1}) on shoot P concentration (A), and shoot P accumulation (B) of pea at the Ellerslie site. Bar represents the mean standard error of treatments.

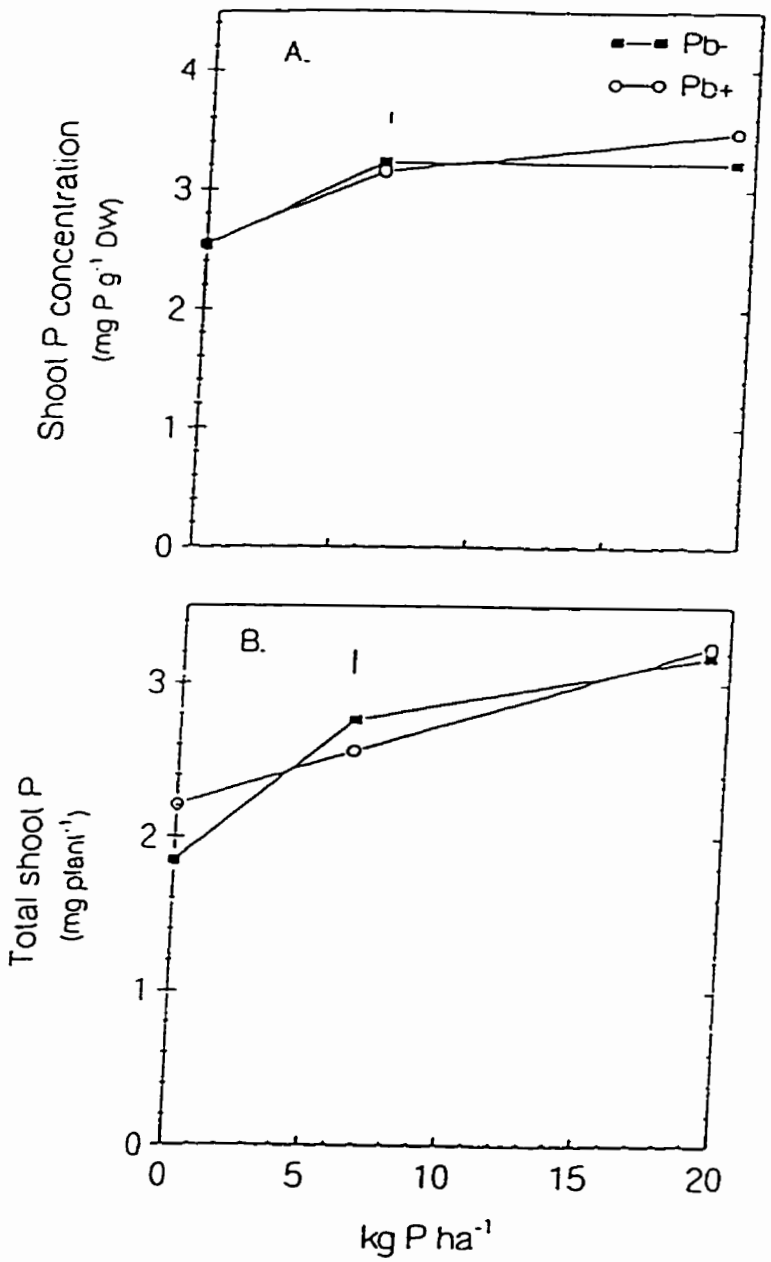

Figure 4.4 The effect of inoculation with (Pb+) or without (Pb-) *Penicillium bilaii* and P fertilizer level (0 P fertilizer added, 6.4 kg P ha^{-1}, and 19.3 kg P ha^{-1}) on shoot P concentration (A), and shoot P accumulation (B) of pea at the Outlook site. Bar represents the mean standard error of treatments.

Table 4.4. Effect of P fertilizer on shoot P concentration and accumulation of pea at the Ellerslie site 36 days after planting.

P fertilizer (kg P ha^{-1})	Shoot P concentration (mg P g^{-1} DW)	Shoot P accumulation (mg $plant^{-1}$)
19.3	4.170	2.042
6.4	3.851	1.831
No added P	3.336	1.437
LSD ($P \leq 0.05$)	0.223	0.312

Table 4.5. Effect of P fertilizer on shoot P concentration and accumulation of pea at the Outlook site 41 days after planting.

P fertilizer (kg P ha^{-1})	Shoot P concentration (mg P g^{-1} DW)	Shoot P accumulation (mg $plant^{-1}$)
19.3	3.347	3.207
6.4	3.183	2.657
No added P	2.536	2.026
LSD ($P \leq 0.05$)	0.235	0.363

However, shoot P accumulation was not affected by *P. bilaii* inoculation (Table 4.3; Figure 4.3). At the Outlook site, *P. bilaii* inoculation did not affect shoot P concentration or accumulation (Table 4.3; Figure 4.4).

4.3.3 Dry Weight Responses

The results of dry weight responses have been summarized on Table 4.6 which presents significance levels for main effects (*P. bilaii* inoculation, P fertilizer) and interactions (*P. bilaii* x P fertilizer) for both the Ellerslie and Outlook sites. Details of treatment effects are presented below.

At the Ellerslie site, there were no detectable treatment effects on shoot or shoot plus nodulated root crown dry weights of pea (Table 4.6; Figure 4.5). However, the main effect of P fertilizer level was significant ($P \leq 0.05$) for root crown dry weight (Table 4.6). Addition of P fertilizer (as 6.4 or 19.3 kg P ha^{-1}) significantly ($P \leq 0.05$) decreased (19 and 20%, respectively) root crown dry weight compared with the no added P fertilizer level (Table 4.7). *Penicillium bilaii* inoculation at the no additional P level significantly ($P \leq 0.05$) increased root dry weight by 21% (Table 4.6; Figure 4.5).

At the Outlook site, the main effect of P fertilizer did not affect dry weight responses of pea (Table 4.6; Figure 4.6). However, *P. bilaii* inoculation at the no added P fertilizer level was significant ($P \leq 0.01$) for shoot and shoot plus root crown dry weight (Table 4.6). Inoculation significantly ($P \leq 0.05$) increased shoot dry weight by 18% and shoot plus root crown by 17% at the no added P fertilizer level at the Outlook site (Table 4.6; Figure 4.6).

Table 4.6. Significance of dry weight responses of pea grown with and without *Penicillium bilaii* (Pb) and at one of three levels of P fertility (no added P, 6.4 and 19.3 kg P ha^{-1}) at the Ellerslie and Outlook sites.

	Ellerslie				Outlook			
	Root crown†	Shoot	Nodule	Shoot + nodulated root crown†	Root crown†	Shoot	Nodule	Shoot + nodulated root crown†
Main effects								
Block	-	-	*	-	-	***	-	***
P level	*	-	-	-	-	***	-	***
P. bilaii	-	-	-	-	-	-	-	-
Interaction								
P level x Pb	-	-	-	-	-	**	-	**
Contrasts								
Pb @ 0P	*	-	-	-	-	**	-	**
Pb @ 6.4 P	-	-	-	-	-	-	-	-
Pb @ 19.3 P	-	-	-	-	-	-	-	-

* $P \leq 0.05$. ** $P \leq 0.01$. *** $P \leq 0.001$. - = not significant.

† root recovered from a core 15 cm in depth, and 6.5 cm in diameter taken at the base of the plants.

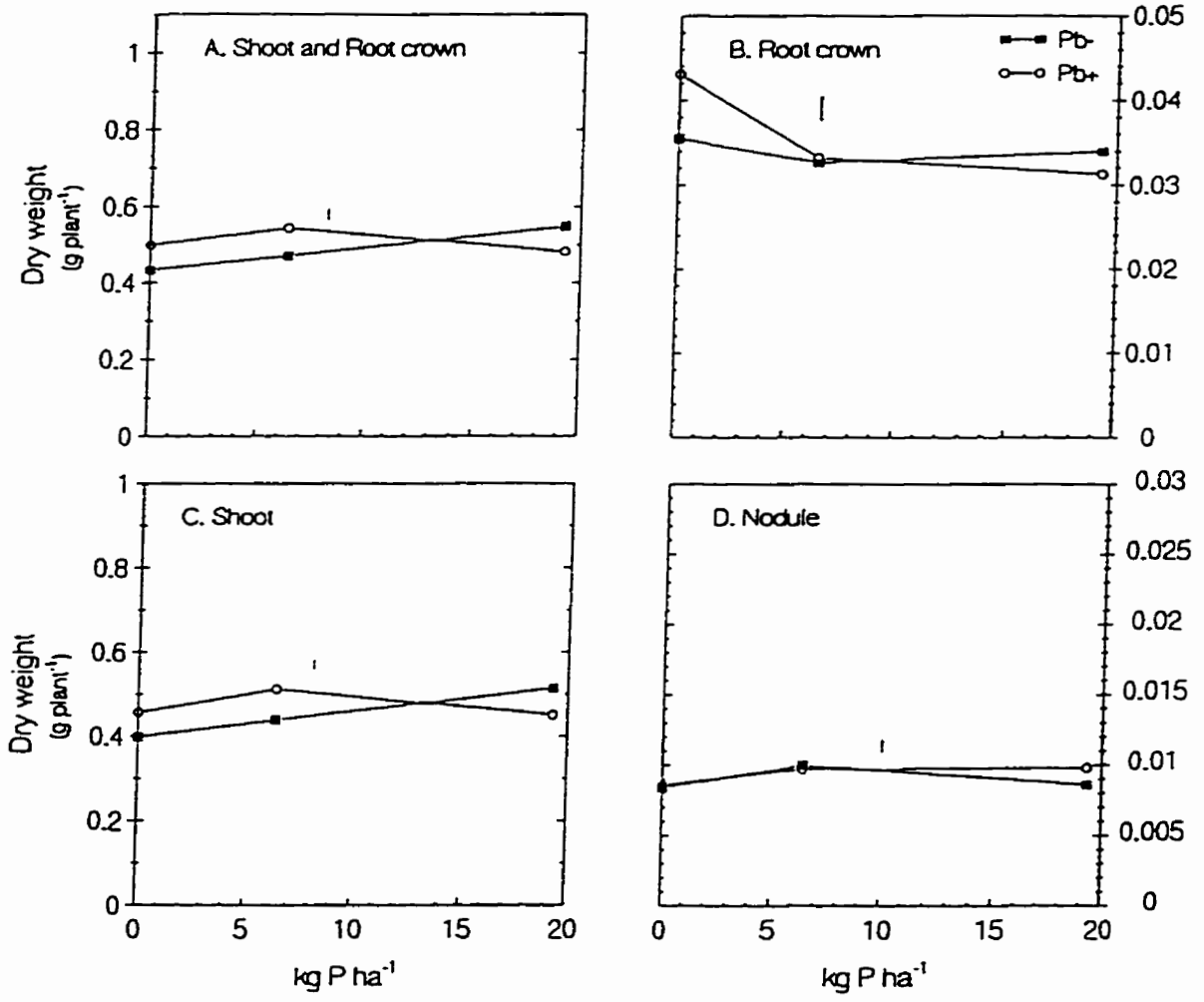

Figure 4.5. The effect of inoculation with (Pb+) or without (Pb-) *Penicillium bilaii* and P fertilizer level (0 P fertilizer added, 6.4 kg P ha^{-1}, and 19.3 kg P ha^{-1}) on shoot and root crown (nodulated) (A), root crown (B), shoot (C), and nodule (D) dry weight of pea at the Ellerslie site. Bar represents the mean standard error of treatments.

Table 4.7. Effect of P fertilizer on upper root crown dry weight of pea at the Ellerslie site, 36 days after planting.

