

**Phosphorus fertilization: effects on asparagus  
yield, and soil microbial parameters**

By

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## ABSTRACT

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*Asparagus* (*Asparagus officinalis*) is a perennial crop well suited to grow in Québec. Despite high demand for this crop, asparagus production is declining because of asparagus root rot disease that is caused by *Fusarium* spp. Phosphorus is one of the most important nutrients for the production of healthy asparagus roots that may resist infection by *Fusarium*. The objective of this study was to determine the effects of phosphorus (P) applications on asparagus yield and soil microbial parameters. Plots receiving 0 to 200 % of the P recommendation, based on the Centre de référence en agriculture et agroalimentaire du Québec (CRAAQ) guidelines, did not differ significantly ( $p < 0.05$ ) in first year yield or plant tissue concentrations. However, asparagus receiving no fertilizer had 2 to 3 times higher arbuscular mycorrhizal colonisation of roots than other treatments in May 2003. Fresh marketable yield was negatively correlated to *Fusarium* incidence ( $p = 0.0091$   $r = -0.51$ ) suggesting that plants with higher yields are less susceptible to *Fusarium* symptoms and that producers should avoid over-harvesting low yielding plants to reduce *Fusarium* spread in the field. Soil microbial activity was not affected consistently by P fertilizers because of high variability in the field.

## RESUMÉ

M.Sc.

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L'asperge (*Asparagus officinalis*) est une plante vivace bien adaptée pour la cultivation au Québec. Malgré une demande élevée dans cette province, la production d'asperges est en baisse à cause de la fusariose causé par *Fusarium* spp. Le phosphore (P) est l'un des éléments les plus important pour la production de racines d'asperge saines pouvant mieux résister l'infection. L'objectif de cette étude est de vérifier l'effet de la fertilisation en P sur le rendement des asperges et des paramètres microbiens du sol d'une aspergeraie. Il n'y avait pas de différence ( $p < 0.05$ ) en rendement de turions frais ou en concentration en P dans les tissus foliaire entre les parcelles recevant 0 % à 200% des apports recommandés en P du CRAAQ. Par contre, les parcelles ne recevant pas de P avaient 2 à 3 fois plus de colonisation mycorhizienne en mai 2003 que les autres traitements. Le poids frais des turions était négativement corrélé à l'incidence de *Fusarium* ( $p = 0.0091$   $r = - 0.51$ ) suggérant que les plants ayant un meilleur rendement sont moins susceptibles à la fusariose. Ces résultats suggèrent que les producteurs devraient éviter de surexploiter les plants avec un faible rendement afin d'éviter la propagation de la fusariose dans le champ. L'effet de la fertilisation en P sur l'activité microbienne du sol était très variable à cause de l'hétérogénéité du champ expérimental.

## PREFACE

This thesis is composed of three chapters. Chapter One is a general introduction to the subject and is followed by the general hypotheses tested in the study. The next two chapters discuss the research that was undertaken for this thesis, and are prepared in manuscript format for submission to scientific journals. Chapter Two describes the effect of P fertilization on soil P saturation and asparagus establishment and yield. Chapter Three investigates the effects of P fertilization on microbial biomass, enzymatic activity, arbuscular mycorrhizal colonization and *Fusarium* incidence. A connecting paragraph between the Chapter Two and Chapter Three clarifies the progression between these two papers. The general conclusions summarize the main results and discuss future areas of research.

The two papers included in this thesis were co-authored by the candidate and his supervisor Dr. J. K. Whalen. Dr. Vladimir Vujanovic contributed to the fusarium diversity and abundance results in Chapter Three. The candidate was responsible for both conducting the research and preparing the manuscripts. Assistance was given by Dr. Whalen through general guidance and editorial corrections during preparation of the manuscripts.

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## Introduction

Asparagus (*Asparagus officinalis* L.) is an economically important perennial crop grown in temperate regions of the world. However, asparagus is susceptible to fungal diseases that can reduce production significantly. One such disease is *Fusarium* root rot, which attacks the root system and crown, impairing root functions and often causing mortality. The factors that predispose asparagus fields to the disease are not well known. In Québec, 45% of asparagus fields are in poor condition due to this disease (Vujanovic, 2003). Currently, methods for reducing asparagus decline are limited and not very efficient (Keulder, 1999). Promoting well developed roots and healthy shoots is therefore important. For this reason, asparagus fields are fertilized with ample quantities of nitrogen, potassium and phosphorus. Of these three major nutrients, phosphorus is the most important for root growth and the Centre de référence en agriculture et agroalimentaire du Québec (CRAAQ) recommends adding up to 310 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> when plantations are established, with as much as 50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> added on rich P soils in subsequent years (CRAAQ, 2003a). The relationship between phosphorus fertilization and soil organisms that are antagonistic (i.e. *Fusarium* spp.) or beneficial (i.e. arbuscular mycorrhizal fungi) to asparagus growth and production are not known. This literature review provides a brief discussion of asparagus production methods and will also review our current knowledge of the impacts of phosphorus fertilization on asparagus shoot production, asparagus pathogens and symbionts.

## **Chapter 1. Literature review**

## **Asparagus life cycle**

Asparagus is an ideal perennial plant to grow in northern climates. During the winter, asparagus is dormant, but in early spring it produces large vigorous shoots that are cut and marketed as a fresh vegetable from May to mid-June. An asparagus plantation is usually planted in the spring as one-year crowns (Figure 1). The crown is the primary root system of asparagus plants. Just above the crown, some of the dormant buds sprout and emerge from the ground as shoots, later developing into ferns (asparagus foliage), that allow the plant to produce carbohydrates. At the end of each growing season, carbohydrates and nutrients are translocated to the crown and the aboveground biomass senesces and dies. These stored carbohydrates and nutrients permit asparagus to get an early start in the spring (shoots generally emerge by early May). Asparagus primary roots can become very extensive, sometimes extending 10 feet underground in older plants (Drost, 1997). Although the roots can extend very deep into the ground, the asparagus fern only grows to be about 6 feet high, thus asparagus have a large part of their biomass underground. After crowns are planted, asparagus grows and accumulates reserves for two years. In the third year, if the crown has accumulated enough reserves, the plant is able to support a harvest of some of its shoots. The shoots are usually harvested every one to two days for a 7 to 10 day period in the first year of harvest, for 3 to 4 weeks in the second year and for 5 to 6 weeks in subsequent years, depending on the vigour of ferns in the previous season (Tessier, 2001). A healthy asparagus field can yield marketable shoots for up to 15-20 years (Figure 2).

## ***Economic importance of asparagus in Québec***

Currently, the market value of asparagus averages \$ 6,800 to \$ 9,000 per hectare, depending on the demand and the market price (Choquette, 2001). The early harvest of the shoots (April to June) provides crop producers with an early source of revenue. Asparagus can therefore be a very profitable crop and a long-term source of income for the farmer. The yearly imports of asparagus in Québec are 3 times higher than the amounts produced and it is estimated that another 1500 ha under asparagus production are needed to supply the demand in this province. Despite this high demand, asparagus production in Québec is declining. In 1987 there were 480 ha of asparagus fields in production but by 2001, only 371 ha were in production (Statistics Canada, 2002).

## ***The asparagus decline and replant problem***

The asparagus decline and replant problem is common in asparagus plantations around the world (Keulder, 1999). The first symptoms of asparagus decline tend to occur one year after a field has been first harvested (Vujanovic, 2003). The symptoms include reddish discolorations in the crown and on the shoots, damaged crowns and dried up, chlorosed small ferns. The reddish discoloration on the crown and shoots is caused by pathogenic strains of *Fusarium* species such as *F. oxysporum asparagii*, *F. moniliform*, *F. redolens* and *F. proliferatum*. Between one and five years after the first symptoms appear, *Fusarium* can take over the whole field, reducing the yield to such an extent that

it is no longer profitable. In a replanted field, symptoms appear much earlier, sometimes only one year after the crowns have been planted. *Fusarium* inoculum present on the old decaying roots often infects the newly planted crowns and reduces their vigour (Singh *et al.*, 1999). One of the most obvious causes in the decline of asparagus is the presence of pathogenic *Fusarium* fungi in the field. Fungicide applications do not control this fungal infection since the fungus lives in soils and asparagus crowns.

Since the 1960's, there have been many attempts to reduce or eliminate the symptoms of the asparagus decline. The asparagus decline symptoms were first described by Grogan and Kimble (1959) and were confirmed to be caused by *Fusarium* pathogens and it is agreed by the scientific community that *Fusarium* is the causative agent producing the symptoms and the death of asparagus plants. However, it is still unclear what factors predispose asparagus to *Fusarium* infection. It is very likely that the asparagus decline and replant problem cannot be attributed to a single factor but is probably due to a combination of stresses that occur during the life of this perennial plant. Since decomposing asparagus roots remain in the soil for many years, the plant must deal with soil organisms and fungal pathogens that accumulate near their roots and their crown each year. The organisms associated with asparagus roots cannot easily be altered. As the populations of pathogenic organisms increases, it is then very difficult to eradicate them from the asparagus plantation. Asparagus are unlike annual plants, which can be rotated to reduce soil borne diseases, and therefore other strategies are needed to protect them from infection by such pathogenic organisms because such infections can intensify through time.



## ***The asparagus plantation***

Establishing an asparagus plantation is a long-term investment and it is not uncommon to devote an entire growing season to preparing a site. After the crop is planted, it is much more difficult and expensive to alter the field conditions. Site preparation includes the removal of perennial weeds, adjustment of pH and incorporation of organic matter to stabilize soil structure and increase water retention.

The most popular cultivars now planted in Québec are the new male varieties, Guelph Millennium and Jersey Giant. These cultivars do not produce seed and therefore allocate more energy to reserves and shoot production. These cultivars are also said to be more resistant to the *Fusarium* disease. However, it has only been about 10 years that these cultivars have been in use and their long-term resistance to asparagus decline disease remains to be assessed.

## ***Asparagus phosphorus requirements***

To help establish healthy asparagus roots, the CRAAQ recommend high initial fertilization rates so the plants obtain adequate nutrient supplies. The initial N, P, K fertilization rates are as high as 150 kg N ha<sup>-1</sup>, 245 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 200 kg K ha<sup>-1</sup> for soils with a medium level of soil fertility (CRAAQ, 2003a). Producers are advised not to

harvest during the first 2 years when the plantation is becoming established (energy reserves are all allocated to root and crown production).

Phosphorus is known to be a limiting nutrient for crop production in Québec soils (CRAAQ, 2003b). Phosphorus must be present in sufficient quantities for cell division to occur, and is also necessary for plant respiration, photosynthesis and many other physiological functions. Phosphorus deficient plants usually present the following symptoms : less developed root system, stunted growth and a bronze or violet colour on the leaves and shoots of young plants. However, if these symptoms are visible, then the crop is already nutrient deficient and will not give an optimum yield. It is therefore important to supply adequate quantities of P before the plant shows these symptoms.

There are very few studies on the effects of phosphorus fertilization in asparagus fields. However, based on the amount of nutrients withdrawn from harvest, the P recommendations seem very high. Giroux and Chamberland (1990) calculated the quantities of P removed from asparagus harvests by calculating the % P removed in the shoots at harvest. They estimated that P removal was approximately 9 kg P ha<sup>-1</sup>. The authors concluded that asparagus is not a very demanding plant in terms of P fertilizer requirements. Another study done in France by Lubet *et al.* (1985) estimated the P removed in asparagus at harvest to be 7.4 kg P ha<sup>-1</sup>. However if we look at the P recommendations for Québec (CRAAQ, 2003a), the quantity of P fertilizer a producer should add is 2 to 28 times (on excessively P rich soil and on poor P soil, respectively) higher than the amount of P removed at harvest! Ontario and Oregon recommend 12 to

50% lower  $P_2O_5$  applications and not to add P fertilizers on excessively rich P soils (Kessel, 2003 ; OSU, 2002). Research is needed to determine whether the P fertilizer recommendations published by CRAAQ (2003a) are appropriate for asparagus plantations in Québec, especially those that have been planted with newer, high yielding varieties.

It is important to optimize fertilizer applications, since higher fertilizer application will not necessarily promote plant health and production. Too high fertilizer rates, for example nitrogen fertilizers, have been found to increase disease incidence and lesion area caused by certain pathogens such as *Blumeria graminis f. sp. tritici* and *Oidium lycopersicum* (Solomon *et al.*, 2003). Thus, inadequate fertilizer rates (ex.: high N fertilizer rates) on asparagus could cause an improper shoot to root ratio and compromise yields in future years (Drost, 1997). High amounts of fertilizers applied also increases the costs for the producers and could also be detrimental to the environment if the nutrients leach in the surrounding waters. Reducing the P rates would be economically beneficial to the producer and would also reduce the risk of environmental degradation from P runoff. In 2002, the Gouvernement of Québec passed a new law restricting P applied to agricultural fields. This law is enforced by the Ministère de l'Environnement du Québec (MEV) and obliges farmers to maintain soil test phosphorus saturation (P/Al ratio) under 7.6% in soils containing more than 30% clay and under 13.1% in soils containing less than 30% clay by 2010.

