THE UNIVERSITY OF MANITOBA

THE EFFECT OF PHOSPHORUS AND VESICULAR-ARBUSCULAR MYCORRHIZA ON GROWTH AND DINITROGEN FIXATION BY LENTIL (*Lens culinaris*)

By

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ABSTRACT

Lentil (*Lens culinaris*), a legume crop introduced into Western Canada in the 1970's, has received much attention in the past few years. However, the knowledge of symbiotic N_2 fixation in lentil is rather sparse, with many of the estimates on the amount of fixation low. Possible reasons for low fixation include, the use of inefficient *Rhizobium* strains not capable of maximum fixation, continuous growth of lentil in adverse environmental conditions which may have weakened the symbiotic association through natural selection, and cropping of lentil on low nutrient status soil where plant growth is not optimized. This last point could be alleviated through better agronomic practices and may be a fundamental approach to improving the fixing ability of this crop.

One nutrient which plays a large role in N_2 fixing legumes is P. This nutrient it seems has a dual function, acting directly on the N_2 fixing system and indirectly via plant growth responses. Legumes infected with Vesicular Arbuscular Mycorrhiza (VAM) often show general improvements in growth, nodulation, and N_2 fixation. This appears to be the result of relief from P stress, as well as the influence of VAM on the legume-*Rhizobium* symbiosis.

Growth chamber, field, and lysimeter experiments were carried out to study the effect of applied or plant available P on growth and N_2 fixation by lentil in two Manitoba soils. Lentil dependency on soil microorganisms (VAM) to aid in the uptake of P was also examined in the growth chamber experiments by using gamma irradiated soil treatments and

VAM inoculant. The effect of inoculation with VAM on lentil growth in a field situation was also assessed in the lysimeter experiment.

Generally, in the growth chamber experiments, lentil responded to P application by increases in dry matter accumulation, shoot P concentration, and N_2 fixation in the non-irradiated and irradiated +VAM soil treatments. The lentil grown in the irradiated soil treatment were unable to grow unless P was applied at high rates, or beneficial microbial populations were placed back in the soil (irradiated +VAM soil treatment).

In the field experiments, the lentil failed to respond to either applied or plant available P. Increase P uptake with P application and/or VAM inoculation was noted in the lysimeter study, however no increase in dry matter accumulation was found. In all these experiments no enhancement on the N_2 fixing system occurred with P application. It seems the amount of plant available P initially present in the soil was adequate for lentil growth. Decreases in Cu and Zn concentration in the lentil with increasing amounts of applied or plant available P were observed, also suggesting that P was not a limiting factor for growth.

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INTRODUCTION

The role of P nutrition in N_2 fixing legume-*Rhizobium* systems has received considerable study. Phosphorus has a dual function, acting directly in the N_2-fixing system (nodulation and nitrogenase activity), and acting indirectly via host plant growth responses (Gates, 1974; Graham and Rosas, 1979; Jakobsen, 1985; Israel, 1987). As well, many authors have described the role of P on the growth and survival of *Rhizobium* in the soil and on the bacterial infection process (Robson, 1983; Israel, 1987). The benefits of adding P fertilizer depend on a number of plant and soil related factors, including the type of soil and legume-root morphology. As well, if P fertilizer is needed, the amount added, the type, and the application method, must be considered.

Legumes infected with Vesicular Arbuscular Mycorrhiza (VAM) often show general improvements in growth, nodulation, and N_2 fixation. This appears to be the result of relief from P stress. Mycorrhizal infection may increase the absorption of trace elements or cause an alteration of plant hormones (Gates, 1974). It may also be possible that VAM influence the legume-*Rhizobium* symbiosis by altering the rhizosphere environment for rhizobia (Crush, 1974). The concentration of available P in the soil appears to play an important role in determining whether or not the colonization of VAM will be beneficial (Paul and Clark, 1989).

Regardless of whether or not the specific functions of P and/or mycorrhiza on the N_2 fixing legume are known, the effect of adding

these soil amendments on plant productivity can be evaluated. Agronomically speaking, this may be the most important question at hand.

Lentil (*Lens culinaris*), a legume crop, introduced into Western Canada in the 1970's, has received much attention in the past few years. Field production in Manitoba was estimated at 60,000 hectares in 1987 (Manitoba Agriculture, 1988), and it is likely acreage will increase as more and more farmers seek alternative cropping systems and crop diversification during hard economic times.

Increasing interest in the production of lentil, and the need to determine its nutritional requirements, has prompted research on this legume. It was the intent of this project to study the effect of adding P fertilizer and/or mycorrhiza inoculant on growth and N_2 fixation by lentil in two Manitoba soils.

LITERATURE REVIEW

Symbiotic relationships between plants and microorganisms are well documented. Of these relationships one of the most important in agriculture is the nodule forming symbiosis between *Rhizobium* species and the roots of some legumes. These Rhizobia have the ability to infect roots, form nodules, and fix atmospheric N_2 which is then made available to the plant. The nodules formed are gall-like lateral outgrowths arising from the root cortex following infection of a root hair. The nodules contain pleomorphic (swollen and distorted) forms of the *Rhizobium* known as bacteroids. These bacteroids actively metabolize N_2 to NH_3.

Nitrogenase, the enzyme responsible for reducing N_2 in the presence of adenosine triphosphate (ATP), reduced ferredoxin, and other enzymes, as well as the substance leghemoglobin, are synthesized during nodule growth (Alexander, 1977). The nitrogenase enzyme is a complex made out of two proteins, the first is an Mo-Fe protein and the second is an Fe protein (Bergersen, 1971). The substance leghemoglobin, found in the bacteroid, has a high affinity for O_2. It protects the nitrogenase enzyme complex from the detrimental effects of O_2, while at the same time allowing adequate amounts of O_2 for bacteroid respiration (Bergersen, 1971).

Once the N_2 is reduced to NH_3, this compound is released from the bacteroid into the nodule cytosol and assimilated into organic forms (initially glutamine). Secondary reactions produce other amino compounds from this glutamine (Pate and Atkins, 1983). The amino

acids are then transferred to the plant via the host vascular connections.

Between infection and the onset of N_2 fixation, there is a lag period of three to five weeks where the relationship between the plant and the *Rhizobium* is not symbiotic but rather the *Rhizobium* are parasitic. During this time, the host is establishing a leaf area large enough to supply photosynthate to the nodules (Marschner, 1986).

In addition to the increased requirement for photosynthate, development and maintenance of the legume-*Rhizobium* symbiosis requires increased inputs of ATP, carbohydrate, and mineral nutrients, all of which must be supplied by the plant. In return, the plant receives all or a portion of the amino acids produced by the *Rhizobium*.

Nutrition of legumes is more complex than that of non-legumes since many of the nutrients required for plant growth, are essential at higher concentrations (as stated above) for N_2 fixation (Robson, 1983). An inadequate supply of the nutrients essential for fixation can often lead to N deficiencies in the legumes dependent on this symbiotic N_2 fixation.

Lowther and Loneragan (1968) found that inadequate Ca supply inhibited nodulation on some acid soils. The stage of nodulation most sensitive to Ca deficiency has been found to correspond to the first three days after inoculation, the time during which the root hairs curl and infection occurs (Munns, 1968). Munns (1970), also reported that the addition of Ca was required for nodule initiation, the concentration needed being much higher than that required for root and shoot growth of the host plant.

Molybdenum and Fe must be supplied to the legume as both are integral parts of the nitrogenase enzyme complex. Iron is also a constituent of leghemoglobin, and Fe-S compounds are needed for the synthesis of both nitrogenase and bacteroidal ferredoxin (Evans and Russel, 1971).

Cobalt is required for the synthesis of vitamin B_{12} (Ahmed and Evans, 1960). Application of this nutrient on deficient soils increased the number and shape of the bacteroids in the nodule, indicating that it is essential in rhizobial cell division (Chatel et al., 1978).

Cartwright and Hallsworth (1970) noted Cu deficient legumes have fewer and smaller nodules, plus the nodules themselves have difficulty incorporating carbon into amino acids. Snowball et al. (1980) also suggested an increased requirement for Cu in legumes since the application of this nutrient on deficient soils or the application of combined N to a legume, corrected for the same deficiency.

The addition of many nutrients such as Zn and S commonly increase plant growth and as a consequence indirectly increase nodule number and size. However, Lo and Reisenauer (1968) showed that the addition of Zn increased N concentration in legumes relying on fixation for their N source.

Phosphorus is needed, as well as the carbon substrate, in the nodules for production of ATP and reductants required for N_2 fixation. Various authors have calculated the amount of ATP required for N_2 fixation. Estimates of 21 ATP (Havelka et al., 1982), 12 to 30 ATP (Hill, 1976), and 15 to 30 ATP (Shanmugam et al., 1978) per molecule

of N_2 fixed have been suggested. However, a definite number of ATP required for the reduction cannot be calculated because the nitrogenase enzyme can reduce several other substrates including H_2, N_2O, and acetylene (Postgate, 1982).

Smith and Daft (1977) suggested P may also be directly responsible for nodule initiation, and Gates and Wilson (1974) showed that like Zn, P additions increased the N concentration more in legumes relying on nitrogen fixation than those receiving nitrogen fertilizer.

In soil, P occurs as organic and inorganic phosphates (P_i), both of which are used by microorganisms and plants. Most studies suggest that before the organic P compounds in the soil can be utilized by microorganisms, they must first be hydrolysed to P_i by various enzymes. Glenn and Dilworth (1979) tested *R. leguminosarium* strains in culture media for the production of hydrolytic enzymes. The rhizobia did not release enzymes into the environment but did produce several periplasmic proteins including alkaline phosphatase, pyrophosphatase, and cyclic phosphodiesterase. Similar proteins were found by Smart et al. (1984b) for Cowpea *Rhizobium* and *R. trifolli*. These enzymes situated between the external and plasma membranes, will hydrolyse organic phosphate esters to P_i. In all cases, the uptake of phosphate was regulated by the P_i concentration in the medium. If the cells were grown in excess P the rate of uptake was slow, however if the cells were grown in limiting P the uptake of P_i was rapid. Thus enzyme activity was repressed by excess P but reactivated when cells were placed in a phosphate poor medium. Thus, the ability to synthesize

hydrolytic enzymes under P limited conditions allowed rhizobia to maintain a high internal level of phosphate.

The storage of phosphate as polyphosphate granules in rhizobia and its reutilization to support subsequent growth has also been shown (Cassman et al., 1981a; Smart et al., 1984c). Cassman et al. (1981b) demonstrated that some strains of *R. japonicum* are more tolerant to P deficient environments than others, indicating an ability for some strains to store and reutilize phosphate better. This must be emphasized since the Rhizobia often inhabit and function in soils where the legume host is phosphate deficient. The reutilization of stored phosphate may be needed on these soils to ensure multiplication of the Rhizobia in the rhizosphere which is needed to induce infection and establish effective nodulation (Cassman et al., 1981b).

Several researchers have studied the transport systems of bacteroids isolated from different legumes, and analyzed for similarities to their free living counterparts (Craig et al., 1973; Smart et al., 1984a and c). The allocation of nutrients to the bacteroids from the plant is important because this is the site of nitrogen fixation. In this study of the transport of phosphate, bacteroids from snake beans plants grown in different P regimes, were isolated and analyzed. It was found that regardless of the level of P supplied to the legume, the *Rhizobium* had a basal alkaline phosphatase activity common to cells grown in phosphate-excess situations and lacked other phosphate repressible proteins. The bacteroids also contained a reserve of phosphate as polyphosphate granules typical of phosphate-excess cells, again suggesting none of the bacteroids was

phosphate limited. Finally the same lack of repression of the P uptake system found in free living *Rhizobium* was found in the bacteroids. These data suggest that regardless of whether or not the host is phosphate deficient, the bacteroid has access to adequate amounts of this nutrient. This probably involves preferential transport from the host via the vascular connections to the nodule (Smart et al., 1984a and c).

Several researchers believe that nitrogenase activity is dependent on the P_i fraction found in the bacteroid cytosol rather than total bacteroid P (Hart, 1988). Experiments using white clover showed all organic P fractions to increase in the nodule when P was supplied to P deficient plants. As the higher rates of addition were applied, little change was seen in the organic P fractions in the nodule but the P_i fraction increased substantially. An increase in acetylene reduction occurred with the increased P_i in the cytosol suggesting that this is the fraction which is directly available to the N_2 fixing system.

Like microorganisms, most plants prefer the inorganic form of phosphorus, taking up the orthophosphate ion (H_2PO_4) present in soil solution. Once taken up and in the plant leaves, P is distributed in lipid, nucleic acid, ester, and inorganic fractions (Marschner, 1986). The lipid, nucleic acid, and ester fractions are essential for cell growth and maintenance, and therefore the amount of each fraction remains stable unless P deficiency is severe. The P_i fraction, found mainly in the plant vacuole, however, fluctuates depending on P supply to the plant.

In healthy plants, the growing leaves typically attain their maximum photosynthate rate and P_i accumulation when they reach about 80% of their maximum leaf area (Milthorpe and Moorby, 1979). Before this point, the leaf shows a net import of P and carbon. Walker (1980) proposed a role for P_i in regulation of the partitioning of photosynthate between starch accumulation and sucrose translocation. This proposal involves the so called P-translocator protein imbedded in the inner membrane of the chloroplast envelope. The protein exchanges one P_i for one triose P, the first reduced product of photosynthesis. Sucrose is formed from triose P in the cytoplasm and is available for translocation from the leaf. Thus the accumulation of P_i within the chloroplast inhibits synthesis of starch and activates starch phosphorylase promoting breakdown of triose P and export to cytoplasm.

Nitrogen fixation is dependent, in part, on the translocation of photosynthate supplied to the bacteroids in the nodule (Layzell et al., 1979). Although the amount of photosynthetically fixed carbon needed to maximize N_2 fixation is different for different legumes-*Rhizobium* associations, for annual legumes up to one third is needed for the energy, reductant, and carbon skeletons for N_2 fixation and assimilation (Pate and Herridge, 1978). In addition nodule function is thought to be dependent on photosynthate recently transported from the leaves rather than on long term storage compounds in the bacteroids (Ta et al., 1987). Marschner (1986) proposed that the role of P_i in the partitioning of photosynthate between source leaves and the roots may limit carbon supply to the nodules in P deficient

plants.

Some workers have also suggested a co-transport of sucrose and P_i via the phloem from source (leaf) to sink may occur. This transport of P_i to the nodule may be very important since N_2 fixation is dependent on the P_i supply to the cytosol. Bergersen (1971) also implied a restricted P supply may affect the formation of ATP in the nodules.

Graham and Rosas (1979) found the nodules in common bean after 42 days of growth, to be a very strong sink for added P. Nodule weight, plant dry weight, and percent P in the shoot increased with added P. The amount of N_2 fixation was significantly correlated with the P added, the concentration of P in the nodules, and the total P content in the nodules. Increases in fixation, they believed, reflected the improved carbohydrate supply to the nodules at the higher rates of P added. Jakobsen (1985) showed through a series of three experiments, that reduced nodulation and N_2 fixation in P deficient pea plants (*Pisum sativum*) was the result of impaired shoot metabolism. Singleton et al. (1985) grew soybean inoculated with effective strains of *R. japonicum*, and showed P deficiencies even when the plants were supplied with adequate amounts of P for growth. They believed the plants remained deficient due to the demand for P needed for nodule development and nitrogenase activity.

Gates (1974) demonstrated the beneficial effects of P in *Subterraneum humilis* on nodule initiation. Nodules were present three days earlier in high-phosphorus than in low-phosphorus plants. Increased percent N in plant when grown at a high P level also

indicated that maximum N_2 fixation requires a higher level of P than that required for maximum growth. Israel (1987) conducted an experiment to study the interaction between P supply and combined N supply on nodulated soybean. The positive interaction between plant N concentration with increasing P nutrition was greater in plants relying on N_2 fixation than those receiving N fertilizer. From the results of this experiment, it was concluded that P plays an important role not only in legume growth, but also nodule initiation, growth, and function. The addition of P increased nodule number more than nodule mass, again suggesting a specific role for P in the early stages of nodule development. Many other researchers have shown legumes to require high levels of phosphate for growth and effective nodulation as well as for enhanced N_2 fixation (Asimi et al., 1980; Bethlenfalvay et al., 1982; Smith and Daft, 1977).

Methods for increasing P availability to legumes might include manipulation of beneficial soil microorganisms. The total population of microorganisms in undisturbed soil fluctuate only minimally to environmental conditions mainly because of lack of an available energy source. Once plants are added to the soil however, the microbial environment is changed drastically since plants are the main supplier of nutrients, especially carbon, to the soil. The microorganisms found in close proximity to the root surface are able to best exploit these nutrients and the microbial gradient which extends from the root surface (rhizoplane) to a centimeter or two away is called the rhizosphere. (Brown, 1975). It is manipulation of the types and number of organisms in this region which is of interest to many

researchers.

Many factors affect the rhizosphere population, and the solubility of P in the soil, one being the changes in pH. Root induced changes in rhizosphere pH are the result of the excretion of H^+ or HCO_3^-, the evolution of CO_2 by respiration, and by the secretion of root exudates (Marschner, 1986). The excretion of H^+ or HCO_3^- ions into the external solution by the plant is the result of the need to approach electrochemical balance both in root cells and in the external solution (Marschner, 1986). This is the main reason for changes in rhizosphere pH. Legumes relying solely on symbiotically fixed N must excrete H^+ ions into the external solution since total cation uptake exceeds anion uptake (Robson, 1983), thus making the rhizosphere acidic. The acidification by nodulating legumes may have an effect on the uptake of plant nutrients as well as the biological activity in the rhizosphere. Phosphorus uptake in particular is enhanced by acidification. Aguilar and Van Diest (1981) showed alfalfa plants dependant on N_2 fixation for their N requirement to utilize rock phosphate better than those fed nitrate-N.

Other processes which affect the solubility of P in the soil, such as soil pH, cation exchange capacity of roots, absorption and adsorption of Ca from calcium phosphate, and the complexing of Fe and Al by organic anions to solubilize Al and Fe phosphates, are also affected by the action of root exudates and the activity of certain microorganisms (Curl and Truelove, 1985). These microorganisms which have phosphate solubilizing ability, are called phosphobacteria (Bagyaraj, 1984). Tinker (1980) suggested the quantity of these

organisms around the plant root system is often low, since they must compete with other rhizosphere microorganisms for the C supplied by root exudates. Thus, under natural conditions it is unlikely that phosphobacteria contribute significantly to the dissolving of sparingly soluble P_i around the root. Barber and Frankenberg (1971) however, showed excised barley (*Hordeum vulgare*) roots grown under non-sterile conditions to have a greater capacity to absorb ions than roots grown in the absence of microorganisms. This effect was particularly pronounced for P uptake. Hayman (1975) also suggests that it would be unrealistic to assess the uptake of soil P without considering the activities of these microorganisms.

A second group of microorganisms associated with plant roots and involved in P nutrition for plants are fungi which form Vesicular Arbuscular Mycorrhizae (VAM). Mycorrhizae translates literally to 'fungus root', and agronomically, the most important type involve the fungi classified as Zygomycetes. These organisms (like *Rhizobium*) have an enormous advantage over free-living organisms because of their niche in plant roots (Hayman, 1975). Mycorrhiza produce structures such as intracellular mycelia, vesicles, and arbuscules in the cortex region of plant roots (Powell and Bagyaraj, 1984). VAM fungi are indigenous in field soil and therefore the mycorrhizal infection is the normal condition in most plant species (Rhodes, 1984).

Plant dependency on VAM fungi depends greatly on their rooting habit and root hair length. Generally, plants with roots greater than 0.5 mm in diameter and lacking root hairs are highly dependent on mycorrhiza. Conversely, plants with dense long root hairs and a

diameter of less than 0.1 mm respond to VAM only in P deficient soils (Barea and Azcon-Aguilar, 1983). Legumes therefore are much more mycotrophic than grasses (Paul and Clark, 1989). Caradus (1982) showed the effect of added mycorrhiza or the effect of increased root hair length in white clover was similar in the uptake of P. It was concluded that selecting white clover genotypes with long root hairs is not necessary since natural mycorrhizal association abolishes any difference in P uptake between populations of long and short root haired clover. In addition, many legumes (such as soybean) supplied with adequate N have a more extensive root system than the comparable N_2 fixing plant (Cassman et al., 1981c). The more extensive root system of the N supplied legume allows for increased ability to absorb P, lowering the P fertilizer requirement.

Development of this symbiotic association is frequently followed by increased absorption of inorganic nutrients, especially P, from the soil (Mosse, 1977). Enhanced uptake is accomplished by the extension of the fungal hyphae (external mycelia) beyond the zone where nutrients have been depleted by diffusion to the roots (Tinker, 1975). It is believed that the external mycelia function analogously to root hairs, increasing the root surface area and therefore increasing the volume of soil from which nutrients can be extracted. If the supply of inorganic nutrients is not limiting to the growth of non-mycorrhizal plants then the association may not be beneficial since VAM increases drain on plant photosynthesis (Rhodes, 1984).

The P is initially translocated from the external mycelia to the internal hyphae as polyphosphate granules, and through cytoplasmic

streaming these granules become deposited in the arbuscules (Cox and Tinker, 1976). Phosphate stored in the arbuscules is then transferred to the host by an active mechanism across the membrane of both symbionts. In return for the phosphate the host plant must supply the fungal endophyte with photosynthate.

The interaction between VAM fungi and phosphate solubilizing bacteria and their effect on plant growth has been studied. Raj et al. (1981) found the phosphate solubilizing bacteria (*Bacillus circulans*) rendered more P soluble while the mycorrhiza (*Glomus fasciculatum*) enhanced more P uptake in finger millet. Azcon-G. De Aguilar and Barea (1978) showed similar enhancement with dual inoculation on *Medicago sativa*. Raj et al. (1981) also noted that phosphate solubilizing bacteria survived for a longer period of time in a rhizosphere of mycorrhizal roots. The synergistic interaction of both organisms may be largely responsible for the beneficial results obtained and often attributed to just mycorrhizal infection (Linderman, 1988).

The uptake of nutrients other than P which diffuse slowly in the soil and give way to depleted zones around the root may also be enhanced by mycorrhizal infection. Lambert et al. (1979) showed both Zn and Cu uptake to increase in soybean infected with mycorrhiza. Even the uptake of nutrients which mainly move through the soil by mass flow, and therefore are rarely rate limiting such a S, Ca, K, Mg, Fe, Mn, Cl, Br, and N, may be enhanced through mycorrhizal infection (Tinker, 1984). Gray and Gerdemann (1973) showed mycorrhizal red clover plants grown under S deficient conditions to be more efficient

in S uptake than non-mycorrhizal plants. The additional uptake of these elements by legumes may be important since many are involved in nodulation and N_2 fixation (Gray and Gerdemann, 1973).

VAM fungi may also promote plant growth via formation of hormones (auxins, gibberellins, cytokinins, etc.) in the plant (Barea and Azcon-Aguilar, 1983). Tinker (1984) also noted that mycorrhizal infection may change the hormonal condition and this in turn may affect the plant's water and nutrient status.

Since VAM may increase the absorption of mineral nutrients from soil and transfer them to the host, and since legumes are often mycotrophic, there has been increasing interest in the use of both VAM fungi and *Rhizobium* together as a means of improving legume production (Asimi et al., 1980).

Most of the studies evaluating VAM-*Rhizobium* interactions within the tripartite association (*Rhizobium*-VAM-legume) show mycorrhiza to significantly improve legume P nutrition thus increasing plant growth and consequently improving nodulation and N_2 fixation (Daft and El-Giahmi, 1974; Carling et al., 1978). The addition of phosphate fertilizer in these experiments, produced a similar effect to that of the mycorrhizal treatment suggesting no direct interaction between the VA fungi and the N_2-fixing bacteria.

A time course study done by Smith and Daft (1977) however, showed mycorrhizal infection to have a direct effect on nodulation and N_2 fixation. They compared mycorrhizal and non-mycorrhizal *Medicago sativa* plants with the same dry weight and the same root:shoot ratios and found increased nitrogenase activity and improved nodulation in

the mycorrhizal plants. At all P additions tested, mycorrhizal plants had higher N content than the non-mycorrhizal controls. This suggested the nodules required P, and this nodule P requirement was stimulated before plant growth. Increased N assimilation in mycorrhizal soybean was found by Asimi et al. (1980). Again, early stimulation of nitrogenase activity before any differences in plant growth between the mycorrhizal and non-mycorrhizal plants indicated a sensitivity of the *Rhizobium* to the presence of mycorrhiza. These papers suggest that nodule function may be stimulated by mycorrhizal infection, making the P directly available to the nodule (Waidyanatha, 1979).

Finally, just as VAM increases P supply to the host, *Rhizobium* may also stimulate the growth of VAM in the rhizosphere through the production of certain compounds which increase root cell permeability and through the synthesis of growth hormones (Dhingra et al., 1988). The result of increased infection is to increase the availability of P to the plant and therefore increase the efficiency of use of applied P (Bieleski, 1973).