P fertilizer (kg P ha^{-1})	Root DW (g)
No added P	0.0393
6.4	0.0329
19.3	0.0327
LSD ($P \leq 0.05$)	0.0054

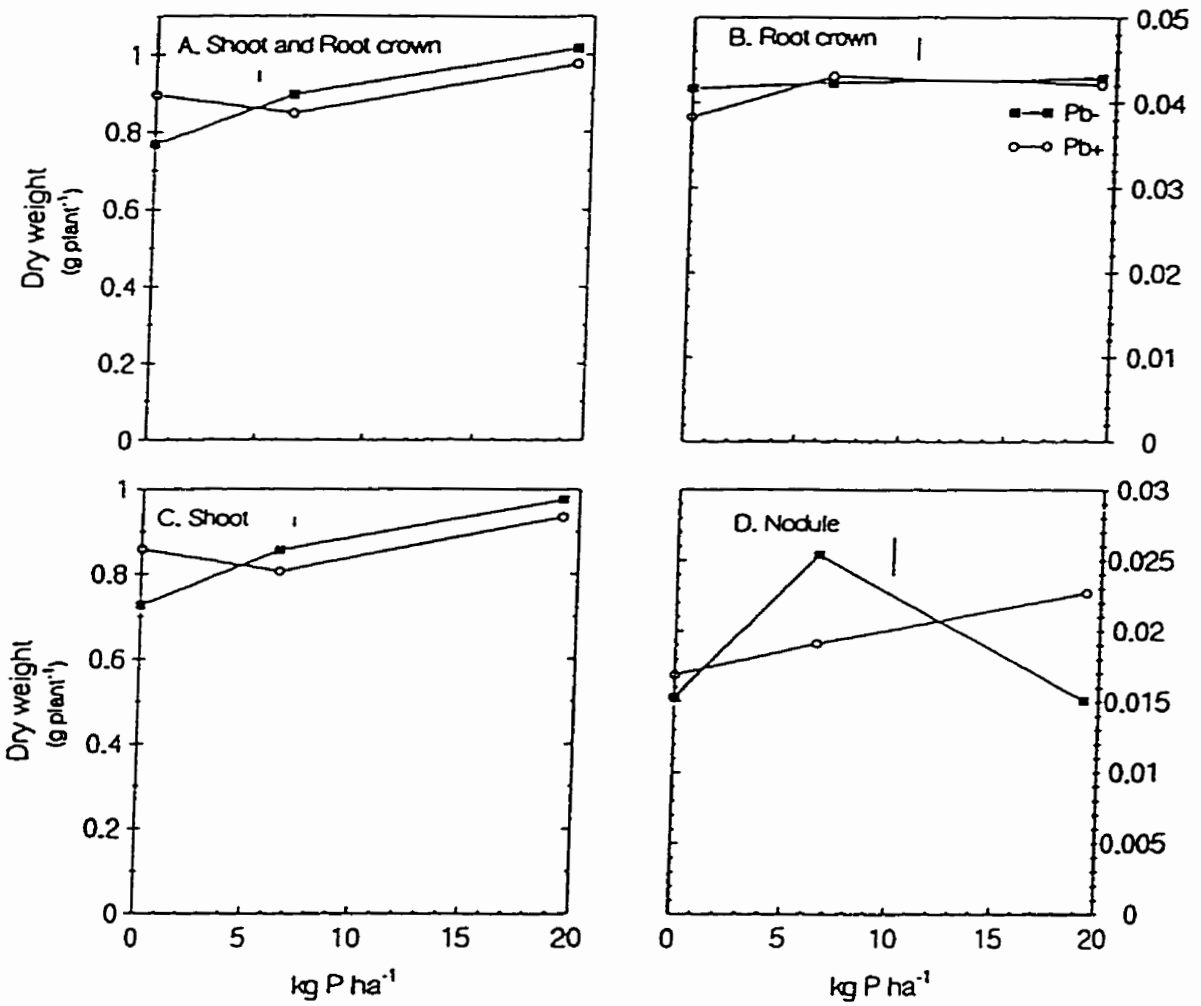

Figure 4.6. The effect of inoculation with (Pb+) or without (Pb-) *Penicillium bilaii* and P fertilizer level (0 P fertilizer added, 6.4 kg P ha^{-1}, and 19.3 kg P ha^{-1}) on shoot and root crown (nodulated) (A), root crown (B), shoot (C), and nodule (D) dry weight of pea at the Outlook site. Bar represents the mean standard error of treatments.

4.3.4 Nodulation Responses

The results of nodulation responses have been summarized on Table 4.8 which presents significance levels for main effects (*P. bilaii* inoculation, P fertilizer) and interactions (*P. bilaii* x P fertilizer) for both the Ellerslie and Outlook sites. Details of treatment effects are presented below.

At both sites, there were no detectable treatment effects on nodulation responses of crown roots of pea (Table 4.8; Figures 4.7, 4.8).

4.3.5 Row Samples

In addition to sampling individual plants (above data), one meter row lengths were sampled from each plot to enable extrapolation to field levels. The results of the meter row samples of above ground biomass taken at each site are presented on Table 4.9, which presents the treatment means, mean standard error of treatments, and the LSD values for main effects.

At the Ellerslie site, there were no detectable treatment effects on the number of plants (based on the number of shoots), the shoot dry weight of the meter samples, or the shoot dry weight per plant (Table 4.9). Shoot dry weight per plant was significant at P = 0.057 when *P. bilaii* was applied alone, and had the second highest average dry weight of all the treatments (Table 4.9). At the Outlook site, application of P fertilizer had a

Table 4.8. Significance of nodulation response (nodule number and specific nodulation) of pea grown with and without *Penicillium bilaii* (Pb) and at one of three levels of P fertility (no added P, 6.4 and 19.3 kg P ha^{-1}) at the Ellerslie and Outlook sites.

	Ellerslie		Outlook	
	Nodule No.	Specific Nodulation	Nodule No.	Specific Nodulation
Main effects				
Block	-	-	-	-
P level	-	-	-	-
P. bilaii	-	-	-	-
Interaction				
P level x Pb	-	-	-	-
Contrasts				
Pb @ 0P	-	-	-	-
Pb @ 6.4 P	-	-	-	-
Pb @ 19.3 P	-	-	-	-

* $P \leq 0.05$. ** $P \leq 0.01$. *** $P \leq 0.001$. - = not significant.

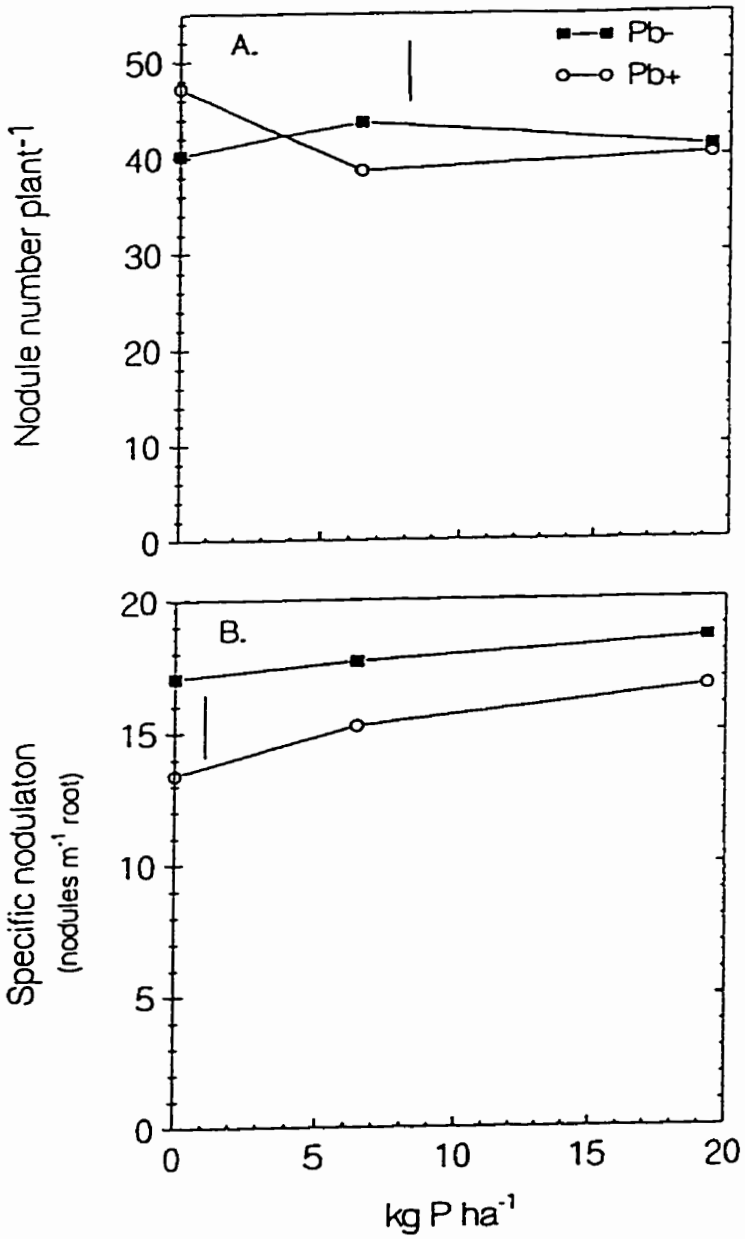

Figure 4.7. The effect of inoculation with (Pb+) or without (Pb-) *Penicillium bilaii* and P fertilizer level (0 P fertilizer added, 6.4 kg P ha^{-1}, and 19.3 kg P ha^{-1}) on nodule number (A), and specific nodulation (B) of pea roots taken from a core 15 cm in depth, and 6.5 cm in diameter, from the base of the plants at the Ellerslie site. Bar represents the mean standard error of treatments.

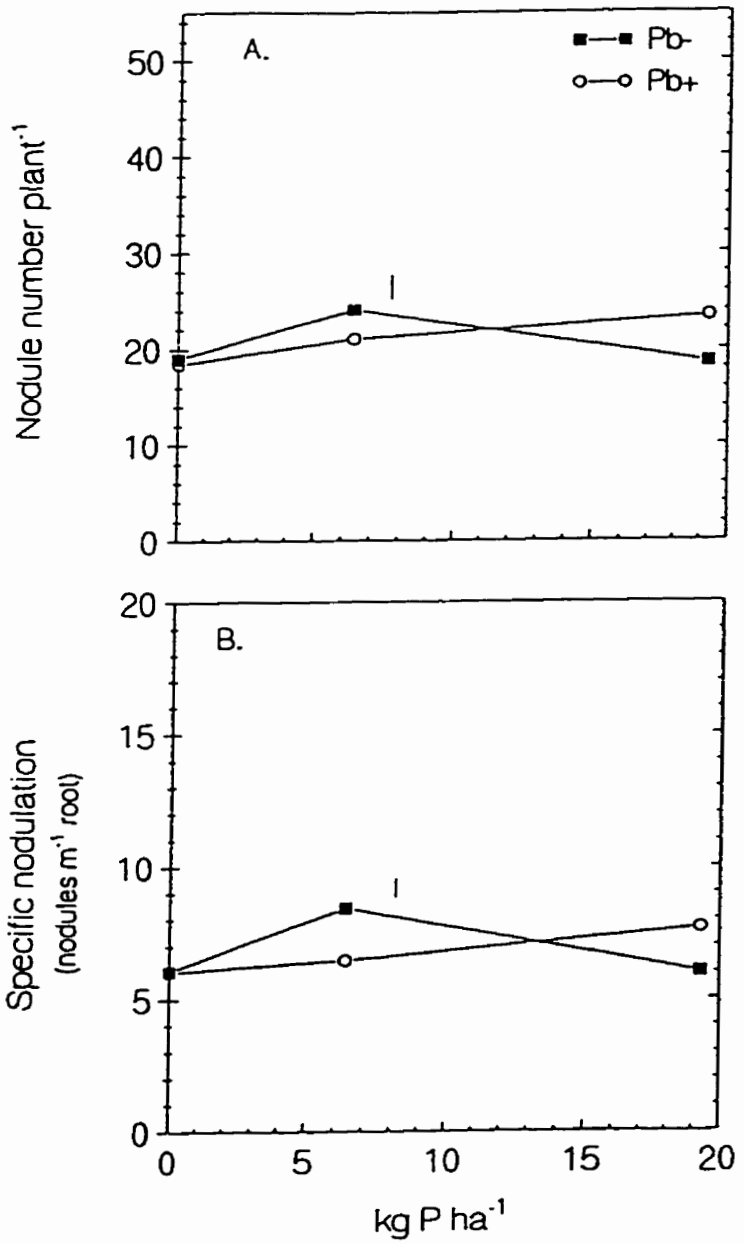

Figure 4.8. The effect of inoculation with (Pb+) or without (Pb-) *Penicillium bilaii* and P fertilizer level (0 P fertilizer added, 6.4 kg P ha^{-1}, and 19.3 kg P ha^{-1}) on nodule number (A), and specific nodulation (B) of pea roots taken from a core 15 cm in depth, and 6.5 cm in diameter, from the base of the plants at the Outlook site. Bar represents the mean standard error of treatments.