## **Phosphorus and *Fusarium* pathogens**

Pathogenic fungi, such as *Fusarium* sp. may be influenced by the amounts of phosphorus in the soil and the tissues in the plants. *Fusarium* pathogenic strains have been identified as opportunistic fungi that are plant parasites under certain conditions. Nigh (1990) observed that reduced irrigation, high insect populations and some cultural practices such as herbicide application increased the incidence of *Fusarium* in asparagus fields of Arizona. He concluded that any factor that increased plant stress could cause asparagus decline. Blok and Bollen (1996a) noted that *Fusarium* pathogenic strains (*F.oxysporum* ; *F.asparagi*) had little saprophytic ability and proliferated only in nutrient-rich media. Thus, highly fertilized plants could be better hosts for fungal pathogens such as *Fusarium* sp. than plants that receive less fertilizer. He *et al.* (2002) succeeded in reducing the amounts of root necrosis caused by pathogenic strains of *Fusarium* sp. by inoculating asparagus with non-pathogenic strains of *Fusarium*. Non-pathogenic *Fusarium* are probably better competitors for nutrients and would therefore be able to reduce pathogenic strain populations by out-competing them for root space. Thus, it seems that *Fusarium* pathogens will not infect healthy asparagus fields but only those which have a certain stress and plant weakness.

It is not known whether phosphorus fertilization can increase or decrease disease in field crops and vegetables (Borowicz, 2001). If P fertilizers influence the incidence of *Fusarium* or other diseases in asparagus plantations, it probably occurs indirectly. Three

possibilities have been suggested by Solomon *et al.* (2003) that could explain higher *Fusarium* pathogenicity in soils that receive more P fertilizer :

- 1) High levels of P fertilization may cause nutrient imbalances in the plant, making it less able to defend itself against *Fusarium* infection.
- 2) High levels of P fertilization may cause a change in the microbial community associated with asparagus roots, making asparagus more susceptible to *Fusarium* infection.
- 3) High levels of P fertilization could increase the survival and proliferation of *Fusarium* pathogens making these organisms more virulent to asparagus.

### ***Phosphorus and arbuscular mycorrhizal fungi (AMF)***

It has been indicated that the soil P level can influence the number and activity of arbuscular mycorrhizal fungi (AMF) (Smith and Reid, 1997 ; Ryan *et al.*, 1994). The impact of phosphorus fertilisation on soil AMF communities in New Zealand grasslands was tested by Johnson (1993). He noted a change in AMF colonisation and that the species of AMF present in grasslands receiving P fertilizer were less efficient at acquiring P from the soil than the species present in the unfertilized grassland. A study by Wacker *et al.* (1990a) found that AMF succession changed over time (from 0 to 15 years) in asparagus fields. They suggested that soil P levels may have influenced the AMF community change. In the first years of the asparagus production, the soil test P levels were 100 mg P kg<sup>-1</sup> however, 5 years later, P levels were lower (40 mg P kg<sup>-1</sup>). The total amount of AMF colonisation did not vary, however, the type of AMF species did change

over time. Other factors, such as plant age, soil compaction, climate, humidity and other soil parameters can contribute to changes in AMF communities.

It is well known that soil P levels are important for controlling the colonization, activity and diversity of AMF associated to higher plants (Smith and Read, 1997). It was observed by Fitter (1989) that AMF are more efficient at extracting P than roots because their smaller hyphae can exploit finer areas in the soil. However, at high soil P levels (around Olsen P = 60 mg kg<sup>-1</sup>), AMF were found to reduce crop biomass and yield of barley and linseed because of too high demands in C from the host plant (Ryan and Graham, 2002). Also, in soils with very low P concentrations (11 mg P kg<sup>-1</sup>), AMF may become parasitic, consume the plant's carbohydrates and compete with plant roots for available nutrients (Bethlenfalavy *et al.*, 1983). The mechanisms controlling beneficial AMF-plant interactions and how these interactions are affected by P fertilization have scarcely been explored.

There is growing evidence that AMFs can protect plants from disease. Research by Jeffries *et al.* (2003) has indicated that AMF could protect plants against certain pathogenic fungi by outcompeting them for root space. Borowicz (2001) concluded that AMF act as a buffer against certain stresses the roots must confront in the soil. For example, when pathogenic nematodes were present, AMF permit good growth of plants despite attacks from pathogenic nematodes. Thus, when plants infected with a parasite were inoculated with AMF, these plants grew just as well, and in some cases better than non-infected plants without AMF. Borowicz (2001) proposed that nutrient (and water)

uptake by AMF compensated for the loss of function in roots damaged by parasitic nematodes. Both Burrows *et al.* (1990) and Wacker *et al.* (1990b) found that asparagus seedlings inoculated with *Glomus* sp. had fewer symptoms of diseases caused by *Fusarium*.

These findings lead us to hypothesize that lower P fertilizer applications in asparagus plantations would promote AMF colonization and protect the plant against harmful pathogens such as *Fusarium*. However, this hypothesis needs to be tested so we can optimize beneficial relationships between asparagus and the AMF symbiosis and reduce the amounts of P fertilizers applied to asparagus plantations.

## **Research Questions**

The primary objective of this study is to better understand the effect of phosphorus fertilization on asparagus establishment and yield. The study site, at the Macdonald Horticultural Center, had 150 mg P kg<sup>-1</sup> soil when the asparagus plantation was established. The CRAAQ (2003a) guidelines recommend an application of 155 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> during establishment, but it is not known whether this rate is optimal to benefit from AMF colonization and adequate for plant growth.

### **Hypothesis 1**

We hypothesize that the application of P fertilizers will not increase asparagus growth and yield (first harvest) at our site.

The second objective of this study was to obtain preliminary data to better understand the effect of phosphorus fertilization on different soil biological properties in the asparagus plantation. The results from this part of the study will orient future research on factors that predispose asparagus plants to *Fusarium* root rot.

### **Hypothesis 2**

We hypothesize that the application of P fertilizers will reduce the population and activity of some beneficial microorganisms, such as AMF, and increase the plant's susceptibility to *Fusarium*.



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Figure 1. One year old asparagus crown and emerging bud. Picture David F. Graper.  
<http://hflp.sdstate.edu/images/Asparagus%201%20yr%20old%20crown.JPG>

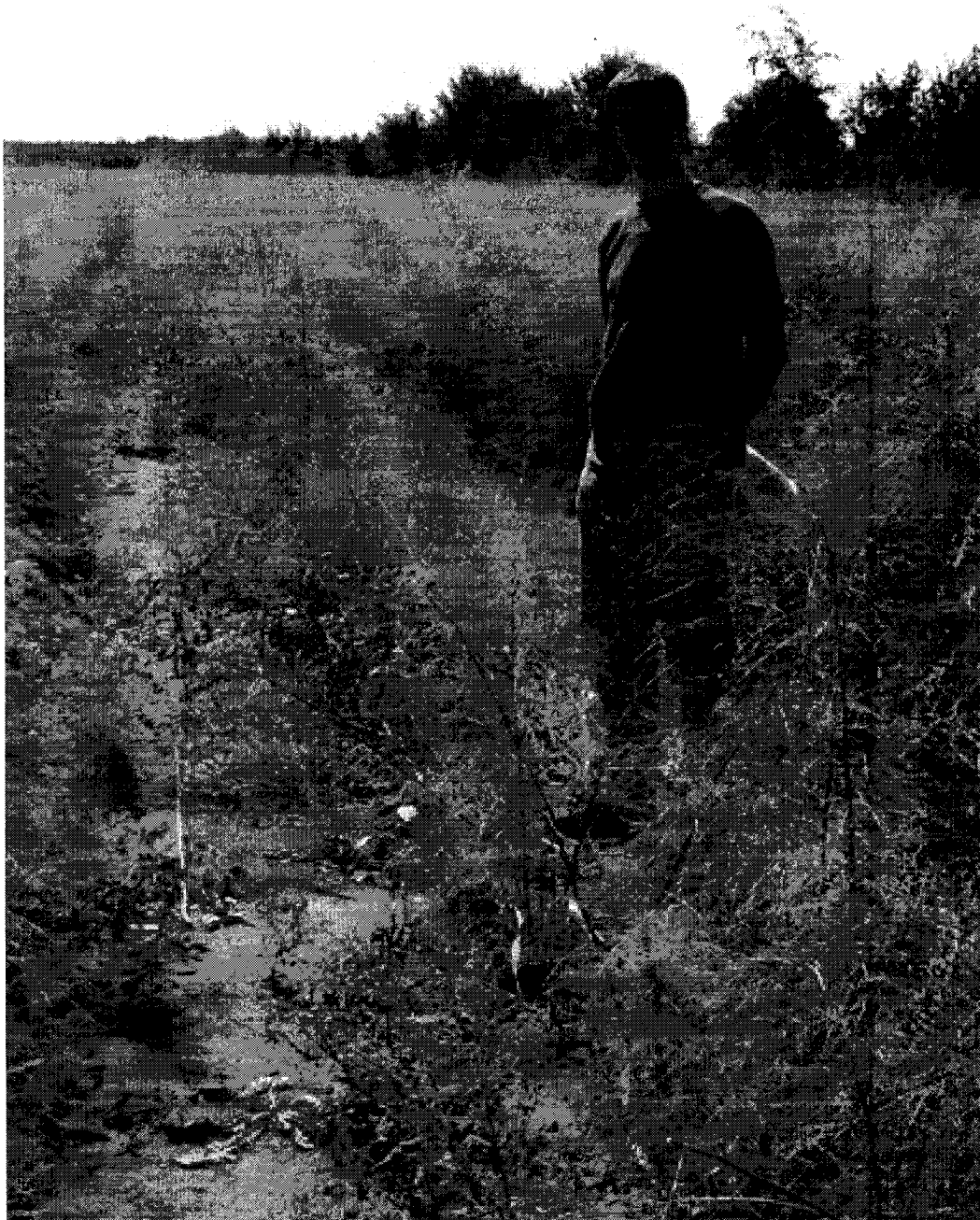


Figure 2. Two year old asparagus field in Saint-Liguori, Quebec. Photo : Vladimir Vujanovic, August 2002.

**CHAPTER 2. Effect of phosphorus fertilizer on the harvest of an  
asparagus plantation after establishment on a rich P soil in  
southern Quebec**



## **Introduction**

Asparagus (*Asparagus officinalis* L.) is a popular vegetable grown in Québec. In 2002, there were 300 ha of asparagus under the management of 117 producers, but the amount of asparagus imported to the province was three times higher than the quantity produced (MAPAQ, 2002). Despite high consumer demand, asparagus production in Québec is declining partly because of imports and competition from other countries (Mongrain, 2003) and because of asparagus root rot disease. Vujanovic (2003) reported that 45% of asparagus fields in Québec have suboptimal shoot production because of this disease.

To compete in the fresh market, Québec producers have adopted male cultivars such as Guelph Millenium, Jersey Giant and Jersey Knight that are more resistant to root rot and more productive than older varieties. The biggest advantage of using these male cultivars is that they do not produce seeds and therefore allocate more energy to roots and vegetative growth than female cultivars. Coulombe and Lamarre (2001) found that Guelph Millenium yields were 10 to 50 % higher than other varieties tested in Québec when there was no rust in the fields. Guelph Millenium has gained in popularity since the early 1990's and is now grown by about half of the producers in Québec. It will be important to determine whether the recommended practices for asparagus management and fertilization are adequate for this cultivar since it has a higher yield potential than other varieties.

One way to achieve optimal asparagus yield is to add the correct amount of fertilizer, yet the N, P and K fertilizer recommendations for asparagus production in

Québec have not changed since 1989 (CPVQ, 1989 ; CRAAQ, 2003a). Fertilizers containing P are perhaps the most important for asparagus production because P is needed to establish a healthy root system in this perennial crop and for normal metabolic functions such as energy relations and photosynthesis. It is not known whether the quantities of fertilizer recommended by the CRAAQ (2003a) fertilization guide will be sufficient for higher yielding cultivars like Guelph Millenium. Agro-environmental laws aimed at reducing the risk of P transport to waterways must also be considered when making P fertilizer recommendations. The Ministère de l'Environnement du Québec (MEV) enforces legislation that will compel farmers to reduce the P saturation (P/Al ratio) to 7.6% in soils with more than 30% clay and to 13.1% in light textured soils ( $\leq$  30% clay) by 2010 (MEV, 2002). It is essential to establish P fertilization practices that optimize asparagus production and quality without harm to the environment.

The objective of this study was to determine the effect of P fertilization on the nutrient content and yield of *A. officinalis* L. cv. Guelph Millenium during establishment and the first year of production (the third year after establishing the asparagus plantation).

## **Materials and Methods**

### **Site description**

The study site was located at the Horticultural Centre of the Macdonald Research Farm, Sainte-Anne de Bellevue, Québec. Mean monthly temperatures range from -10.3°C in January to 18.0 °C in July, with mean annual precipitation of 940 mm (Environment Canada, 1998). The soil is a fine, loamy, mixed, frigid Typic Endoaquent of the Chicot series (Humic Gleysol). The soil was a loamy sand (pH = 6.0) with 530 g kg<sup>-1</sup> sand, 300 g kg<sup>-1</sup> silt and 170 g kg<sup>-1</sup> clay containing 48.4 g organic C kg<sup>-1</sup> and 147 mg Mehlich III P kg<sup>-1</sup>. In 2000, the year before asparagus was planted, cucumber was grown at the site.

### **Site preparation and management**

Prior to planting asparagus, the field was limed (target pH = 6.5), tilled to a depth of 30 cm, and sprayed with herbicides Sin-bar (0.34 kg active ingredient ha<sup>-1</sup>) and Devrinol (6.7 kg active ingredient ha<sup>-1</sup>). In June 2001, one year old *A. officinalis* cv. Guelph Millenium crowns were hand-planted at approximately 30 cm spacing in 15 cm deep trenches, and the trenches were ridged gradually during the summer. For the first two years after crowns were planted (2001 and 2002), the field was weeded by hand because a new asparagus plantation can be affected negatively by herbicide applications (CRAAQ, 2003b). In 2003, weed control was achieved by applying Sin-bar and Devrinol

approximately one week before asparagus emergence at the same rates as above. In mid-June 2003, the fungicide myclobutanil (Nova W 40, 250 g active ingredient ha<sup>-1</sup>) was applied to control rust in the plantation.