METHODS AND MATERIALS

The Effect of Phosphorus on Growth and N_2 Fixation by Lentil

To determine the effect of P on growth and N_2 fixation by lentil (*Lens culinaris* var. Eston), a growth chamber experiment and a field experiment were conducted. The growth chamber experiment was conducted in 1985 using an Almasippi (Gleyed Rego Black) very fine sandy loam soil (soil #1) collected from Haywood, MB. The field experiment was conducted during the summers of 1985 and 1986 at two sites, one located north of Haywood MB, on an Almasippi (Gleyed Rego Black) loamy fine sand soil [soils #2 (1985) and #3 (1986)] and the other north of St. Claude, MB, on an Elm creek (Gleyed Black) loam soil [soils #4 (1985) and #5 (1986)]. The characteristics of the soils used are reported in Table 1. The pH values were determined with a glass electrode (soil:water ratio, 1:2.5) on <2 mm air-dry soil (McLean, 1982). Organic C was determined by a dichromate oxidation method (Walkley and Black, 1934) and inorganic C was determined by a gravimetric method (Allison and Moodie, 1965). Nitrate-N was determined by the phenoldisulfonic acid method (Bremner, 1965a), P was extracted using HCO_3 and phosphate determined by a colorimetric method using acid molybdate-ascorbic acid (Olsen and Sommers, 1982), and DTPA-extractable Cu and Zn were determined using an atomic absorption spectrophotometer (Follett and Lindsay, 1971).

Growth Chamber Experiment

The experiment was arranged in a completely randomized design

Table 1. Analysis of soils.†

Soil No.	pH	Organic carbon	Inorganic carbon	NO_3^--N	PO_4^{3-}-P	Cu	Zn
		%		$\mu g\ g^{-1}$ soil			
1	7.8	4.4	4.1	16.4	7.8	0.8	2.2
2	7.8	3.9	4.5	1.0	1.3	0.6	1.0
3	8.2	4.1	4.3	5.0	AP‡	0.2	0.3
4	7.1	1.9	0.0	15.4	10.3	0.5	0.7
5	7.3	1.7	0.0	4.5	AP	0.3	0.9

† Analyses were done on surface samples of soil (0-15 cm).
‡ AP=Available P, initial levels of P dependent on previous 1985 treatments (see Table 2).

(Little and Hills, 1978) with two crops, lentil (test crop) and winter rape (*Brassica napus* var. Regent) (reference, non-mycorrhizal crop); two soil treatments, non-irradiated and irradiated soil; and eight P treatments, six for the test crop and two for the reference crop. All treatments were replicated three times. The soil irradiation treatment was used to test the ability of lentil to utilize P in the absence of phosphate solubilizing microorganisms and Vesicular-Arbuscular Mycorrhiza (VAM).

The soil was air dried, ground, sieved (<2 mm), and stored at room temperature until use. Samples of air-dried soil (2500 g) were placed in polyethylene bags and treated with 10 mL of deionized water containing 200 μg K g^{-1} soil as K_2SO_4, 5 μg Cu g^{-1} soil as $CuSO_4 \cdot 5H_2O$, and 10 μg Zn g^{-1} soil as $ZnSO_4 \cdot 7H_2O$. The resulting solution also contained 108 μg S g^{-1} soil. Eight treatments of P were also applied

to the soil samples in 10 mL solutions containing 0, 10, 20, 40, 80, or 100 μg P g^{-1} soil as H_3PO_4 for the test crop, and 0 or 100 μg P g^{-1} soil as H_3PO_4 for the reference crop.

After the addition of the nutrients, the soil samples were mixed thoroughly, and one half of the soil samples were irradiated using a cobalt 60 source at a dose of 10,000 Gy (A.E.C.L. Whiteshell Research Station in Pinawa, MB). This resulted in the two soil treatments, non-irradiated and irradiated soil. After irradiation, all bags were placed in pots, and the bags were opened and the soil was allowed to aerate at room temperature for nine days before seeding.

Lentil and winter rape seeds were pre-germinated for four days on moist paper towels prior to planting. The lentil seeds were inoculated with a suspension of *Rhizobium leguminosarium* culture (lot number NC26, Urbana Culture Superior Inoculant) during germination and again when the seedlings were placed in the pots. Eight lentil seedlings per pot were planted at a depth of 2.5 cm and eight winter rape seedlings per pot were planted at a depth of 0.5 cm. After emergence the pots were thinned to two plants per pot.

After planting, the pots were placed in a growth chamber that was maintained at a 16 hour (22 C, 50% R. H.) - 8 hour (15 C, 70% R. H.) day - night cycle. The light source consisted of General Electric cool whites supplemented with 10% incandescent lamps. Photosynthetically active radiation was measured at 565 μmol photon m^{-2} s^{-1} at the top of the lentil crop canopy. The pots were maintained near field capacity (by weight) by adding water to the soil surface as required.

The plants were harvested when the lentil reached the mid-pod growth stage at 64 days after planting. The shoots were oven-dried for two days at 80°C, weighed for dry matter yield determination, and ground to pass a 2 mm mesh screen (Wiley mill). The dry material was analyzed for total N (Nelson and Sommers, 1973), P (Murphy and Riley, 1962), and Cu and Zn contents (Isaac and Kerber, 1980). N_2 fixation was determined using the classical difference method. Experimental data were statistically analyzed using the general linear models procedure of the Statistical Analysis System Package (Spector et al., 1985).

Field Experiment, 1985

The experiment was arranged in a split-block design with four rates of P as the main treatments and an additional 18 kg P ha^{-1} applied as a band to one half of the block, which was considered to be the subtreatment. The main plots, 8 m by 8 m, were seeded to lentil (test crop), and replicated four times. Another two replicates, 2 m by 32 m, were incorporated into the split block to accommodate wheat (*Triticum aestivum* var. Columbus) (reference crop).

Potassium at a rate of 200 kg K ha^{-1} was applied as K_2O and P was applied at rates of 0, 50, 100, or 200 kg P ha^{-1} as $Ca(H_2PO_4)_2$. The two replicates set aside for the reference crop received 200 kg K ha^{-1} as K_2O and 200 kg P ha^{-1} as $Ca(H_2PO_4)_2$. Gypsum ($CaSO_4 \cdot 2H_2O$) was added at a rate of 20 kg S ha^{-1}, at the St. Claude site only, to all treatments. All fertilizer treatments except the banded P treatment, were broadcast and incorporated into the top 15 cm of soil by using a Rototiller™, one day after application.

Prior to planting, the lentil seeds were adequately inoculated with *R. leguminosarium* culture (lot number NC26, Urbana Culture Superior Inoculant). Plots were sown to lentil using an 8 row Versatile 2200 Zero Till drill with a 25.4 cm spacing between rows, at a rate of 64 kg ha-1 and to a depth of 2.5 cm. For the banded P subtreatment, 18 kg P ha^{-1} as $Ca(H_2PO_4)_2$ was placed 2.5 cm directly below the seed, at the time of planting. The reference crop was sown at a rate of 107 kg ha^{-1} and at a depth of 5 cm, with 18 kg P ha^{-1} as $Ca(H_2PO_4)_2$ banded 2.5 cm directly below the seed.

Weed control at Haywood site was initially carried out by recommended pre-seeding application of glyphosate (Round-up™). At both sites, glyphosate was spot sprayed at a rate of 1 L ha^{-1}, before emergence, and post-emergence Diclofop methyl (Hoegrass 284™) was applied at recommended rates. In addition, weed control was maintained by hand weeding at various times throughout the season.

Plant samples were collected from all plots of the test crop, five times throughout the growing season. Each sampling corresponded to a specific legume growth stage [vegetative development, bud to early bloom, full bloom, pod (green seed), and maturity (harvest)]. The reference crop was also sampled at each of these times. The samples covered a 1.0 m^2 area, consisting of four rows with about 24 plants/row.

The samples were placed in burlap bags and allowed to air dry for one week before being oven dried for at least 48 hours at 65 C. Samples were weighed for dry matter yield determination. Final harvest samples were separated into seed and straw components in order

to determine grain and dry matter yield. All samples were ground to pass a 2 mm mesh screen (Wiley mill). The dry material was analyzed for total N (Nelson and Sommers, 1973), P (Murphy and Riley, 1962), and Cu and Zn contents (Isaac and Kerber, 1980). Nitrogen fixation was determined using the classical difference method. Experimental data were statistically analyzed using the general linear models procedure of the Statistical Analysis System Package (Spector et al., 1985).

Field Experiment, 1986

The field experiment was repeated at the same two sites during the summer of 1986. Only those plots which did not receive banded P in 1985 were used in this second year, to investigate the effects of available P (Table 2) on lentil growth and N_2 fixation. Therefore, the design was altered from a split-block to a randomized complete block (Little and Hills, 1978) and all treatments were replicated four times.

Table 2. Available P on main plot treatments of the 1985 field experiment.†

	Available P on the 1985 Main Plot Treatments‡			
Site	1	2	3	4
	---------- $\mu g\ g^{-1}$ soil ----------			
1	0.0	8.6	21.8	55.0
2	5.0	11.7	20.2	28.7

† Analyses were done on surface samples of soil (0-15 cm).
‡ The main plot treatments of the 1985 field experiment were 1 = 0 kg P ha^{-1}, 2 = 50 kg P ha^{-1}, 3 = 100 kg P ha^{-1}, and 4 = 200 kg P ha^{-1}; and this available P (PO_4^{3-}-P) in 1986 is averaged over the four replicates of each treatment.

Fertilizers were not applied, with the exception of 20 kg S ha^{-1} as $CaSO_4.2H_2O$ at the St. Claude site only. Prior to planting, the lentil seeds were inoculated at the recommended rate with *R. leguminosarium* (Nitragin Company, lot number C26). All plots were sown to lentil using an Allis Chalmers 9 row double disk press drill with an 18 cm spacing between rows, at a rate of 60 kg ha^{-1}. The broadcast plus banded subtreatments from the previous year were used as guard rows. The same reference plots were seeded to wheat at a rate of 90 kg ha^{-1}.

For weed control at both sites, post-emergence Diclofop methyl (Hoegrass 284™) was applied at recommended rates. At the Haywood site, post-emergence metribuzin (Sencor™) was also applied at recommended rates. In addition, weed control was maintained by hand weeding at various times throughout the season.

Plant samples were collected from all plots of the test crop, five times throughout the growing season. Each sampling corresponded to a specific legume growth stage [late vegetative development, bud to early bloom, full bloom, pod (green seed), and maturity (harvest)]. The reference crop was also sampled at each of these times. The samples covered a 1.0 m^2 area, consisting of five rows with about 18 plants/row.

The samples were placed in burlap bags and allowed to air dry for one week before being oven dried for at least 48 hours at 65 C. Samples were weighed for dry matter yield determination. Final harvest samples were separated into seed and straw components in order to determine grain and dry matter yield. All samples were ground to pass a 2 mm mesh screen (Wiley mill). The dry material was analyzed

for total N (Nelson and Sommers, 1973), P (Murphy and Riley, 1962), and Cu and Zn contents (Isaac and Kerber, 1980). Nitrogen fixation was determined using the classical difference method. Experimental data were statistically analyzed using the general linear models procedure of the Statistical Analysis System Package (Spector et al., 1985).

The Effect of Phosphorus and Vesicular-Arbuscular Mycorrhiza on Growth and N_2 Fixation by Lentil

To determine the effect of P and/or VAM on growth and N_2 fixation by lentil, a growth chamber experiment and a lysimeter experiment were conducted. The growth chamber experiment was conducted in 1986 using an Almasippi (Gleyed Rego Black) very fine sandy loam (soil #1) collected from Haywood, MB. The lysimeter experiment was conducted during the summer of 1986 at one site located north of St. Claude on a Willowcrest (Gleyed Orthic Black) loamy fine sand soil (soil #3). The characteristics of the soils used are reported in Tables 3 and 4. The analyses for pH, organic C, inorganic C, NO_3-N, PO_4-P, Cu, and Zn were performed as described in the previous experiment on the effect of P on growth and N_2 fixation by lentil. Sulfate was determined by a turbidimetric method (Hamm et al., 1973). DTPA-extractable Mn and Fe were determined using an atomic absorption spectrophotometer (Follett and Lindsay, 1971). The exchangeable K, Ca, and Mg were extracted with ammonium acetate and analyzed using an atomic absorption spectrophotometer (Isaac and Kerber, 1980).

Table 3. pH and nutrient analysis of soils.†

Soil No.	pH	Organic carbon	Inorganic carbon	NO_3^--N	PO_4^{3-}-P	SO_4^{2-}-S
		%		$\mu g\ g^{-1}$ soil		
1	7.8	3.6	2.1	6.0	7.5	19.4
2‡	7.7	3.8	1.9	10.8	6.9	19.1
3	7.3	2.6	2.1	2.0	5.4	2.8

† Analyses were done on surface samples of soil (0-15 cm).
‡ Soil #2: Analysis of soil #1 after irradiation treatment with Co 60.

Table 4. Nutrient analysis of soils.†

Soil No.	Nutrient						
	Cu	Zn	Mn	Fe	K	Ca	Mg
	$\mu g\ g^{-1}$ soil						
1	0.8	1.0	14.9	40.9	67	7100	1190
2	0.7	1.0	11.8	36.0	60	7940	1300
3	0.6	0.8	12.7	19.4	94	3040	320

† Analyses were done on surface samples of soil (0-15 cm).

Growth Chamber Experiment

The experiment was arranged in a completely-randomized design (Little and Hills, 1978) with two crops, lentil (test crop) and winter rape (reference, non-mycorrhizal crop); three soil treatments, non-irradiated soil, irradiated -VAM soil, and irradiated +VAM soil; and seven P treatments, five for the test crop and two for the reference crop. All treatments were replicated three times. Because winter

rape is a non-mycorrhizal crop, this reference crop did not receive the third soil treatment, irradiated +VAM.

The soil was air dried, ground, sieved (<2 mm), and stored at room temperature until use. Samples of air-dried soil (2500 g) were placed in polyethylene bags and treated with 10 mL of deionized water containing 30 μg N g^{-1} soil as labelled $(^{15}NH_2)_2CO$, 5.07 atom %; and with 10 mL of deionized water containing 100 μg K g^{-1} soil as KCl, 5 μg Cu g^{-1} soil as $CuSO_4 \cdot 5H_2O$, and 10 μg Zn g^{-1} soil as $ZnSO_4 \cdot 7H_2O$ (the resulting solution adding 7 μg S g^{-1} soil, as well). Seven treatments of P were also applied to the soil samples in 10 mL solutions containing 0, 20, 60, 80, or 120 μg P g^{-1} soil as H_3PO_4 for the test crop, and 0 or 120 μg P g^{-1} soil as H_3PO_4 for the reference crop.

After the addition of the nutrients, the soil samples were mixed thoroughly, and two thirds of the samples for the test crop and one half of the samples for the reference crop were irradiated using a cobalt 60 source at a dose of 10,000 Gy (A.E.C.L. Whiteshell Research Station in Pinawa, MB). This resulted in the two soil treatments, non-irradiated and irradiated soil. After irradiation, all bags were placed in pots, and the bags were opened and the soil was allowed to aerate at room temperature for 13 days before seeding.

Lentil and winter rape seeds were surface sterilized with 95% ethanol (30 sec), 1% sodium hypochlorite solution (4 min), and 3 rinsings in double distilled sterile water, and then allowed to dry. Once dry the lentil seeds were inoculated with *R. leguminosarum* (Nitragin Company, lot number C26). Four lentil seeds per pot were planted at a depth of 2.5 cm and four winter rape seeds per pot were

planted at a depth of 0.5 cm. After emergence the pots were thinned to two plants per pot.

At planting, one half of the irradiated samples for the test crop were inoculated with 1 g subsamples of sweet corn (*Zea mays*) roots infected with mycorrhiza (*Glomus intraradicis*), by placing pads of corn roots directly below the lentil seed. This constituted the irradiated +VAM soil treatment. The remaining irradiated samples for the test crop and all irradiated samples for the reference crop received 4 g of autoclaved sweet corn roots and 10 mL of root washings, mixed into the top 3 cm of soil. This constituted the irradiated -VAM soil treatment.

To obtain the VAM infected roots for the irradiated soil +VAM soil treatment, sweet corn was grown in the growth chamber in a coarse grade of calcined montmorillonite (Zorball™) to which an inoculum of *G. intraradicis* had been added. The plants were grown for five weeks using a modified Hoagland's solution (low P) (Tuite, 1978). The roots were harvested and checked for infection. Infected roots were chopped (1 cm fragments), washed, and placed below the lentil seed for the irradiated +VAM soil treatment. For the irradiated -VAM soil treatment, the autoclaved roots were obtained by autoclaving sweet corn roots which were not infected when grown for the irradiated +VAM soil treatment. A filtered effluent obtained from washing VAM infected sweet corn roots was the root washings used in the irradiated -VAM soil treatment.

After planting, all pots were placed in a growth chamber that was maintained at a 16 hour (25 C, 55% R. H.) - 8 hour (16 C, 70% R. H.)

day - night cycle. The light source consisted of General Electric cool whites supplemented with 10% incandescent lamps. Photosynthetically active radiation was measured at 550 μmol photon m^{-2} s^{-1} at the top of the lentil crop canopy. The pots were maintained at field capacity (by weight) by adding water to the soil surface as required.

The plants were harvested when the lentil reached the mid-pod growth stage at 65 days after planting. The shoots were oven-dried for two days at 80 C, weighed for dry matter yield determination, and ground to pass a 2 mm mesh screen (Wiley mill). The roots were collected from each pot and rinsed with distilled water. Twenty 1-cm root segments of lentil were selected and stained to check for VAM infection (Phillips and Hayman, 1970). The dry shoot material was analyzed for total N (Nelson and Sommers, 1973), P (Murphy and Riley, 1962), Cu, Zn, K (Isaac and Kerber, 1980), and S contents (Hamm et al., 1973), and for isotope-ratio analysis of ^{15}N (Bremner, 1965b), modified to use H_2SO_4 in place of H_3BO_3. Nitrogen fixation was determined using the dilution technique (McAuliffe et al., 1958). Experimental data were statistically analyzed using the general linear models procedure of the Statistical Analysis System Package (Spector et al., 1985).

Lysimeter Experiment

The experiment using lysimeters was arranged in a randomized complete-block design (Little and Hills, 1978) with four P treatments and two soil treatments, non-inoculated and inoculated, for lentil (test crop). An additional 8 lysimeters were incorporated into the

design to accommodate winter rape (reference, non-mycorrhizal crop) with two P treatments and one soil treatment (non-inoculated). Each treatment for the test and references crops was replicated 4 times.

The open-ended steel lysimeters were 34 cm in height, with a cross sectional area of 0.10 m^2. Each lysimeter was pressed into the soil with a front end loader until only the top 3 cm remained above ground. The top 10 cm of soil in each lysimeter was removed, and was treated with 1 L of solution containing 200 kg K ha^{-1} as KCl and 30 kg N ha^{-1} and 34 kg S ha^{-1} as ^{15}N enriched (5.07%) $(NH_4)_2SO_4$; and with 1 L of solution containing 0, 60, 120 or 200 kg P ha^{-1} as H_3PO_4 for the test crop and 0 or 200 kg P ha^{-1} as H_3PO_4 for the reference crop. The soil was then mixed thoroughly, and placed back into the lysimeter. The lysimeters were seeded the following day.

Lentil seeds were inoculated with four times the recommended rate (2.8 kg/700 kg seed) of *R. leguminosarium* (Nitragin Company, lot number C26). For the non-inoculated soil treatment, the top 2.5 cm of soil was removed from the lysimeter and mixed with about 10 g of autoclaved corn roots. For the test crop, 20 lentil seeds were planted at the 2.5 cm depth, using a 0.10 m^2 template. The template was then removed, and the soil containing the autoclaved roots was placed back in the lysimeter, covering the seeds. For the reference crop, the soil containing the autoclaved roots was placed back in the lysimeter, and then the winter rape was planted, again using the template. The template was simply placed in the lysimeter, on top of the soil, and seeds inserted at a depth of 0.5 cm.

For the inoculated soil treatment, for the test crop, the top 3 cm

of soil was removed from the lysimeter, the template inserted, and 0.5 g subsamples of sweet corn roots infected with mycorrhiza (*G. intraradicis*) placed in each hole. The template was removed, a thin layer of soil placed over the mycorrhiza, and then 20 lentil seeds were planted using the 0.10 m^2 template. The template was then removed, and the remaining soil was used to cover the seeds.

The autoclaved sweet corn roots for the non-inoculated soil treatment and the sweet corn roots infected with mycorrhiza for the inoculated soil treatment were obtained as described in the procedure for the irradiated soil -VAM and irradiated soil +VAM soil treatments in the growth chamber experiment on the effect of P and VAM on growth and N_2 fixation by lentil.

Guard rows containing either lentil or winter rape seeds were sown around the lysimeters to ensure a crop canopy.

Lack of moisture caused poor germination and resulted in all lysimeters, including those seeded with winter rape, to be resown one week later. Three days after emergence, the lysimeters were thinned to 12 lentil plants or 15 winter rape plants per lysimeter.

Weed control was maintained by handweeding throughout the growing season. Diclofop Methyl (Hoegrass 1™) at a rate of 12 mL L^{-1} was used to control some of weeds, by spot spraying with a back pack sprayer.

All lysimeters were harvested when the lentil had reached the pod stage at 65 days after planting. The above ground portions of the plants in each lysimeter were collected. The samples were placed in burlap bags and allowed to air dry for 1 week before being oven dried for at least 48 hours at 65 C. Samples were then weighed for dry

matter yield determination, and ground to pass a 2 mm mesh screen (Wiley mill). The lysimeters, with their contents, were removed, and roots were collected by running water through each lysimeter until all soil was removed. Twenty 1-cm root segments of lentil were selected and stained to check for VAM infection (Phillips and Hayman, 1970). The dry shoot material was analyzed for total N (Nelson and Sommers, 1973), P (Murphy and Riley, 1962), Cu, and Zn contents (Isaac and Kerber, 1980), and for isotope-ratio analysis of ^{15}N (Bremner, 1965b), modified to use H_2SO_4 in place of H_3BO_3. Nitrogen fixation was determined using the dilution technique (McAuliffe et al., 1958). Experimental data were statistically analyzed using the general linear models procedure of the Statistical Analysis System Package (Spector et al., 1985).

RESULTS AND DISCUSSION

The Effect of Phosphorus on Growth and N_2 Fixation by Lentil

To determine the effect of P on growth and N_2 fixation by lentil, a growth chamber experiment and a field experiment were conducted.

Growth Chamber Experiment

With the lentil crop, no differences in growth were observed among treatments during the first 10 days after emergence. At 15 days, all the lentil plants showed tip-burning on the lower leaves. In the non-irradiated soil treatments and in the irradiated soil treatments of 80 and 100 μg P g^{-1} soil, the lentil recovered from this symptom with time. In the other irradiated P treatments, the lentil crop did not recover. With the lower P treatments on the irradiated soil treatment, the lower leaves became more chlorotic until finally they dropped off. The upper leaves and stem remained pale green but very little growth occurred. After about 40 days, the lentil in the 40 μg P g^{-1} soil irradiated soil treatments started to grow, and after about 60 days the lentil in the other P irradiated soil treatments also developed new growth. These increases were negligible however, since the harvest was conducted at 64 days.