Table 4.9. Number and dry weight means with mean standard errors and least significant difference (LSD) values of pea grown with (+) and without (-) *Penicillium bilaii* (Pb) and at one of three levels of P fertility (no added P, 6.4 and 19.3 kg P ha^{-1}) in the row samples at the Ellerslie and Outlook sites.

Ellerslie				Outlook			
Treatment	No. Plants	DW Plants (g)	DW Per Plant (g $plant^{-1}$)	Treatment	No. Plants	DW Plants (g)	DW Per Plant (g $plant^{-1}$)
0 / -	11.8	4.326	0.3653	0 / -	11.2	7.086	0.6436
0 / +	11.6	4.832	0.4199	0 / +	11.4	7.876	0.6988
6.4 / -	13.2	4.848	0.3752	6.4 / -	11.4	9.252	0.8117
6.4 / +	14	5.196	0.3746	6.4 / +	10.8	7.584	0.7206
19.3 / -	12.6	5.35	0.426	19.3 / -	13	10.09	0.7834
19.3 / +	13.2	5.316	0.4003	19.3 / +	12.2	10.226	0.8671
STD ERR	1.2	0.455	0.0191	STD ERR	0.841	0.6939	0.0454
P LEVEL LSD[1]	2.4	0.949	0.0398	P LEVEL LSD[1]	1.75	1.448	0.0948
P. bilaii LSD[1]	1.9	0.774	0.0325	*P. bilaii* LSD[1]	1.43	1.182	0.0774

[1]LSD ($P \leq 0.05$)

significant effect ($P \leq 0.01$) on the shoot dry weight of meter samples and shoot dry weight per plant (Table 4.9). Shoot dry weight of the meter samples was significantly ($P \leq 0.05$) greater for plants at the 19.3 kg P ha^{-1} fertilizer level than the other fertilizer levels (Table 4.9). The addition of P fertilizer (as 6.4 or 19.3 kg P ha^{-1}) produced significantly greater shoot dry weight per plant than when no P fertilizer was added (Table 4.9).

Overall, the row sample shoot dry weight responses reflect those of individual plant samples at the Ellerslie site (i.e., no response observed). However, responses to P fertilizer at the Outlook site were not observed with individual samples but were observed with the row samples. This is likely due to the larger sample size (more plants) of the row samples which would increase the accuracy of estimates of treatment effects.

4.4 Discussion

This experiment was conducted to investigate whether the rhizospheric fungus *Penicillium bilaii* increases root surface area for phosphate absorption. In research related to the present study (Chapter 3), *P. bilaii* under controlled conditions, was associated with increased P concentration and accumulation of pea shoot and root tissue, but this increase was not a direct result of an increased root surface area for phosphate absorption. In fact, the observed effect of this fungus on root morphology was that inoculation resulted in a reduction of total root length and specific root length (i.e., shorter roots with greater diameters) while maintaining the P concentration of the pea tissue to that of control plants.

In the present study, we used a subsample of the root system (root crown) for analysis. The sample core removed the roots directly under the shoot to a depth of 15 cm

and a diameter of 6.5 cm. At the Ellerslie site, the observed effect of the fungus on root morphology was that when no P fertilizer was applied, *P. bilaii* inoculation resulted in a 48% increase in root crown length and a 21% increase in specific root length (i.e., longer, finer roots in the core sample) (Table 4.2; Figure 4.2). This alteration of root morphology may have been associated with the 13% increase in shoot P concentration observed for those inoculated plants (Figure 4.3). Sensitivity analysis of models for phosphate uptake into plant roots has indicated that increased phosphate uptake may result from plants that have a finer and longer root system (Silverbush and Barber, 1983) (Figure 4.2). However, we only analysed part of the root system (crown area). Plant roots are known to proliferate in P-rich regions of soil (Drew *et al.*, 1973). Drew *et al.* (1973) have attributed increases in root growth in response to local concentrations of N and P to the fact that lateral root growth only occurs at the point on the root if adequate external nutrient concentrations exist there. Although root proliferation is stimulated in a P-rich zone, the addition of P may lead to a reduction in the amount of roots per unit weight of top (Christie and Moorby, 1975; Ozanne *et al.*, 1969). Total root length and dry weight may be reduced when the levels of P in the soil are increased (Asher and Loneragan, 1967; Powell, 1974). A possible interpretation of the observed root morphology of inoculated plants when no P fertilizer was applied, is that *P. bilaii* may be increasing the availability of P to the plants in the sampled area, thus resulting in a proliferation of roots in the sampled area. The favoured hypothesis of how *P. bilaii* promotes plant growth is by increasing the availability of soluble phosphate to the plant (Asea *et al.*, 1988; Kucey, 1987, 1988). *Penicillium bilaii* has shown P-solubilizing ability (Asea *et al.*, 1988;

Kucey, 1983, 1988) and produces oxalic and citric acid in pure culture (Cunningham and Kuiack, 1992). Production of organic compounds such as citrate can release phosphate from soil particles (Bolan, 1991; Bar-Yosef, 1996). Previous studies utilizing ^{32}P indicated that plants inoculated with *P. bilaii* are able to utilise soil P sources that are unavailable to control plants (Chambers, 1992) as well as rock phosphate (Asea *et al.*, 1988). The increase in shoot P concentration of the inoculated plants at the no added P fertilizer level also suggests a more direct effect of the fungus on increasing the uptake of phosphate (Figure 4.3). In addition, if the fungus was increasing the availability of phosphorus to the inoculated plants, the effect would be more pronounced when no P fertilizer was added; which was the case at the Ellerslie site (Figure 3).

At the Outlook site, *P. bilaii* inoculation when no P fertilizer was applied, did not result in increased pea tissue P concentration, nor were there any observed effects on root morphology (Tables 4.2, 4.3; Figures 4.1, 4.4). However, there was an 18% increase in shoot dry weight when no P fertilizer was added (Table 4.6; Figure 4.6). Other researchers have observed increased dry matter production with inoculation with *P. bilaii* that was not associated with increased P content of the plants (Chambers, 1992; Downey and van Kessel, 1990; Gleddie, 1992; Keyes, 1990). This evidence suggests that *P. bilaii* may stimulate plant growth by other mechanisms, possibly in addition to increasing the availability of P to plants. Keyes (1990) has suggested that *P. bilaii* may promote plant growth through passive biocontrol or growth-promoting effects. However, Allaway (1971) has concluded that the effect of adding a nutrient as a fertilizer may range from no increase in the concentration of the element in the plant although a marked yield increase

may be obtained, to significant increases in the level of the element in plant tissue without change in yields.

Another objective of this study was to determine if *P. bilaii* inoculation affected nodulation of peas under field conditions. There were no observed treatment effects on nodule number, specific nodulation or nodule dry weight (Tables 4.6, 4.8; Figures 4.5, 4.6, 4.7, 4.8). Gleddie (1992) reported inoculation with *P. bilaii* resulted in increased nodulation of pea roots by *Rhizobium leguminosarum* in a growth room study, and promoted increased N uptake by pea in growth chamber and P responsive field trials. However, Downey and van Kessel (1990) observed that inoculating with *P. bilaii* decreased total N accumulation of pea plants in a growth chamber experiment. They proposed that the production of P-solubilizing organic acids by the fungus may have reduced the rhizosphere pH to a degree that would inhibit *Rhizobium* function.

Results of the row samples indicated there were no detectable treatment effects on the number of plants that emerged on either site. At the Ellerslie site, there were no detectable treatment effects on the row sample shoot dry weights. Although there was an increase in P concentration of the shoot tissue with each increase in P level of individual plant samples, this did not translate into a dry weight response at this site. Application of *P. bilaii* alone was significant at $P = 0.057$ for increasing the shoot dry weight per plant of the meter samples. However, this lack of dry weight response of pea plants is consistent with observations that the effect of *P. bilaii* is only beneficial in P-responsive soils (Chambers, 1992; Gleddie *et al.*, 1991). At the Outlook site, there was a shoot dry weight response of the row samples to the addition of P fertilizer and the P concentration of the

individual shoot samples was also increased with the addition of P fertilizer. *Penicillium bilaii* inoculation at the no added P fertilizer level for individual samples resulted in increased shoot dry weight but did not affect the P concentration of the shoot tissue.

In conclusion, along with the results of a previous related study (Chapter 3), which suggested that *P. bilaii* inoculated pea plants require less root surface area to produce shoot dry matter and for P absorption, it is likely that the observed effects of *P. bilaii* on root morphology at the Ellerslie site are a result of roots proliferating in the sampled area as a result of increased P availability. The increase in shoot P concentration of the inoculated plants supports this hypothesis. However, total root length was not measured in this study. If we assume that the results from the Ellerslie site are consistent with those of the growth cabinet work in Chapter 3, then total root length of inoculated plants when no P fertilizer was added may in fact have less total root length than non-inoculated plants. The observed increase in dry weight of inoculated plants that was not associated with increased P-uptake at the Outlook site when no P fertilizer was applied, suggests that *P. bilaii* may promote plant growth by other mechanism (s) in addition to increasing the availability of P. However, this manifestation of non-P related enhancement of growth only occurred when the P supply was low, and therefore may also be related to low P-availability. There were no detectable treatment effects on nodulation responses of pea in this study, suggesting that *P. bilaii* inoculation does not affect the nodulation of pea crown roots under field conditions.

5.0 Effect of *Penicillium bilaii* on Root Morphology and Architecture of Pea (*Pisum sativum* L.) - Growth Pouch study

5.1 Introduction

The relationship between root growth and absorption of phosphate (P) is influenced by soil and plant factors. Sensitivity analysis of models for P uptake into plant roots has indicated that plant factors that are most sensitive for P uptake are those that affect root surface area (i.e., root length and diameter) (Silverbush and Barber, 1983). The most sensitive soil parameter is the initial soil solution concentration (C_l), whereas diffusion coefficient (D_e) and buffer power (b) have greater effects if their values are diminished than if they are increased (Silverbush and Barber, 1983). Therefore, the most effective way to increase the rate of P uptake by plant roots is to increase the rate of root growth or to increase the concentration of P in the soil solution.

Although root biomass is the most commonly reported parameter of root growth, this measurement does not consider how assimilates are allocated (Hetrick, 1991). It is the alteration in root morphology and architecture which dictates the nutrient absorbing power of the root system, which cannot be assessed by root weight measurements (Hetrick, 1991). Root morphology will change in response to the soil environment to reduce the metabolic cost of maintaining the root system while maximizing nutrient acquisition (Hetrick, 1991). Under nutrient-limiting conditions, plants may increase root fineness or specific root length (root length per gram of root dry weight), root:shoot ratio, or root hair length and number; each resulting in a different metabolic cost to the plant

(Hetrick, 1991). The extent to which a root system branches will affect the volume of soil explored for a nutrient (Fitter, 1987). Plants that produce the greatest interface with the soil have the greatest nutrient uptake potential, but this is balanced against the cost of the plant growing and maintaining roots (Fitter, 1987). Generally, increased branching is a response to increased supply of mineral nutrients (Fitter, 1982). Fitter (1982) concluded the main effect of fertility is on the branching pattern of roots rather than rates of elongation of laterals or the production of new branches. Differences in root topology or architecture will influence both the exploration and transport characteristics of the root system (Fitter, 1982). Topological analysis first described by Fitter (1985), can be used to quantify the branching patterns of root systems. This type of root analysis considers root systems as mathematical trees. P_e, total exterior pathlength of the tree (root system), is assessed by summing the number of links from the root crown to each lateral root tip (terminal end point). A link represents the line connecting two branch points. Total exterior pathlength is a measure of branching patterns when root systems with the same number of terminal end points are compared. Root systems with high pathlength (P_e) values have elongate, sparingly branched root systems, which allows maximum exploration of soil volume for nutrients, but this morphology is less efficient for nutrient transport to the shoot and represents the greatest energy expense to the plant (Fitter, 1987). These root systems are referred to having an exploratory, herringbone root architecture. Root systems with low P_e values have a highly branched, absorbing root morphology, which limits the volume of soil explored, but maximizes nutrient transport to the shoot and cost efficiency (Fitter, 1987). This type of root system may develop in soils

where resources are abundant and are often referred to as dichotomous, absorptive root systems. These concepts are thoroughly reviewed by Fitter (1985, 1987).