### **Experimental design**

The experimental design was a completely randomized block design with 5 blocks and 5 treatments, for a total of 25 plots ( figure 1 in chapter 3). Each block was 7 m by 20 m and contained alternating guard and treatment rows (7 m by 1.8 m each). The blocks were separated by 2 m tilled alleys. The quantities of fertilizer applied to guard and treatment rows during this study (2001 to 2003) supplied the recommended amounts of N and K according to soil test (table 1), and between 0 and 200% of the P recommendation, based on the CRAAQ (2003a) guidelines.

The N, P and K fertilizers applied in 2001 were broadcast prior to planting crowns in May. In 2002, fertilizers were banded 5 cm from the row about one week after the shoots emerged in MONTH. In 2003, fertilizers were band applied one wk after the harvest ended (May 18).

### **Total and marketable asparagus yield**

Asparagus shoots were harvested on eight days from May 8 to May 18, 2003. Shoots were cut with a steak knife when they measured at least 20 cm. The total fresh weight harvested from each plot was recorded and the shoots were classified according to

size and general appearance. Asparagus shoots having a base diameter between 0.4 and 0.9 cm were classified as small, while medium shoots were from 0.9 to 1.3 cm and large shoots were greater than 1.3 cm. Shoots that were crooked, curved or that had other visible defects were rejected, and the marketable Grade A yield (g fresh weight basis) for each plot was then determined (table 2).

### **Plant nutrient analysis**

Plant tissue was collected from three randomly selected plants per plot at full fern. We removed the third, fifth, eighth and eleventh leaf from the most recently developed leaf on the dominant stem. In 2002, all foliage from the plants in each plot were combined, dried at 60°C and finely ground (< 1 mm mesh) before analysis. In 2003, the younger leaves (third and fifth leaves) were processed (dried and ground) separately from older leaves (eighth and eleventh leaves). Plant tissue samples were digested with H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub> (Robarge and Fernandez, 1986). Total N and P concentrations were determined on a Lachat Quick-Chem flow injection autoanalyser (Lachat Instruments, Milwaukee, WI), while total K, Ca, Mg, Fe, Zn and Mn concentrations in plant tissue digests were analysed on an atomic absorption spectrophotometer.

### **Soil analysis**

Soil samples were collected in August 2002, May 2003 and August 2003. Seven soil cores (each 15 cm long, 3 cm internal diameter) were collected from within rows in

each plot and then composited. Soils were dried at 60°C for 48 h, finely ground (< 2 mm mesh) and extracted with Mehlich III solution (1:10 soil:solution) after shaking for 5 min at 130 rpm (Tran and Simard, 1993). The P concentration in Mehlich III extracts was analyzed colorimetrically within 2 h on a Lachat Quick-Chem flow injection autoanalyser, while Al concentration in Mehlich III extracts was determined by atomic absorption spectrometry. The P saturation ratio (% P/Al) was calculated from equation 1:

$$\% \text{ P/Al} = (\text{Mehlich III-P} / \text{Mehlich III-Al}) \times 1.12 \times 100 \% \quad (1)$$

where Mehlich III-P and Mehlich III-Al are the concentrations (mg kg<sup>-1</sup>) of P and Al in Mehlich III extracts and the factor 1.12 is used for comparison with plasma emission spectrometry systems (CRAAQ 2003a).

### **Statistical analysis**

Normality of the raw data was assessed using the Proc UNIVARIATE function of SAS 6.12 for Windows (SAS Institute Inc., Cary, North Carolina, USA) and descriptive statistics (mean, standard deviation, maximum and minimum) were calculated. The effect of P fertilization on crop and soil parameters was assessed by one-way analysis of variance (ANOVA) using SAS software. The P saturation ratio (% P/Al) was log transformed before ANOVA was performed. Significant treatment effects were compared statistically using a Scheffe's multiple range test at the 95% confidence level.

## **Results and Discussion**

### **Asparagus yield and nutrient analysis**

We harvested shoots 8 times during a 10 d period, following CRAAQ (2003b) recommendations. In the first year, shoots are usually harvested every one to two d during a 7 to 10 d period because over-harvesting will cause lower yields in subsequent years (CRAAQ 2003b). Most of the asparagus shoots harvested from May 8 to May 18, 2003 had medium to large base diameter ( $> 0.9$  cm). However, the proportion of small shoots tended to increase during the 10 d period, while the proportion of medium shoots stabilized after the third day of harvest (Figs. 1a, 1b). The proportion of large shoots harvested decreased after the second day of harvest (Fig. 1c). The proportion of rejected shoots increased steadily during the harvest period, reaching 23% on May 18 (Fig. 1d). Harvest of shoots was ended when the weight of small and rejected shoots approximately equalled the weight of large shoots (S. Roy, personal communications 2003).

The mass of marketable asparagus shoots harvested increased from May 8 to May 10 and then peaked on May 14 (Fig. 2). Marketable asparagus yield was not affected by P fertilization on most harvest dates, although treatments receiving 100% and 200% of the recommended P fertilizer rate had significantly ( $P < 0.05$ ) higher yields than the 0% and 50% treatments on May 18 (Fig. 2). Total marketable yield in the 2003 harvest period ranged from 1658 to 3937 g fresh weight (fw) per plot, which is equivalent to between 1305 and 3100 kg fw ha<sup>-1</sup>, and was not affected by P fertilization (Table 3). In Québec,

asparagus yield during the first year of harvest typically ranges from 1000 to 2000 kg fw ha<sup>-1</sup> (CRAAQ, 2003), indicating that our yield was similar or slightly higher than the average.

Generally, the nutrient concentrations in asparagus tissue were not affected by P fertilization, so we present the mean, minimum and maximum nutrient concentrations in asparagus foliage (Tables 3 and 4). In 2002, nutrient concentrations were usually within the sufficient range, but the N concentration was below the sufficient range (Table 4). When foliage was separated into old and young leaves in 2003, we found that there were significant differences ( $P < 0.05$ ) in the nutrient concentrations of old and young leaves for all nutrients except Zn (Table 5). The N, P, Ca and Mg concentrations in old leaves were below the sufficiency range, while the P concentration in young leaves was below the sufficiency range (Table 5). One difficulty encountered in 2003 was an outbreak of rust after a dry spell in July 2003. Many plots were heavily infested with the disease and it was not possible to collect healthy leaves from those plots. Since plant diseases can interfere with normal growth and nutrient uptake (Walworth and Sumner, 1988), the nutrient analysis from 2003 should be interpreted cautiously.

Differences in the nutrient concentrations of old and young leaves in asparagus plantations indicate that sampling methods must be standardized before plant nutrient analysis can be interpreted correctly. Hartmann et al. (1990) suggested that foliar tissue testing may be an inappropriate method to assess asparagus nutrient requirements since they found a better correlation between root tissue samples and asparagus nutrient status.



However, it is more time consuming and potentially damaging to the plant to collect root tissue than foliar tissue samples. Ferland (2003) suggested that sampling the terminal ends of newly developed asparagus leaves at the end of July or beginning of August could be used as a standard method for asparagus nutrient diagnosis in Québec. However, there is considerable climatic variability among the regions of Québec where asparagus is grown, which makes this approach impractical because not all fields will reach a similar growth stage at this period of time. We suggest that samples of newly developed leaves at an easily identifiable growth stage of the asparagus fern, such as after flowering, could be more easily collected and compared. Further work is needed to standardize plant nutrient analysis methods for asparagus in Québec and elsewhere.

### **P uptake by asparagus**

At our field site, increasing the quantity of P fertilizer applied did not affect asparagus yield or nutrient analysis. Other workers have found that only a small proportion of nutrients required by asparagus are removed at harvest (Drost, 1997; Giroux and Chamberland, 1990; Lubet et al. 1985). In France, Lubet et al. (1985) estimated that withdrawal of P in established asparagus plantations in southeastern France (Landes) was  $7.4 \text{ kg P ha}^{-1}$ . Giroux and Chamberland (1990) surveyed three 5-8 year old fields in Quebec over 3 years and obtained yields between 2653 and 3262 kg fw  $\text{ha}^{-1}$ . Harvested shoots contained between 0.70% and 0.80% P (Giroux and Chamberland, 1990). Assuming a humidity of 92% in fresh shoots (CRAAQ, 2003a), the amount of P withdrawal in harvested shoots was between 1.5 and 2.1 kg P  $\text{ha}^{-1}$ . When we use these

values, we found that between 0.7 and 2.0 kg P ha<sup>-1</sup> were removed in the asparagus shoots harvested in 2003 from our site, which is lower than the values reported by study of Giroux and Chamberland (1990). The P uptake by asparagus was equivalent to between 0.3 and 2.5 % of the P fertilizer applied to these soils in 2001 and 2002. Thus, it seems that a relatively small proportion of the P fertilizer applied was transformed into asparagus shoots. We conclude that there was sufficient P in all treatments to sustain asparagus growth during establishment and the first year of harvest at our site.

### **Soil analysis**

The Mehlich III P concentration and P saturation ratio was significantly ( $P < 0.05$ ) lower in soils receiving no P fertilizer than those receiving the 200% P treatment (Figs. 3, 4). The P saturation ratio in the 0% P treatment ranged from 12.5 to 13.8 % P/Al during the study (Fig. 4), which is close to the acceptable level of 13.1% P/Al for light textured (< 30% clay) soils set by the MEV (2002). However, soils receiving the 200% P treatment had P saturation ratios between 25 and 30% P/Al (Fig. 4), which are approximately 2 times higher than the MEV (2002) limit. Our results indicate that the P saturation ratio increases significantly within 2 to 3 y of applying P fertilizer at double the recommended rate in an asparagus plantation.

It is not surprising that the application of P fertilizers increased the Mehlich III P concentration and the P saturation ratio so rapidly. Each year we applied P fertilizers, but only in 2003 was there P withdrawal from the site since senesced asparagus residues were

retained on site from every year to protect crowns from freezing, based on CRAAQ (2003b) recommendations. The estimated P removal at harvest in 2003 was less than 5 kg  $\text{P}_2\text{O}_5 \text{ ha}^{-1}$ , while annual P fertilizer inputs in 2001 and 2002 ranged from 78 to 410 kg  $\text{P}_2\text{O}_5 \text{ ha}^{-1}$  (Table 1).

When soils have excessive soil test P levels or a high P saturation ratio, producers can reduce the risk of P pollution in waterways by matching P fertilizer inputs with crop P requirements. The MEV (2002) guidelines for Québec suggest that soils should not receive more P fertilizer than necessary for annual root and shoot production when the soil test P level exceeds 150 kg Mehlich III P  $\text{ha}^{-1}$  or when the P saturation ratio is greater than 13.1% P/Al.

We did not measure the annual root production in our asparagus plantation, but the root/shoot ratio of asparagus is reported to range from 4.3 to 5.6 (Guo et al., 2002). When we combine the root/shoot ratio of 5.6 with the highest shoot production obtained at our site (3093 kg fw  $\text{ha}^{-1}$ , Table 2) and assume 92% humidity and 0.8% P in asparagus roots and shoots, it appears that as much as 13 kg P  $\text{ha}^{-1}$  is required for asparagus root and shoot production. Therefore, an application of 30 kg  $\text{P}_2\text{O}_5 \text{ ha}^{-1} \text{ y}^{-1}$  of P fertilizer may supply the P needed for asparagus production at our field site. If senesced ferns are not returned to the soil, then the P removal from the plots will be slightly higher than we have assumed. It should be noted that our estimated P fertilizer requirement is 40% lower than the recommended P fertilizer input of 50 kg  $\text{P}_2\text{O}_5 \text{ ha}^{-1}$  for an asparagus field in production (CRAAQ, 2003a).

Further investigation is needed to validate these results at the field scale across different soil types and agricultural regions. Such research is necessary to verify the accuracy of P fertilizer recommendations in Québec and ensure that the application of fertilizer will improve asparagus yield and quality, leading to economic benefits for producers without compromising agroenvironmental objectives.

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Table 1. Fertilizers applied to the asparagus field during establishment of the plantation (2001 and 2002) and during first year of harvest (2003).

Treatment <sup>a</sup>	N <sup>b</sup> (kg N ha <sup>-1</sup> )	P <sup>c</sup> (kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )	K <sup>d</sup> (kg K <sub>2</sub> O ha <sup>-1</sup> )
2001 <sup>e</sup>			
0 % P	110	0	150
50 % P	110	50	150
75 % P	110	75	150
100 % P	110	100	150
200 % P	110	200	150
2002 <sup>f</sup>			
0 % P	60	0	100
50 % P	60	50	100
75 % P	60	75	100
100 % P	60	100	100
200 % P	60	200	100
2003 <sup>g</sup>			
0 % P	100	0	95
50 % P	100	25	95
75 % P	100	38	95
100 % P	100	50	95
200 % P	100	100	95

<sup>a</sup> Guard rows received the same quantities of N, P, K fertilizers as the 100 % treatment.