In the non-irradiated treatments, shoot dry matter increased significantly with the addition of 40 and 80 μg P g^{-1} soil (Figure 1a and Table 5). However, no further increase in yield was observed with the addition of 100 μg P g^{-1} soil. The addition of P in the irradiated soil treatment did not increase yield significantly until 80 μg P g^{-1}

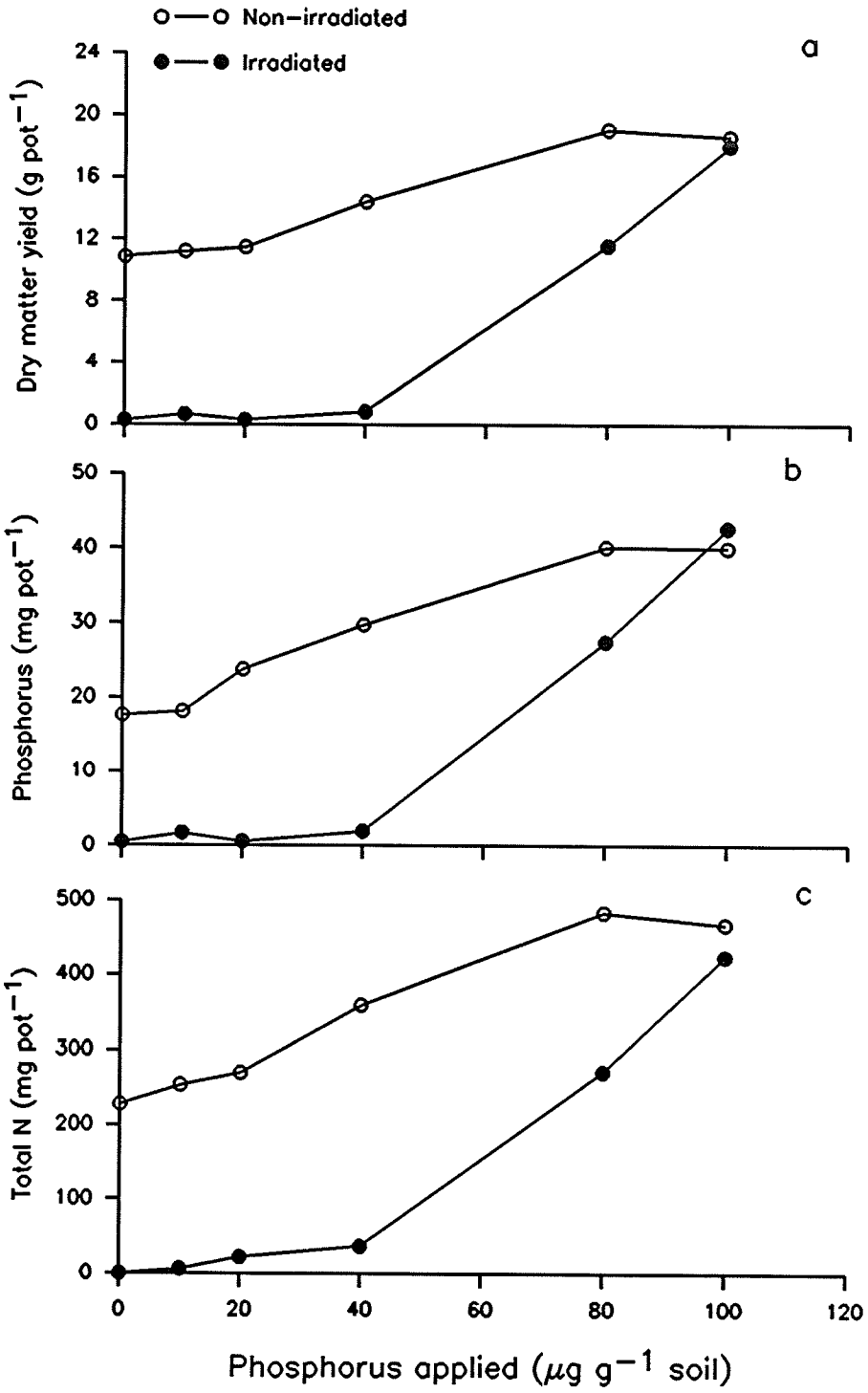

Figure 1. The effect of phosphorus application on dry matter yield (a), P uptake (b), and total N uptake (c) by lentil.

Table 5. The effect of phosphorus application on dry matter yield, phosphorus and total nitrogen uptake, and N_2 fixation by lentil.

Phosphorus applied	Dry Matter yield	Phosphorus uptake	Total N uptake	N_2 fixed
$\mu g\ g^{-1}$ soil	g pot^{-1}	---------- mg pot^{-1} ----------		
Non-irradiated soil treatment				
0	10.9 C†	17.6 C	227.6 C	149.1 C
10	11.2 C	18.2 C	253.2 C	174.7 C
20	11.5 C	23.8 BC	269.4 C	190.9 C
40	14.4 B	29.8 B	359.4 B	280.9 B
80	19.1 A	40.2 A	483.3 A	404.9 A
100	18.6 A	40.0 A	467.3 A	388.9 A
Irradiated soil treatment‡				
0	n.d.	n.d.	n.d.	n.d.
10	n.d.	n.d.	n.d.	n.d.
20	n.d.	n.d.	n.d.	n.d.
40	n.d.	n.d.	n.d.	n.d.
80	11.6	27.5	270.8	40.6
100	18.0	42.7	424.0	193.4

† Means followed by the same letter for each soil treatment are not significantly different at P=0.05 (Tukey's Studentized Range Test).
‡ Statistical analyses were not calculated for this soil treatment.

soil was added (Figure 1a and Table 5). Increases in dry matter yield with added P, for both the non-irradiated and the irradiated 80 and 100 $\mu g\ P\ g^{-1}$ soil treatments, were more the result of increase branching rather than increases in plant height.

Increased P uptake in the lentil shoots resulted with each addition of P up to 80 $\mu g\ g^{-1}$ soil in the non-irradiated soil treatment (Figure 1b and Table 5). Shoot P concentration ranged from 0.16% for the 0 $\mu g\ P\ g^{-1}$ soil treatment to 0.21% for the 80 $\mu g\ P\ g^{-1}$ soil treatments. Significant increases in shoot P uptake were observed at the 20, 40 and 80 $\mu g\ P\ g^{-1}$ soil treatments (Figure 1b and Table 5). No further increase in P uptake was observed with the addition of 100 $\mu g\ P\ g^{-1}$ soil. The lentil grown in the irradiated soil treatments also responded to the addition of P (Figure 1b and Table 5) with a statistically significant increase in shoot P uptake at both the 80 and 100 $\mu g\ P\ g^{-1}$ soil treatments. The concentration of shoot P, at both P treatments, was 0.24%.

Total plant N increased with each addition of P up to 80 $\mu g\ P\ g^{-1}$ soil in the non-irradiated soil treatment. The concentration of N in the plant also increased with increasing rates of P up to 80 $\mu g\ g^{-1}$ soil, from 2.1% to 2.5%. No additional uptake of N was observed with the addition of 100 $\mu g\ P\ g^{-1}$ soil (Figure 1c and Table 5). The lentil in the irradiated soil treatment also responded with increased N uptake for both the 80 and 100 $\mu g\ P\ g^{-1}$ soil applications (Figure 1c and Table 5).

The winter rape crop showed brown and chlorotic leaves in both the non-irradiated and irradiated soil treatments, and at both levels

of P applied. Those plants grown in the irradiated soil treatment showed more branching and more vegetative growth than the crop grown in the non-irradiated soil treatment. The application of P resulted in a slightly earlier maturation date, as compared to those plants which did not receive P.

Unlike the lentil, growth of the winter rape was not increased with added P. Dry matter yield, P uptake, and total N uptake were not significantly affected by the addition of 100 μg P g^{-1} soil (Figure 2 and Table 6). This crop did, however, respond favourably to soil irradiation, with at least a two fold increase in yield, P uptake, and total N uptake, as compared to the non-irradiated treatment (Figure 2 and Table 6).

The ability of lentil to utilize P in the absence of phosphate solubilizing microorganisms and VAM was also measured by using a soil irradiation treatment, in addition to a P treatment, as discussed above. However, when selecting the dose of irradiation to use in this experiment, generalities were made regarding the radiosensitivity of the different groups of organisms (fungi being the most sensitive, actinomycetes intermediate, and bacteria following). The nature of the microflora which survives irradiation however, depends on a number of factors including soil moisture (at the time of irradiation), amount of soil organic matter (protective effect for some microorganisms), soil type, and the initial microbial population (Cawse, 1975). As a result, it was virtually impossible to predict the level of irradiation needed to selectively sterilize the soil.

The dose of irradiation decided on, 10,000 Gy (1.0 Mrad),

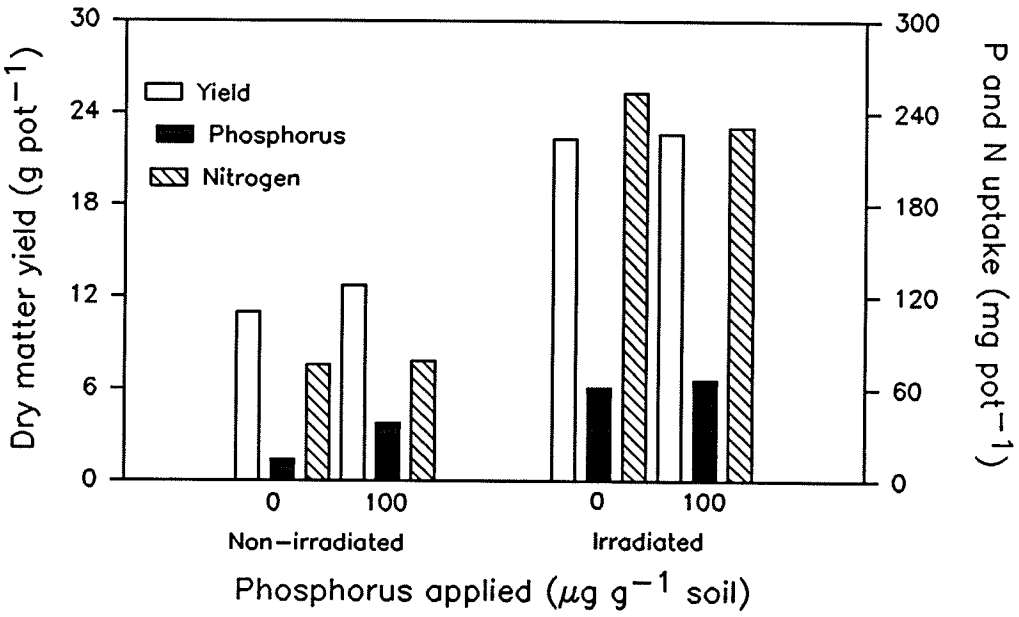

Figure 2. The effect of phosphorus application on dry matter yield, P uptake, and total N uptake by winter rape.

Table 6. The effect of phosphorus application on dry matter yield, and phosphorus and total nitrogen uptake by winter rape.

Phosphorus applied	Dry Matter yield	Phosphorus uptake	Total N uptake
$\mu g\ g^{-1}$ soil	g pot^{-1}	---------- mg pot^{-1} ----------	
Non-irradiated soil treatment			
0	10.95	14.13	75.71
100	12.70	37.59	78.36
Irradiated soil treatment			
0	22.31	60.90	252.93
100	22.65	65.91	230.59

although similar to doses generally applied to soils to eliminate VAM, (Jakobsen and Anderson, 1982) resulted in a substantial release of plant available nutrients, mainly P and N. This release can be seen from the soil analysis done at the end of the experiment (Table 7). The release was probably brought about by lysed organisms, primarily fungal, and the action of irradiation on the organic compounds (humus) (Mclaren, 1969).

It appears that the winter rape was quite efficient in absorbing the P and other nutrients released upon irradiation, without the aid of microbial interactions. In addition, the irradiated treatment released sufficient P in the 0 μg P g^{-1} soil treatment to satisfy plant growth. The differences in winter rape growth between the non-irradiated and irradiated soil treatments suggests that N, not P, was limiting growth. With increased N released by irradiation (Table 7), the winter rape yields doubled (Figure 2), but still there was no response to P application. The ability to take up the nutrients released by irradiation, resulted in larger winter rape plants compared to those grown in the non-irradiated soil treatment (Figure 2). Conversely, the lentil root system was not able to take up the nutrients released by irradiation, resulting in yield depression at the 0 to 40 μg P g^{-1} soil irradiated soil treatments. The potential benefits of soil sterilization, i.e., release of nutrients, seem to be masked by the inability of the root system to uptake these nutrients. Perhaps the lack of microbial associations to aid in the uptake of essential nutrients contributed to the growth depression.

Graham (1988) showed VAM colonization to occur after seed

Table 7. Soil analysis after crop growth.

Phosphorus added	Non-irradiated soil		Irradiated soil	
	PO_4^{3-}-P	$(NO_3^- + NO_2^-)$-N	PO_4^{3-}-P	$(NO_3^- + NO_2^-)$-N
	$\mu g\ g^{-1}$ soil			
Lentil				
0	4.0	1.4	13.0	66.7
10	5.6	1.1	15.0	74.9
20	6.7	0.9	17.9	72.0
40	9.7	0.9	24.4	58.9
80	16.8	0.7	32.6	1.5
100	18.7	1.0	41.0	0.4
Winter rape				
0	4.0	1.4	8.0	1.1
100	19.9	1.2	30.9	2.3

germination, when the radicle is growing quickly and seed P reserves are depleted. In the non-irradiated treatments, mycorrhizal infection was stimulated in the zone of elongation where root exudation is known to be greatest. Once the infection occurred, root P levels may have increased (increased uptake from the soil) causing root permeability to decrease along with root exudation (Graham et al., 1981). These plants, therefore, responded to P application after the 10 days. As stated previously, in this experiment at the low P application rates, differences between treatments (non-irradiated and irradiated soil treatment) for lentil were noted after 10 days of growth. Perhaps at this point, seed nutrient reserves became limiting and the lentil plants, grown in the irradiated low P soil, were unable to take up P from the soil without the mycorrhiza present and hence, showed growth

depression. Lack of P uptake may have resulted in P (phospholipid) depletion in the root membranes (Ratnayake et al., 1978). The increased permeability due to lipid depletion may have resulted in increased root exudation and consequently the decrease in plant growth. Nutrient retention therefore may have been impaired in this treatment at the lower level of applied P.

In addition, without mycorrhiza present in the irradiated soil treatment, susceptibility to pathogen invasion may have increased because of this "leaky roots" problem (Graham, 1988). Furthermore, VAM can reduce plant response to soil stresses such as minor element toxicities (Linderman, 1988). Without VAM present, the lentil may have developed some minor element imbalance resulting in suppressed growth.

The shape of the curves for both the non-irradiated and irradiated treatments for the variable dry matter yield should also be noted (Figure 1a). The lentil grown in the non-irradiated soil treatment shows a typical sigmoidal growth response to added P, the curves reaching a plateau at the higher rates of P. On the other hand, the lentil grown in the irradiated soil treatment showed a linear response at the higher rates of P, indicating that increased growth would have continued with further additions of P. Also, the threshold level of P needed to obtain a growth response was less in the non-irradiated (mycorrhizal) soil treatment, suggesting that the mycorrhiza reduced the time required for lentil to respond to low soil P levels.

Bolan et al. (1983) grew subterranean clover in soil low in

available P. With the addition of VAM, the clover growth was promoted at the low P levels. Conversely, ryegrass growing in the same soil, was not affected by presence or absence of VAM. They suggested that the extensive, fairly divided root system of ryegrass provided a large surface area resulting in efficient P uptake even when the P concentration in the soil solution is low. The thicker roots of subterranean clover were not effective unless VAM increased the surface area of the roots. Bolan et al. (1983) also noted that the rate of transport of P to the plant by diffusion depends on its concentration at the plant root surface, the distance it must move, and the concentration of P in the bulk soil. If by adding VAM, root surface area and root density is increased, this in turn would enhance uptake and allow greater desorption of P from the soil particles thus establishing a steeper gradient for P movement to the root.

In this experiment, the lentil grown in the non-irradiated (mycorrhizal) soil treatment, may have been able to increase the concentration of P near the roots reducing the lag time required to get a response to P when it was added at low levels, thereby reducing the initial phase of the sigmoidal curve. The lentil grown in the irradiated soil treatment, without the mycorrhizal association, took longer to get established. Once established however, the benefits of irradiation can be seen. Perhaps at the higher P additions, the concentration of P near the root surface was adequate for plant growth.

As the amount of P added reached 100 $\mu g\ g^{-1}$ soil in the non-irradiated soil treatment, no additional benefit in plant growth or P

uptake was seen. Figures 1a and 1b actually show slight decreases in both growth and P uptake at this level. Perhaps at 100 μg P g^{-1} soil added, membrane permeability decreased substantially and P concentration near the roots was adequate, consequently mycorrhizal infection neither enhanced P uptake or reduced exudation. The mycorrhizal association may even have caused growth depression since it requires as much as 10% of the carbon allocated to the roots (Koch and Johnson, 1984).

At the termination of the experiment, the lentil roots were not checked for mycorrhizal infection. However, before the start of the experiment, the soil was checked for native VAM spores using a modified version of a flotation and bubbling apparatus for collecting spores developed by Furlan and Fortin (1975). VAM spores were found, which was expected as the soil was obtained from a field infested with quack grass, a weed that is known to be heavily mycorrhizal (Dr. Krikun, personal communication). The data obtained from the non-irradiated soil treatment also suggests the lentil roots were infected.

It should be noted that radiation may only inhibit cell division and not affect microbial metabolic activity, and that soil enzymes rarely decline after irradiation even at doses sufficient to inactivate all microorganisms (Cawse, 1975). Thus it is possible to increase certain enzymatic processes by irradiation, such as nitrification, without the proliferation of nitrifier cells (McLaren, 1969). Cawse and Crawford (1967) noticed that organic calcareous soils irradiated with 0.6 to 2.5 Mrads (6000 to 25000 Gy) showed a

sharp rise in nitrite (NO_2^-) concentration. They concluded that the rise was due to the reduction of nitrate and not the inhibition of *Nitrobacter*. This NO_3^- reduction was thought to occur through radioresistant enzyme systems of denitrifying bacteria (i.e., *Bacillus* and *Pseudomonas* spp.) which favour soils with high pH. In this experiment the production of NO_2^- may have occurred in the irradiated soil treatment, and the sensitivity to NO_2^- accumulation may have been different for the two crops grown. This may also help to explain the differences in growth observed between the two crops.

Each addition of P enhanced N_2 fixation in the non-irradiated treatments (Figure 3a). Statistically significant increases were noted with the application of 40 and 80 μg P g^{-1} soil (Table 5). No further increase was seen with the addition of 100 μg P g^{-1} soil. In the irradiated soil treatment, little N_2 fixation was observed with P additions less than 80 μg P g-1 soil (Figure 3a and Table 5). Under these conditions, plants growth was not stimulated (Figure 1a).

A linear regression (Figure 3b) shows that N_2 fixation had a high correlation with P application, in the non-irradiated soil treatment. The correlation was lower with the irradiated soil treatment, because plant growth, and hence N_2 fixation, did not occur at P rates less than 80 μg g-1 soil (Figure 3b).

Figure 4a shows the relationship between dry matter accumulation and N_2 fixation. For the non-irradiated treatment, the addition of 10 and 20 μg P g^{-1} soil did not increase dry matter accumulation or N_2 fixation significantly over the 0 μg P g^{-1} soil treatment. The addition of 40 μg P g^{-1} soil lead to a 25% increase in dry matter yield

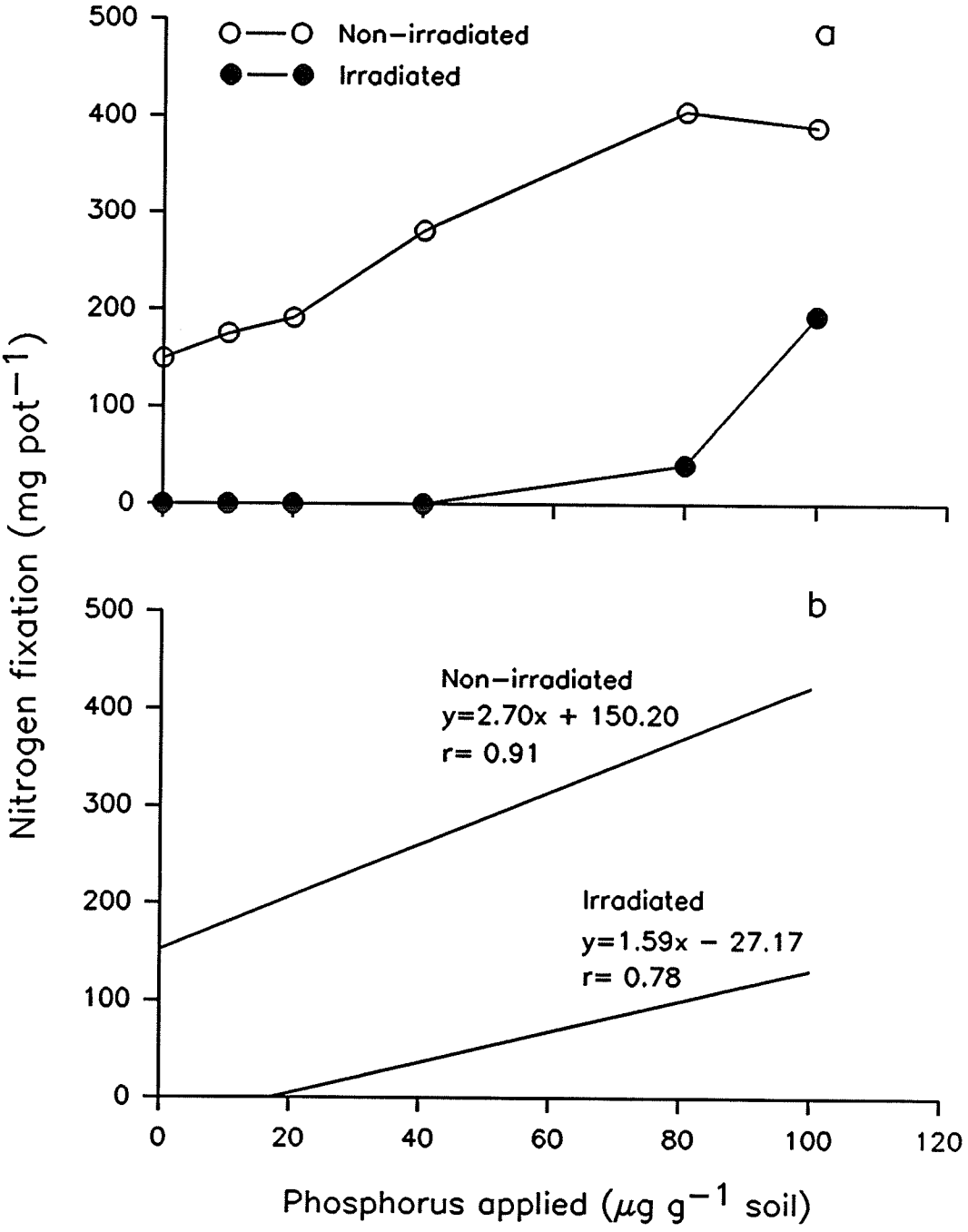

Figure 3. The effect of phosphorus application on N_2 fixation by lentil (a), and the correlation between P applied and N_2 fixation (b).

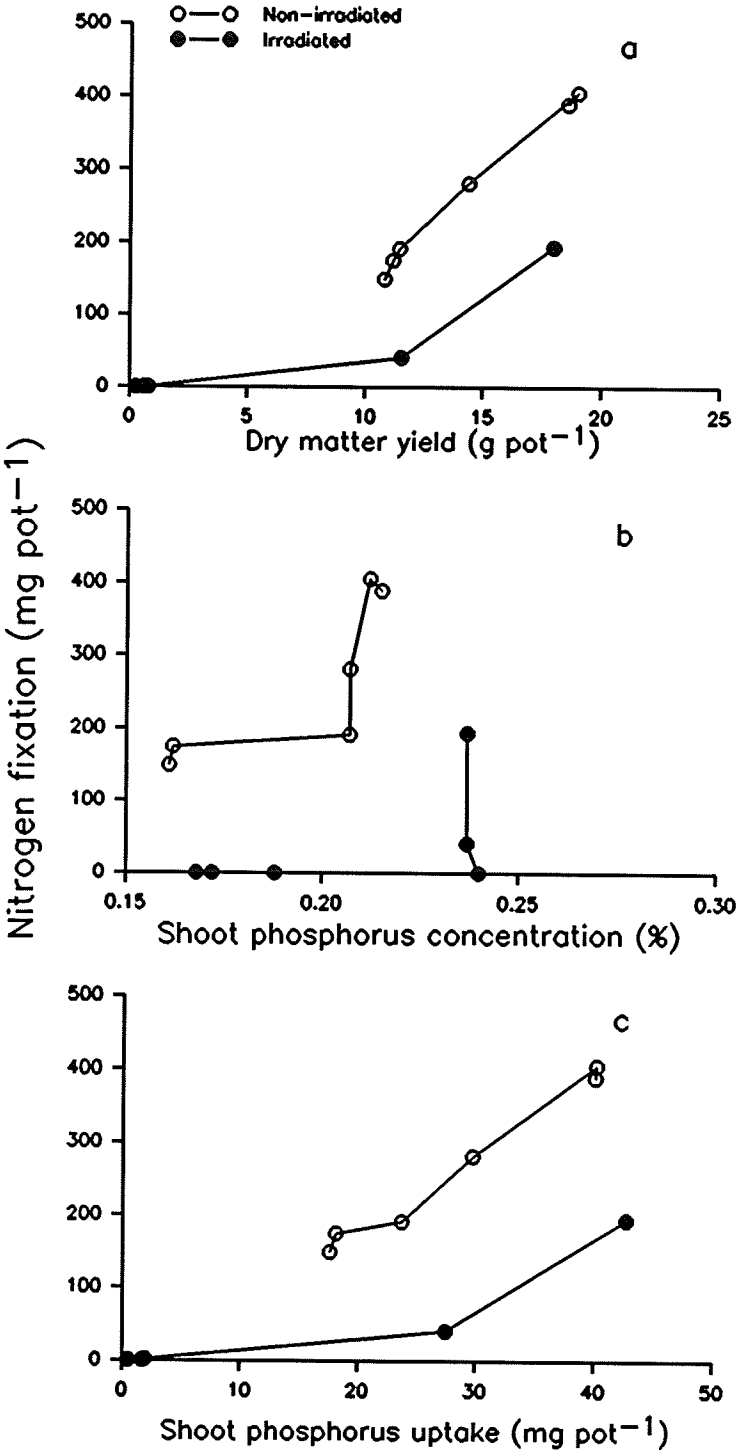

Figure 4. The effect of dry matter accumulation (a), shoot P concentration (b), and shoot P accumulation (c) on dinitrogen fixation by lentil. (Data points represent a stepwise increase in applied P from 0 to 100 μg g-1 soil.)

and a 47% increase in N_2 fixation over the 20 μg P g^{-1} soil treatment. A further increase in P added from 40 to 80 μg g^{-1} soil lead to a 33% increase in dry matter accumulation and a 44% increase in N_2 fixation. The addition of P had a greater effect on N_2 fixation than on dry matter accumulation. No further increase in dry matter yield or N_2 fixation occurred with the addition of 100 μg P g^{-1} soil.