Soil fertility and rhizosphere microorganisms have been shown to significantly affect root growth and architecture (Hetrick, 1991; Hetrick *et al.*, 1988; Schenk and Barber, 1979b). Mycorrhizal fungi and soil microbes significantly alter root architecture by reducing root branching (Hetrick *et al.*, 1988). Mycorrhizal plant roots appear to adopt an exploratory root system architecture (high P_e) in soils low in available P, which allows the mycorrhizal root system to exploit a greater volume of soil for nutrients (Hetrick, 1991). Allowing the fungal hyphae to perform nutrient absorption is more cost effective than expending energy to generate roots that may be unable to acquire adequate P (Hetrick, 1991). When evaluating the effect of rhizosphere microorganisms on plant root architecture, it is important to distinguish between direct effects of the organism on plant rooting strategy, and those changes which may occur indirectly as a result of improved nutrient status of the plant (Hetrick, 1991). Hetrick (1991) has observed differences in pathlength as a result of mycorrhizal infection (VAM), and to some degree other soil microorganisms, that were not simulated by P fertilizers, implying that the effects of these organisms are not strictly nutritional but could perhaps be hormonal.

Penicillium bilaii (ATCC strain no. 20851) is a rhizospheric fungus reported to increase dry matter production, grain yield, and P uptake of wheat, canola, bean, pea, and lentil in growth chamber and field experiments alone, or in combination with rock phosphate or monoammonium phosphate (MAP) (Asea *et al.*, 1988; Chambers, 1992; Gleddie, 1993; Gleddie *et al.*, 1991, 1993; Kucey, 1987, 1988; Kucey and Leggett,

1989). The mechanism (s) underlying the stimulation of plant growth and P-uptake is not known. Indirect evidence suggests that *P. bilaii* may increase the availability of phosphate to the plant by releasing organic acids, which may act to acidify localised areas of the rhizosphere and/or act as a chelator of cationic partners of the phosphate anion (Asea *et al.*, 1988; Cunningham and Kuiack, 1992; Kucey, 1983, 1988). However, there is alternative evidence that suggests *P. bilaii* may stimulate plant growth by other mechanisms. Researchers have observed increased dry matter production with inoculation with *P. bilaii* that was not associated with increased P content of the plants (Chambers, 1992; Downey and van Kessel, 1990; Gleddie, 1992; Keyes, 1990). Keyes (1990) has suggested that *P. bilaii* may promote plant growth through passive biocontrol or growth-promoting effects.

In other research related to the present study (Chapter 4, under field conditions using soil core samples), *P. bilaii* inoculation when no P fertilizer was applied, resulted in a 48% increase in root crown length and a 21% increase in specific root length. This absorptive root morphology may have been related to the 13% increase in shoot P concentration of inoculated plants. However, it is proposed that the observed changes in root morphology are an indirect result of the fungus increasing the availability of soluble P to the plant, causing the roots to proliferate in the area of increased P availability, rather than a direct effect of the fungus on plant rooting strategy.

The objective of this study was to determine, under controlled conditions using growth pouches, the effect of *P. bilaii* and solution P concentration on root morphology and architecture of pea (*Pisum sativum* L.). We also determined the effect of this fungus

and solution P concentration on assimilate partitioning (dry matter) and nodulation response of pea.

5.2 Materials and Methods

5.2.1 Growth Pouch Preparation

Pea (*Pisum sativum* L. cv. Express) was grown in a growth chamber experiment using disposable growth pouches (Scientific Products, catalog no. B1220, Evanston, Illinois). The layout of the experiment was a completely randomized design, with a factorial (2 x 4) treatment structure. The two factors were *P. bilaii* with two levels (inoculated, non-inoculated) and P concentration with four levels (0, 0.1, 1.0, and 10 mg P l^{-1}) resulting in eight treatments with six replicates.

Growth pouches (16.5 cm x 16.5 cm), constructed from heat sealing plastic, were sterilized at 121° C for 20 minutes. Sterile nutrient solution (30 ml) was added to the growth pouch under sterile conditions. The concentrations of the nutrients in the solution were as follows: 1.376 mM SO_4^{2-}, 0.5 mM Mg^{2+}, 0.375 mM Ca^{2+}, 3.7 µM Fe^{3+} as 300 Fe-sequestrene (Ciba-Geigy Corp.), 18 µM $B(OH)_4^-$, 4 µM Mn^{2+}, 8 µM Cl^-, 0.4 µM Cu^{2+}, 0.35 µM Zn^{2+}, 0.3 µM MoO_4^{2-}, and 0.1 µM Co^{2+}. The P concentrations of the nutrient solutions for the different treatments were 0, 0.1, 1.0, and 10 mg P l^{-1}. Phosphorus was added as a buffered solution of HPO_4^{2-} and $H_2PO_4^-$ at a ratio of 12:5, respectively, and the K^+ concentration varied from 1 mM (no added P) to 1.54 mM (10 mg P l^{-1}). A 5 M KOH solution was used to adjust the nutrient solution pH (6.5-6.8). The composition of the nutrient solution used in this experiment was based on a standard solution with varying quantities of phosphate (P) and K^+. In other work on root growth and development, K is

considered less important than P or NO_3^- (Barber, 1979; Drew, 1975; Price *et al.*, 1989). The influence of these differences in K^+ concentration in this study are considered minimal.

Pea seeds were treated for 5 m in 1% hyporchlorite solution and rinsed thoroughly with distilled water. Under sterile conditions, three 1-cm perforations were cut into the trough of the growth pouch and the seeds were placed on top of the perforations. All seeds were inoculated with 1 ml of yeast mannitol broth containing a minimum of 1.3 x 10^9 colony forming units (CFU) ml^{-1} *Rhizobium leguminosarum bv. viceae* (Hup^- strain 128A1, Liphatech, Milwaukee) as determined by dilution plating. Pouches not receiving *P. bilaii* treatment had 1 ml sterilized water placed on the seed after inoculation with *Rhizobium*. Seeds that received the *P. bilaii* treatment (ATCC strain no. 20851) were inoculated with 1 ml of a diluted inoculum supplied by Philom Bios Inc., Saskatoon, SK, containing a minimum of 1.16 x 10^4 CFU ml^{-1} as determined by dilution plating. Growth pouches were encased in black plastic pouches (18.5 cm x 23 cm) to exclude light and the tops were fastened with sterilized paper clips (clips were removed 2 days after planting). The pouches were placed into holders, which held the pouches vertical, and were randomly placed into the growth chamber.

5.2.2 Growth Pouch Maintenance

Growth pouches were placed into a controlled environment cabinet (model GRV36-Econaire, Winnipeg, MB) with a day/night temperature regime of 20/16° C with the lights turned off to allow germination. On the 4th day after planting, the lights were turned on and the plants were under a 16/8hr, 20/16° C day/night regime and exposed to an average photon flux density of 600 ± 40 µmol $m^{-2}s^{-1}$ provided by a combination of cool

white VHO and Grolux fluorescent lamps (Sylvania, Inc., Drummonville, PQ) at a ratio of 4:1, respectively. On the 5th day after planting, an additional 10 ml of the appropriate sterilized nutrient solution was added to the growth pouches in the growth chamber. Seven days after planting, under sterile conditions, growth pouches were thinned to 1 plant per pouch; the nutrient solution was removed and replaced using a sterile pipette. The nutrient solution was similarly replaced every day for the duration of the experiment.

5.2.3 Sampling

Plants were harvested 12, 19, and 26 days after planting. Shoots were removed, and frozen at -20°C, then later freeze dried and weighed. The pathlengths (P_e) and the terminal end points (TEP) of the root systems were determined using topological analysis first described by Fitter (1985), to quantify the branching patterns of the root systems. This type of root analysis considers root systems as mathematical trees. P_e, total exterior pathlength of the tree (root system), is assessed by summing the number of links from the root crown to each lateral root tip (terminal end point). A link represents the line connecting two branch points. It is important to note that a link is counted every time it is included in a path to a different root tip. Terminal end points are considered external links (those terminating in a meristem) which have a magnitude of 1 according to Fitter (1985). For ease of analysis, P_e was determined by starting at the main axis apex and counting the number of laterals (links) back to the root crown. By doing this, the number of primary laterals (links) above the next lateral root being assessed was known.

Total exterior pathlength is a measure of branching patterns when root systems with the same number of terminal end points are compared. To compare root systems

with different numbers of terminal end points, a ratio of P_e:terminal end points was used for analysis.

Root systems with high P_e are elongate, sparingly branched, and explore a large volume of soil. On the other hand, root systems with low P_e are highly branching, and are considered absorptive in nature rather than exploratory (see Figure 5.1).

After determination of pathlength, the roots were then stained with methylene blue (0.1% w/v distilled water) by placing the roots into the dye solution for 30 s, and then rinsing with distilled water for 30 s. Root lengths were determined using IMAGEX, a digital analysis system, developed by L. Lamari, Dept. of Plant Science, Winnipeg, MB, as described previously in Chapter 3. The roots were frozen after analysis at -20° C and then later thawed to remove and count the nodules. The samples were then refrozen, freeze dried, and weighed.

Data was analysed using the General Linear Model procedure of the Statistical Analysis System package (SAS Institute Inc., 1986) and single degree of freedom contrasts were made. Treatment means were separated using the Fisher protected least significant difference test (LSD) at $\alpha = 0.05$ level, after the analysis of variances indicated significant differences at the same level.

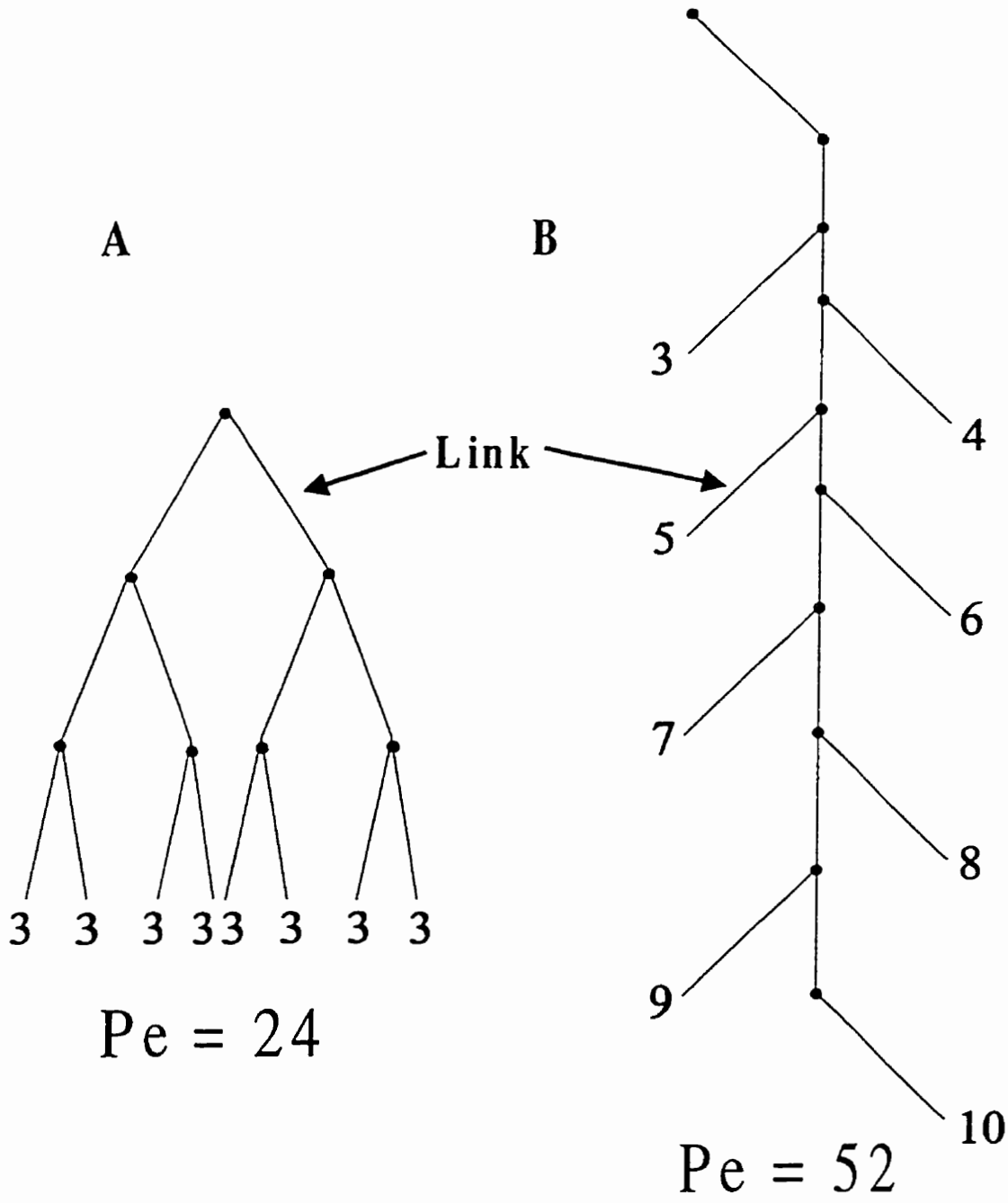

Figure 5.1. Topological analysis of branching patterns. Two root systems with 8 terminal end points are depicted. The number below each root tip is the number of links between root crown and tip. The sum of these numbers, the total pathlength (P_e), is lower for the highly branched root system A, than for the sparingly branched root system B. Figure adapted from Hetrick (1991).