<sup>b</sup> Ammonium nitrate, 34-0-0

<sup>c</sup> Triple superphosphate, 0-46-0

<sup>d</sup> Muriate of potash, 0-0-60

<sup>e</sup> P and K recommendations based on Mehlich III soil test in May 2001 (P= 329 ± 10 and K= 336 ± 11)

<sup>f</sup> P and K recommendations based on Mehlich III soil test in May 2002 (P= 342 ± 20 and K= 422 ± 7)

<sup>g</sup> P and K recommendations based on Mehlich III soil test in May 2002 (P= 352 ± 24 and K= 483 ± 18)

Table 2. Criteria for rejecting asparagus shoots from the marketable Grade A category.

Category	Name	Criteria
1	Open	Shoot tip is open and the first leaves have appeared
2	Curve	Curve is too pronounced and the shoot is not marketable
3	Rust	Rust is apparent (orange or reddish discoloration on shoot)
4	Small	Diameter of the shoot is smaller than 0.4 cm
5	Deformed	The shoot is deformed, not straight
6	Flat	Shoot more flat than rounded
7	Hollow	Shoot is hollow, hole in the center when opened
8	Grade B	Shoots have a slight curve and cannot be sold at same price as Grade A asparagus

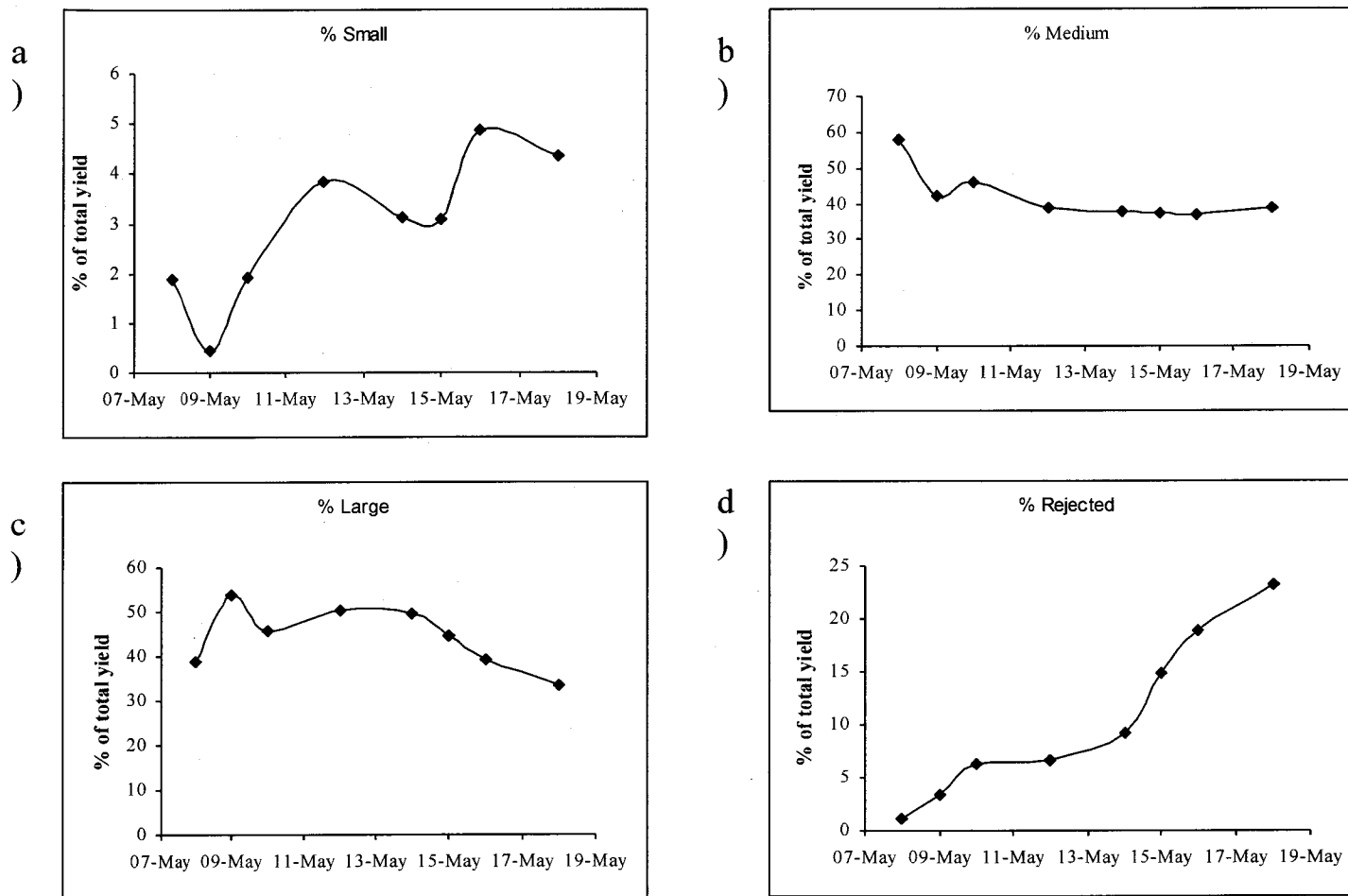


Fig. 1. Proportion of asparagus shoots in each category. a) Small (0.4-0.9 cm) b) Medium (0.9-1.3 cm) c) Large (>1.3cm) d) Rejected shoots

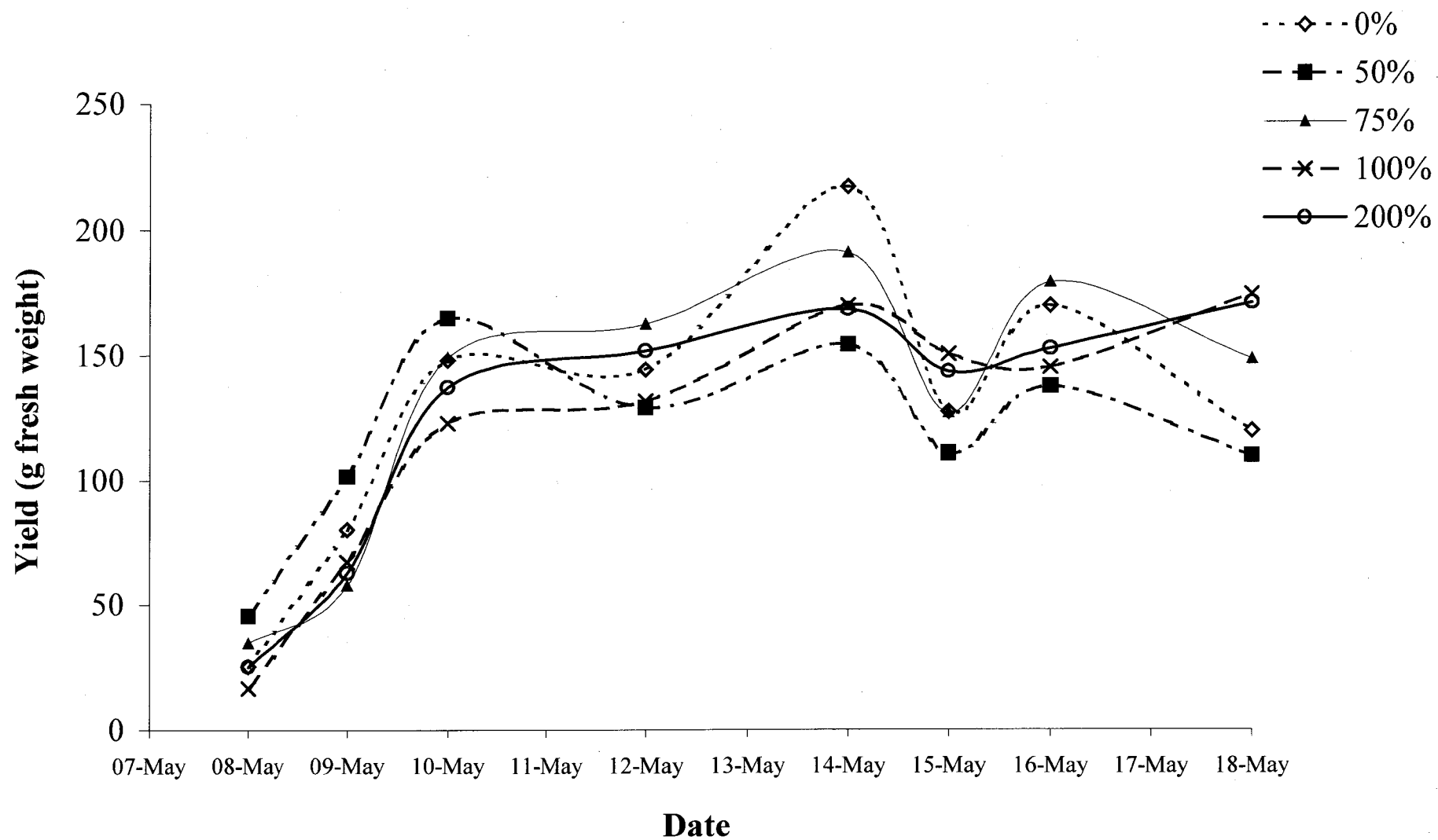


Fig. 2 : Daily marketable yield (g fresh weight) for each phosphorus treatment (% P recommendation based on CRAAQ 2003 guidelines). Shoots were harvested from May 8<sup>th</sup> to May 18<sup>th</sup>, 2003.

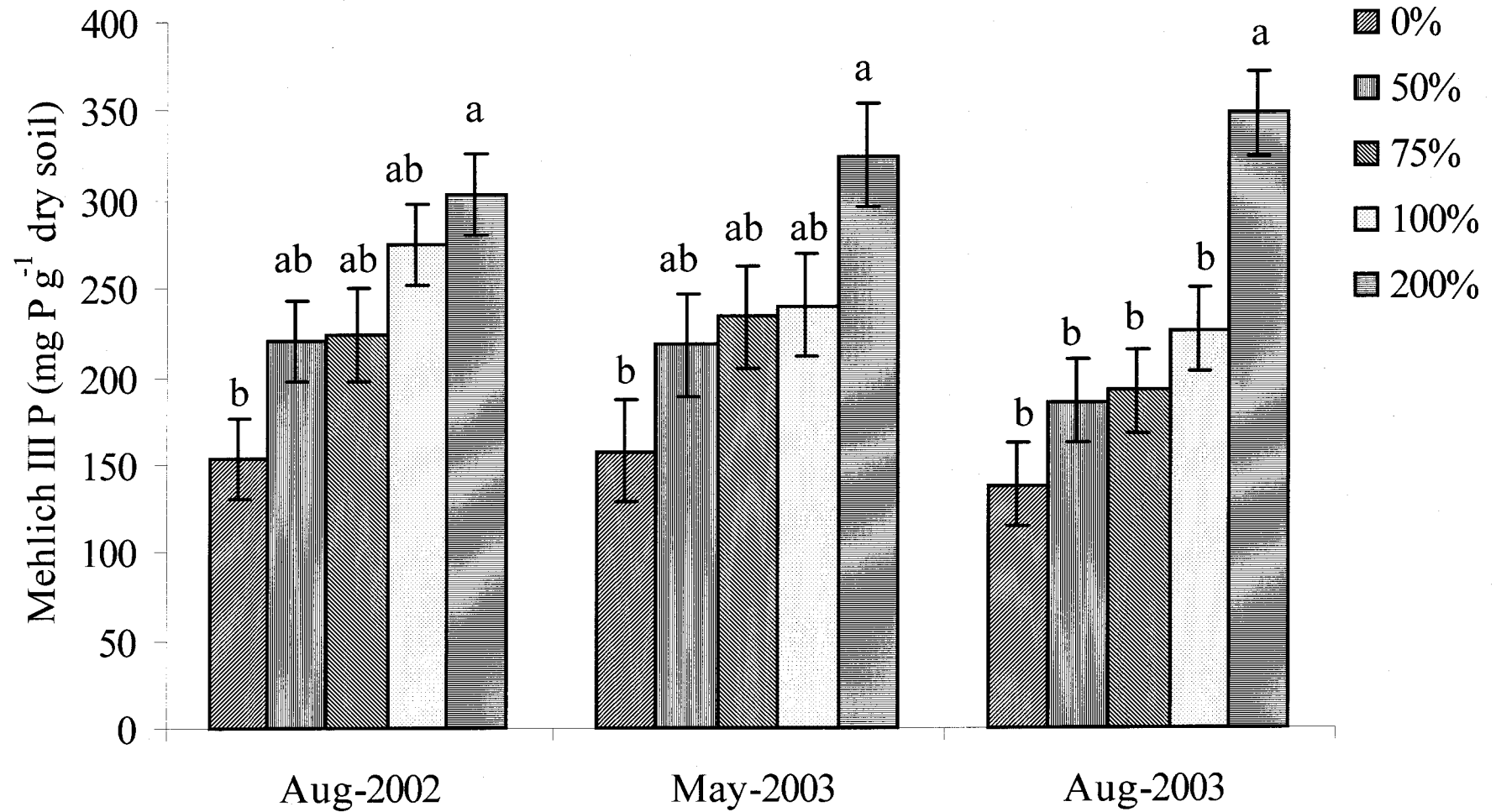


Fig. 3. Effect of P fertilization on soil test P on three sampling dates (August 2002, May and August 2003) in an asparagus field. Treatments are 0 to 200 % of the P recommendation based on CRAAQ (2003) guidelines. Treatments with the same letters, within the same sampling date, are not significantly different at  $P=0.05$  (Scheffe multiple range test).