The relationship between shoot P concentration and N_2 fixation is shown in Figure 4b. The shoot P levels increased significantly with the addition of 20 μg P g^{-1} soil as compared to the 0 and 10 μg P g^{-1} soil non-irradiated treatments, however, this did not result in significant increases in N_2 fixation. Above this threshold value, with the addition of 40 and 80 μg P g^{-1} soil, N_2 fixation was stimulated. No further increase in shoot P concentration occurred with the addition of 100 μg P g^{-1} soil and consequently no further enhancement of the N_2 fixing system was observed. In the irradiated soil treatment, the threshold value of P for increased N_2 fixation could not be determined as growth occurred only at P application rates greater than 80 μg g^{-1} soil. The threshold value of P for increased N_2 fixation was not evident in the relationship between shoot P uptake and N_2 fixation (Figure 4c).

As reported, for the lentil grown in the non-irradiated soil treatment, the effect of P on N_2 fixation was examined. Many researchers have suggested the main effect of P in legumes is in shoot metabolism (Jakobsen, 1985, Graham and Rosas, 1979). At higher levels of added P, plant growth and photosynthetic capacity increase along with carbohydrate supply to the nodule. The amount of P_i in the plant

may also be important since the level available to the cytoplasm probably regulates partitioning of photosynthate between accumulation of starch and formation of translocated sucrose (Heldt et al., 1977).

Other researchers have suggested the main effect of P is its direct effect of the N_2 fixing system (Gates, 1974; Isreal, 1987). Within the nodule, nitrogenase activity requires high levels of P_i and photosynthate to support production of ATP and reductant required by the enzyme (Bergensen, 1971). Growth and maintenance of the nodule itself and of enclosed bacteroids also generate a high energy (P) demand.

In this experiment the addition of P resulted in larger percent increases in N_2 fixation than dry matter accumulation. This could simply be due to a bigger plant with a bigger nitrogen sink, causing N_2 fixation to increase faster than dry matter accumulation. Plant response to P addition in the non-irradiated soil treatment was noted about 10 days after emergence, and was seen as an increase in dry matter accumulation. With this response occurring so early in the experiment it would have been virtually impossible to do a time course study to find if the addition of P increased nitrogenase activity (acetylene reduction) before increasing plant growth. Percent P in shoot tissue and total P accumulation increased with increased levels of P and this was associated with increases in N_2 fixation. As well percent N in tissue showed a modest increase (perhaps because carbon/nitrogen ratio was fairly constant) with increasing levels of P from 2.1 to 2.5%.

The accumulation of Cu and Zn, as affected by the level of P

added can be seen Figures 5a and 5b and Table 8. It appears that the differences in total uptake largely reflect the lentil response to P additions by an increase in dry matter accumulation. Plant Cu concentration remained at about 14 $\mu g\ g^{-1}$ tissue regardless of P level in the non-irradiated soil treatments. The concentration in the lentil grown in the irradiated soil treatment decreased over the P additions from 22 $\mu g\ g^{-1}$ tissue with no P added to 15 $\mu g\ g^{-1}$ tissue at the highest (100 $\mu g\ P\ g^{-1}$ soil) addition.

Plant Zn concentration remained constant regardless of P treatment at about 50 $\mu g\ g^{-1}$ tissue in the non-irradiated soil. The lentil grown in the irradiated soil treatment showed slight increases in Zn concentration with increasing rates of P, from 40 $\mu g\ g^{-1}$ tissue to 60 $\mu g\ g^{-1}$ tissue.

Field Experiment, 1985

Plant samples were collected, after visual observations were done, at both sites five times throughout the growing season, each sampling corresponding to a specific legume growth stage [vegetative development, bud to early bloom, full bloom, pod (green seed), and maturity (harvest)]. For the first four samplings, there was little or no effect of added P on yield and nutrient uptake (see Appendix A for data and statistical analysis).

Emergence was noted at both sites, and for both crops, 10 days after seeding. Adequate soil moisture initially gave the seedling a good start, but low precipitation throughout the growing season (<20

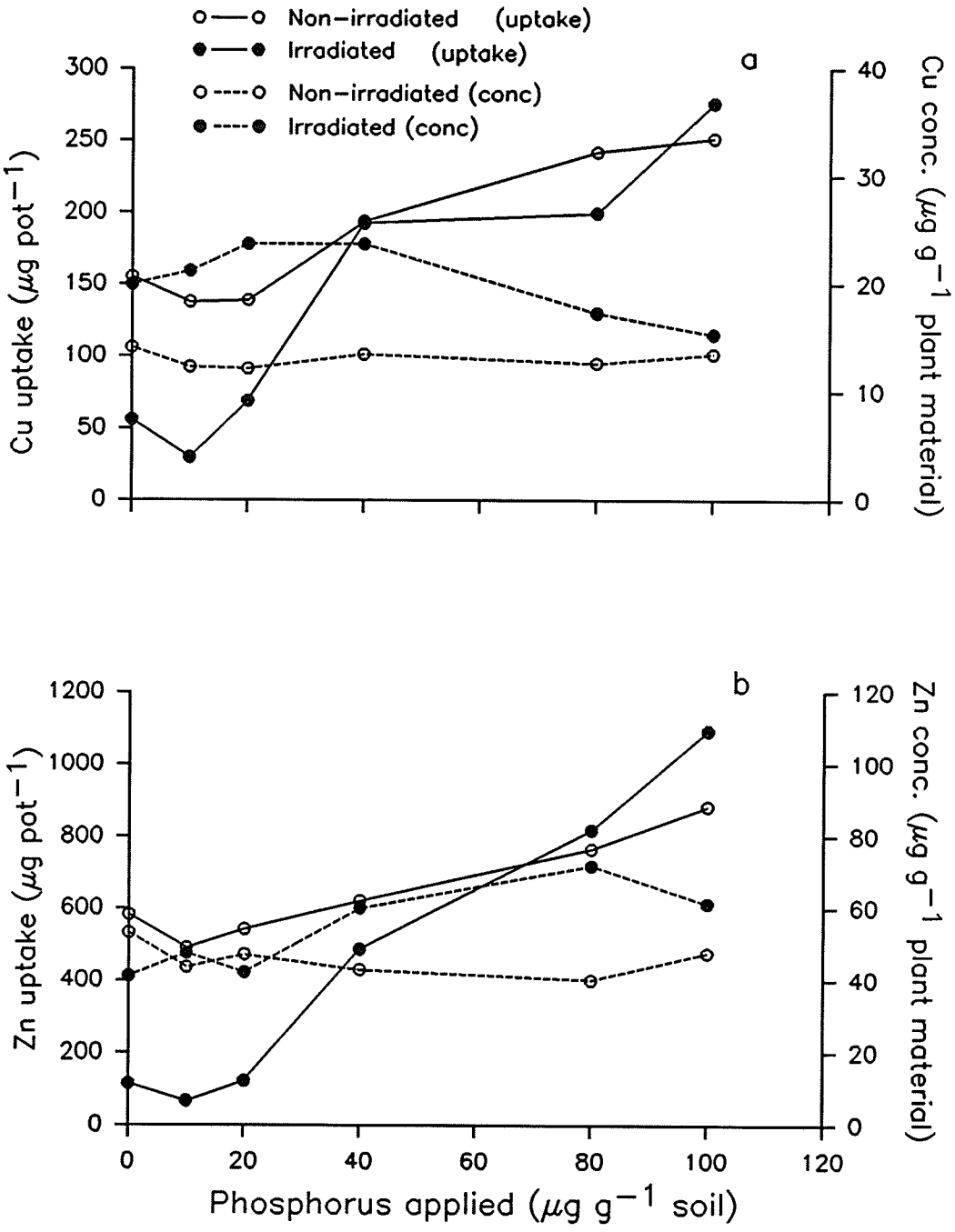

Figure 5. The effect of phosphorus application on Cu uptake and concentration (a), and Zn uptake and concentration (b) by lentil.

Table 8. The effect of phosphorus application on Cu and Zn uptake by lentil.

Phosphorus applied	Cu uptake	Zn uptake
$\mu g\ g^{-1}$ soil	---------- $\mu g\ pot^{-1}$ ----------	
Non-irradiated soil treatment		
0	156 CD†	582 BC
10	138 D	490 C
20	139 D	541 C
40	194 BC	620 BC
80	242 AB	765 AB
100	251 A	883 A
Irradiated soil treatment‡		
0	n.d.	n.d.
10	n.d.	n.d.
20	n.d.	n.d.
40	n.d.	n.d.
80	200	831
100	275	1111

† Means followed by the same letter for each soil treatment are not significantly different at P=0.05 (Tukey's Studentized Range Test).
‡ Statistical analyses were not calculated for this soil treatment.

mm rainfall), as well as an infestation of weeds, caused considerable stress to the crops. The lentil crop seemed to adapt to the low precipitation by becoming more determinant in its growth. Herbicide application during vegetative development resulted in an apparent yellowing of the plants, for approximately one week. An infestation of grasshoppers severely damaged the reference crop, and as a result, N_2 fixation of the lentil crop was not determined.

Although some shattering of pods occurred at final harvest, regardless of P treatment, the lentil grain yields were comparable to those obtained by Manitoba producers (Manitoba Agriculture, 1982).

Total dry matter increased significantly with the addition of 50 kg P ha^{-1} with the broadcast P treatment and with the addition of 68 kg P ha^{-1} with the broadcast plus banded P treatment, but at the Haywood site only (Figure 6a and Table 9). There was no significant increase in total dry matter yield, with the application of either P treatment, at the St. Claude site (Figure 6b and Table 9). As well, lentil grain yield, at either site, did not respond significantly to the application of P (Figure 6 and Table 9).

At the Haywood site, a significant increase in P uptake by the lentil straw occurred with the application of 100 and 200 kg P ha^{-1} with the broadcast P treatment and with the application of 218 kg P ha^{-1} with the broadcast plus banded P treatment (Figure 7a and Table 10). The P uptake in the lentil grain also showed a significant response to P application, at the 50 kg P ha^{-1} with the broadcast P treatment and at the 68 kg P ha^{-1} with the broadcast plus banded P treatment (Figure 7a and Table 10). At the St. Claude site, as at the

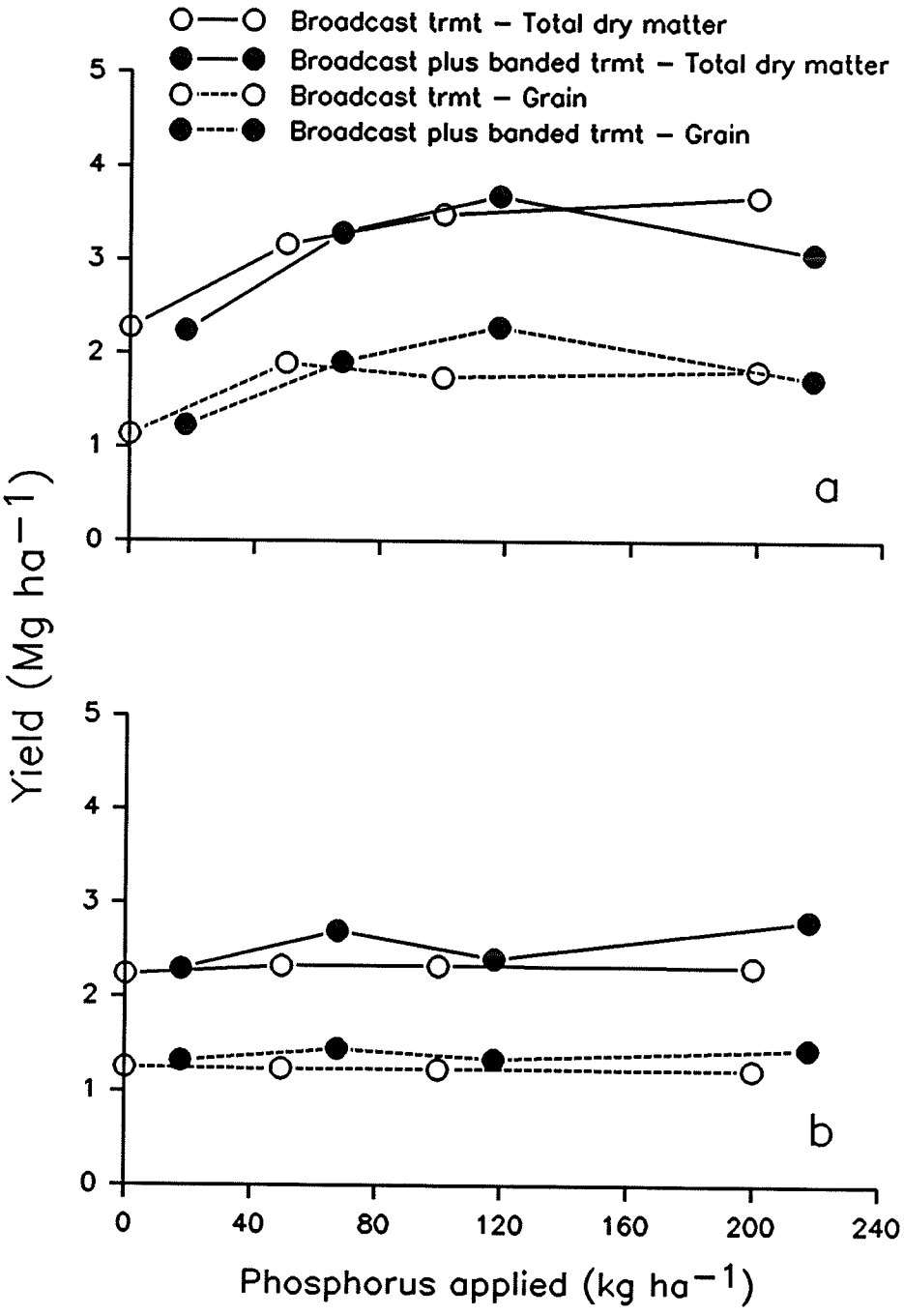

Figure 6. Effect of applied P on total dry matter and grain yield of lentil, at the Haywood site (a), and at the St. Claude site (b).

Table 9. Effect of phosphorus application on total dry matter and grain yield of lentil, at the two field sites.

Phosphorus applied	Haywood site		St. Claude site	
	Total dry matter	Grain	Total dry matter	Grain
kg ha^{-1}	---------- Mg ha^{-1} ----------			
Broadcast treatment				
0	2.275 B†	1.136 A	2.240 A	1.255 A
50	3.165 A	1.894 A	2.328 A	1.240 A
100	3.488 A	1.746 A	2.328 A	1.234 A
200	3.673 A	1.821 A	2.318 A	1.227 A
Broadcast plus banded treatment				
18	2.243 B	1.234 A	2.295 A	1.325 A
68	3.290 A	1.911 A	2.698 A	1.453 A
118	3.683 A	2.285 A	2.403 A	1.341 A
218	3.070 AB	1.727 A	2.813 A	1.451 A

† Means followed by the same letter for each site and within treatments, are not significantly different at P=0.05 (Tukey's Studentized Range Test).

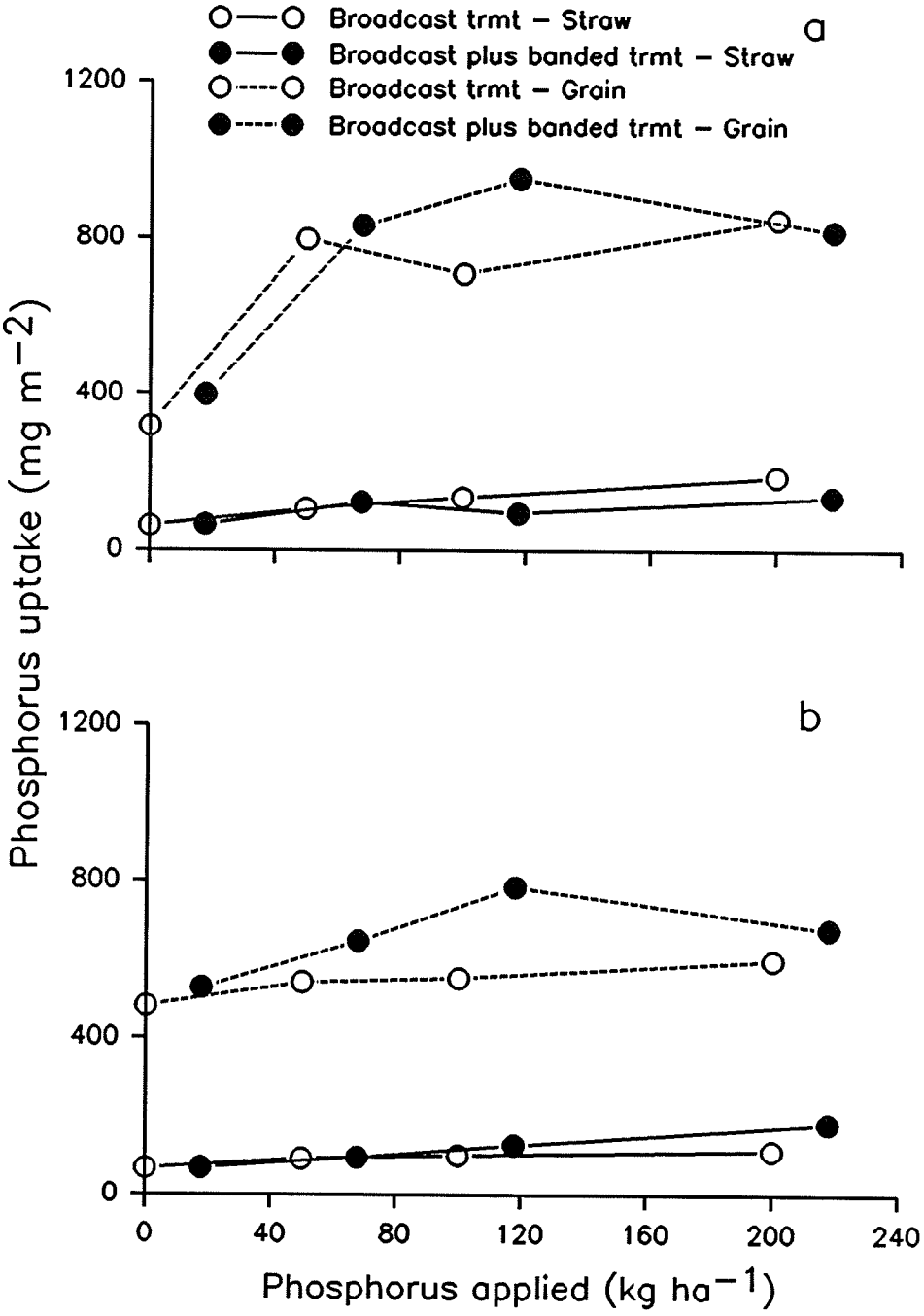

Figure 7. Effect of applied P on P uptake by straw and grain of lentil, at the Haywood site (a), and at the St. Claude site (b).

Table 10. Effect of phosphorus application on P uptake by straw and grain of lentil, at the two field sites.

Phosphorus applied	Haywood site		St. Claude site	
	Straw	Grain	Straw	Grain
kg ha^{-1}	---------- mg m^{-2} ----------			
Broadcast treatment				
0	63 C†	318 B	66 A	481 A
50	106 BC	797 A	91 A	540 A
100	135 AB	709 AB	99 A	551 A
200	188 A	847 A	112 A	596 A
Broadcast plus banded treatment				
18	66 B	398 B	68 C	527 A
68	123 AB	832 A	94 BC	645 A
118	96 AB	952 A	126 B	783 A
218	136 A	817 A	181 A	675 A

† Means followed by the same letter for each site and within treatments, are not significantly different at P=0.05 (Tukey's Studentized Range Test).

Haywood site, a significant increase in P uptake by the lentil straw occurred, but only with the application of 118 and 218 kg P ha^{-1} with the broadcast plus banded P treatment (Figure 7b and Table 10). Lentil grain P uptake, at the St. Claude site, did not respond significantly to the application of either P treatment (Figure 7b and Table 10).

Total plant N in the dry matter was significantly increased with the application of 200 kg P ha^{-1} with the broadcast P treatment, but at the Haywood site only (Figure 8a and Table 11). With the broadcast plus banded P treatment, total plant N in the dry matter and grain was significantly increased with the application of 68 kg P ha^{-1}, but again at the Haywood site only (Figure 8a and Table 11). Total plant N in dry matter or grain was not significantly affected by the application of P, at the St. Claude site (Figure 8b and Table 11). As with the samples from the first four growth stages, N_2 fixation by the lentil crop at final harvest was not measured at either site, due to an infestation of grasshoppers which destroyed the reference crop.

The Cu and Zn uptake in lentil straw and grain generally decreased with increased P application (Figures 9 and 10 and Tables 12 and 13). However, only the decrease in Zn uptake in lentil grain at the St. Claude site, observed with the broadcast P treatment, was statistically significant (Figure 10b and Table 13).

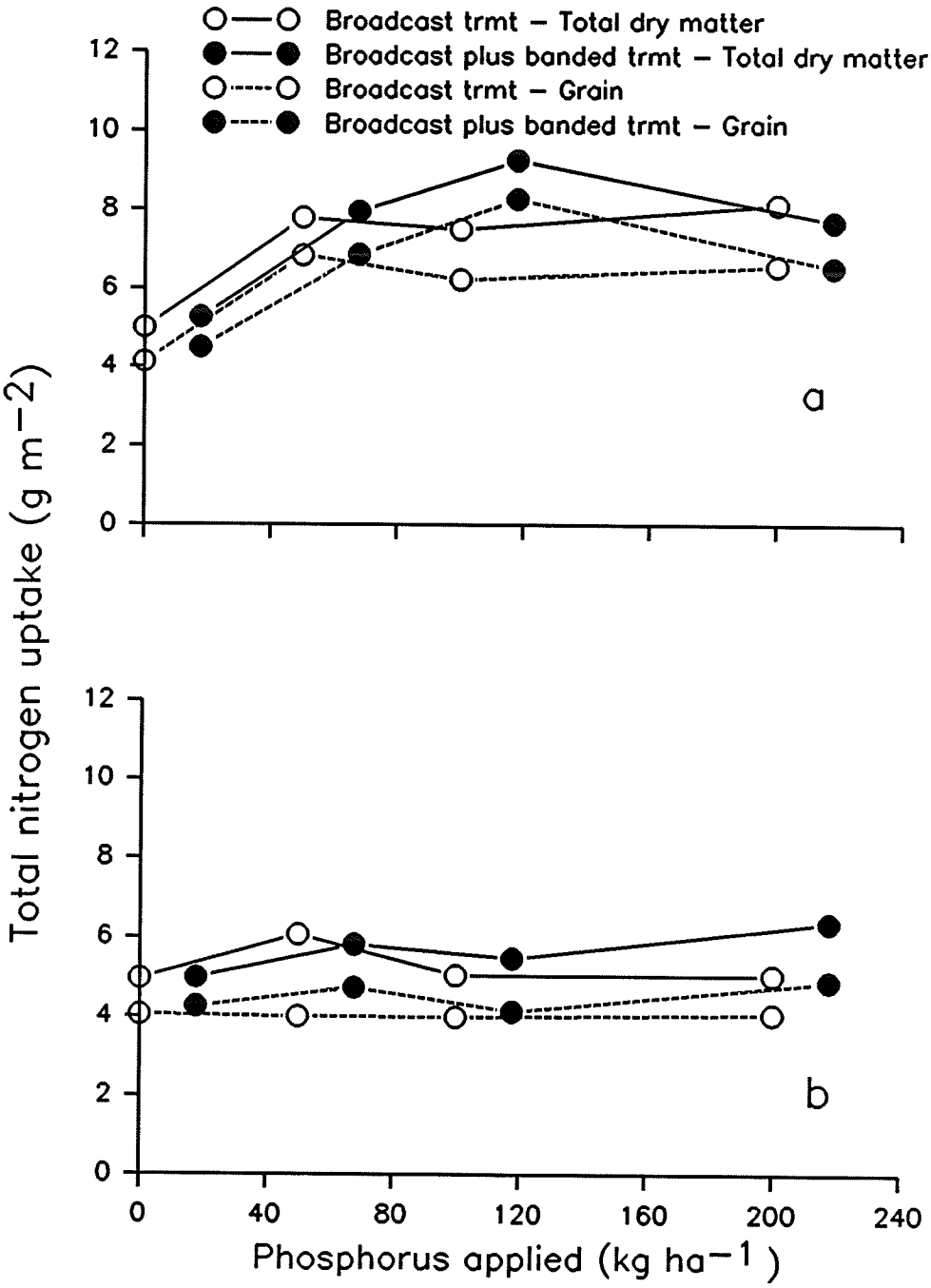

Figure 8. Effect of applied P on total N uptake by total dry matter and grain yield of lentil, at the Haywood site (a), and at the St. Claude site (b).