5.3 Results

This growth chamber experiment utilizing growth pouches was conducted to investigate the effect of *Penicillium bilaii* and solution P fertilizer level on root morphology and architecture (P_e ratio) of pea. The sampling dates were 12, 19, and 26 days after planting (DAP). Since the effects of *P. bilaii* and P availability may be influenced by time, treatment effects will be compared within sampling dates.

5.3.1 Root Morphology and Architecture

The results on root morphology and P_e ratio of pea have been summarized on Table 5.1 which presents significance levels for main effects (*P. bilaii* inoculation, P fertilizer) and interactions (*P. bilaii* x P fertilizer) for all harvest dates. Individual treatment effects within sampling dates are examined below.

5.3.1.1 12 DAP

There were no detectable treatment effects on root length of pea at 12 DAP (Table 5.1; Figure 5.2). However, the main effect of *P. bilaii* was significant ($P \leq 0.05$) for specific root length (SRL) (length of root per gram dry weight root) over all P fertilizer levels (Table 5.1, 5.2). *Penicillium bilaii* inoculation resulted in a 12% increase in SRL (Table 5.2; Figure 5.2). The main effect of *P. bilaii* inoculation was also significant ($P \leq 0.05$) for P_e ratio (Table 5.1, 5.2). Contrast statements indicated a significant ($P \leq 0.05$) effect of *P. bilaii* inoculation at the 10 mg P l^{-1} fertilizer level. Inoculation resulted in a 28% increase in P_e ratio (Table 5.1; Figure 5.2).

Table 5.1. Significance of root responses [pathlength ratio (P_e), root length and specific root length (SRL)] of pea grown with and without *Penicillium bilaii* (Pb) and at one of four levels (0, 0.1, 1 and 10 mg P l^{-1}) of P fertility in the growth pouches.

	12 DAP			19 DAP			26 DAP		
	P_e Ratio	Length	SRL	P_e Ratio	Length	SRL	P_e Ratio	Length	SRL
Main effects									
P fertilizer	-	-	-	*	***	-	***	-	-
P. bilaii	*	-	*	-	-	-	-	**	**
Interaction									
P x Pb	-	-	-	-	-	-	-	-	-
Contrasts									
Pb @ 0 P	-	-	-	-	-	-	-	-	-
Pb @ 0.1 P	-	-	-	-	-	-	-	*	*
Pb @ 1 P	-	-	-	-	-	-	-	**	-
Pb @ 10 P	*	-	-	-	-	-	-	-	-

* $P \leq 0.05$. ** $P \leq 0.01$. *** $P \leq 0.001$. - = not significant.

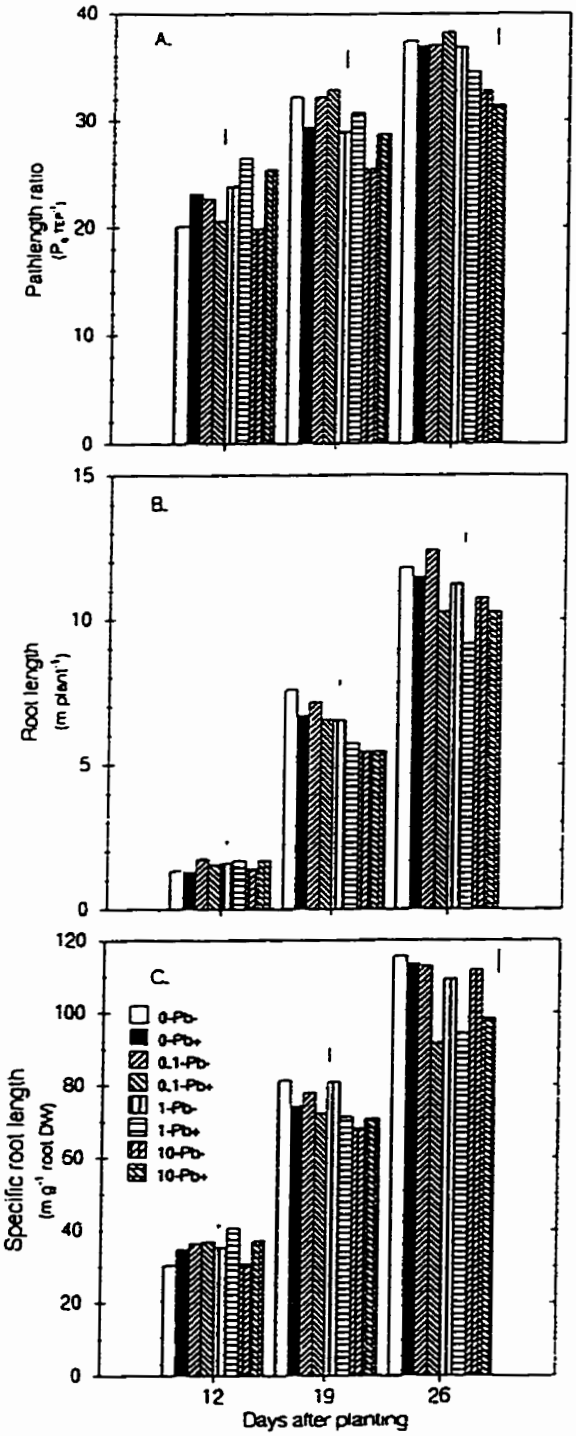

Figure 5.2. The effect of inoculation with (Pb+) or without (Pb-) *Penicillium bilaii* and P fertilizer level (0, 0.1, 1, 10 mg P l^{-1}) on P_e ratio [pathlength (Terminal End Points)$^{-1}$] (A), root length (B), and specific root length (C) of pea in the growth pouches. Bar represents the mean standard error of treatments within dates.

Table 5.2. Effect of *Penicillium bilaii* inoculation on P_e ratio [pathlength (terminal end points)$^{-1}$] and specific root length (SRL) of pea in the growth pouches, 12 days after planting.

P. bilaii	P_e ratio	SRL (m g^{-1} DW root)
-	21.7	33.433
+	23.9	37.462
LSD ($P \leq 0.05$)	2.1	3.910

5.3.1.2 19 DAP

There were no detectable treatment effects on SRL of pea at 19 DAP (Table 5.1; Figure 5.2). However, the main effect of P fertilizer was significant ($P \leq 0.05$) for P_e ratio and highly significant ($P \leq 0.001$) for root length (Table 5.1). The main effect of *P. bilaii* inoculation was significant at $P = 0.06$ for reducing root length. Pathlength ratios generally decreased as the level of P fertilizer increased (Table 5.3; Figure 5.2). Pathlength ratios at the no added P and 0.1 mg P l^{-1} fertilizer levels were significantly ($P \leq 0.05$) higher than the 10 mg P l^{-1} level, while the 1 mg P l^{-1} was not significantly different from either group (Table 5.3; Figure 5.2). Root length values followed a similar trend; as the level of P fertilizer increased, root length decreased (Table 5.4; Figure 5.2). Root lengths at the no added P fertilizer level produced significantly ($P \leq 0.05$) greater root length than those produced at 10 mg P l^{-1} level (Table 5.4; Figure 5.2). Root lengths at the 0.1 mg P l^{-1} level were not significantly different from those at the no added P or 1 mg P l^{-1} fertilizer levels, while root lengths at the 1 mg P l^{-1} fertilizer level were not significantly different from those at the 10 mg P l^{-1} level (Table 5.4; Figure 5.2).

5.3.1.3 26 DAP

At 26 DAP, there was still a significant ($P \leq 0.001$) effect of P fertilizer on P_e ratio of pea (Table 5.1; Figure 5.2). However, at this sampling date, there was only a significant ($P \leq 0.05$) difference between P_e ratio at 10 mg P l^{-1}, and the rest of the P fertilizer levels (Table 5.5; Figure 5.2). The main effect of P fertilizer was significant for reducing root length at $P = 0.0584$, while the main effect of *P. bilaii* was significant ($P \leq 0.01$) for reducing root length by 14% and SRL by 13% (Tables 5.1, 5.6).

Table 5.3. Effect of P level on P_e ratio [pathlength (terminal end points)$^{-1}$] of pea in the growth pouches, 19 days after planting.

P level (mg P l^{-1})	P_e ratio
0.1	32.6
0	30.9
1	29.8
10	27.0
LSD ($P \leq 0.05$)	3.6

Table 5.4. Effect of P level on root length of pea in the growth pouches, 19 days after planting.

P level (mg P l^{-1})	Root length (m)
0	7.155
0.1	6.891
1	6.181
10	5.457
LSD ($p \leq 0.05$)	0.821

Table 5.5. Effect of P level on P_e ratio [pathlength (terminal end points)$^{-1}$] of pea in the growth pouches, 26 days after planting.

P level (mg P l^{-1})	P_e ratio
0.1	37.5
0	37.3
1	35.6
10	32.1
LSD ($p \leq 0.05$)	2.7

Table 5.6. Effect of *Penicillium bilaii* on root length and specific root length (SRL) of pea in the growth pouches, 26 days after planting.

P. bilaii	Root length (m)	SRL (m g^{-1} DW root)
-	11.610	112.530
+	10.182	99.313
LSD ($P \leq 0.05$)	0.821	8.151

Contrast statements indicated that *P. bilaii* inoculation at the 0.1 mg P l^{-1} fertilizer level significantly ($P \leq 0.05$) reduced root length 21% and at the 1 mg P l^{-1} significantly ($P \leq 0.01$) reduced root length 23% (Table 5.1; Figure 5.2). Further, *P. bilaii* inoculation at the 0.1 mg P l^{-1} fertilizer level significantly ($P \leq 0.05$) reduced SRL 23% while SRL at the 1 mg P l^{-1} was significant at $P = 0.06$ for reducing SRL (Tables 5.1; Figure 5.2).

5.3.2 Dry Weight and Root:Shoot Ratio Responses

The results on dry weight responses and root:shoot ratios of pea have been summarized on Table 5.7 which presents significance levels for main effects (*P. bilaii* inoculation, P fertilizer) and interactions (*P. bilaii* x P fertilizer) for all harvest dates. Individual treatment effects within sampling dates are examined below.

5.3.2.1 12 DAP

The only observed dry weight response of pea at 12 DAP occurred for root dry weight (Table 5.7; Figure 5.3). The main effect of *P. bilaii* inoculation was significant ($P \leq 0.05$) for reducing root dry weight (9%) over all P fertilizer levels (Table 5.8; Figure 5.3). There were no detectable treatment effects on root:shoot ratio (Table 5.7; Figure 5.4).

5.3.2.2 19 DAP

At 19 DAP, there were no detectable treatment effects on shoot or plant dry weights of pea (Table 5.7; Figure 5.3). However, the main effect of P fertilizer was significant ($P \leq 0.05$) for root dry weight (Table 5.7). Root dry weights were significantly ($P \leq 0.05$) greater at the no added P and 0.1 mg P l^{-1} fertilizer levels than the dry weights produced at the 1 and 10 mg P l^{-1} levels (Table 5.9; Figure 5.3). The only significant

Table 5.7. Significance of dry weight and root:shoot ratio (RSR) responses of pea grown with and without *Penicillium bilaii* (Pb) and at one of four levels (0, 0.1, 1 and 10 mg P l^{-1}) of P fertility in the growth pouches.