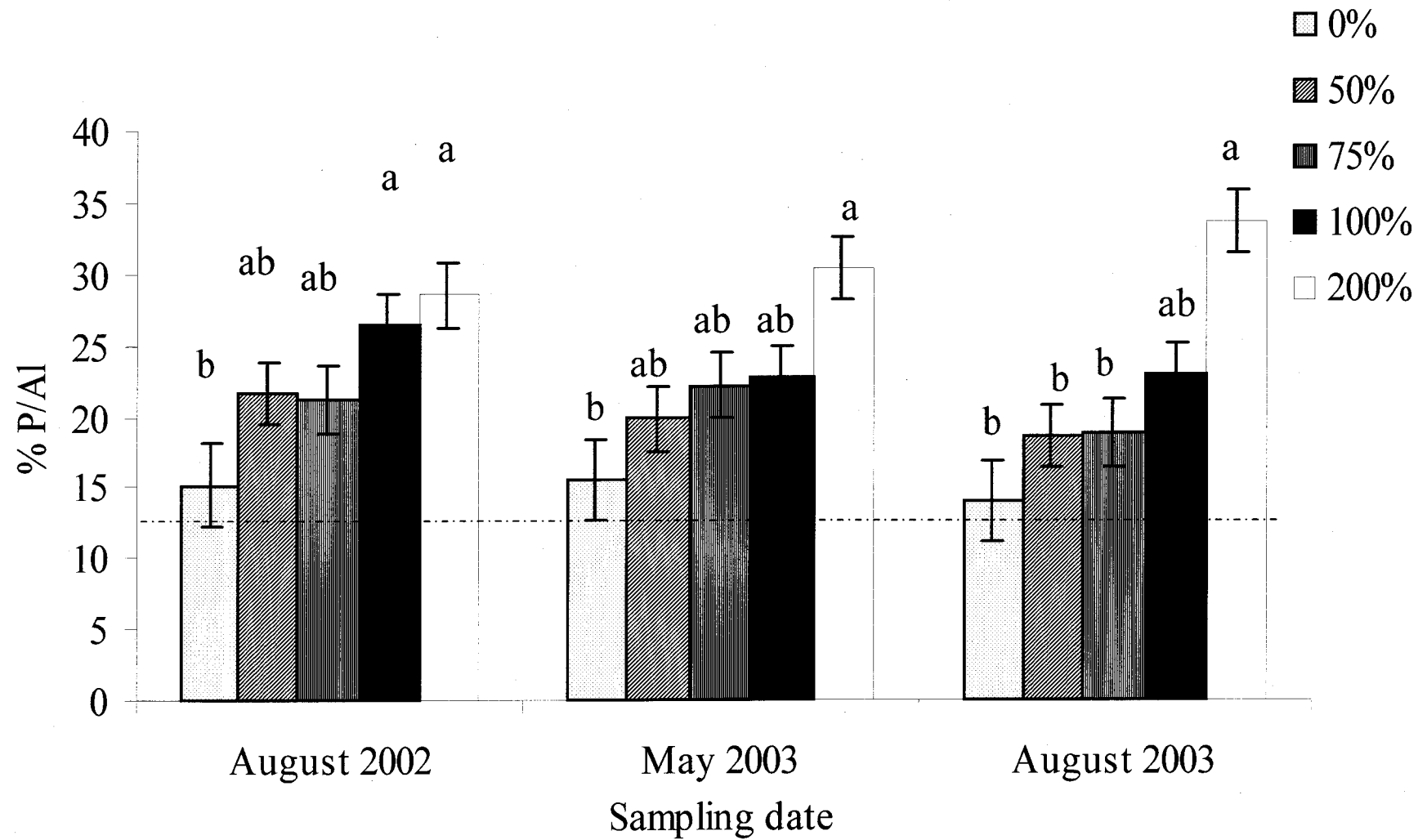


Fig. 4. The P saturation ratio (% P/Al) in soils under asparagus production receiving between 0 and 200 % of the recommended P fertilizer rate. The agroenvironmental limit for sandy soils is 13.1 % P/Al. Treatments with the same letter, within the same sampling date, are not significantly different at  $P = 0.05$  (Scheffe multiple range test).

Table 3. Total marketable yield (kg fresh weight ha<sup>-1</sup>) of asparagus shoots harvested from May 8 to May 18, 2003. Treatments supplied 0 to 200% of P recommended by the CRAAQ (2003a) guidelines.

Treatment	N	Mean	Standard Deviation	Minimum	Maximum
0% P	5	2435	287	1792	2841
50% P	5	2247	521	1657	2965
75% P	5	2474	324	2168	2979
100% P	5	2307	578	1303	2769
200% P	5	2388	484	1980	3093

Table 4. Nutrient analysis of asparagus leaves collected at the full fern stage in 2002.

Values in bold indicate nutrient concentrations below the sufficiency range.

Nutrient	N	Mean	Std Dev	Minimum	Maximum	Sufficiency <sup>z</sup> range
N mg g <sup>-1</sup>	25	<b>23</b>	2	20	27	2.4 – 4.0
P mg g <sup>-1</sup>	25	2.4	0.2	2.0	2.9	0.21 - 0.40
K mg g <sup>-1</sup>	25	20.7	1.7	18.3	24.4	1.5 – 3.0
Ca mg g <sup>-1</sup>	25	4.0	1.0	2.0	6.0	0.4 – 1.0
Mg mg g <sup>-1</sup>	25	1.5	0.2	1.2	0.19	0.15 – 0.30
Fe (µg g <sup>-1</sup> )	25	124.5	66.9	48.3	292.6	20 – 150
Mn (µg g <sup>-1</sup> )	25	47.5	9.9	0.0	50.8	15 – 160
Zn (µg g <sup>-1</sup> )	25	30.7	17.2	15.0	99.0	20 – 80

<sup>z</sup> Ferland (2003)



Table 5. Nutrient analysis of asparagus leaves collected at the full fern stage in 2003.

Values in bold indicate nutrient concentrations below the sufficiency range

Nutrient	N	Mean	Std Dev	Minimum	Maximum	Sufficiency <sup>z</sup> range
Old asparagus leaves <sup>y</sup>						
N mg g <sup>-1</sup>	25	<b>18</b>	2.0	15	23	24 – 40
P mg g <sup>-1</sup>	25	<b>1.9</b>	0.2	1.5	2.4	2.1 – 4.0
K mg g <sup>-1</sup>	25	18.6	1.5	15.8	21.7	1.5 – 3.0
Ca mg g <sup>-1</sup>	25	<b>3.0</b>	1.0	2.0	5.0	4.0 – 10.0
Mg mg g <sup>-1</sup>	25	<b>1.2</b>	0.2	0.9	1.5	1.5 – 3.0
Fe µg g <sup>-1</sup>	25	134.6	63.4	49.3	298.5	20 – 150
Mn µg g <sup>-1</sup>	25	49.6	0.4	49.0	50.5	15 – 160
Zn µg g <sup>-1</sup>	25	26.8	12.7	14.7	69.3	20 – 80
Young asparagus leaves <sup>x</sup>						
N mg g <sup>-1</sup>	25	25	2.0	22	28	24 – 40
P mg g <sup>-1</sup>	25	<b>1.8</b>	0.2	1.6	2.5	2.1 – 4.0
K mg g <sup>-1</sup>	25	16.8	1.9	11.9	19.9	15 – 30
Ca mg g <sup>-1</sup>	25	7.0	1.0	5.0	8.0	4.0 – 10.0
Mg mg g <sup>-1</sup>	25	1.6	0.2	1.2	1.9	1.5 – 3.0
Fe µg g <sup>-1</sup>	25	123.3	32.1	98.0	197.0	20 – 150
Mn µg g <sup>-1</sup>	25	70.6	24.4	48.8	102.0	15 – 160
Zn µg g <sup>-1</sup>	25	25.7	10.8	14.7	54.5	20 – 80

<sup>z</sup> Ferland (2003)

<sup>y</sup> eighth and eleventh leaf from dominant stem

<sup>x</sup> third and fifth leaf from dominant stem

### **Connecting paragraph**

In the previous chapter, we found that P fertilization increased soil P saturation to levels that posed environmental risks but P did not affect asparagus fresh yield or plant tissue concentrations. In the next chapter, the effect of P fertilization on soil microbial biomass, enzymatic activity, arbuscular mycorrhizal colonisation and *Fusarium* incidence are investigated. The relationship between the different parameters is also evaluated.

### **CHAPTER 3. Soil biological parameters in an asparagus plantation as influenced by phosphorus fertilization**

## **Introduction**

Asparagus (*Asparagus officinalis* L.) is a popular vegetable grown in Québec. In 2002, there were 300 ha of asparagus under the management of 117 producers in Québec (MAPAQ, 2002). Canadian asparagus consumption has increased by 28 % since the 1990's (Mongrain, 2003) and yearly fresh imports for this vegetable are three times higher than the amounts produced in the province of Québec. Despite the high demand, asparagus production is declining across North America because of a widespread disease, asparagus root rot. Vujanovic (2003) reported that 45 % of asparagus fields in production have suboptimal shoot production because of this disease.

Establishing an asparagus plantation is a long term investment, since it takes at least two years after crowns are planted for the plantation to produce marketable shoots. A well established plantation, free of disease, can be productive for 15 to 20 years. For this reason, producers must maintain good management practices in order to help reduce root rot disease. Adequate fertilization, especially with P fertilizer, is essential for the growth of a healthy root system that will support above-ground production of this perennial crop.

There is interest in determining how P fertilizers affect the diversity and activity of soil biota. Root rot is caused by a pathogenic soil borne fungi of the *Fusarium* spp. (Elmer *et al.*, 1996). *Fusarium* spp. infect asparagus crowns and roots, causing feeder roots to rot (reddish brown discoloration). The shoots of infected plants are small and

chlorotic, and the plant eventually dies. Unfortunately, there are no pesticides available to control this pathogen, but Wacker *et al.*, (1990) found that arbuscular mycorrhizal fungi (AMF) may prevent *Fusarium* from colonizing roots by competing for available root space. It is well known that the percentage of AMF root colonization and activity declines when P fertilizers are applied (Smith and Reid, 1997 ; Borowicz, 2001). Studies on how P fertilization affects AMF and soil microbial activity in the rhizosphere of asparagus are needed to understand whether asparagus plantations may be susceptible to infestation by *Fusarium* spp.

The objective of this study was to determine how AMF root colonization, microbial biomass, soil enzymatic activity and *Fusarium* incidence in asparagus plantations were influenced by P fertilization.

## **Materials and Methods**

### **Site description**

The study site was located at the Macdonald Research Farm (Horticultural Center), Sainte-Anne de Bellevue, Québec. Mean monthly temperatures range from – 10.3 °C in January to 18.0 °C in July, with mean annual precipitation of 940 mm (Environment Canada, 1998). The soil is a fine, loamy, mixed, frigid Typic Endoaquent of the Chicot series (Humic Gleysol). The soil was a loamy sand (pH = 6.0) with 530 g kg<sup>-1</sup> sand, 300 g kg<sup>-1</sup> silt and 170 g kg<sup>-1</sup> clay containing 48.4 g organic C kg<sup>-1</sup> and 147 mg

Mehlich III P kg<sup>-1</sup>. In 2000, the year before asparagus was planted, cucumber was grown on the site.

### **Site preparation**

Prior to planting asparagus, the field was limed (target pH = 6.5), tilled to a depth of 30 cm, and sprayed with Sin-bar (0.34 kg a.i. ha<sup>-1</sup>) and Devrinol (6.7 kg a.i. ha<sup>-1</sup>). One year old *A. officinalis* cv. Guelph Millenium crowns were hand-planted at approximately 30 cm spacing in 15 cm deep trenches, and the trenches were ridged gradually during the summer. For the first two years after crowns were planted, the field was weeded by hand because a new asparagus plantation can be affected negatively by herbicide applications (CRAAQ, 2003a). Weed control in the third year of the study was achieved by applying Sin-bar and Devrinol approximately 1 week before asparagus emergence at the same rates as in 2001. In mid-June 2003, the fungicide myclobutanil (Nova W 40 ; 250 g active ingredient ha<sup>-1</sup>) was applied to control rust that was appearing in the field. It was relatively successful in reducing rust to a tolerable level.

### **Experimental design**

The experimental design was a completely randomized block design with 5 blocks and 5 treatments, for a total of 25 plots (Fig. 1). Each block was 7 m by 20 m and contained alternating guard and treatment rows (7 m by 1.8 m each). The blocks were separated by 2 m tilled alleys. The quantities of fertilizer applied to guard and treatment

rows during this study (2001 to 2003) supplied the recommended amounts of N and K, and between 0 and 200 % of the P recommendation, based on the CRAAQ (2003b) guidelines (Table 1).

In 2001, we broadcast N, P and K fertilizers, while in 2002, the fertilizer was banded 5 cm from the row about one week after the shoots emerged from the ground. In 2003, fertilizers were band-applied one week after the harvest ended.

### **Collection of soil and root samples**

Soil and root samples was collected on three dates : August 18<sup>th</sup> 2002, May 21<sup>st</sup> 2003 and August 28<sup>th</sup> 2003. Soil samples were composites of seven cores (3 cm diameter by 15 cm depth) collected between plants within the crop row in each plot. Soils were sieved (5 mm mesh) and stored at 4 °C until analysed (within 2 weeks). Root samples were live, secondary roots (0-15 cm depth) clipped from the primary roots of three plants per plot. Roots were refrigerated until further analysis.