Table 11. Effect of phosphorus application on total N uptake by dry matter and grain of lentil, at the two field sites.

Phosphorus applied	Haywood site		St. Claude site	
	Total dry matter	Grain	Total dry matter	Grain
kg ha^{-1}	---------- g m^{-2} ----------			
Broadcast treatment				
0	5.0 B†	4.1 A	5.0 A	4.1 A
50	7.8 AB	6.8 A	5.1 A	4.0 A
100	7.5 AB	6.2 A	5.0 A	4.0 A
200	8.1 A	6.6 A	5.0 A	4.1 A
Broadcast plus banded treatment				
18	5.3 B	4.5 B	5.0 A	4.3 A
68	8.0 A	6.9 A	5.8 A	4.7 A
118	9.3 A	8.3 A	5.5 A	4.1 A
218	7.7 A	6.5 A	6.4 A	4.9 A

† Means followed by the same letter for each site and within treatments, are not significantly different at P=0.05 (Tukey's Studentized Range Test).

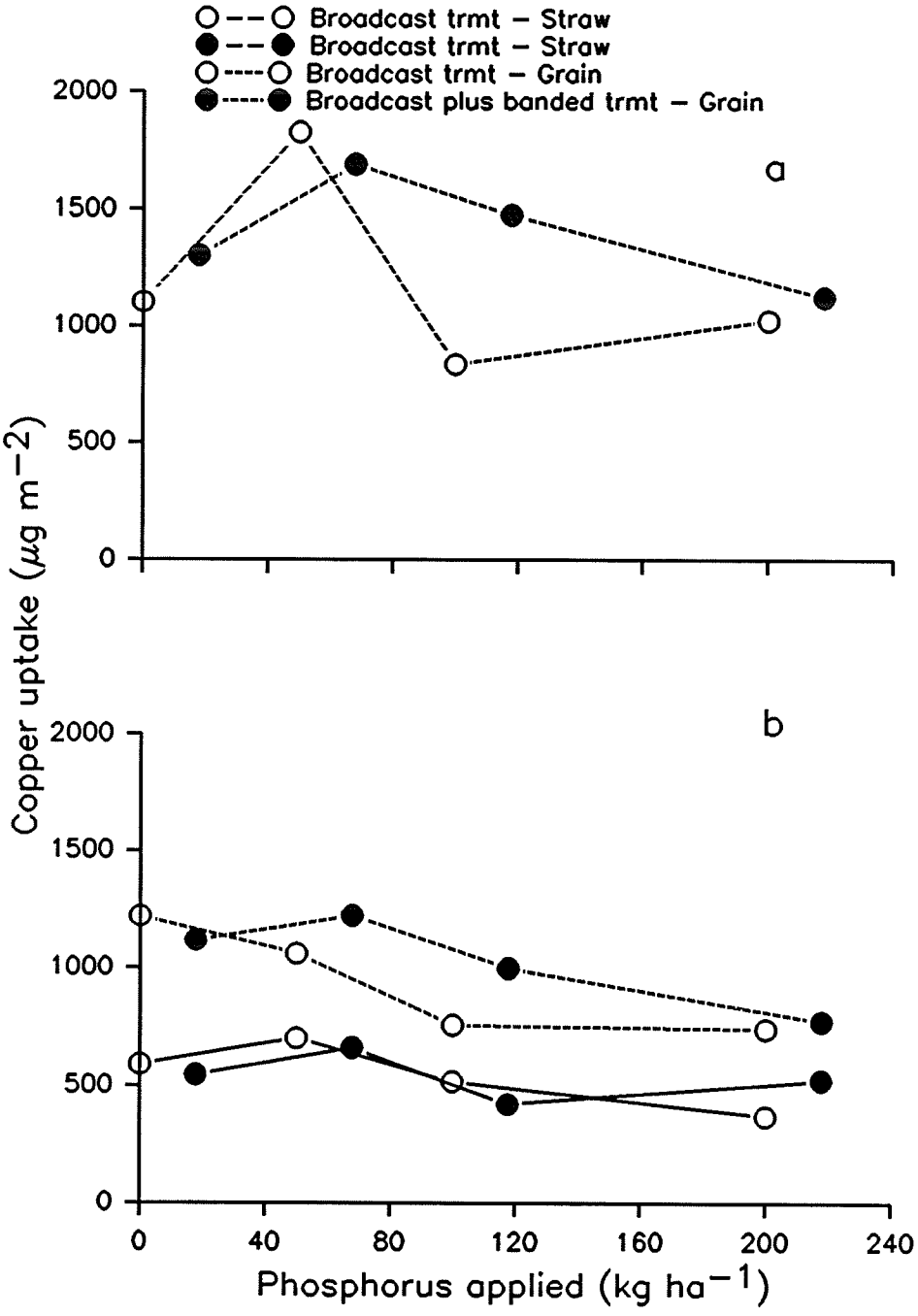

Figure 9. Effect of applied P on Cu uptake by straw and grain of lentil, at the haywood site (a), and at the St. Claude site (b).

Table 12. Effect of phosphorus application on Cu uptake by straw and grain of lentil, at the two field sites.

Phosphorus applied	Haywood site		St. Claude site	
	Straw	Grain	Straw	Grain
kg ha^{-1}	---------- $\mu g\ m^{-2}$ ----------			
Broadcast treatment				
0	n.d.	1104 AB†	590 A	1219 A
50	n.d.	1827 A	701 A	1059 A
100	n.d.	835 B	517 A	756 A
200	n.d.	1024 AB	369 A	740 A
Broadcast plus banded treatment				
18	n.d.	1303 A	546 A	1117 A
68	n.d.	1690 A	660 A	1221 A
118	n.d.	1475 A	421 A	998 A
218	n.d.	1125 A	523 A	772 A

† Means followed by the same letter for each site and within treatments, are not significantly different at P=0.05 (Tukey's Studentized Range Test).

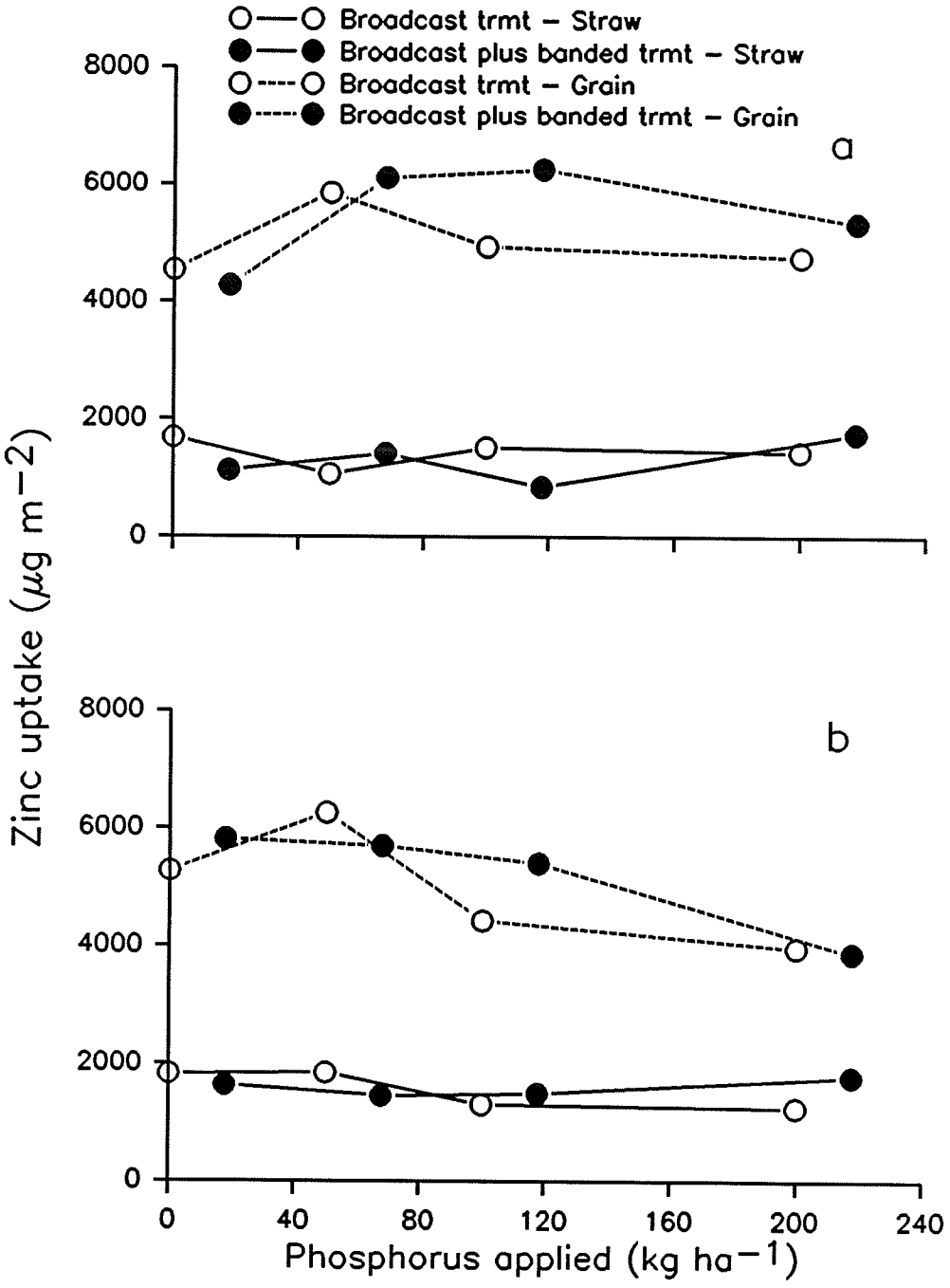

Figure 10. Effect of applied P on Zn uptake by straw and grain of lentil, at the Haywood site (a), and the St. Claude site (b).

Table 13. Effect of phosphorus application on Zn uptake by straw and grain of lentil, at the two field sites.

Phosphorus applied	Haywood site		St. Claude site	
	Straw	Grain	Straw	Grain
kg ha^{-1}	---------- $\mu g\ m^{-2}$ ----------			
Broadcast treatment				
0	1692 A†	4542 A	1823 A	5280 A
50	1069 A	5848 A	1839 A	6259 A
100	1516 A	4929 A	1301 A	4422 B
200	1439 A	4741 A	1243 A	3959 B
Broadcast plus banded treatment				
18	1128 A	4274 A	1635 A	5814 A
68	1414 A	6108 A	1452 A	5697 A
118	844 A	6253 A	1486 A	5403 A
218	1751 A	5329 A	1771 A	3872 A

† Means followed by the same letter for each site and within treatments, are not significantly different at P=0.05 (Tukey's Studentized Range Test).

Field Experiment, 1986

In the second year, the field experiment was conducted without the application of P, so only the P that was present from the previous year was available to the crop. Plant samples were again collected, after visual observations were done, at both sites five times throughout the growing season, each sampling corresponding to a specific legume growth stage [late vegetative development, bud to early bloom, full bloom, pod (green seed), and maturity (harvest)]. As in 1985, for the first four samplings, there was little or no effect of available P on yield and nutrient uptake (see Appendix A for data and statistical analysis).

Emergence was noted at both sites, and for both crops, 10 days after seeding. However, both sites were initially under water, due to their location, and so planting was delayed. Also, the rainfall in this growing season was approximately 10X greater than in 1985 (200 mm) and so the lentil crop remained very indeterminant in their growth habit. The lentil crop remained in the vegetative stage for about two weeks longer than in the previous year. Definite distinctions were not visible between growth stages, and bud, bloom, and green pod appeared to occur almost simultaneously. Final harvest was conducted when at least 75% of the pods were mature, at this time, some plants were still flowering. Considerable shattering of pods occurred at final harvest, regardless of P treatment, but the grain yields were still comparable to those obtained by Manitoba producers (Manitoba Agriculture, 1982).

Total dry matter and grain yield were not significantly affected

by the available P in the soil, at either site (Figure 11 and Table 14). It is interesting to note, however, that the total dry matter yields (particularly the straw yields) in this field experiment were higher than those obtained in the field experiment in 1985 (Figure 6 and Table 9).

At the Haywood site, a significant increase in P uptake by the lentil straw occurred with the available 9 kg P ha^{-1}. However, the available P had no significant effect on P uptake by lentil straw at the St. Claude site and no significant effect on P uptake by lentil grain at either site (Figure 12 and Table 14). It is noteworthy that, at both sites, the P uptake by the lentil straw in the 1986 experiment was 5 to 15 times higher than P uptake by lentil straw in the 1985 experiment (Figures 7 and 12). This can be explained in part by the 2 to 4 times higher straw yields obtained in the 1986 field experiment.

At both sites, the total plant N in the dry matter and grain was not significantly increased with the P available to the lentil crop (Figure 13 and Table 15). As with the P uptake, the total N uptake by the lentil straw in 1986, at both sites, was higher than the N uptake by lentil straw in the 1985 experiment (Figures 8 and 13), and once again could be explained in part by the higher straw yields obtained in the 1986 field experiment.

The lentil crop exhibited N_2 fixation at both sites in 1986 (Figure 14 and Table 15). However, the plants did not respond to the P available, as there were no significant differences in N_2 fixation with increased available P.

The Cu and Zn uptake in lentil grain, at both sites, were

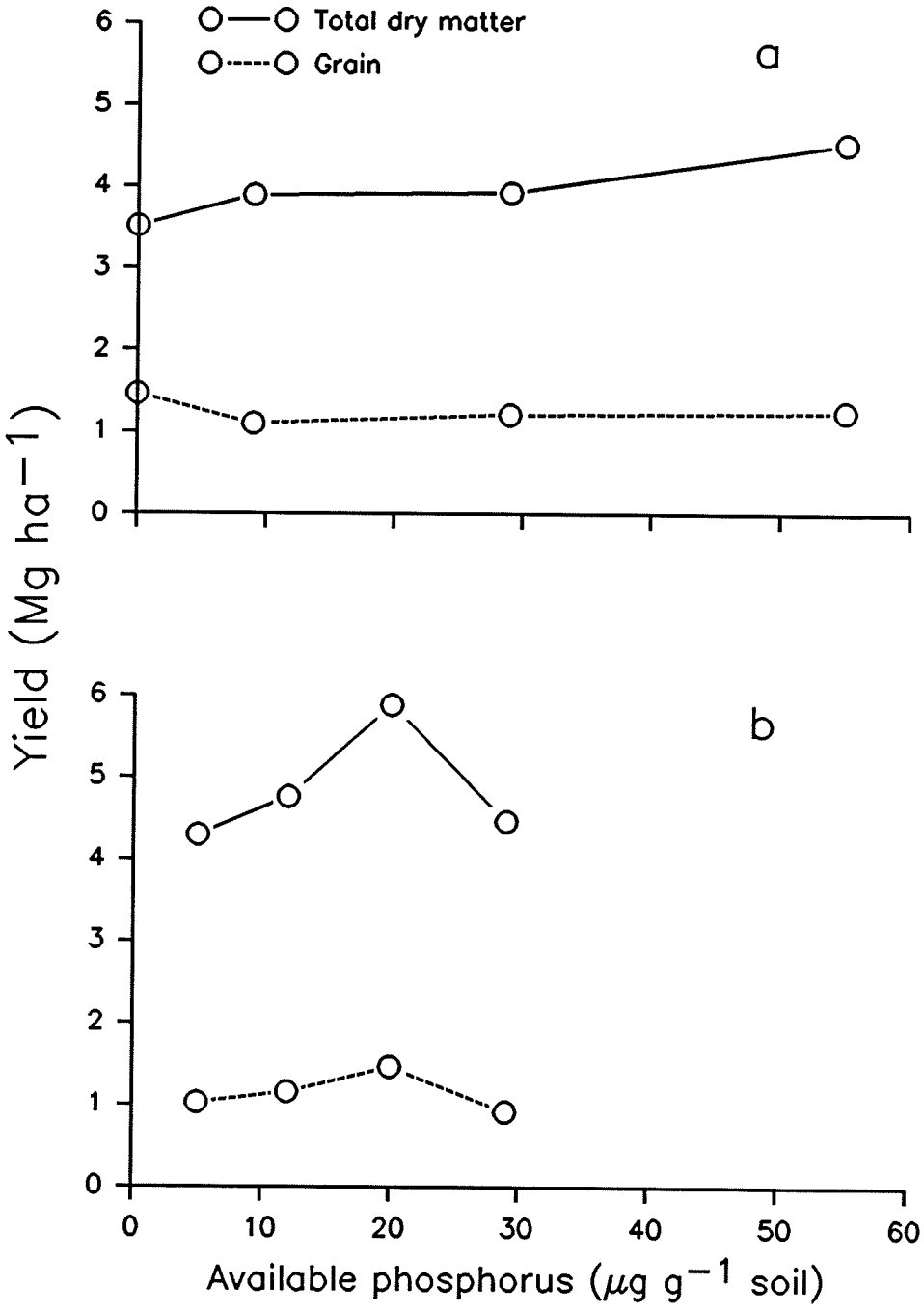

Figure 11. Effect of available P on total dry matter and grain yield of lentil, at the Haywood site (a), and at the St. Claude site (b).

Table 14. Effect of available P on total dry matter and grain yield, and P uptake by straw and grain, of lentil, at the two field sites.

Available phosphorus	Yield		P uptake	
	Total dry matter	Grain	Straw	Grain
$\mu g\ g^{-1}$ soil	-------- Mg ha^{-1} --------		--------- mg m^{-2} -------	
Haywood site				
0	3.513 A†	1.468 A	312 B	696 A
9	3.893 A	1.105 A	782 A	662 A
29	3.923 A	1.213 A	828 A	746 A
55	4.525 A	1.248 A	1115 A	765 A
St. Claude site				
5	4.300 A	1.030 A	976 A	597 A
12	4.760 A	1.160 A	1151 A	670 A
20	5.883 A	1.465 A	1337 A	808 A
29	4.463 A	0.920 A	1099 A	582 A

† Means followed by the same letter for each site are not significantly different at P=0.05 (Tukey's Studentized Range Test).

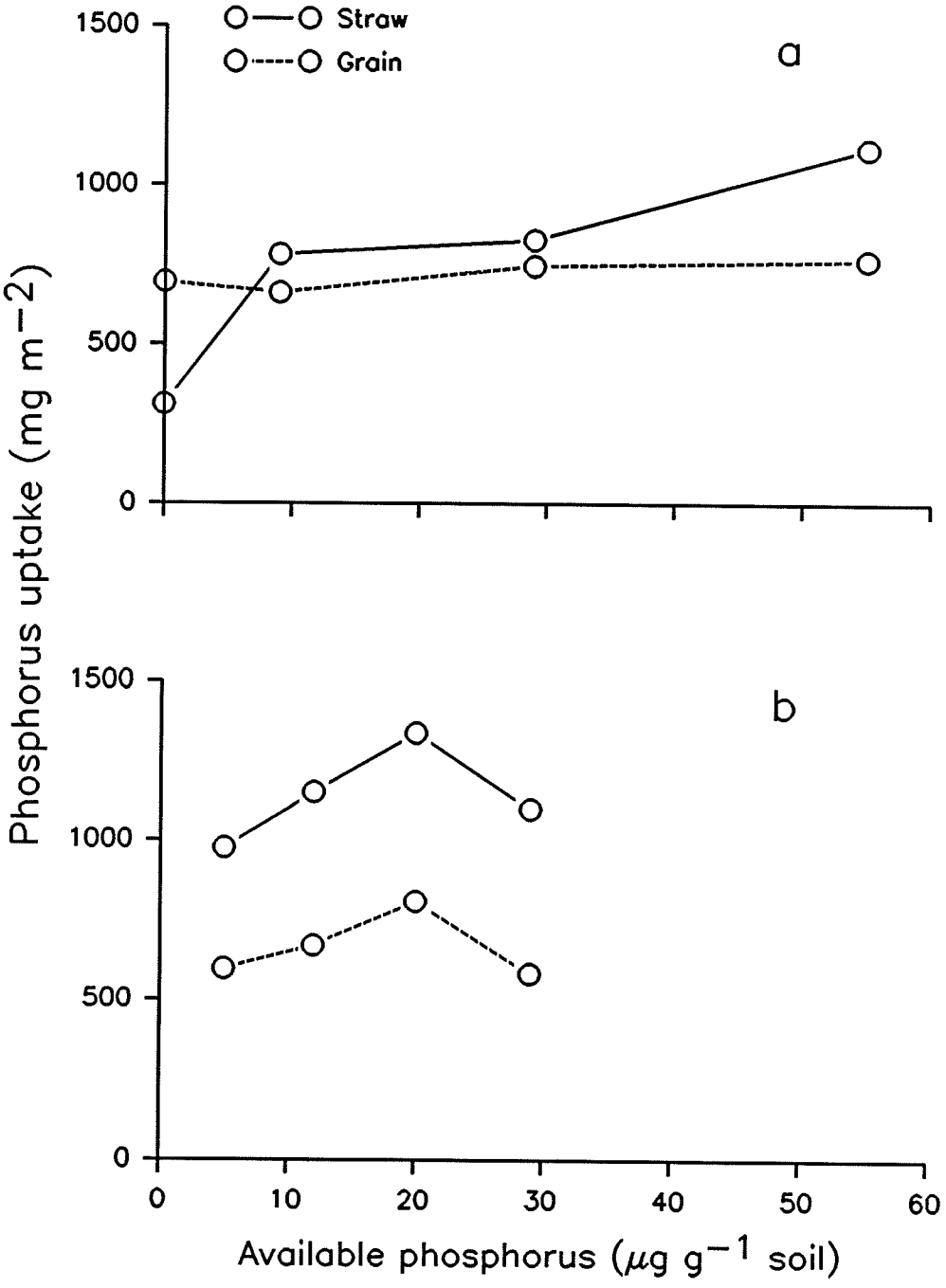

Figure 12. Effect of available P on P uptake by straw and grain of lentil, at the Haywood site (a), and at the St. Claude site (b).

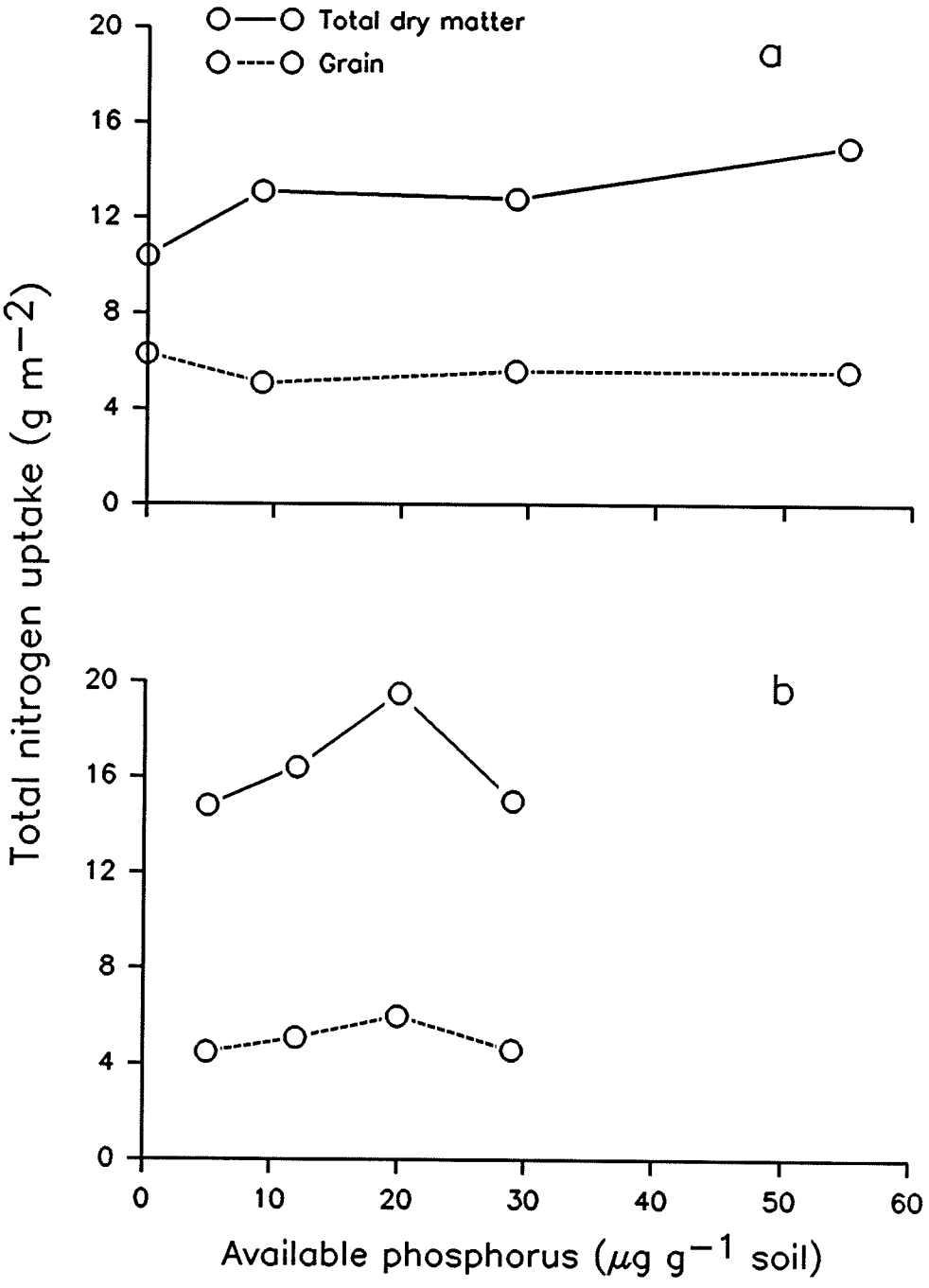

Figure 13. Effect of available P on total N uptake by total dry matter and grain yield of lentil, at the Haywood site (a), and at the St. Claude site (b).