	12 DAP					19 DAP					26 DAP				
	Root	Shoot	Nodule	Plant	RSR	Root	Shoot	Nodule	Plant	RSR	Root	Shoot	Nodule	Plant	RSR
Main effects															
P fertilizer	-	-	NA	-	-	*	-	-	-	-	-	-	***	-	-
P. bilaii	*	-	NA	-	-	-	-	-	-	-	-	-	-	-	-
Interaction															
P x Pb	-	-	NA	-	-	-	-	-	-	-	-	-	-	-	-
Contrasts															
Pb @ 0 P	-	-	NA	-	-	-	-	-	-	-	-	-	-	-	-
Pb @ 0.1 P	-	-	NA	-	-	-	-	-	-	-	-	-	-	-	-
Pb @ 1 P	-	-	NA	-	-	-	-	-	-	-	-	-	-	-	-
Pb @ 10 P	-	-	NA	-	-	-	-	-	-	*	-	-	-	-	-

* P ≤ 0.05. ** P ≤ 0.01. *** P ≤ 0.001. - = not significant. NA = not applicable.

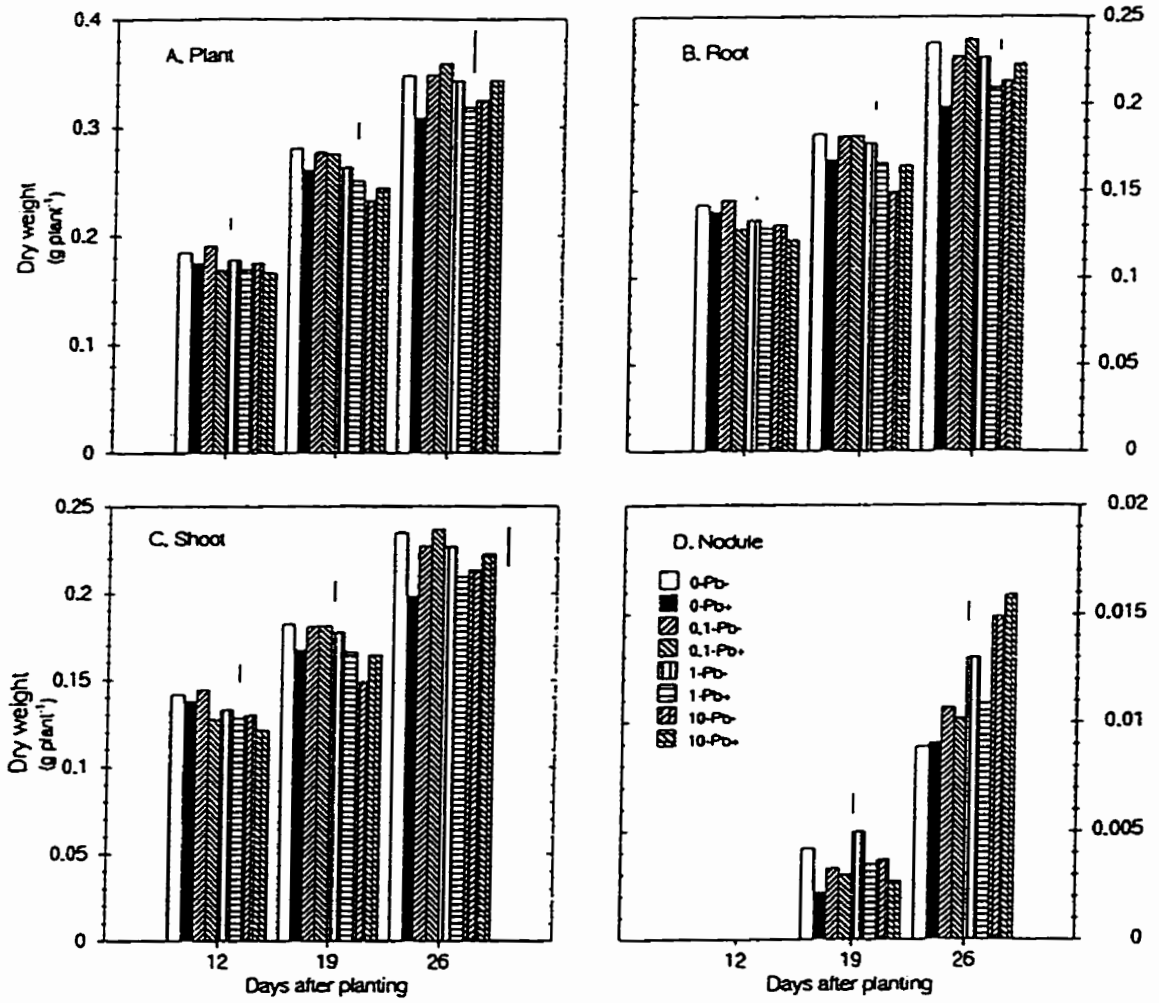

Figure 5.3. The effect of inoculation with (Pb+) or without (Pb-) *Penicillium bilaii* and P fertilizer level (0, 0.1, 1, 10 mg P l^{-1}) on plant (A), root (B), shoot (C), and nodule (D) dry weight of pea in the growth pouches. Bar represents the mean standard error of treatments within dates.

Table 5.8. Effect of *Penicillium bilaii* inoculation on root dry weight of pea in the growth pouches, 12 days after planting.

P. bilaii	Root dry weight (g)
-	0.0456
+	0.0417
LSD ($P \leq 0.05$)	0.0033

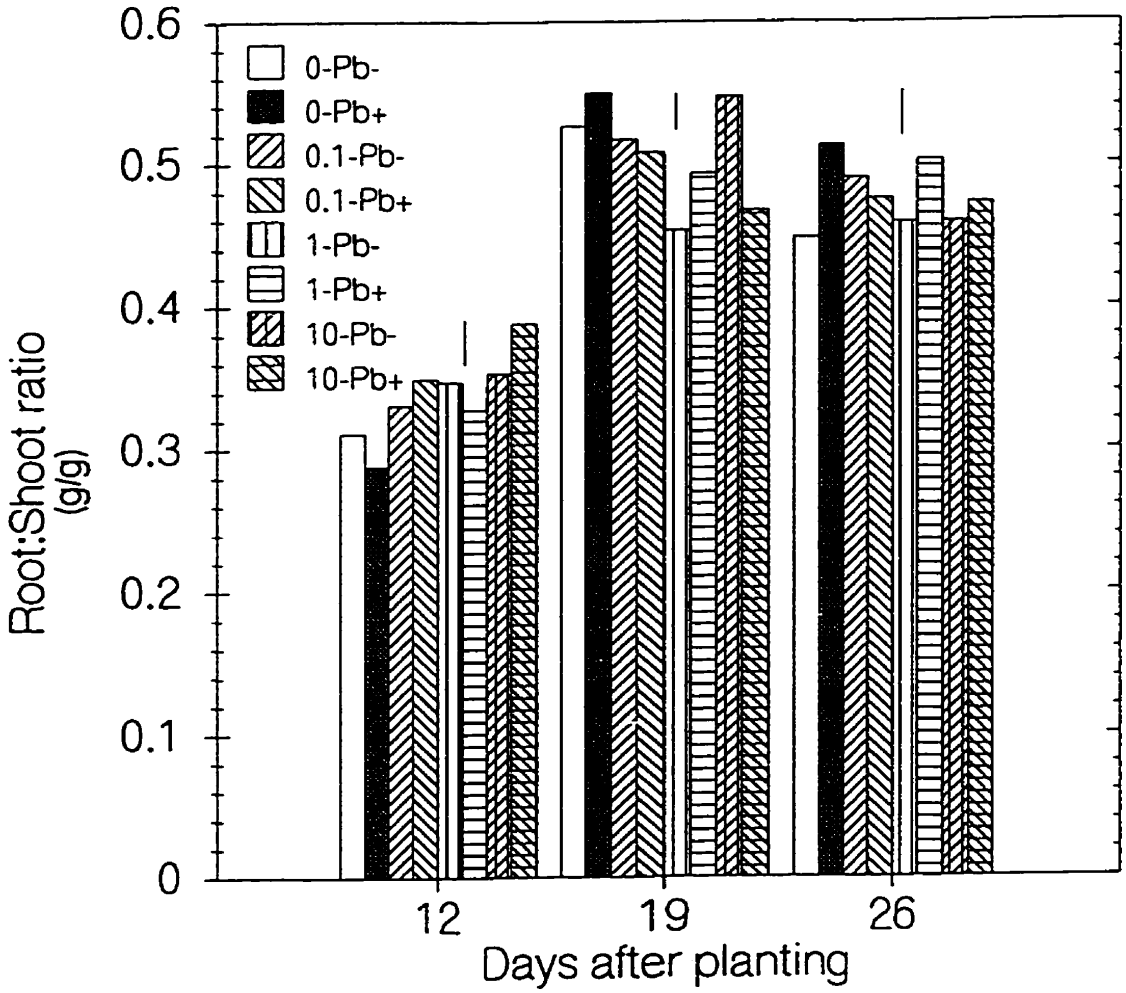

Figure 5.4. The effect of inoculation with (Pb+) or without (Pb-) *Penicillium bilaii* and P fertilizer level (0, 0.1, 1, 10 mg P l^{-1}) on root:shoot ratio of pea in the growth pouches. Bar represents the mean standard error of treatments within dates.

Table 5.9. Effect of P level on root dry weight of pea in the growth pouches, 19 days after planting.

P level (mg P l^{-1})	Root dry weight (g)
0	0.0930
0.1	0.0923
1	0.0807
10	0.0787
LSD ($p \leq 0.05$)	0.0109

treatment effect on root:shoot ratio occurred for *P. bilaii* inoculation at the 10 mg P l^{-1} fertilizer level (Table 5.7; Figure 5.4). Inoculation resulted in a significant ($P \leq 0.05$) reduction in root:shoot ratio of 17% for inoculated plants at the 10 mg P l^{-1} level (Table 5.7; Figure 5.4).

5.3.2.3 26 DAP

At 26 DAP, there were no detectable treatment effects on dry weight responses or root:shoot ratios of pea (Table 5.7; Figures 5.3, 5.4).

5.3.3 Nodulation Responses

The results on nodulation responses of pea have been summarized on Table 5.10 which presents significance levels for main effects (*P. bilaii* inoculation, P fertilizer) and interactions (*P. bilaii* x P fertilizer) for all harvest dates. Individual treatment effects within sampling dates are examined below.

5.3.3.1 12 DAP

There were no distinguishable nodules formed on the roots of pea plants at this sampling date.

5.3.3.2 19 DAP

There were no detectable treatment effects on nodule number or dry weight at 19 DAP (Tables 5.7, 5.10; Figures 5.3, 5.5). However, the main effect of P fertilizer was significant ($P \leq 0.05$) for specific nodulation (nodule no. m^{-1} root) (Table 5.10). Specific nodulation was significantly ($P \leq 0.05$) greater at the 1 and 10 mg P l^{-1} fertilizer levels than for the no added P and 0.1 mg P l^{-1} levels (Tables 5.10, 5.11; Figure 5.5). This increase in specific nodulation is likely a result of the fact that there was less root length

Table 5.10. Significance of nodulation response of pea grown with and without *Penicillium bilaii* (Pb) and at one of four levels (0, 0.1, 1 and 10 mg P l^{-1}) of P fertility in the growth pouches.

	12 DAP		19 DAP		26 DAP	
	Nodule No.	Specific Nodulation	Nodule No.	Specific Nodulation	Nodule No.	Specific Nodulation
Main effects						
P fertilizer	NA	NA	-	*	-	-
P. bilaii	NA	NA	-	-	-	-
Interaction						
P x Pb	NA	NA	-	-	-	-
Contrasts						
Pb @ 0 P	NA	NA	-	-	-	-
Pb @ 0.1 P	NA	NA	-	-	-	-
Pb @ 1 P	NA	NA	-	-	-	-
Pb @ 10 P	NA	NA	-	-	-	-

* $P \leq 0.05$. ** $P \leq 0.01$. *** $P \leq 0.001$. - = not significant. NA = not applicable.