### **Soil analysis**

Soils were dried at 60 °C for 48 h, finely ground ( 2 mm mesh sieve) and extracted with Mehlich III solution (1 : 10 soil:solution) after shaking for 5 min. at 130 rpm (Tran and Simard, 1993). The Mehlich III P concentration was analyzed colorimetrically within 2 h on a Lachat Quick-Chem flow injection autoanalyser (Lachat Instruments Milwaukee,

WI). Mehlich III – extractable Al concentrations were determined by atomic absorption spectrometry. The P saturation ratio was calculated from equation 1 as :

$$\% \text{ P/Al} = \text{Mehlich III - P} / \text{Mehlich III - Al} \times 1.12 \times 100 \% \quad (1)$$

where Mehlich III - P and Mehlich III – Al are the concentrations ( $\text{mg kg}^{-1}$ ) of P and Al in Mehlich III extracts and the constant of 1.12 is a conversion factor for comparison with plasma emission spectrometry systems (CRAAQ, 2003b).

Soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were estimated using the chloroform fumigation-direct extraction method (Voroney and Bayaert, 1993). Extractable C (0.5 M  $\text{K}_2\text{SO}_4$ , 1 : 5 soil : extractant ratio) in unfumigated and fumigated soils was measured by wet combustion on a Shimadzu TOC-V carbon analyzer (Shimadzu Corporation, Kyoto, Japan). Microbial biomass C (MBC) was calculated as :

$$\text{MBC} = \text{Extractable } C_{\text{fumigated}} - \text{Extractable } C_{\text{unfumigated}} \quad (2)$$

Persulfate digestion of soil extracts (0.5 M  $\text{K}_2\text{SO}_4$  extracts, 1 : 5 soil : extractant ratio) was done, and the N concentration of persulfate digests was determined on a Lachat Quick-Chem AE flow injection autoanalyser (Lachat Instruments Milwaukee, WI). Microbial biomass N (MBN) was calculated as :



$$\text{MBN} = \text{Digested NO}_3\text{-N}_{\text{fumigated}} - \text{Digested NO}_3\text{-N}_{\text{unfumigated}} \quad (3)$$

Soil monophosphatase,  $\beta$ -D-glucosidase and dehydrogenase activities were assessed on 3 replicate samples, including controls, from each plot. For monophosphatase activity, 1 g of field-moist soil was incubated at 37 °C for 1 h in 4 ml modified universal buffer with 1 ml of 0.05 M p-nitrophenyl phosphate. The reaction was stopped and color was developed by adding 4 ml of 0.5 M NaOH and 1 ml 0.5 M CaCl<sub>2</sub>, and the reaction product in filtered extracts was measured at 420 nm (Tabatabai, 1994).

For  $\beta$ -D-glucosidase activity, 1 g of field-moist soil was incubated for 1 h at 37 °C in 4 ml MUB and 1 ml of 0.05 M p-nitrophenyl-  $\beta$ -D-glucoside. Color was then developed with 4 ml of 0.1 M Tris(hydroxymethyl)aminomethane (THAM) pH 12 and 1ml of CaCl<sub>2</sub>, and filtered extracts were analysed at 420 nm (Tabatabai, 1994). The dehydrogenase assay was modified from Casida *et al.* (1964). Briefly, 1 g of field-moist soil was incubated for 6 h at 40 °C in 1 ml 0.5 % 2,4,5-triphenyl tetrazolium chloride and 1 ml 0.5 M THAM (pH= 7.6). To stop the reaction, samples were placed in a freezer at – 10 °C. Samples were thawed, extracted with 10 ml methanol for 1 h and then placed overnight in the fridge to allow sediments to settle. The supernatant was then analysed at 480 nm.

## **Root analysis**

Root samples were cut into 1 cm pieces, placed in small plastic cartridges and cleared for 25 minutes in a 10% (w/v) KOH solution in the autoclave. After clearing, roots were rinsed with water and arbuscular mycorrhizal tissues were stained with 0.02 % acid fuschin for 2 to 3 days. Next, the roots were rinsed with water and preserved in 50% glycerol : water solution until analysis. AMF colonisation (% of root colonization) was estimated using a dissecting microscope and the gridline intersect method (Giovannetti and Mosse 1980).

## **Fusarium symptoms and diversity**

*Fusarium* symptoms were evaluated for all plants in each plot on September 3, 2003. Plants demonstrating clear signs of *Fusarium* infection (small, chlorosed shoots, reddish discoloration on the lower part of the shoots, soggy shoot base) were counted as being infected by *Fusarium* pathogenic fungi. The percentage of infected plants was determined for each plot.

*Fusarium* diversity was determined by destructively sampling six healthy plants and six plants infected with *Fusarium* from plots receiving no P fertilizer (0 % treatment) and plots receiving the recommended amount of P fertilizer (100 % P treatment). The roots of each plant was collected and kept at 4 °C until transported to the laboratory. Then, roots were washed with tap water to remove excess soil particles, surface-sterilised

with 70 % ethanol for 10 s, rinsed in sterile distilled water (SDW) for 10 s, submerged for 3 min in 2.5% NaOCl containing 0.1% Tween 20, and rinsed three times in SDW. Each root was cut into five small sections with a sterile scalpel and each section was placed together on separate plates. There was approximately 1 cm<sup>2</sup> of roots (~ 2 mm thick) per 9 cm-Petri plate. Pieces were distributed evenly on a plate containing 20 ml of myclobutanil agar (MBA), a *Fusarium* selective medium (Vujanovic *et al.*, 2002), supplemented with antibiotics (100 mg l<sup>-1</sup> streptomycin sulphate and 12 mg l<sup>-1</sup> neomycin sulphate). Three replicates were prepared for each sample. Results are presented in colony forming units (CFU) per cm<sup>2</sup>.

#### **Total and Marketable asparagus yield**

Asparagus shoots were harvested on eight days from May 8 to May 18, 2003. Shoots were cut with a steak knife when they measured at least 20 cm. The total fresh weight harvested from each plot was recorded and the shoots were then graded according to size and general appearance. The marketable (Grade A) asparagus yield (g fresh weight) for each plot was then determined.

#### **Statistical analysis**

Normality of the raw data was assessed using the Proc UNIVARIATE function of SAS 6.12 for Windows (SAS Institute Inc. Cary, North Carolina, USA). Data was analysed by ANOVA in a general linear model (GLM) using SAS. Data using

percentages (AMF colonisation and *Fusarium* incidence) were transformed using an arcsin transformation to normalize data. Effects of treatment were compared statistically using a Scheffe multiple range test at the 95% confidence level. Pearson correlation coefficients between soil and plant parameters were evaluated using the Proc CORR function of SAS. Descriptive statistics (mean, maximum, minimum, standard deviation) were calculated when treatment effects were not significant.

## ***Results and Discussion***

### **Soil analysis**

Plots receiving no P fertilizer had lower STP concentrations than plots receiving the highest amounts of fertilizer (Fig. 2). The % P/Al saturation ratio is an index used by the Ministère de l'Environnement du Québec (MEV) to evaluate the environmental risk of phosphorus transport from agricultural soils in leachates and surface runoff. For fields with less than 30 % clay, like those investigated in this study, the saturation limit is 13.1% P/Al. The % P/Al saturation ratio was within the environmentally safe level for the 0% P treatment in 2003, but plots receiving 50% P, 75% P, 100% P and 200% P were oversaturated in P at all sampling dates (Fig. 3). Thus, adding P fertilizer increased P saturation in these soils to a level that poses an environmental risk.

Treatments receiving 100 % P had significantly ( $p < 0.05$ ) higher MBC than the 200 % P treatments in August 2002, but the 0, 50 and 75 % P treatments had an intermediate MBC level that was not different from the 100 % P or 200 % P treatment. There was substantial variation in microbial biomass and enzyme activity at each sampling date. While MBC was relatively stable between sampling dates, the mean MBN was 44 % to 48 % lower in 2003 than 2002 (Table 2). It is not known why such variation occurred in MBC and MBN. Factors such as seasonal temperature and moisture fluctuations, soil pH, root exudation, plant development, tillage and fertilization affect the biomass of soil microorganisms. Soil enzymatic activity was influenced more by the

sampling time than by P fertilizer treatments. Monophosphatase activity was higher in May 2003 and dehydrogenase activity was lowest in May 2003, while  $\beta$ -D-glucosidase activity tended to be greater in August 2002 than other sampling dates (Table 2).

Monophosphatase is known to be necessary for hydrolysing organic P into inorganic forms more easily available to plants and microorganisms (Dick and Tabatabai, 1993). There was about 2 times more monophosphatase activity in May 2003 than on other sampling dates, suggesting that there was more substrate (organic P compounds) available at that time. Thus, the high monophosphatase activity in May indicates that there will be higher proportions of organic P mineralized and available for plants and microorganisms in the spring than at the end of the growing season.

Dehydrogenase activity is affected by the redox potential in soils and is an indicator of microbial growth (Dick and Tabatabai, 1993). Asparagus rows are not tilled, and oxygen availability decreases as the growing season progresses because soil organisms and roots require O<sub>2</sub> for biological activity. This could explain why dehydrogenase activity is lower in the spring (Table 2), when redox potential is greatest (high O<sub>2</sub> concentration in soil) than at the end of the growing season.

$\beta$ -D-glucosidase is known to be an indicator of fungal growth and cellulolytic bacterial biomass (Tabatabai, 1994 ; Aon and Colaneri, 2001). Glucosidase enzymes degrade complex sugars (such as cellobiose and maltose) and are also used by some organisms to degrade or lyse cell walls (Dick and Tabatabai, 1993). When soils are not

tilled (i.e. within asparagus rows), organic residues tend to accumulate on the soil surface and may not be available for microorganisms in the soil profile. Thus,  $\beta$ -D-glucosidase may have declined because of a reduction in the quantity of readily decomposable organic substrates in the soil.

We found that MBC was negatively correlated with STP, while monophosphatase activity was positively related to STP (Table 3). The % P/Al ratio was positively correlated with MBN and  $\beta$ -D-glucosidase (Table 3). We cannot explain these correlations, but note that they do not indicate a cause and effect relationship. MBC was generally negatively correlated with enzymatic activity. It is difficult to explain the relationship between MBC and extracellular enzymes such as phosphatase and  $\beta$ -D-glucosidase since extracellular enzymes function independently from living cells. Continued monitoring at the experimental site may reveal clearer relationships between P fertilization and these soil biological parameters. The negative correlation between the dehydrogenase activity and MBC could indicate that there is an increase in the proportion of metabolically active microorganisms when the soil microbial community is small. Seasonal changes in weather and soil conditions are expected to influence this relationship.

### **AMF colonisation**

Phosphorus fertilization had a significant ( $p < 0.05$ ) effect on AMF colonisation in May 2003. Treatments receiving 0 % P had 14 to 18 % AMF colonisation, which was

higher than the other P treatments (2 to 8 % colonization). AMF colonization did not vary among treatments in August 2002 or August 2003, but AMF colonisation was negatively correlated with STP during the study (Table 3). Much literature has demonstrated that P fertilization reduces the amount of AMF colonization (Smith and Read, 1997). O'Keefe and Sylvia (1990) indicate that AMF colonisation can occur very rapidly in soils (within three weeks of initial root growth) and colonisation is even more rapid when the surface is not tilled and hyphae remain in place. Thus, asparagus in plots with no P fertilizer may have faster AMF colonisation than asparagus receiving P fertilizer. The possibility of earlier AMF colonization in unfertilized soils was not investigated during this study, but should be measured in the future.

### **Fusarium incidence**

*Fusarium* incidence was not affected by STP level (data not shown) or the % P/Al saturation (Fig. 4a). However, a significant negative correlation was found between *Fusarium* incidence and asparagus shoot yield of plants (Fig. 4b). Weaker plants (lower yielding plants) exhibited more *Fusarium* symptoms than plants with higher yields. Also, there were fewer *Fusarium* CFUs in soil from fertilized (100 %) plots than on unfertilized (0 % P) plots (Fig. 5). The *Fusarium* diversity in unhealthy and healthy plants was also different. Infected asparagus roots had a higher proportion of *F. proliferatum* (11%) but lower amounts of *F. oxysporum* (85%), than healthy plants (*F. proliferatum* 3% and *F.oxysporum* 91%). Also, healthy plants had more unidentified *Fusarium* spp. (6%) than



unhealthy plants (2%). These results suggest symptoms of asparagus root rot we observed may be caused by *F. proliferatum*.

*Fusarium* incidence seemed to be affected by plant general health. Plants that gave higher yields were more healthy and seemed to be less susceptible to *Fusarium* symptoms. Other studies have demonstrated that weaker, stressed plants were more susceptible to disease (Nigh, 1990 ; Elmer *et al.*, 1996). One of the major stresses for asparagus is shoot removal at harvest, indicating that asparagus producers must take care not to over-harvest their plantation. Since yield is correlated to *Fusarium* incidence, it could be possible to evaluate, after some days (4 or 5 days) of harvest, which parts of the field are more susceptible to *Fusarium* and to stop harvesting those plants. This management practice could prevent the disease from spreading in the field.

Phosphorus fertilization seemed to affect *Fusarium* abundance in roots, but further work is needed to determine whether this trend occurs in all fertilized plots. A second area for future research is to determine the pathogenicity of *Fusarium* found on asparagus roots since high levels of *Fusarium* colonization, such as were found in the plants from the 0 % P treatment, is not necessarily detrimental to asparagus unless the fungus are pathogenic.