Table 15. Effect of available P on N uptake by total dry matter and grain, and on N_2 fixation, by lentil, at the two field sites.

Available phosphorus	N uptake		N_2 fixation
	Total dry matter	Grain	
$\mu g\ g^{-1}$ soil	------- $g\ m^{-2}$ -------		-------- $kg\ ha^{-1}$ -------
Haywood site			
0	10.4 A†	6.3 A	53 A
9	13.1 A	5.1 A	80 A
29	12.8 A	5.6 A	77 A
55	15.0 A	5.6 A	99 A
St. Claude site			
5	14.8 A	4.5 A	75 A
12	16.4 A	5.1 A	91 A
20	19.5 A	6.0 A	122 A
29	15.0 A	4.6 A	77 A

† Means followed by the same letter for each site are not significantly different at P=0.05 (Tukey's Studentized Range Test).

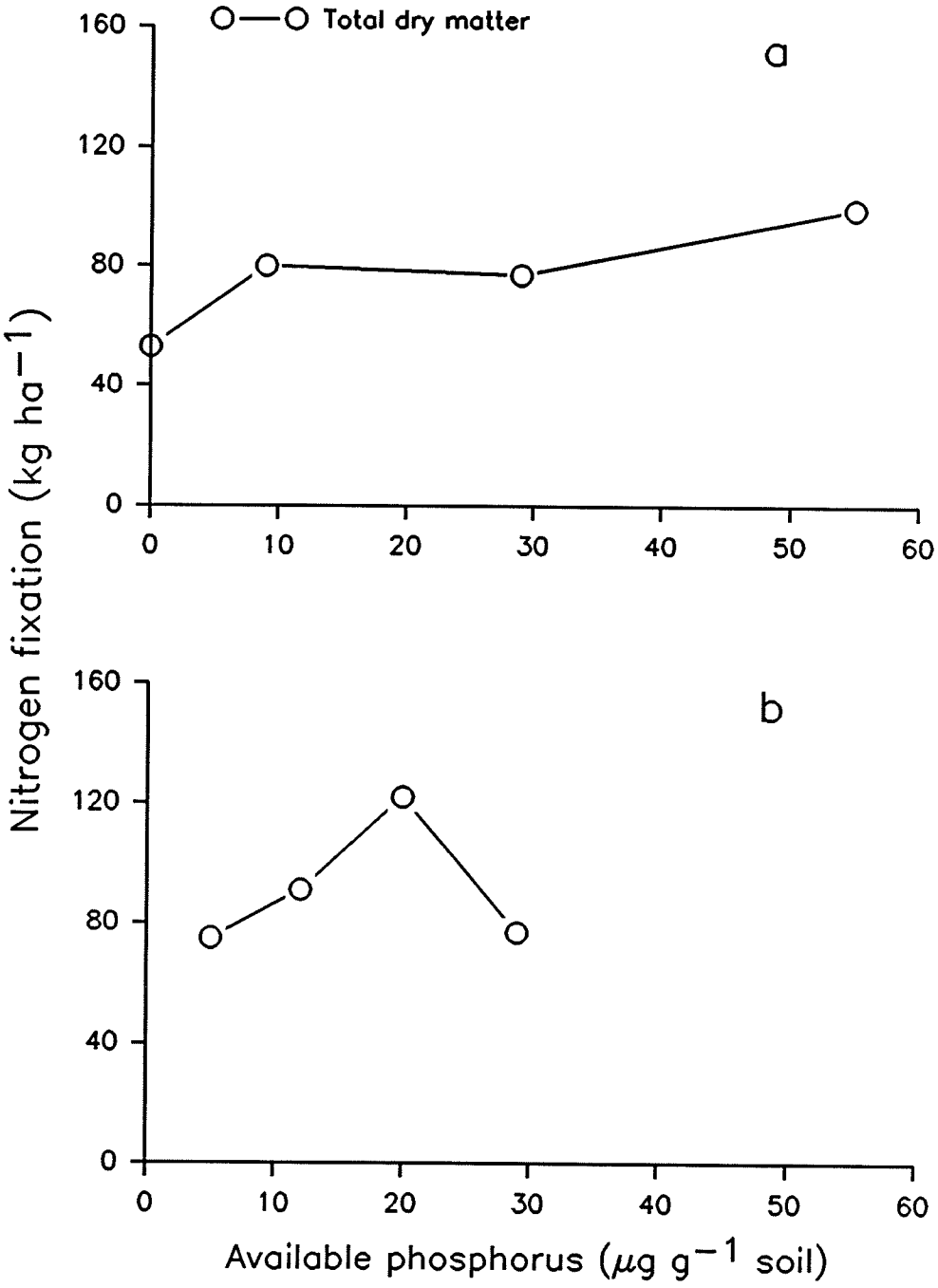

Figure 14. Effect of available P on N_2 fixation by lentil, at the Haywood site (a), and at the St. Claude site (b).

decreased in the presence of the available P, and these decreases were significant at the Haywood site (Figures 15 and 16 and Table 16). Decreases in Cu and Zn uptake by lentil straw were also noted at the St. Claude site, but only the decrease in Cu at the highest level of available P was significant.

The field experiments were conducted to determine the effect of P fertilizer application on growth and N_2 fixation by lentil. It was hoped that the results would help to establish a field recommendation for P application to lentil, on sandy textured soils. In most cases, there was no response by lentil to either P application, or to available P.

The lack of a consistent response to P by lentil in these field experiments is in agreement with other researchers who have reported that lentil does not respond positively to P application (Singh and Singh, 1986; Asghar et al., 1988). Also, Olsen and Sommers (1982) state that the relationship between the P soluble in sodium bicarbonate and the expected yield response to added P fertilizer is as follows: <5 μg P g^{-1} soil, a response; between 5 and 10 μg P g^{-1} soil, a probable response; and >10 μg P g^{-1} soil, a response unlikely. In Table 1, for field experiment 1985, the data show that the soil at the Haywood site contained 1.3 μg P g^{-1} soil while the soil at the St. Claude site contained 10.3 μg P g^{-1} soil. Therefore, a lack of response to P, particularly at the St. Claude site, is not surprising in light of the fact that the level of P in the soil prior to the addition of treatments, was high. In the second year of the field experiment (1986), the available P levels were all, except for one

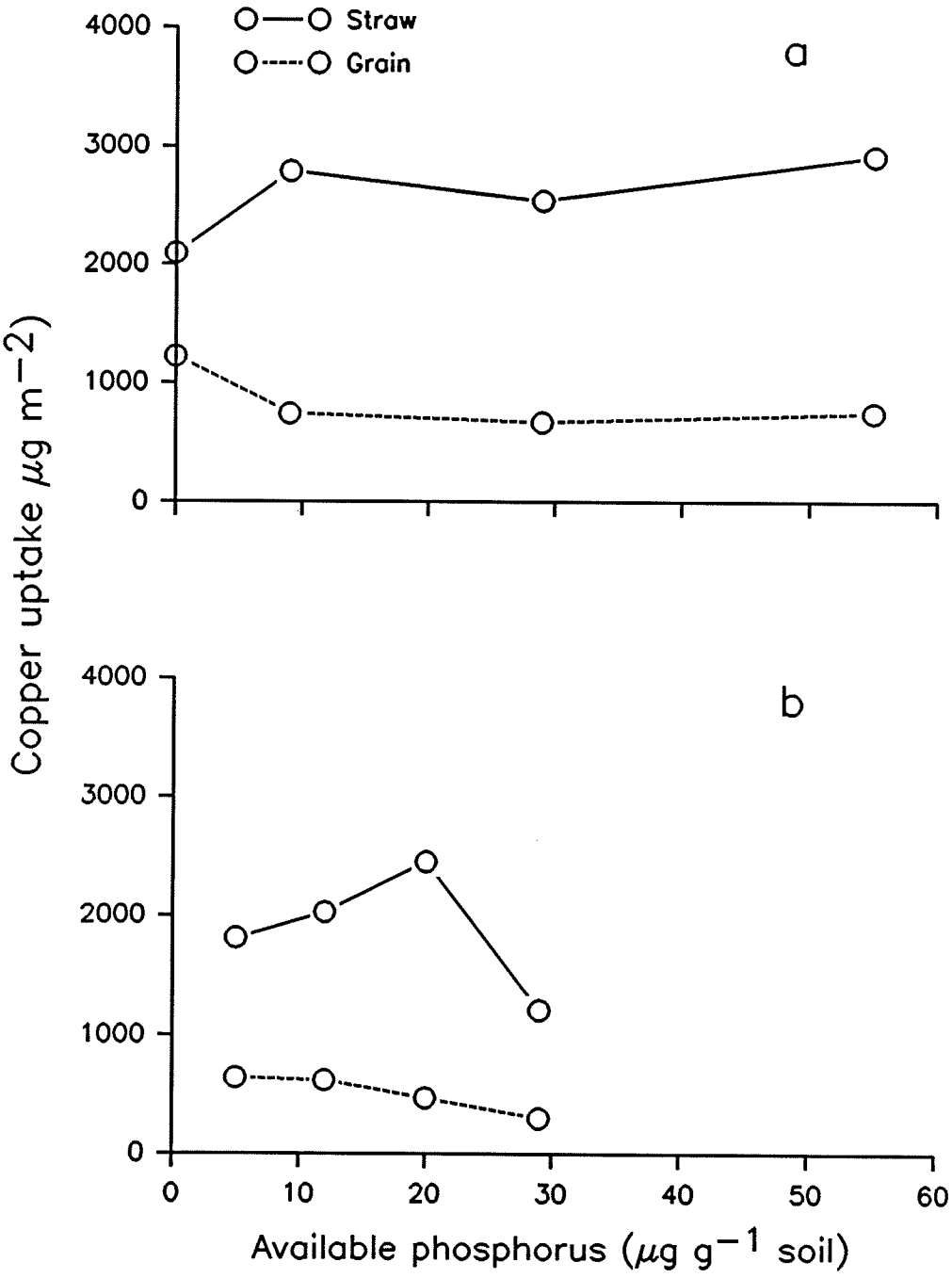

Figure 15. Effect of available P on Cu uptake by straw and grain of lentil, at the Haywood site (a), and at the St. Claude site (b).

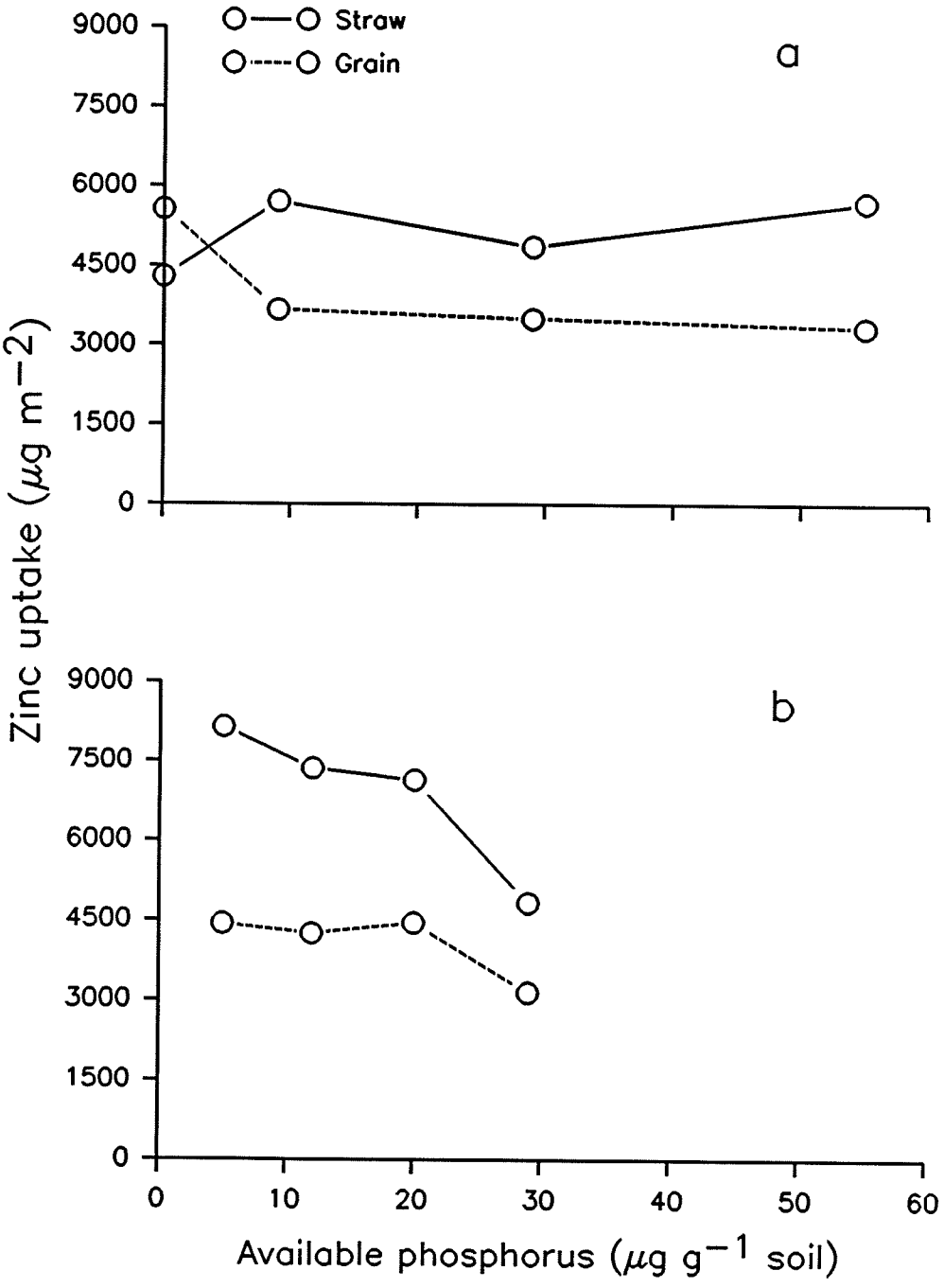

Figure 16. Effect of available P on Zn uptake by straw and grain of lentil, at the Haywood site (a), and at the St. Claude site (b).

Table 16. Effect of available P on Cu and Zn uptake by straw and grain of lentil, at the two field sites.

Available phosphorus	Cu uptake		Zn uptake	
	Straw	Grain	Straw	Grain
$\mu g\ g^{-1}$ soil	----------		$\mu g\ m^{-2}$ ----------	
Haywood site				
0	2096 A†	1223 A	4300 A	5564 A
9	2789 A	742 B	5714 A	3672 B
29	2537 A	668 B	4863 A	3507 B
55	2912 A	746 B	5695 A	3336 B
St. Claude site				
5	1815 AB	642 A	8148 A	4437 A
12	2029 AB	619 A	7361 A	4251 A
20	2454 A	471 A	7138 A	4453 A
29	1213 B	300 A	4827 A	3146 A

† Means followed by the same letter for each site are not significantly different at P=0.05 (Tukey's Studentized Range Test).

treatment at the Haywood site, in excess of 5 μg P g^{-1} soil (Table 2), indicating that a response to P, at either site, would be unlikely.

One noticeable trend in both years, and at both sites, was a decrease in Cu and Zn concentration in the lentil crop. It has been reported that excessive P concentration in the lentil can result in Zn deficiency, one of the few trace element deficiencies identified in lentil (Summerfield, 1981). This further indicates that P availability was not a limiting factor in the lentil growth in these field experiments.

The Effect of Phosphorus and Vesicular-Arbuscular Mycorrhiza on Growth and N_2 Fixation by Lentil

To determine the effect of P and/or VAM on growth and N_2 fixation by lentil, a growth chamber experiment and a lysimeter experiment were conducted.

Growth Chamber Experiment

With the lentil crop, no differences in growth were observed among treatments, during the first 12 days after emergence. At 18 days, all the lentil plants showed tip-burning on the lower leaves. The plants grown in the non-irradiated soil treatments and the irradiated +VAM soil treatments recovered from this symptom with time, however, the plants growing in the irradiated -VAM soil treatment became very chlorotic and resembled those grown in the irradiated soil in 1985. However, unlike the plants in the 1985 experiment, none of the lentil plants recovered, regardless of level of P applied.

Because of this limited growth, not enough dry matter was obtained to do any type of analysis for this treatment.

In the non-irradiated treatments, shoot dry matter of lentil increased significantly with the addition of 20 μg P g^{-1} soil (Figure 17a and Table 17). However, no further significant increases in yield were observed with the increased P application up to 120 μg P g^{-1} soil. The lentil grown in the irradiated +VAM soil treatment had significant increases in shoot dry matter with each addition of P up to 80 μg P g^{-1} soil (Figure 17a and Table 17). No further significant increase in yield was observed with the addition of 120 μg P g^{-1} soil. At all P levels, dry matter production was greater in the irradiated +VAM soil treatments as compared to the lentil grown in the non-irradiated soil treatment.

Uptake of P by the lentil shoots was significantly increased with each addition of P in the non-irradiated soil treatment (Figure 17b and Table 17). Shoot P concentration ranged from 0.12% for the 0 μg P g^{-1} soil to 0.24% for the 120 μg P g^{-1} soil treatment. The lentil grown in the irradiated +VAM soil treatment also responded to increased P applications, a statistically significant increase was observed with the addition of 60, 80 and 120 μg P g^{-1} soil (Figure 17b and Table 17). The shoot P concentration ranged from 0.15% for the 0 μg P g^{-1} soil to 0.24% for the 120 μg P g^{-1} soil treatment. Lentil total N in the shoot increased with each addition of P for both the non-irradiated and irradiated +VAM soil treatments (Figure 17c and Table 17). In the non-irradiated soil treatment, only the 20 μg P g^{-1} soil treatment caused a statistically significant increase. The

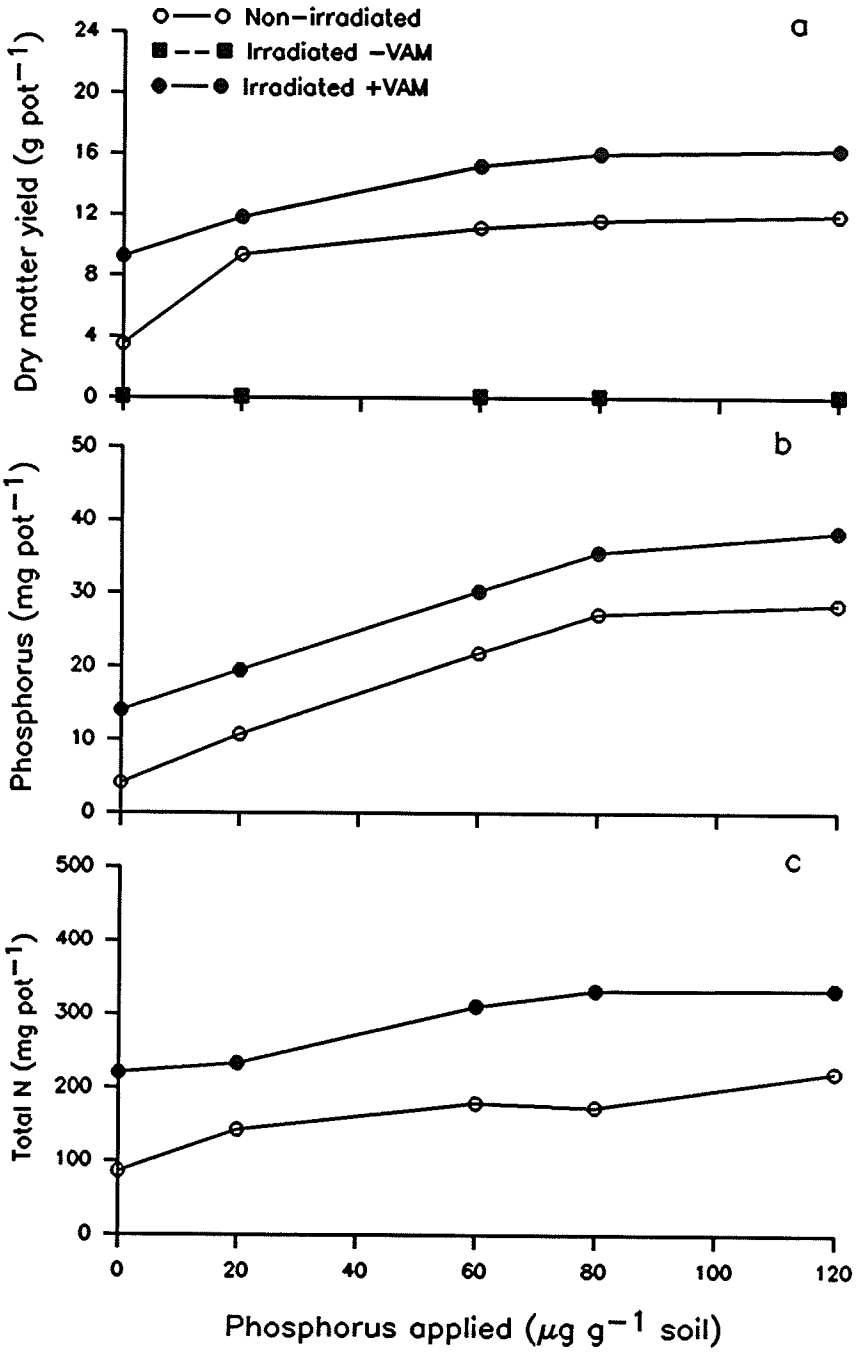

Figure 17. The effect of phosphorus application on dry matter yield (a), phosphorus uptake (b), and total nitrogen uptake (c) by lentil.

Table 17. The effect of phosphorus application on dry matter yield, phosphorus and total nitrogen uptake, and N_2 fixation by lentil.

Phosphorus applied	Dry Matter yield	Phosphorus uptake	Total N uptake	N_2 fixed
$\mu g\ g^{-1}$ soil	g pot^{-1}	----------	mg pot^{-1}	----------
Non-irradiated soil treatment				
0	3.5 B†	4.2 D	86.0 B	18.7 C
20	9.3 A	10.8 C	142.9 A	33.4 BC
60	11.1 A	21.8 B	173.0 A	67.5 ABC
80	11.6 A	27.1 AB	178.8 A	72.1 AB
120	11.9 A	28.4 A	196.3 A	111.6 A
Irradiated +VAM soil treatment				
0	9.2 C	14.0 B	220.4 B	17.9 B
20	11.8 BC	19.4 B	232.8 B	47.4 B
60	15.1 AB	30.2 A	310.4 A	123.3 AB
80	16.0 A	35.5 A	331.7 A	157.7 A
120	16.2 A	38.3 A	333.1 A	161.1 A

† Means followed by the same letter for each soil treatment are not significantly different at P=0.05 (Tukey's Studentized Range Test).

percent N in the tissue decreased with increasing P applications from 2.4% at the 0 μg P g^{-1} soil treatment to 1.6% at the 120 μg P g^{-1} soil treatment. With the irradiated +VAM treatment, a statistically significant increase in total N uptake was observed with the application of 60, 80 and 120 μg P g^{-1} soil. The percent N in the tissue decreased slightly with increasing P applications, from 2.4% at the 0 μg P g^{-1} soil treatment to 2.1% at the 120 μg P g^{-1} soil treatment.