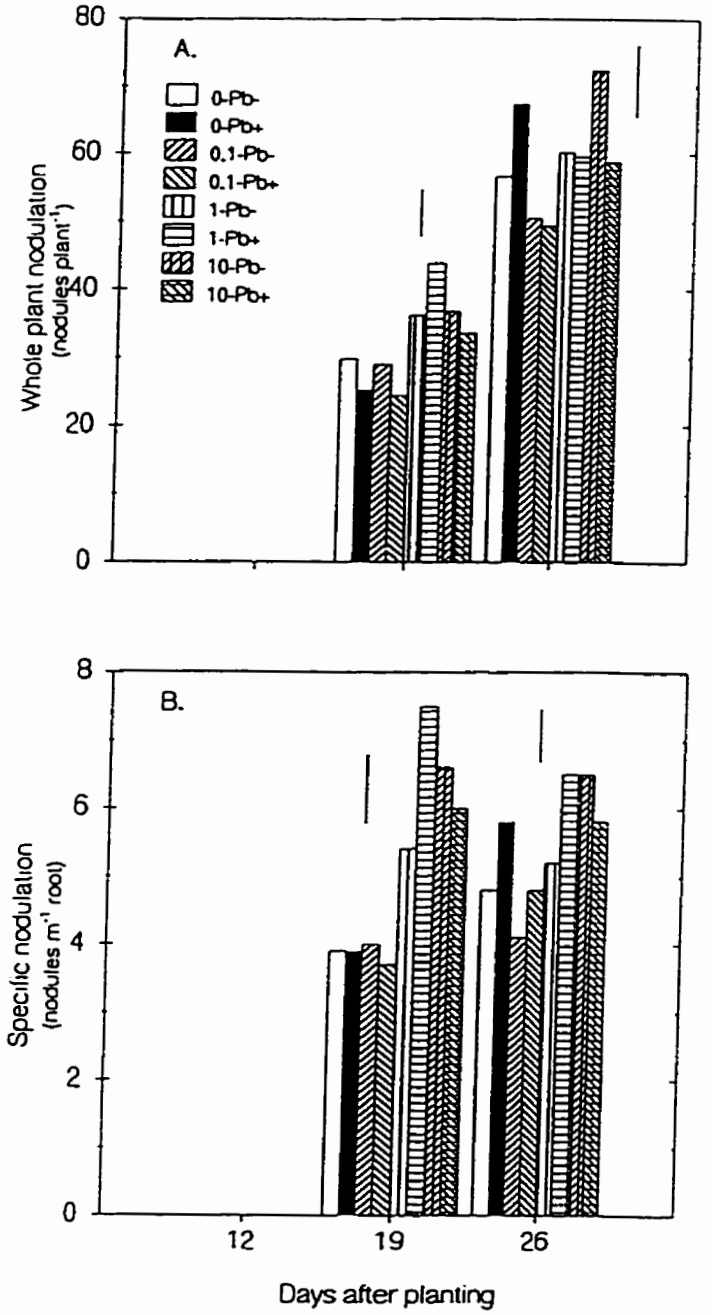

Figure 5.5. The effect of inoculation with (Pb+) or without (Pb-) *Penicillium bilaii* and P fertilizer level (0, 0.1, 1, 10 mg P l^{-1}) on whole plant nodulation (A), and specific nodulation (B), of pea in the growth pouches. Bar represents the mean standard error of treatments within dates.

Table 5.11. Effect of P level on specific nodulation of pea in the growth pouches, 19 days after planting.

P level (mg P l^{-1})	Specific nodulation (no. m^{-1} root)
1	6.382
10	6.371
0	3.923
0.1	3.903
LSD ($p \leq 0.05$)	2.024

produced at the 1 and 10 mg P l^{-1} fertilizer levels compared with the no added P and 0.1 mg P l^{-1} levels as nodule number was not affected (Table 5.4; Figures 5.2, 5.5).

5.3.3.3 26 DAP

No detectable treatment effects on nodule number or specific nodulation of pea at 26 DAP were observed (Tables 5.7, 5.10; Figures 5.3, 5.5). However, the main effect of P fertilizer was highly significant ($P \leq 0.001$) for nodule dry weight (Table 5.7, Figure 5.3). Nodule dry weight was significantly ($P \leq 0.05$) greater for plants at the 10 mg P l^{-1} fertilizer level, while plants at the no added P level produced the lowest nodule dry weights (Table 5.12; Figure 5.3). Plants at the 1 mg P l^{-1} fertilizer level were significantly different from the 10 mg P l^{-1} and the no added P level, but were not significantly from plants at the 0.1 mg P l^{-1} level (Table 5.12; Figure 5.3). Plants at the 0.1 mg P l^{-1} level were not significantly different from plants at the no added P fertilizer level (Table 5.12; Figure 5.3).

Table 5.12. Effect of P level on nodule dry weight of pea in the growth pouches, 26 days after planting.

P level (mg P l^{-1})	Nodule dry weight (g)
10	0.0154
1	0.0118
0.1	0.0105
0	0.0090
LSD ($p \leq 0.05$)	0.0021

5.4 Discussion

The configuration of pea root systems in this study was greatly influenced by the level of P in the nutrient solution rather than the presence of *Penicillium bilaii* (Table 5.1; Figure 5.2). At 19 DAP, plants at the lower two fertility levels had larger P_e ratios, indicative of a more exploratory type root system (Tables 5.1, 5.4; Figure 5.2). The higher two fertility levels produced root systems with lower P_e ratios indicative of a more branched, absorptive type root system (Tables 5.1, 5.4; Figure 5.2). This trend continued to 26 DAP, except that at the last harvest date only the P_e ratios for plants at the highest level of P fertility were significantly lower than those produced at the other P regimes (Table 5.1; Figure 5.2). Fitter (1982) reported that root systems growing in conditions of higher fertility were more branching (absorptive in nature) than those growing at lower fertilities. Price *et al.* (1989) have attributed increased branching under higher P fertility to the increased extension and higher frequency of initiation of lateral roots. Plants with higher values of P_e are thought to be exploratory in nature, allowing roots to access a greater volume of soil to search for mineral nutrients (Fitter, 1985).

The only observed effect of *P. bilaii* on P_e ratios occurred at 12 DAP at the highest level of P fertilization (Table 5.1; Figure 5.2). Inoculation significantly increased P_e ratio by 28%, which translates into inoculated plants having a more exploratory root system in early plant growth (Tables 5.1, 5.2; Figure 5.2). At this harvest date, the overall effect of *P. bilaii* inoculation resulted in higher SRL values (i.e., finer roots), most likely a result of reduced root dry weights (Tables 5.1, 5.2, 5.7; Figures 5.2, 5.3). At 26 DAP, the overall effect of *P. bilaii* inoculation resulted in a reduction in root length, and for the 0.1

and 1 mg P l^{-1} fertilizer levels significantly reduced root length 21 and 23%, respectively (Tables 5.1, 5.6; Figure 5.2). Furthermore, the overall effect of *P. bilaii* inoculation was significant for reducing SRL, and at the 0.1 mg P l^{-1} fertilizer level significantly reduced SRL 23% (Tables 5.1, 5.6; Figure 5.2). This translates into shorter roots with greater diameters. Specific root length is known to be greater in young plants, which have a high number of fine roots, plants in culture medium of low P-availability, or those generally experiencing low soil fertility (Anghinoni and Barber, 1980; Christie, 1975; Fitter, 1985; Hetrick, 1991). It has been observed that P fertilization of P-deficient soils results in plants with thicker roots with lower SRL (Powell, 1974). In the present study, higher SRL values were observed when no P was added to the nutrient solution, and SRL values decreased as the P concentration increased. Inoculation with *P. bilaii* further reduced SRL at 26 DAP (Table 5.1; Figure 5.2). This may have been a result of the improved P status of inoculated plants; unfortunately this was not confirmed in this study. In research related to the present study (Chapter 3), *P. bilaii* inoculation under controlled conditions resulted in a reduction in the total root length and SRL while maintaining the P concentration of the tissue to that of control plants. This alteration in root morphology in early plant growth did not affect the subsequent P status or dry weight of inoculated plants. *P. bilaii* inoculation was also associated with higher shoot and root tissue P concentration and accumulation in that study.

Sensitivity analysis of models for phosphate uptake has indicated that the most important plant factors for P uptake into roots are root length and diameter as these factors will greatly influence the root surface area for P absorption (Silverbush and Barber,

1983). If *P. bilaii* is promoting the uptake of P by plant roots (either directly or indirectly), it seems conceivable that there would be an effect on root morphology. Root architecture (P_e ratio) was affected by P concentration in this study, but root morphology (i.e., root length and diameter) is considered more important for the uptake of immobile nutrients such as P, while root architecture is more important for mobile resources (eg. water, nitrate) (Fitter, 1987). In other research related to this present study (Chapter 4), under field conditions using soil core samples, *P. bilaii* inoculation when no P fertilizer was added, resulted in a 48% increase in root crown length and a 21% increase in SRL (i.e., longer, finer roots in sampled area). This root morphology may have been related to the 13% increase in shoot P concentration for inoculated plants. Under P-limiting conditions, increased phosphate uptake may result from plants that have a finer and longer root system as this morphology would maximize root surface area for P absorption (Silverbush and Barber, 1983). Plants that produce the greatest interface with the soil generally will have the greatest nutrient uptake potential, but this must be balanced against the cost of the plant growing and maintaining these roots (Fitter, 1987). It was proposed in the field study that *P. bilaii* may have been increasing the availability of soil P to the inoculated plants (no additional P level), thus resulting in a proliferation of roots in the sampled area. Plant roots are known to proliferate in P-rich regions of soil (Drew *et al.*, 1973). Although root proliferation is stimulated in a P-rich zone, the addition of P may lead to a reduction in the actual amounts of roots per unit weight of shoot (Christie and Moorby, 1975; Ozanne *et al.*, 1969). Total root length and dry weight may be reduced when the levels of P in the soil are increased (Asher and Loneragan, 1967; Powell, 1974).

If *P. bilaii* was affecting the availability of P, or influencing the uptake of P by plant roots, this would likely result in plants that have shorter, thicker roots as further investment into root production for soil exploration would be superfluous.

The favoured hypothesis of how *P. bilaii* stimulates plant growth is that the fungus secretes organic acids, which cause the solubilization of precipitated phosphate, thereby increasing the availability of soluble phosphate to the plant (Kucey, 1988). However, in this study the P was in a plant available form (dissolved in the solution). Therefore, if *P. bilaii* was only able to promote or alter plant growth by increasing the availability of P to the plant, there should have been no observed effects of the fungus on root morphology. It is also important to point out that there generally was no effect of the fungus on root morphology when there was no P available, or when it was in abundance (10 mg P l^{-1}) in the nutrient solution. The greatest effects were observed at the intermediate P fertility regimes. This suggests that the effect of *P. bilaii* on plant growth is P-related because to see any effect, P must be limiting.

Penicillium bilaii has been associated with increased P concentration and accumulation of pea tissue in previous research (Chapters 3, 4) when no added P was added to supplement soil P reserves or when rock phosphate fertilizer was added. In addition, the observed reduction in SRL of inoculated plants suggests improved P status of the plants (Figure 5.2) (Powell, 1974). It is unfortunate that P analysis was not performed, as it would have provided a better interpretation of the results. However, despite this limitation, it appears that the mechanism of growth promotion by *P. bilaii* is P-related, but not exclusively. In the previously described field study (Chapter 4), at a

different field site, *P. bilaii* applied alone increased shoot dry weight of inoculated plants without an observed increase in P concentration; suggesting that *P. bilaii* may promote plant growth by other mechanisms. Other researchers have observed growth promotion (dry matter yields) that was not directly related to P (Chambers, 1992; Downey and van Kessel, 1990; Gleddie, 1992; Keyes, 1990). Keyes (1990) has suggested that *P. bilaii* may promote plant growth through passive biocontrol or growth-promoting effects.

Another objective of this study was to determine if *P. bilaii* affected nodulation responses (nodule number, dry weight, specific nodulation) of pea. *Penicillium bilaii* under the conditions of this study did not affect nodulation of pea (Tables 5.7, 5.10; Figures 5.3, 5.5). Other researchers have observed effects of this fungus on pea root nodulation responses. Gleddie (1992) reported inoculation with *P. bilaii* resulted in increased nodulation of pea roots by *Rhizobium leguminosarum* in a growth room study, and promoted increased N uptake by pea in growth chamber and P responsive field trials. Downey and van Kessel (1990) concluded that inoculating with *P. bilaii* decreased total N accumulation by pea plants in a growth chamber experiment. They proposed that the production of P-solubilizing organic acids by the fungus may have reduced the rhizosphere pH to a degree that would inhibit *Rhizobium* function.