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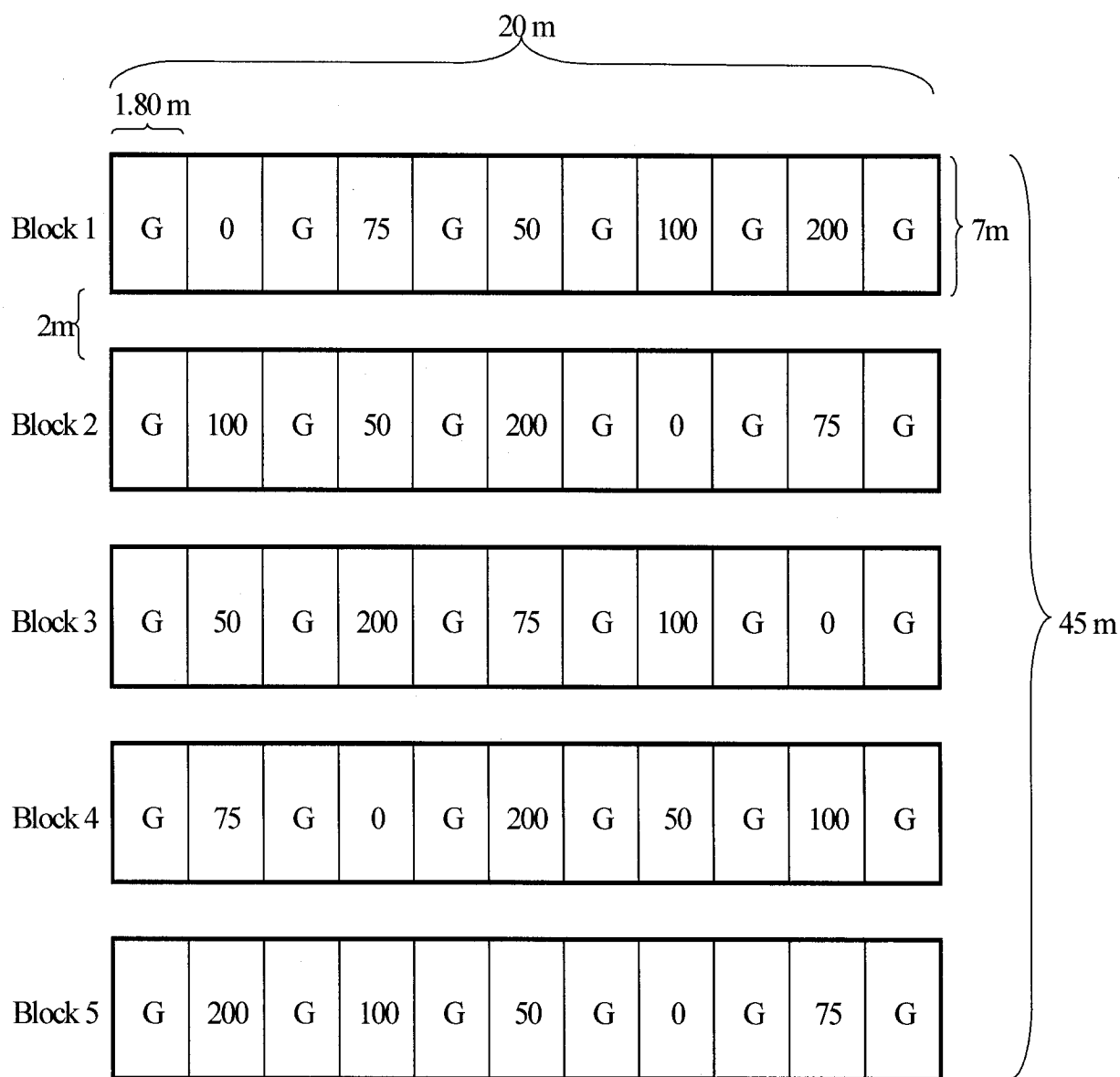


Fig. 1. Experimental design of the asparagus plantation, established at the Macdonald Horticultural center (Sainte-Anne-de-Bellevue, Quebec) in May, 2001. Treatments are 0 to 200% of the recommendation based on CRAAQ (2003) guidelines. Guard rows (G) received 100% of the P recommendation.

Table 1. Fertilizers applied to the asparagus field during establishment of the plantation (2001 and 2002) and during first year of harvest (2003).

Treatment <sup>a</sup>	N <sup>b</sup> (kg N ha <sup>-1</sup> )	P <sup>c</sup> (kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )	K <sup>d</sup> (kg K <sub>2</sub> O ha <sup>-1</sup> )
2001 <sup>e</sup>			
0 % P	110	0	150
50 % P	110	50	150
75 % P	110	75	150
100 % P	110	100	150
200 % P	110	200	150
2002 <sup>f</sup>			
0 % P	60	0	100
50 % P	60	50	100
75 % P	60	75	100
100 % P	60	100	100
200 % P	60	200	100
2003 <sup>g</sup>			
0 % P	100	0	95
50 % P	100	25	95
75 % P	100	38	95
100 % P	100	50	95
200 % P	100	100	95

<sup>a</sup> Guard rows received the same quantities of N, P, K fertilizers as the 100 % treatment.

<sup>b</sup> Ammonium nitrate, 34-0-0

<sup>c</sup> Triple superphosphate, 0-46-0

<sup>d</sup> Muriate of potash, 0-0-60

<sup>e</sup> P and K recommendations based on Mehlich III soil test in May 2001 (P= 329 ± 10 and K= 336 ± 11)

<sup>f</sup> P and K recommendations based on Mehlich III soil test in May 2002 (P= 342 ± 20 and K= 422 ± 7)

<sup>g</sup> P and K recommendations based on Mehlich III soil test in May 2002 (P= 352 ± 24 and K= 483 ± 18)

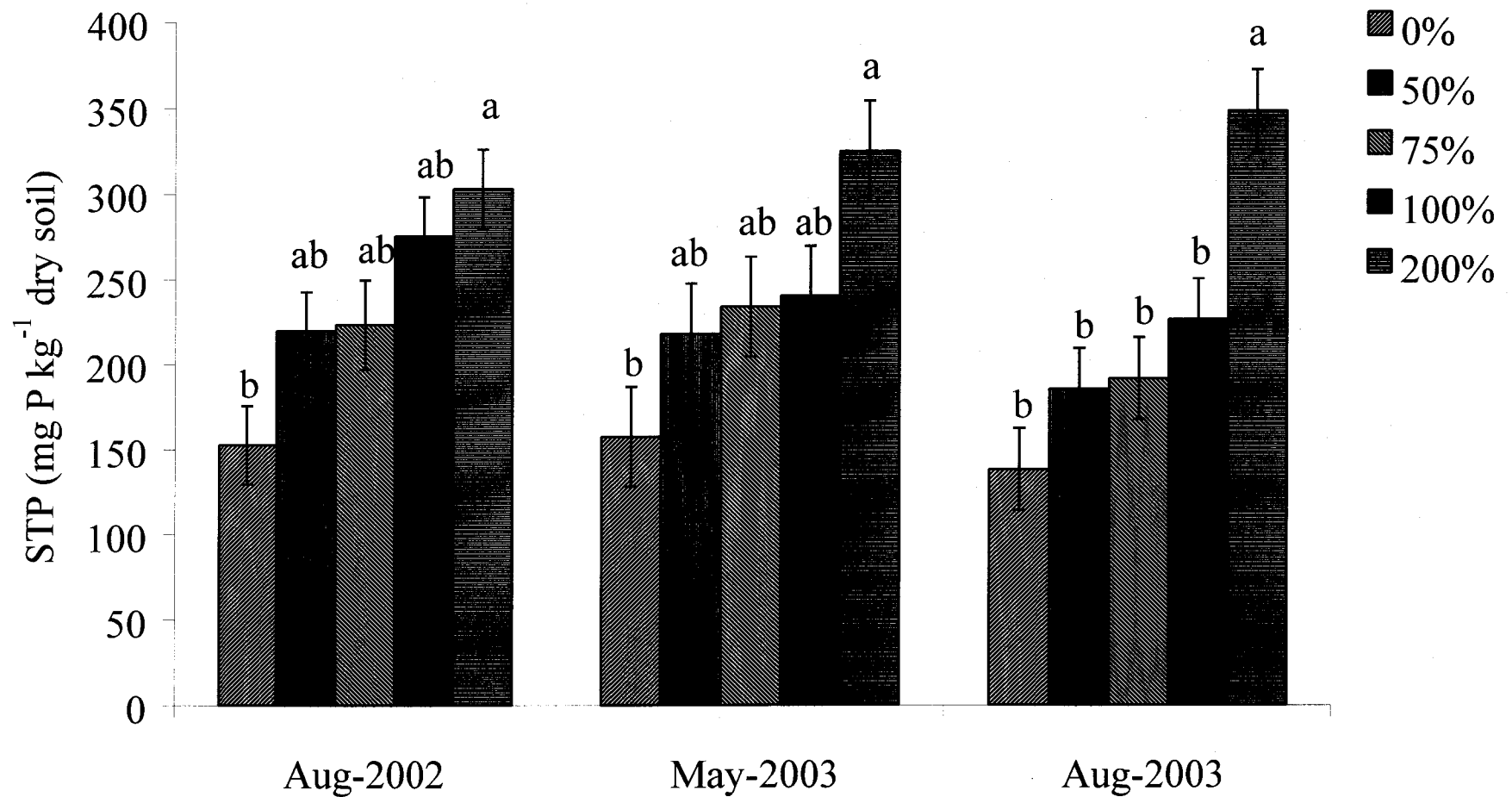


Fig. 2. Effect of P fertilization on soil test P on three sampling dates (August 2002, May and August 2003) in an asparagus field. Treatments are 0 to 200 % of the P recommendation based on CRAAQ (2003) guidelines. Treatments with same letters, within a sampling date are not significantly different at  $P = 0.05$  (Scheffe multiple range test).



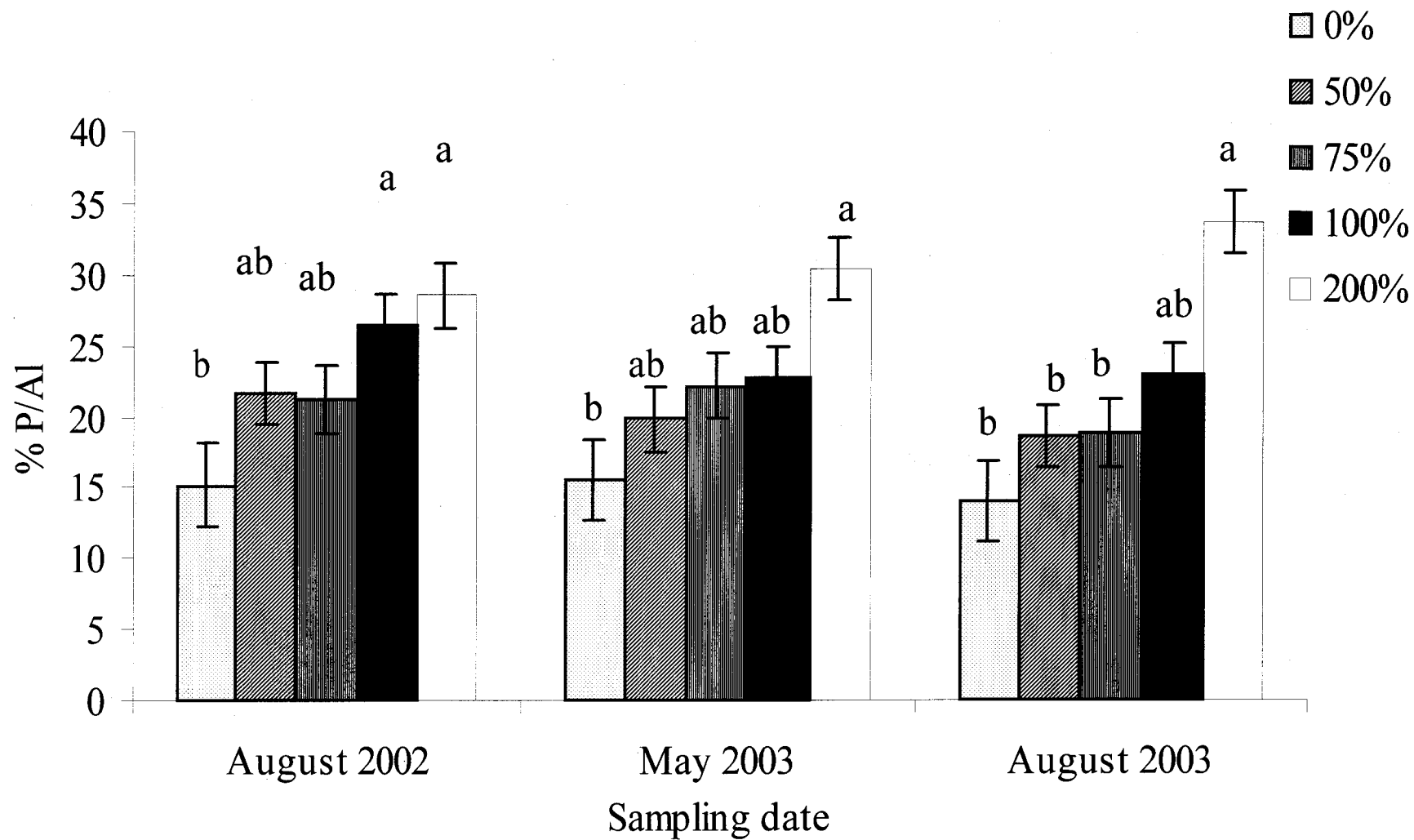


Fig. 3. The P saturation ratio (% P/Al) in soils under asparagus production receiving between 0 and 200 % of the recommended P fertilizer rate. The agroenvironmental limit for sandy soils is 13.1 % P/Al.