VAM infection was measured for lentil grown with the non-irradiated soil treatment and the irradiated +VAM soil treatment (Table 18). The number of root segments infected increased with inoculation and no suppression of VAM infection was noted with increasing rates of P. However, the type of mycorrhiza present

Table 18. Lentil root VAM infection†

Phosphorus applied	Soil treatment	
	Non-irradiated	Irradiated +VAM
μg g^{-1} soil	% infection	
0	25	95
20	40	100
60	30	100
80	45	100
120	40	85

† Percent infection based on incidence of infection on 20, one centimetre root segments (average of three replicates).

differed among the treatments. In the non-irradiated soil treatment, native VAM species produced spores on the outside of the roots whereas the roots grown in the irradiated +VAM soil treatment, inoculated with

Glomus intraradices, bore intracellular spores. No further attempt was made to quantify the amount of infection since information concerning the type and amount of VAM infection needed to maximize growth is unavailable.

The winter rape crop showed larger plants with more branching, at all stages of growth, when grown in the irradiated soil as compared to the non-irradiated soil. However, the plants grown in both soil treatments appeared healthy. Also with both soil treatments, the application of P resulted in slightly earlier maturation of the crop.

The winter rape grown in the non-irradiated soil treatment showed only a slight increase in dry matter yield with the addition of 120 μg P g-1 soil (Figure 18 and Table 19). The addition of P also increased P uptake by the winter rape, but did not increase total N uptake by the crop. With the irradiated soil treatment, the winter rape responded favourably to the P application, almost doubling its yield as well as plant P uptake (Figure 18 and Table 19). However, there was only a slight increase in total N uptake by the winter rape with the application of 120 μg P g-1 soil, in the irradiated soil treatment.

As in the 1985 growth chamber experiment (Figure 2), the winter rape responded favourably to the soil irradiation treatment, with increases in yield, P uptake, and total N uptake, as compared to the non-irradiated soil treatment, at both levels of P application (Figure 18 and Table 19). It appears that the winter rape was responding favourably to the release of nutrients which occurred when the soil was irradiated.

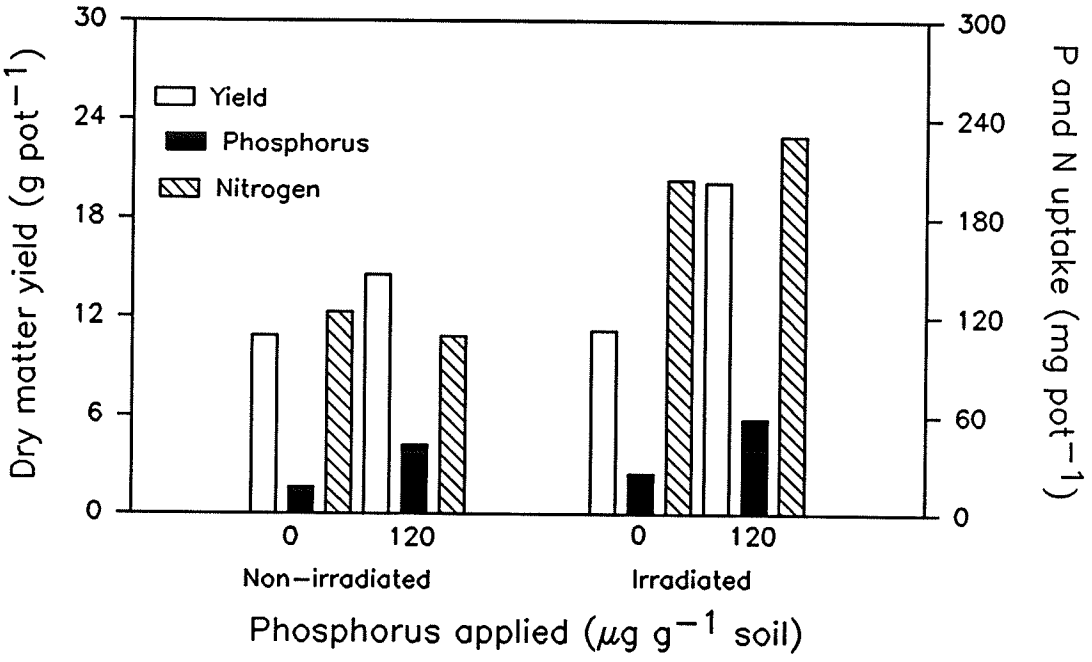

Figure 18. The effect of phosphorus application on dry matter yield, phosphorus uptake, and total nitrogen uptake by winter rape.

Table 19. The effect of phosphorus application on dry matter yield, and phosphorus and total nitrogen uptake by winter rape.

Phosphorus applied	Dry Matter yield	Phosphorus uptake	Total N uptake
$\mu g\ g^{-1}$ soil	g pot^{-1}	---------- mg pot^{-1} ----------	
Non-irradiated soil treatment			
0	10.87	16.41	122.96
120	14.51	42.40	108.23
Irradiated soil treatment			
0	11.17	24.91	202.82
120	20.14	58.20	229.62

The ability of lentil to utilize P in the absence of phosphate solubilizing microorganisms and VAM was again measured by using a soil irradiation treatment, in addition to a P treatment, as discussed previously. However, a third treatment, reinoculating the irradiated soil with a source of VAM, was included to determine if the detrimental effects of irradiation could be overcome. The same dose of irradiation was used as in the previous growth chamber experiment of 1985 (10,000 Gy). Once again, the irradiation treatment resulted in a substantial release in plant available nutrients (Table 20), as evidenced by the presence of nutrients at the termination of the experiment, in the irradiated -VAM soil treatment where the lentil crop did not grow.

The winter rape was capable of absorbing the nutrients released upon irradiation (Figure 18 and Table 19, 20). The increase in yield and nutrient uptake was not as dramatic in this growth chamber experiment, as observed in the 1985 growth chamber experiment, between the non-irradiated and irradiated soil treatments. This may be due to the fact that a small amount N fertilizer was added to the pots in the 1986 experiment, so N was not as limiting as it was in the 1985 growth chamber experiment. Also, a P response was observed in this experiment, again suggesting that N was not as limiting.

Unlike the winter rape, the lentil crop could not make use of the nutrients made available by the irradiation -VAM soil treatment (Figure 17a and Table 20), even at the highest rates of P application. In contrast, in the 1985 experiment, the detrimental effects of soil irradiation were overcome by high applications rates of P. Similar

results were obtained in this experiment with the reintroduction of a microbial population, VAM, to the irradiated soil. The lentil crop was then able to make use of all available nutrients, and showed a significant response to P application.

Table 20. Soil analysis after crop growth.†

Phosphorus added	Non-irradiated		Irradiated -VAM		Irradiated +VAM	
	P	N	P	N	P	N
	---------- $\mu g\ g^{-1}$ soil ----------					
Lentil						
0	4.1	35.5	6.8	95.9	6.7	5.4
20	7.5	6.5	22.0	85.9	10.8	2.4
60	15.7	6.4	37.3	83.6	18.5	2.6
80	17.7	4.3	63.0	69.8	18.8	2.7
120	20.3	4.4	80.2	56.7	22.4	2.1
Winter rape						
0	5.4	2.7	6.0	3.5		
100	21.5	3.2	22.1	2.7		

† $P=PO_4^{3-}$-P and $N=(NO_3^- + NO_2^-)$-N

These differences between the two growth chamber experiments can be explained by differences in the experimental design. In this experiment, both the lentil and winter rape were grown in soil which received autoclaved corn roots and VAM root washings (irradiated -VAM soil treatment), mixed into the top 3 cm of soil. The irradiated soil treatment in the 1985 experiment did not receive these additions. In this experiment, the roots were added to keep the soil C:N ratio the same as the lentil grown in the irradiated soil which received fresh

mycorrhizal corn roots (irradiated +VAM soil treatment). Root washing were added to reintroduce a microbial population into the soil similar to the microbial population associated with the VAM inoculant. The addition of a carbon source and microorganisms into a soil rich in nutrients and low in microbial competition (due to irradiation) may have caused large increases in microbial population (McLaren, 1969). This would have allowed for increased decomposition of the organic matter present and immobilization of the nutrients released by irradiation. Any available P, either released due to irradiation or introduced as a P application, would be used by the microbial population and would not be available to either crop. These nutrients would probably be released with time through mineralization, as evidenced by the high PO_4^{3-}-P and (NO_3^-+NO_2^-)-N levels in the irradiated -VAM soil treatment at the termination of the experiment (Table 20).

The addition of VAM into the irradiated soil did assist the lentil root system in the uptake of P. However, the favourable response to nutrients released by the irradiation treatment could have been lessened by immobilization of P. This would explain the lower yields and P uptake values at the higher rates of P application when compared to data obtained in the 1985 experiment.

It is also interesting to note, that in the irradiated -VAM soil, available N decreased almost linear with added P although there was essentially no plant growth (Table 20). This may indicate that the irradiated soil was reinoculated with bacteria and fungi which took advantage of the C made available from microorganisms killed by irradiation. The fact that available P increased linear but less than

the combined total of initial soil P level plus applied P would also support this idea.

The lentil grown in either the non-irradiated soil treatment or the irradiated +VAM soil treatment responded at each P addition by an increase in N_2 fixation (Figure 19a and Table 17). At all rates of P applied, the plants grown in the irradiated +VAM soil treatment symbiotically fixed more N_2 than in the non-irradiated treatments. A linear regression (Figure 19b) shows that N_2 fixation had a high correlation with P application, in both the non-irradiated and the irradiated +VAM soil treatments.

The lentil plants grown in the non-irradiated soil treatment showed simultaneous increases in both dry matter accumulation and N_2 fixation until dry matter accumulation reach 11.0 g (Figure 20a). At this point, further additions of P to the soil (80 and 120 μg P g^{-1} soil) resulted in increases in N_2 fixation but no further increase in dry matter accumulation. The plants grown in the irradiated +VAM soil treatment showed increases in dry matter and N_2 fixation with each P application simultaneously (Figure 20a). Comparing the two soil treatments, the plants grown in the irradiated +VAM soil treatment showed both more growth potential and more N_2 fixation than the plants grown in the non-irradiated soil treatment when P was applied at a rate greater than 60 μg P g^{-1} soil.

The relationship between shoot P concentration and N_2 fixation are shown in Figure 20b. For the lentil grown in the non-irradiated soil treatment, shoot P concentration increased with each addition of P up to 80 μg P g-1 soil, however, this did not result in an increase in

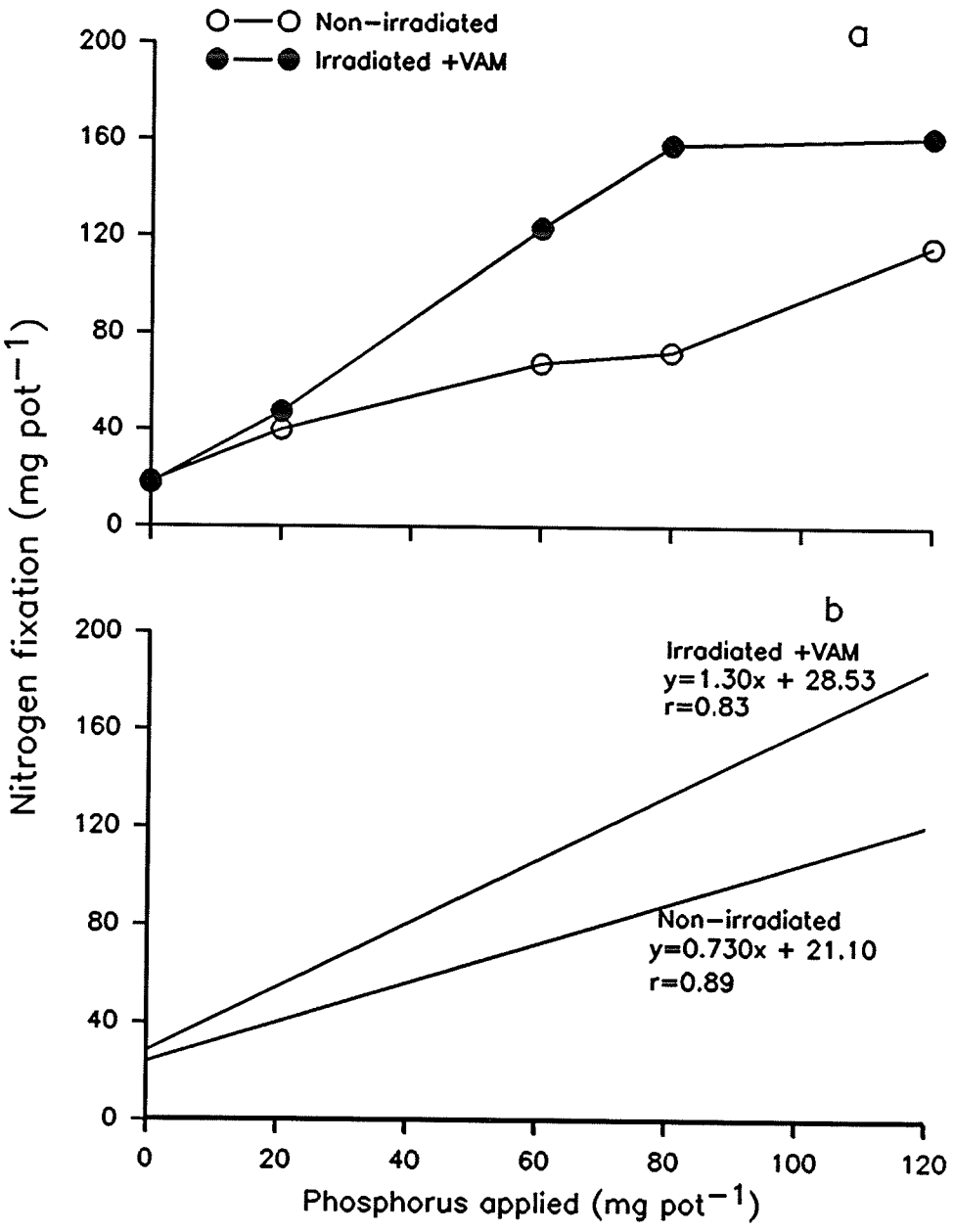

Figure 19. The effect of phosphorus application on N_2 fixation by lentil (a), and the correlation between P application and N_2 fixation (b).

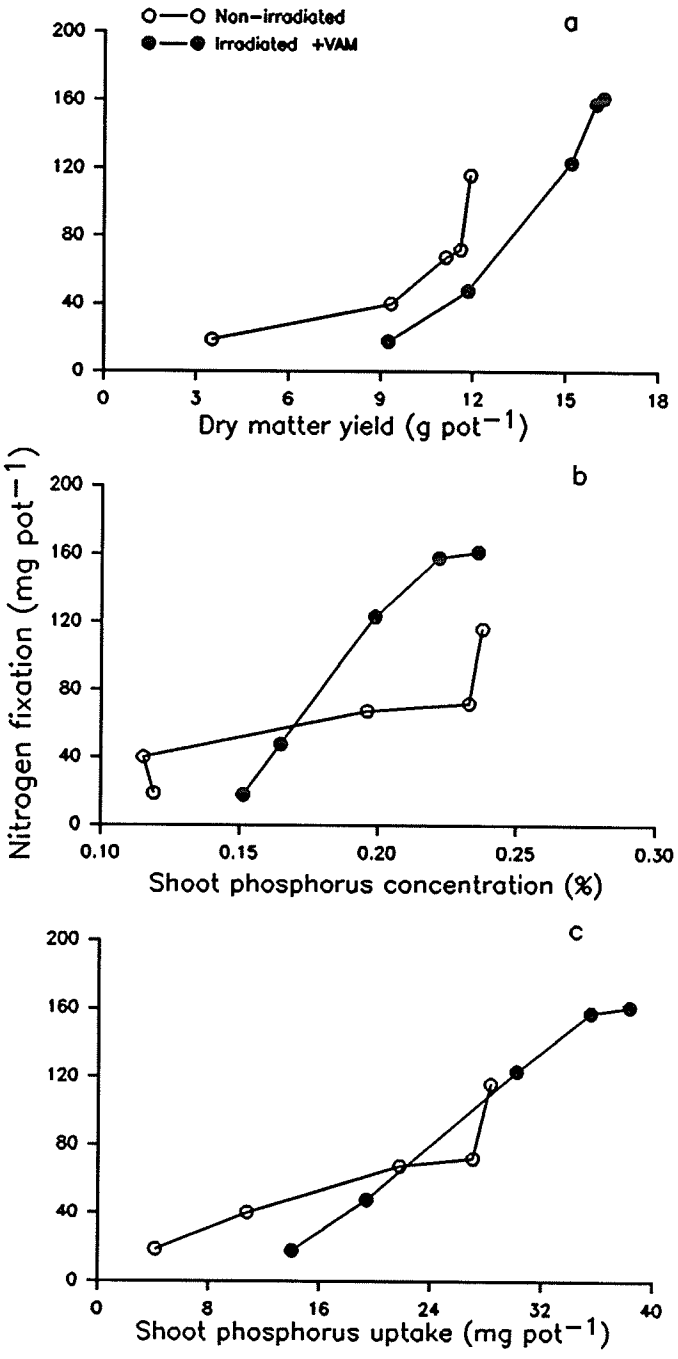

Figure 20. The effect of dry matter accumulation (a), shoot P concentration (b), and shoot P accumulation (c) on dinitrogen fixation by lentil. (Data points represent a stepwise increase in applied P from 0 to 120 μg g^{-1} soil.)

N_2 fixation by the lentil, particularly between 20 and 80 μg P g^{-1} soil applied. Above this threshold value of between 60 and 80 μg P g^{-1} soil applied (shoot P concentration of 0.24%), N_2 fixation was stimulated. In the irradiated +VAM soil treatment, the lentil appeared to be able to fix N_2 at a much lower shoot P concentration and no threshold value of P was evident (Figure 20b).

The effect of shoot P uptake on N_2 fixation also showed a threshold value in the non-irradiated soil treatment (shoot P uptake of 28 mg pot^{-1}) but again a threshold value was not evident with the irradiated +VAM soil treatment (Figure 20c).

The lower P concentration in the tissue observed with the application of 0 and 20 μg P g^{-1} soil may have limited photosynthesis and inhibited photosynthate translocation into the nodule. At the higher P applications (between 60 and 80 μg P g^{-1} soil applied), the high tissue P level seemed to favour translocation of sucrose (and P_i) into the nodule and increased N_2 by the plant (Figure 20b). Perhaps the amount of photosynthate produced at this P level could not support both growth and N_2 fixation, and was preferentially translocated to the nodule.

In this experiment, the addition of P resulted in increases in N_2 fixation for both the non-irradiated and the irradiated +VAM soil treatments however, accumulation of N derived from N_2 fixation was drastically lower than was observed in the 1985 growth chamber experiment in the non-irradiated soil treatment. Percent N due to fixation reached 85% with the addition of 100 μg P g^{-1} soil in 1985 while the maximum percent N due to fixation in 1986 was 48%. This

suggests a substantial contribution of N from the soil in 1986. Perhaps the amount of combined N released due to irradiation and the addition of 30 μg N g^{-1} soil did not enhance fixation by reducing the lag phase between infection at the onset of fixation, but resulted in a drastic reduction in nitrogenase activity and hence the plants' potential for N_2 fixation.

The percent N in the lentil tissue was increased substantially with the application of VAM at the higher P application rates, compared to the non-irradiated treatments (1.6% N in the non-irradiated soil treatment and 2.1% in the irradiated +VAM soil treatment). This suggests a direct effect of VAM on the N_2-fixing system. However, the irradiation process released plant available N which was subsequently taken up by the irradiated +VAM treatments thus contributing to tissue N. Since all the lentil plants grown in irradiated -VAM soil treatment were stunted, percent N in this comparable treatment was not analyzed. Therefore, any direct benefit of VAM on the N_2-fixing system could not be determined.

In the non-irradiated treatments, percent N in the plant tissue decreased with the addition of P in 1986 from 2.4 to 1.6%, while in 1985 percent N increased from 2.1 to 2.6%. In 1985, no N was applied, suggesting these plants solely reliant on N_2 fixation were able to give the largest increases in %N. Similar results were obtained by Gates and Wilson (1974) while looking for optimum application rate of N and P to maximize *S. humilis* growth parameters.

Plant Cu, Zn, K, and S concentrations were also measured. Although the Cu and Zn uptake by the lentil tended to increase with

increased P application, both the Cu and Zn concentrations in the non-irradiated soil treatment and the irradiated +VAM soil treatment decreased with increasing P application (Figure 21 and Table 21). Plant K and S uptake also tended to increase with increased P application (Figure 22 and Table 21). However, the plant K concentration (1.2%) and S concentration (0.17%) remained fairly constant, regardless of P application or soil treatment. It appears that the differences in total uptake of these four nutrients, largely reflect the lentil response to P additions by an increase in dry matter accumulation (Figures 21 and 22 and Table 21).

Lysimeter Experiment

The lentil crop did not respond with an increase in dry matter accumulation, to either P application or VAM inoculation (Figure 23a and Table 22). In the inoculated soil treatment, the addition of 60 kg P ha^{-1} caused a slight increase in dry matter yield, but the increase was not significant.

The lentil crop, with both the non-inoculated and the inoculated soil treatments, responded to the addition of P by increasing P uptake (Figure 23b and Table 22). With the non-inoculated soil treatment, the increase in P uptake was not significant. The P concentration in the tissue increased with added P, from 0.20% to 0.26%. For the inoculated soil treatment, a statistically significant increase in P uptake was observed with the addition of 60 kg P ha^{-1}. Tissue P

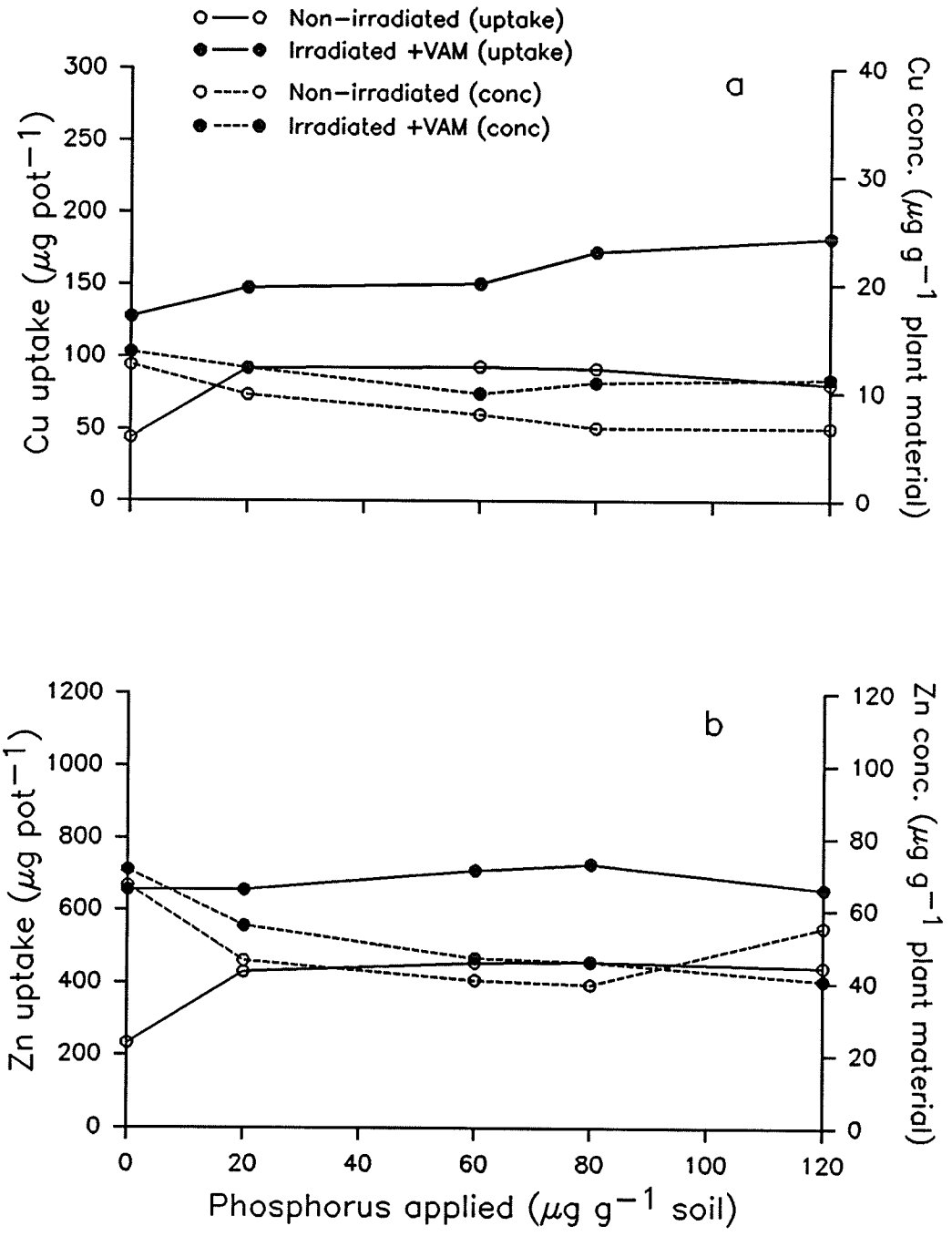

Figure 21. The effect of phosphorus application on Cu uptake and concentration (a), and Zn uptake and concentration (b) by lentil.