Although nodulation responses were not affected by the presence of *P. bilaii*, there were observed effects of P fertilizer on specific nodulation and nodule dry weights (Tables 5.7, 5.10, 5.11, 5.12; Figures 5.3, 5.5). Specific nodulation at 19 DAP at the two higher fertility regimes was almost twice that of the plants at the two lower P fertility regimes (Tables 5.10, 5.11; Figure 5.5). This may have been a result of the observed reduction in

root length as the P concentration in the nutrient solution increased (Table 5.4). Nodule dry weight at 26 DAP was also significantly affected by the level of P fertility (Tables 5.7, 5.12; Figure 5.3). As the level of P increased, nodule dry weights increased (Tables 5.7, 5.12; Figure 5.3). Nodule number and mass have been shown to increase with increased supply of P (Cassman *et al.*, 1981; Dhingra *et al.*, 1988; Gates, 1974; Graham and Rosas, 1979; Pereira and Bliss, 1987).

In conclusion, it appears that *P. bilaii* has a greater effect on root morphology (root length and diameter) than root architecture (P_e ratio). This study also suggests that *P. bilaii* may affect plant growth by other mechanism (s) besides increasing the availability of P to plants, as all of the P used in this study was plant available. However, it appears that the limitation of P to plants plays a key role in determining the ability of this fungus to affect root morphology and plant growth. Results from present and past work show the most significant effects are seen when P is present, but limiting. Root morphology of *P. bilaii* inoculated plants in this study suggest an improved P status of the plant, unfortunately this was not confirmed. We also determined, under the conditions of this experiment, that *P. bilaii* had no significant positive or negative effect on nodulation responses of pea.

6. General Conclusions

This section will outline the rationale for this work, highlight the significant findings from the three manuscripts, suggest possible explanations of these findings, and conclude with suggestions for future research.

Inoculation of various crop plants with *Penicillium bilaii* has resulted in increased phosphorus (P)-uptake, dry matter production, and grain yields (Asea *et al.*, 1988; Chambers, 1992; Gleddie, 1993; Gleddie *et al.*, 1991, 1993; Kucey, 1987, 1988; Kucey and Leggett, 1989). However, the mechanism (s) underlying the stimulation of plant growth and P-uptake is not known. The favoured hypothesis of how *P. bilaii* promotes plant growth is through solubilization of sparingly soluble phosphates. It is suggested that *P. bilaii* secretes organic acids, which acidify the surrounding soil and/or by cation chelation, thereby increasing the availability of soluble phosphate to the plant, thus promoting plant growth (Asea *et al.*, 1988; Kucey, 1987, 1988). This fungus has been shown to produce oxalic and citric acids in pure culture (Cunningham and Kuiack, 1992), and can solubilize calcium phosphate in agar medium (Kucey, 1983) and rock phosphate in liquid culture (Asea *et al.*, 1988). However, it has been observed that *P. bilaii* promotes plant growth without promoting the uptake of P (i.e., is not P-related) (Chambers, 1992; Downey and van Kessel, 1990; Gleddie, 1992; Keyes, 1990). This suggests that *P. bilaii* may promote plant growth via other mechanisms, possibly beyond increasing plant available P. It has been suggested that *P. bilaii* may promote plant growth through passive biocontrol or growth-promoting effects (Keyes, 190).

An alternative mechanism by which *P. bilaii* may promote plant growth and P-

uptake is through stimulation of root growth (i.e., increase root length), thereby increasing the root surface area for phosphate absorption. Sensitivity analysis of models for phosphate uptake into plant roots has indicated the most important plant properties for P-uptake are root length and diameter (Silverbush and Barber, 1983). This is the possible mechanism by which *P. bilaii* stimulates plant growth and P-uptake investigated in this thesis.

In the controlled environment studies, we observed that application of *P. bilaii* alone resulted in increased shoot P concentration and accumulation. When rock phosphate fertilizer was added, *P. bilaii* inoculation increased both root and shoot P concentration and accumulation. However, these increases in tissue P concentration and accumulation were not accompanied, or preceded by increased root length, and therefore was not a result of increased root surface area for P absorption. In fact, it was observed that *P. bilaii* inoculation resulted in decreases in root length. This reduction in root length did not affect the P concentration of roots or shoots, or shoot P accumulation. This alteration of root morphology in early growth did not affect subsequent P concentration and accumulation, or dry matter. This suggests that *P. bilaii* inoculated plants in early growth may require less root surface area to produce shoot dry matter and possibly for phosphate absorption. Plant roots of *P. bilaii* inoculated plants when no P fertilizer was added, at the final harvest, were thicker (greater mean diameters) than non-inoculated plants. Usually, increased average root diameters (thickness) suggests improved P status of the plant. However, *P. bilaii* did not affect P concentration or accumulation. Results from this study indicate that *P. bilaii* does not stimulate root growth and thus, root

surface area for phosphate absorption. Inoculation with this fungus may effect the morphological properties of the root system (root length and diameter) by affecting (improving) the availability of P to the plant, and thus, the resulting P status of the plant. Changes in P availability, and the resulting plant uptake may result in the alteration of the morphological properties of the root system, as P status is known to affect root morphology.

In the field study, *P. bilaii* inoculation at one field site, when no P fertilizer was applied, resulted in longer and finer roots in the sample core. This increase in root length may have contributed to the 13% increase in shoot P concentration of inoculated plants, as increased root surface area can lead to increased P absorption. A possible interpretation of the observed root morphology, is that *P. bilaii* may have increased the availability of P to inoculated plants, resulting in a proliferation of roots in the sampled area; rather than a direct effect of the fungus stimulating root growth. Furthermore, shoot P concentration was increased, but had no effect on dry matter was observed, further supporting this hypothesis. At another field site, *P. bilaii* inoculation promoted shoot dry matter production when no P fertilizer was applied. However, the increase in dry matter production was not a result of improved P status of the plant. This suggests that *P. bilaii* may stimulate plant growth by other mechanisms, possibly beyond increasing the availability of P.

It was thought that if *P. bilaii* could alter the morphological properties of the root, it may also influence root architecture (i.e., branching patterns). Alteration of branching patterns could result as a direct effect of the fungus through growth promoting effects, or

through its possible ability to improve P availability, as the nutritional status of the plant can greatly influence branching patterns. Our results from Chapter 5 indicated that *P. bilaii* has a greater effect on root morphology, than branching patterns of peas. However, branching patterns of pea roots were greatly influenced by the level of P in the nutrient solution. Plants at the higher fertility regimes were more branching compared with plants at lower fertility regimes. It was observed that *P. bilaii* inoculation, similar to results from the controlled environment study, reduced pea root length, and increased mean root diameters. This suggests improved P status of inoculated plants, however, this was not confirmed in this study. The fact that all the P in the nutrient solution was available to the plants, and that effects of *P. bilaii* inoculation were observed, suggests that *P. bilaii* may affect plant growth by other mechanisms, and is not strictly associated with increased P availability. However, it appears that the limitation of P plays a key role in determining the ability of this fungus to alter root morphology and plant growth. The results show that the most significant and positive effects are seen when P is present, but limiting.

In the field and growth pouch studies, *P. bilaii* inoculation did not affect nodulation response of peas. Results on nodulation responses from the controlled environment study were inconclusive as the CV values were quite high.

This work will likely prompt more questions and investigations into possible mechanisms of how *P. bilaii* promotes plant growth and P-uptake. Suggested areas for future research include studying the effect of *P. bilaii* inoculation on: (1) root morphology under field conditions, using larger sampling equipment or through root excavation to obtain a larger portion of the root system; (2) determine and quantify organic acid

production or other metabolites produced *in situ*, and examine their effect on plant nutrient availability and possible growth promoting effects; (3) examine the possible stimulation of root hair production (numbers) and morphology (length, and diameter).

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IMAGE EVALUATION
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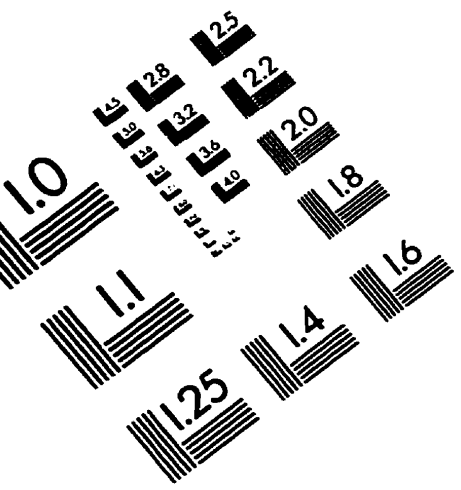

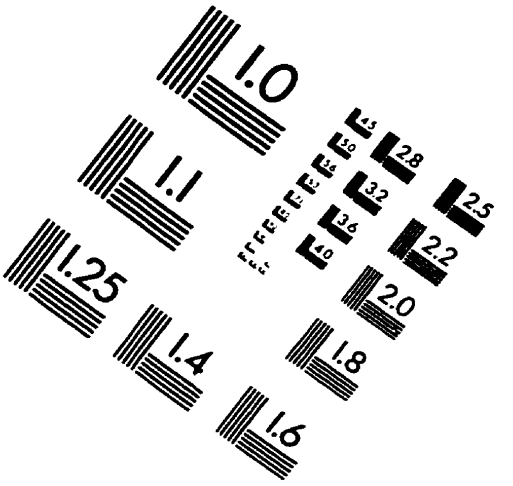

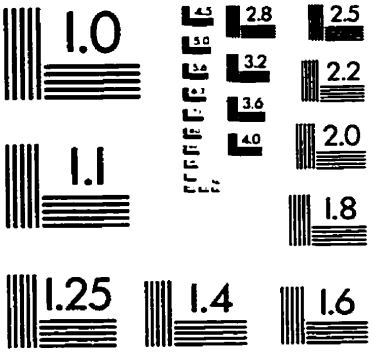

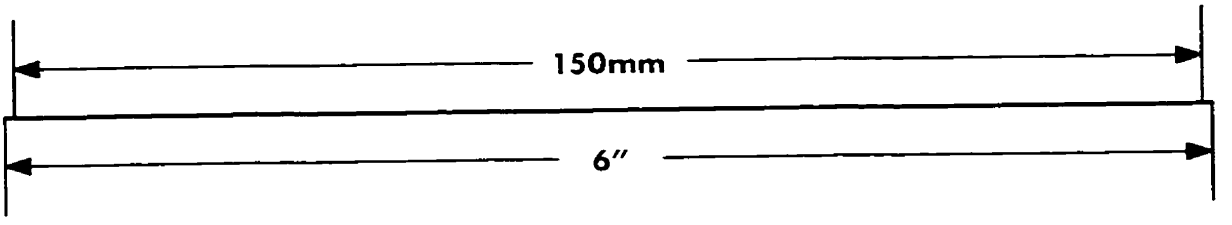

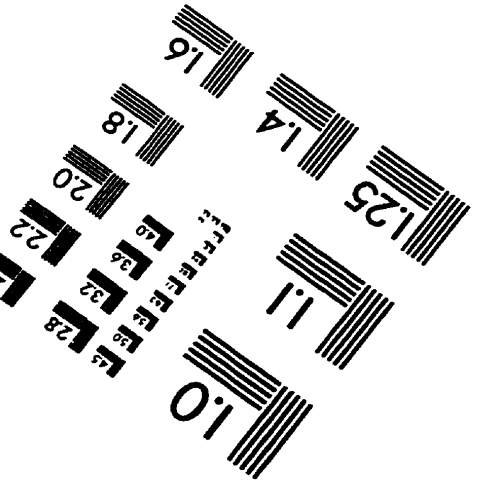

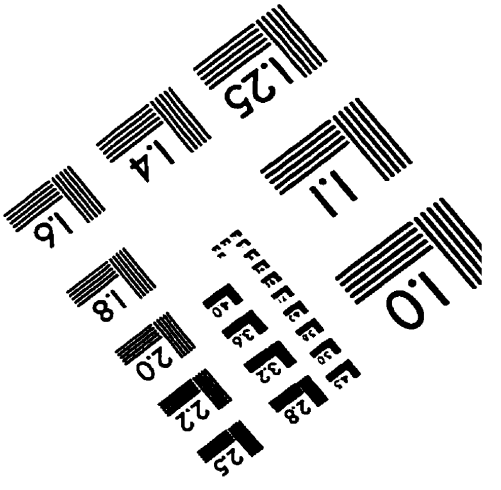

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