Table 2. Soil microbial biomass, enzyme activity and AMF colonization in asparagus plots. Values are the mean, standard deviation (std), min (min) and maximum (max) of 25 plots. Numbers in bold indicate significant ( $p < 0.05$ ) effects due to fertilization.

Parameter	Mean	Std	Min	Max	F value	Pr > F
August 2002						
MBC (mg kg <sup>-1</sup> )	194.3	56.3	99.8	31	5.65	<b>0.005</b>
MBN (mg kg <sup>-1</sup> )	28.2	7.2	17.1	45.0	1.25	0.33
Monophosphatase(μg g <sup>-1</sup> )	286.6	62.1	154.2	412.4	0.67	0.62
B-D-glucosidase (μg g <sup>-1</sup> )	16.1	3.4	9.2	22.9	0.55	0.70
dehydrogenase (μg g <sup>-1</sup> )	4.11	1.5	2.3	8.3	0.50	0.73
AMF colonisation (%)	7.0	6.2	0	25.0	0.26	0.90
May 2003						
MBC (mg kg <sup>-1</sup> )	166.9	61.0	74.7	275.0	2.39	0.09
MBN (mg kg <sup>-1</sup> )	12.6	2.9	7.7	15.6	2.14	0.12
Monophosphatase(μg g <sup>-1</sup> )	472.2	82.4	345.5	700.2	0.09	0.98
B-D-glucosidase (μg g <sup>-1</sup> )	142.6	67.8	42.0	266.2	0.36	0.84
dehydrogenase (μg g <sup>-1</sup> )	2.23	0.61	0.11	0.32	2.68	0.07
AMF colonisation (%)	7.5	6.8	1.1	26.3	4.28	<b>0.02</b>
August 2003						
MBC (mg kg <sup>-1</sup> )	224.1	80.0	117.1	384.1	0.48	0.75
MBN (mg kg <sup>-1</sup> )	13.6	2.6	9.3	21.6	0.14	0.97
Monophosphatase(μg g <sup>-1</sup> )	251.2	44.2	177.8	349.1	0.73	0.58
B-D-glucosidase (μg g <sup>-1</sup> )	8.0	1.9	3.4	11.5	2.18	0.12
dehydrogenase (μg g <sup>-1</sup> )	5.61	1.41	0.34	0.90	0.73	0.59
AMF colonisation (%)	4.5	3.5	0	11.5	1.03	0.42

Table 3. Pearson correlation coefficients between P saturation and soil biological parameters in asparagus plots (n=75). Asterisks (\*, \*\*, \*\*\*) indicate Pearson correlation coefficients were significant at  $p < 0.1$ ,  $p < 0.01$  and  $p < 0.001$ , respectively.

	%myc <sup>a</sup>	MBC <sup>b</sup>	MBN <sup>c</sup>	P/Al <sup>d</sup>	P <sup>e</sup>	Deh <sup>f</sup>	Mono <sup>g</sup>	Bg <sup>h</sup>
%myc	1							
MBC	0.07	1						
MBN	0.10	0.20*	1					
P/Al	-0.13	-0.19	0.53***	1				
P	-0.23*	-0.30*	0.06	0.80***	1			
Deh	-0.19	-0.46***	0.09	-0.08	-0.17	1		
Mono	0.15	-0.22*	-0.23*	-0.04	0.20*	-0.60***	1	
Bg	0.03	-0.19*	0.37**	0.26*	0.017	-0.22*	0.20*	1

<sup>a</sup> % mycorrhizal colonisation in asparagus roots

<sup>b</sup> Microbial biomass carbon from within the asparagus row (mg kg<sup>-1</sup>)

<sup>c</sup> Microbial biomass nitrogen from within the asparagus row (mg kg<sup>-1</sup>)

<sup>d</sup> P/Al saturation ratio of soil (%)

<sup>e</sup> P concentration, mg Mehlich-3 P kg<sup>-1</sup>

<sup>f</sup> Dehydrogenase activity (μg g<sup>-1</sup>)

<sup>g</sup> Monophosphatase activity (μg g<sup>-1</sup>)

<sup>h</sup> β-D-glucosidase activity (μg g<sup>-1</sup>)

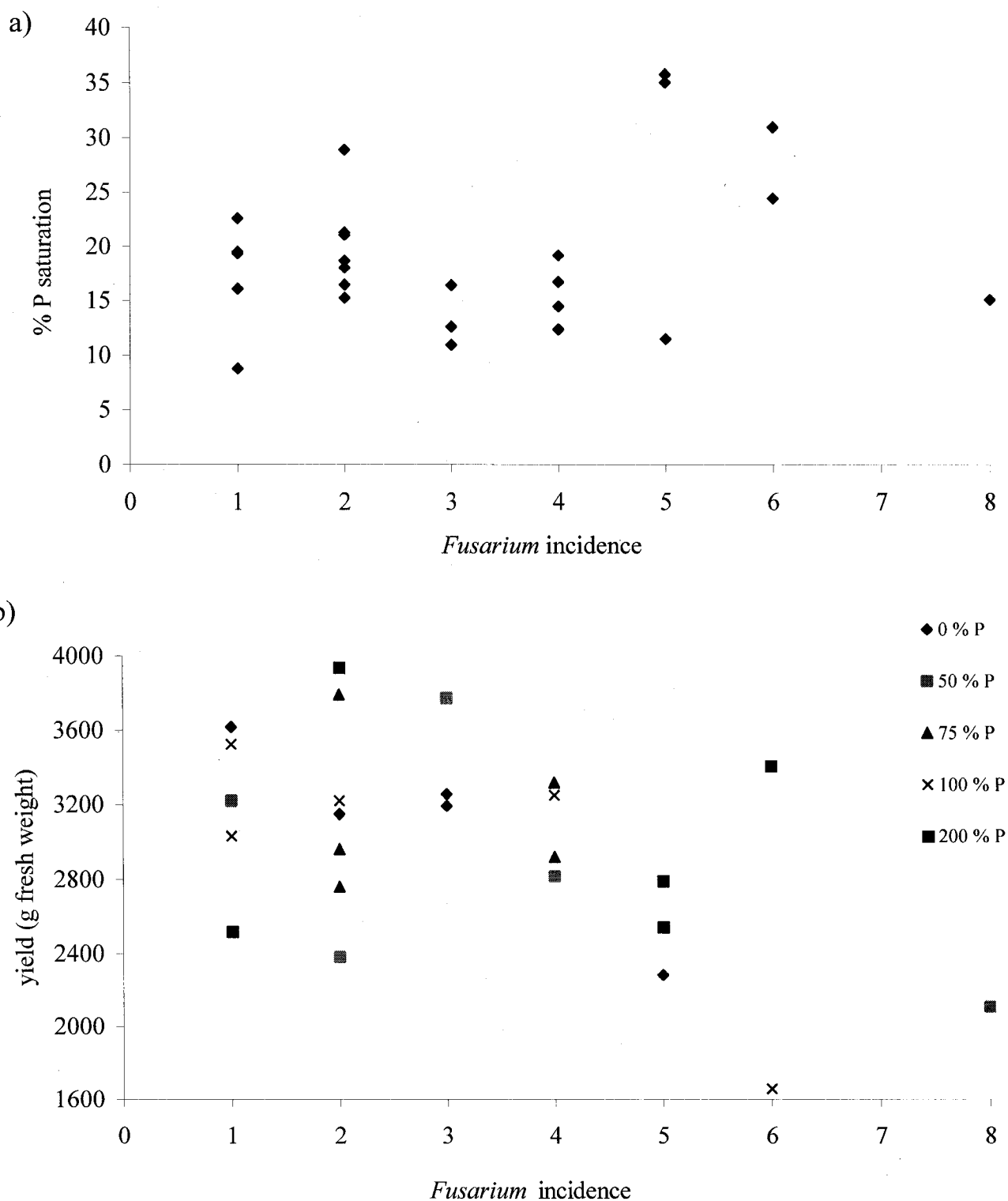


Fig. 4. a) Correlation between % P saturation (P/Al ratio) and *Fusarium* incidence in an asparagus field (symptomatic plants plot<sup>-1</sup>)  $p=0.23$   $r=0.25$  and b) Correlation between asparagus shoot yield and *Fusarium* incidence (g fresh weight plot<sup>-1</sup>).  $p=0.0091$   $r=-0.51$ .

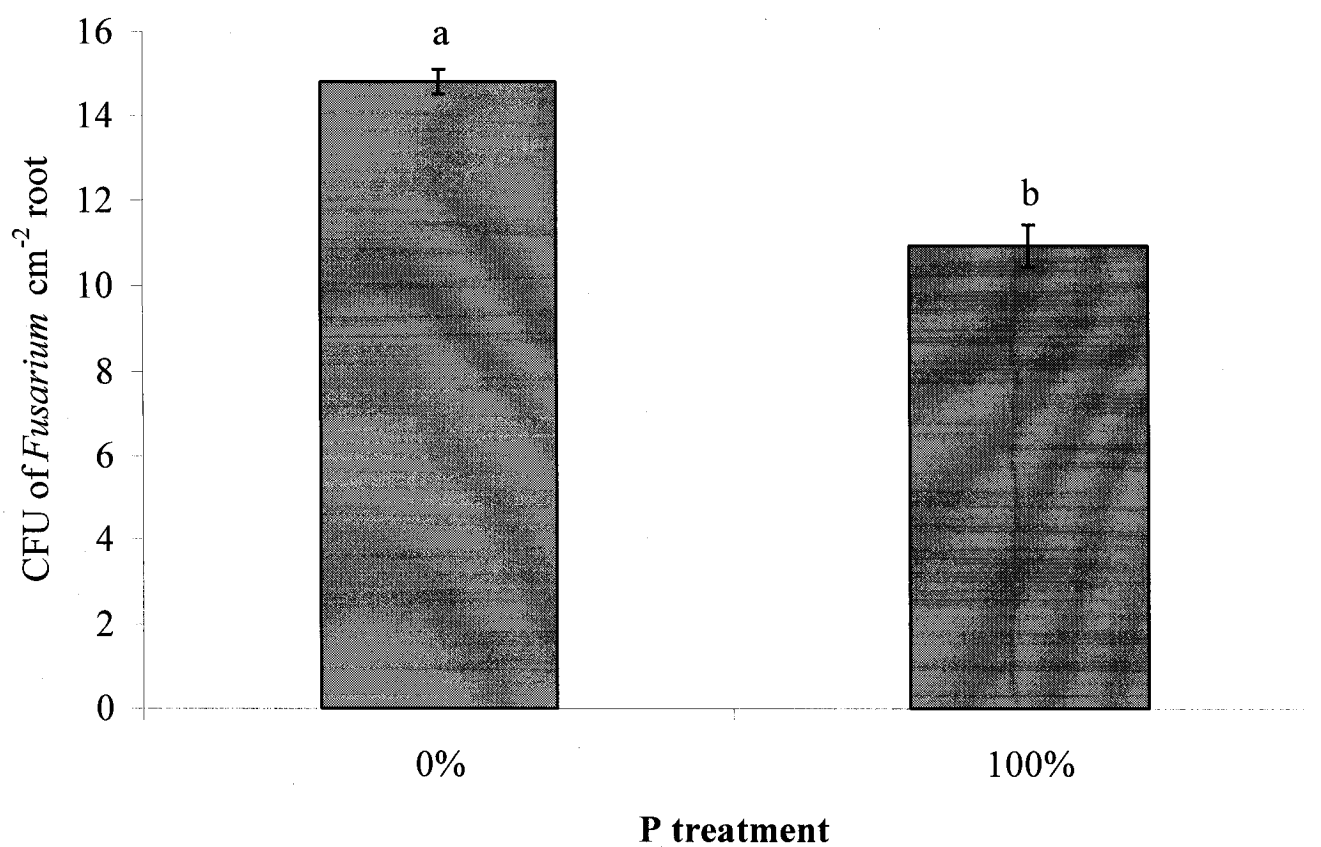


Fig. 5. *Fusarium* sp. colony forming units present on 1 cm<sup>2</sup> of asparagus roots. Treatments are 0 % and 100 % of the P recommended P fertilizer rate. Error bars represent standard error. Significant at  $p=0.001$  using a simple t-test.

## General conclusions

Phosphorus fertilization did not significantly affect asparagus establishment and yield. The plants seemed to have sufficient quantities of available phosphorus in the soil for growth. Plant tissue nutrient concentrations were not affected by P fertilization.

Phosphorus fertilization did not influence soil biological parameters (microbial biomass, enzyme activity) in a consistent manner. Microbial biomass and enzymatic activity were likely affected more by seasonal variation than by P fertilization. Some significant correlations seem difficult to explain, such as the negative correlation between MBC and dehydrogenase activity. Further investigation is needed to determine the cause of these relationships. However, AMF colonisation of roots was negatively affected by P fertilization in May 2003, and negatively correlated with soil P concentrations throughout the study. We propose that AMF colonization is faster in asparagus roots growing in soils receiving no P fertilizer, but this hypothesis remains to be tested.

*Fusarium* incidence was not significantly affected by P fertilization, but plants with the highest level of *Fusarium* tended to have the lowest yield. The relationship between *Fusarium* incidence and asparagus shoot yield is of importance to producers. Plants that produce the fewest shoots early in the season may be more susceptible to root rot and should not be over-harvested. If pathogenic *Fusarium* infection can be managed by reducing harvest on weak plants, it could be possible to greatly reduce root rot damage and spread in the field. Further work is needed to optimize management practices and increase the potential lifetime of the asparagus plantation. Such research is urgently required by asparagus growers in Quebec and elsewhere in North America.