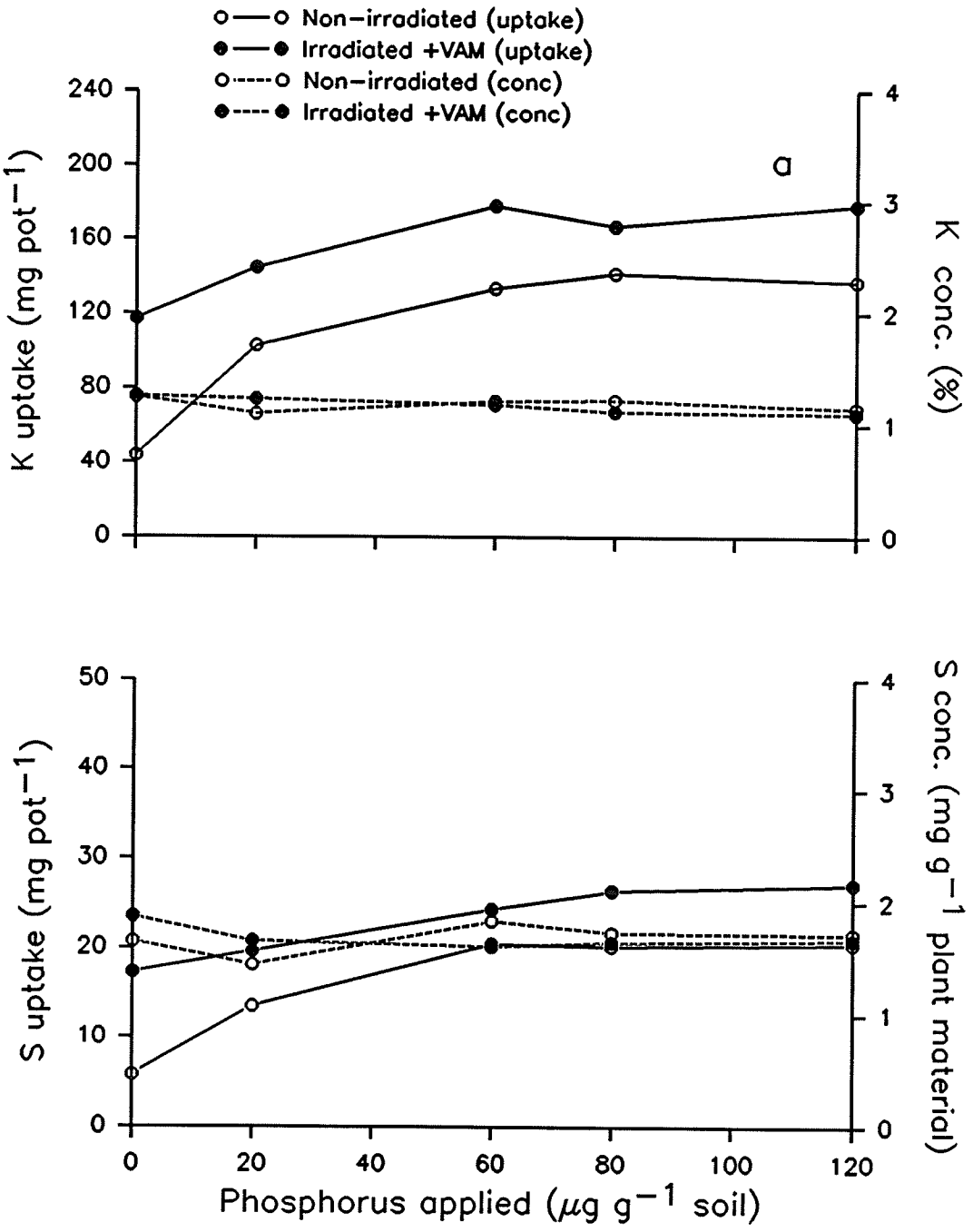

Figure 22. The effect of phosphorus application on K uptake and concentration (a), and S uptake and concentration (b) by lentil.

Table 21. The effect of phosphorus application on Cu, Zn, K, and S uptake by lentil.

Phosphorus applied	Cu uptake	Zn uptake	K uptake	S uptake
$\mu g\ g^{-1}$ soil	-------- $\mu g\ pot^{-1}$ --------		------- mg pot^{-1} -----	
Non-irradiated soil treatment				
0	44 B†	233 B	44 C	6 B
20	92 A	430 A	103 B	14 AB
60	92 A	455 A	133 A	20 A
80	92 A	457 A	142 A	20 A
120	81 AB	443 A	137 A	21 A
Irradiated +VAM soil treatment				
0	127 A	656 A	117 C	17 B
20	147 A	656 A	145 B	20 AB
60	150 A	710 A	178 A	24 AB
80	172 A	728 A	167 A	26 A
120	182 A	658 A	178 A	27 A

† Means followed by the same letter for each soil treatment are not significantly different at P=0.05 (Tukey's Studentized Range Test).

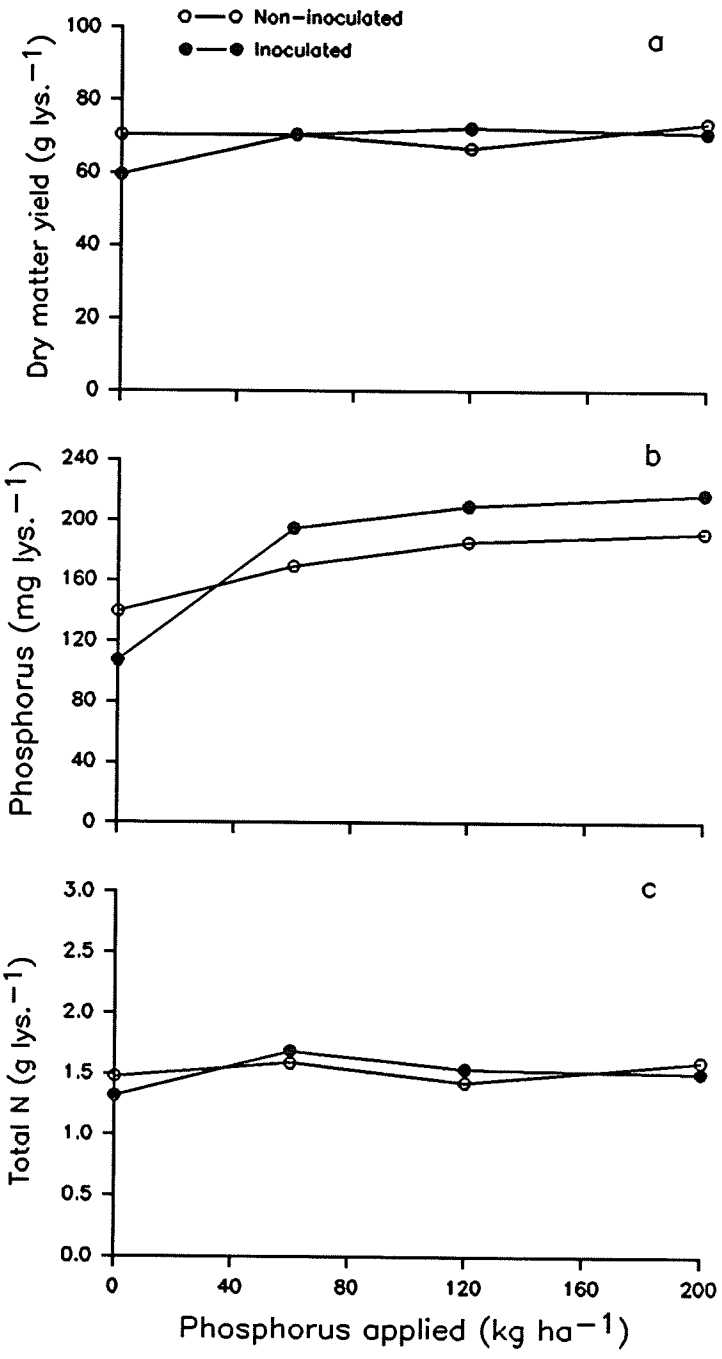

Figure 23. The effect of phosphorus application on dry matter yield (a), P uptake (b), and total N uptake (c) by lentil.

Table 22. The effect of phosphorus application on dry matter yield, and phosphorus and total nitrogen uptake by lentil.

Phosphorus applied	Dry Matter yield	Phosphorus uptake	Total N uptake
kg ha^{-1}	g lys^{-1}	---------- mg lys^{-1} ----------	
Non-inoculated soil treatment			
0	71 A†	140 A	1480 A
60	71 A	169 A	1590 A
120	67 A	186 A	1430 A
200	74 A	192 A	1600 A
Inoculated soil treatment			
0	60 A	107 B	1320 A
60	70 A	195 A	1690 A
120	72 A	209 A	1540 A
200	71 A	217 A	1510 A

† Means followed by the same letter for each soil treatment are not significantly different at P=0.05 (Tukey's Studentized Range Test).

Table 23. The effect of phosphorus application on dry matter yield, and P, total N, Cu, and Zn uptake, by winter rape.

Phosphorus applied	Dry Matter yield	Phosphorus uptake	Total N uptake	Cu uptake	Zn uptake
kg ha-1	g lys^{-1}	------ mg lys^{-1} ------		--- μg lys^{-1} ---	
0	83	108	1203	282	1470
200	123	332	1967	381	1320

concentration increased from 0.17% to 0.31%, with the increasing P application rates. Except when P was not added, the lentil grown in the inoculated soil treatment was higher in both P uptake and tissue P concentration, over the comparable non-inoculated soil treatments.

No differences in lentil total N uptake were observed with either the application of P or VAM inoculation (Figure 23c and Table 22). The concentration of total N at 65 days of growth also did not show any variation among the treatments (2.2%).

The winter rape responded favourably to P application. The amount of dry matter accumulation was increased substantially with the addition of 200 kg P ha^{-1} (Table 23). The addition of P also resulted in an increase in both P and total N uptake (Table 23). The P concentration increased from 0.13% to 0.27%, however, the total N concentration remained relatively constant at approximately 1.5%.

The addition of VAM resulted in infection by both native and inoculant types of mycorrhiza, in the inoculated treatments. (See growth chamber experiment 1986 for a description of the endophytes.) No attempt was made to exclude VAM from the lentil grown in the non-

inoculated soil treatment. As a result, both treatments contained fungal associations on their root systems (Table 24). No decrease in the percent infection was observed with the addition of the P treatments.

The benefits of adding VAM in greenhouse and growth chamber experiments, where plants are grown in potted, sterilized soil, can not be disputed. However, little information is available on the

Table 24. Lentil root VAM infection.†

Phosphorus applied	Soil treatment	
	Non-inoculated	Inoculated
kg ha^{-1}	% infection	
0	50	60
60	50	65
120	40	55
200	40	75

† Percent infection based on incidence of infection on 20, one centimetre root segments (average of four replicates).

effect of field inoculation. There are several reasons to inoculate non-sterilized soils with VAM: the natural inoculant level in the soil may be low (previous crops grown on the soil may have been non-mycorrhizal or the land may have been left fallow thus suppressing spore populations), the indigenous endophytes may be less efficient than those planned to be introduced, or some crops will show host

specificity to certain strains or species of VAM (Rhodes, 1984).

Lentil have been shown to respond to VAM in potted experiments when grown on unsterilized sandy soil (Singh and Singh, 1986). Their response in field situations however, have not been addressed adequately. Since this crop is able to adapt to a wide range of soil types and environmental conditions throughout the world (Saxena, 1985) and are often grown on soils unable to sustain other crops, growing lentil on marginal land for soil reclamation or as green manure crops is becoming feasible. Therefore, the importance of microbial associations such as VAM and *Rhizobium* in crop production on these soils must also be considered. Although the benefit of VAM inoculation was minimal in this experiment (perhaps because the quantity and types of native endophytes present in the soil was adequate), the effect of a low native VAM population should be considered when attempting to grow lentil on marginal soils. (At the present time, field inoculation with VAM, is not economically feasible except for crops which are transplanted from nursery beds.)

Dinitrogen fixation by the lentil crop was not detected, using either the dilution or the difference method to determine the amount fixed, and winter rape as the reference crop. However, nodules were present on the test crop root system, and were coloured red inside suggesting leghemoglobin synthesis, thus indicating that the N_2 fixation was in fact occurring. But, percent utilization of the N fertilizer was calculated to be approximately 25% for the lentil crop and no variation in utilization was associated with the P treatments (Table 25). The winter rape percent utilization of fertilizer N was

lower than for the lentil and averaged about 20%, suggesting that the winter rape obtained a higher percentage of its N from the soil, as compared to the lentil crop. For the lentil crop, the majority of its root system was found in the top ten centimetres of the soil, the layer to which the nutrients had been applied. The winter rape root system penetrated the whole length of the lysimeter and therefore the soil zone explored for N, and other nutrients, was different than for the test crop.

Table 25. Percent ^{15}N abundance and percent utilization of fertilizer nitrogen.

Phosphorus applied	Soil treatment			
	Non-inoculated	Inoculated	Non-inoculated	Inoculated
kg ha^{-1}	---- % ^{15}N Abundance ----		% Utilization Fertilizer N	
Lentil				
0	0.62	0.60	24.3	23.7
60	0.57	0.60	20.4	25.9
120	0.60	0.63	22.1	26.8
200	0.59	0.66	23.9	28.0
Winter rape				
0	0.60		20.0	
200	0.52		20.3	

Copper uptake for the lentil crop, grown in either the non-inoculated or the inoculated soil treatments, showed similar trends with added P (Figure 24a and Table 26). Total Cu uptake decreased with increased P application up to 120 kg P ha^{-1}, however, a slight increase in Cu uptake was noted with the addition of 200 kg P ha^{-1}. The decrease was statistically significant in the non-inoculated soil treatment. The Cu concentration in shoot tissue with the non-inoculated soil treatment, decreased from 6.5 $\mu g\ g^{-1}$ with no P added to 3.5 $\mu g\ g^{-1}$ with the addition of 120 kg P ha^{-1}. Then the Cu concentration increased to 4.5 $\mu g\ g^{-1}$ with the addition of 200 kg P ha^{-1}. In the inoculated soil treatment, Cu concentration also decreased from 6.8 to 3.3 $\mu g\ g^{-1}$ when P was applied to a rate of 120 kg ha^{-1}, with the concentration showing an increase to 5.0 $\mu g\ g^{-1}$, with 200 kg P ha^{-1} application rate.

Zinc uptake in the lentil was also depressed with the application of P, in both soil treatments (Figure 24b and Table 26). The depression was greatest in the non-inoculated soil which showed a statistically significant decrease in Zn uptake with the addition of 120 kg P ha-1. As with the Cu uptake, a slight increase in Zn uptake was observed with the addition of 200 kg P ha^{-1}. Zinc concentration in the lentil crop was depressed with each addition of P, tissue levels decreasing from 17 to 11 $\mu g\ g^{-1}$, with the non-inoculated soil treatment and decreasing 20 to 14 $\mu g\ g^{-1}$, with the inoculated soil treatment.

The concentration of nutrients in the tissue may be lower than the above results suggest because the plant material was not separated into straw and grain components before analysis (plants were harvested

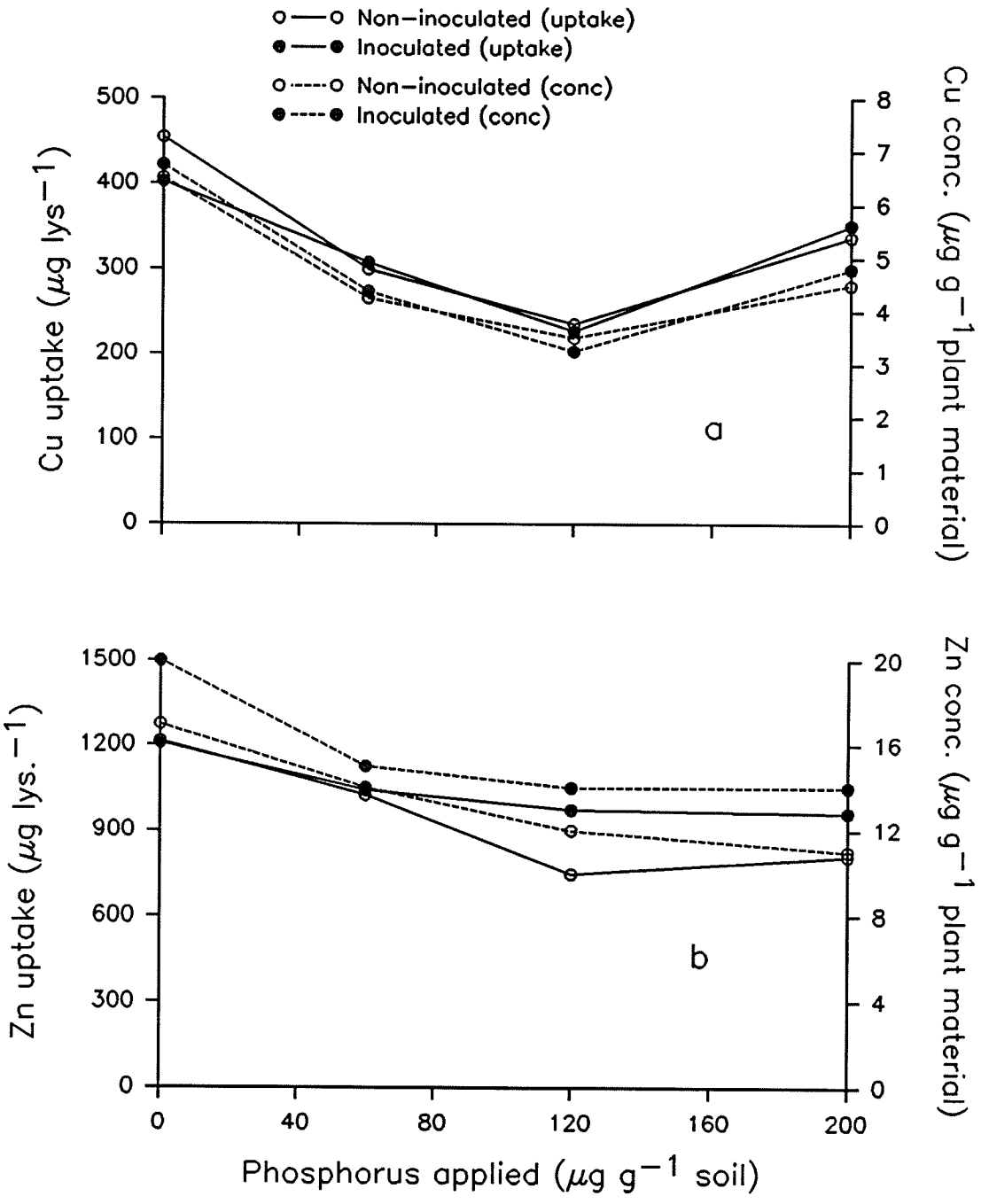

Figure 24. The effect of phosphorus application on Cu uptake and concentration (a), and Zn uptake and concentration (b) by lentil.

Table 26. The effect of phosphorus application on Cu, Zn, and K uptake by lentil.

Phosphorus applied	Cu uptake	Zn uptake	K uptake
kg ha^{-1}	---------- $\mu g\ lys^{-1}$ ----------		mg lys^{-1}
Non-inoculated soil treatment			
0	454 A†	1214 A	1049 A
60	299 AB	1024 AB	1061 A
120	235 B	748 B	1005 A
200	337 AB	809 AB	1161 A
Inoculated soil treatment			
0	402 A	1208 A	849 A
60	307 A	1041 A	1126 A
120	227 A	972 A	1087 A
200	351 A	961 A	1105 A

† Means followed by the same letter for each soil treatment are not significantly different at P=0.05 (Tukey's Studentized Range Test).

when the lentil reached the pod fill developmental stage). Instead, a representative sample including both was ground together and analyzed. Since the seed is a strong sink for nutrients including Cu and Zn, the concentration in the vegetative material may have been much lower. In the winter rape crop, Cu uptake increased with the addition of P (Table 23), and the concentration in the tissue decreased slightly from 3.4 to 3.1 $\mu g\ g^{-1}$. However, Zn uptake decreased (Table 23), as did the concentration of Zn in the tissues, from 18 to 11 $\mu g\ g^{-1}$ soil.

Loneragan et al. (1982) studied the interaction between P and Zn supply in okra (*Abelmoschus esculentus*). When the plants were grown in low levels of Zn, increasing the rate of P application resulted in P toxicity. When the level of Zn applied increased, the resulting increase in Zn concentration appeared to suppress P accumulation. Zinc seemed to affect P absorption and transport, since the addition depressed percent P transport to the tops but increased concentration in the roots. Thus, under conditions of high P, this nutrient accumulated to toxic levels in leaves, showing similar symptoms to Zn deficiency.

Singh et al. (1986) studied P induced Zn deficiency on wheat. The addition of Zn resulted in a grain yield greater than with the addition of P alone, but only when P was added with the application of the Zn, did it result in an increase in yield. The P level in the tissue did not reach toxic levels in this experiment, even with the addition of 160 kg P ha^{-1}, but with the high application rate, the concentration of Zn in the tissue decreased to near critical levels. High Zn levels in the tissue were associated with high percent VAM

infection in the roots, which was suppressed at the high P levels. It was concluded that a portion of the decrease in Zn concentration was due to the dilution effect and the remaining portion due to reduced mycorrhizal infection in the roots at the higher application rates of P.

Lambert et al. (1979) demonstrated two mechanisms for P induced reductions in Cu and Zn concentration in soybean (*Glycine max*) and corn (*Zea mays*) in an experiment which included both mycorrhizal and non-mycorrhizal treatments, and various P fertilization rates. The first mechanism involves the suppression of the mycorrhizal uptake of Cu and Zn with the application of P. The application of P fertilizer reduced the Cu and Zn concentration in the mycorrhizal soybeans but the concentration in the non-mycorrhizal treatments were not affected. The second mechanism is simply the dilution effect, a larger, more P sufficient plant does not accumulate elements to the same extent as smaller, deficient plants. They noted that in about 90% of the cases P induced Cu and Zn deficiencies were reported for plants which form mycorrhizae.

Singh et al. (1986) and Lambert et al. (1979) suggested both the dilution and mycorrhizal suppression to be the cause for the Cu and Zn deficiency. However, in my experiment, the lentil plants did not respond to P by an increase in dry matter, therefore the dilution effect can be ruled out. The winter rape did respond to P application, so the dilution effect may explain the decrease in Zn that was observed. The effect of P on the amount of VAM infection in all treatment combinations was not assessed quantitatively, however

visually, no decrease was observed. Thus depression of tissue Cu concentration in the lentil may have been a VAM response however the reduction in Zn concentration seems to be independent of the VAM. Perhaps if Cu and Zn had been applied to the soil and plant response observed, the interaction between these elements and P could have been assessed more accurately.

CONCLUSION

A growth chamber experiment and four field experiments, Haywood 1985, 1986 and St. Claude 1985, 1986, were conducted to obtain information on the effect of available and applied P on growth and N_2 fixation by lentil (*Lens culinaris*) in two Manitoba soils.

The growth chamber experiment showed significant dry matter yield increases with the application of P up to 80 $\mu g\ g^{-1}$ soil in the non-irradiated soil treatment. The relationship between P applied and N_2 fixation was best described by linear regression (r= 0.91). N_2 fixation was stimulated when lentil shoot P concentration reached about 0.24%. The lentil grown in the irradiated soil treatment were not able to grow at low rates of applied P (0 to 40 $\mu g\ g^{-1}$ soil). However, at the two highest rates of P application (80 and 100 $\mu g\ g^{-1}$ soil), dry matter yields increased as well as N_2 fixation. The relationship between P applied and N_2 fixation for this soil treatment was best described by linear regression (r= 0.78). Dinitrogen fixation was stimulated when lentil shoot P concentration reached about 0.21%. It was concluded from this study that the benefits of soil irradiation, ie., the release of plant available nutrients, was masked by the inability of the lentil root system to utilize those nutrients. Perhaps the lack of microbial associations which aid in the uptake of essential nutrients, particularly P contributed to this growth depression.

In the field experiments neither the application of P (broadcasted at rates of 0, 50, 100, and 200 kg P ha^{-1} or broadcast at the same rates plus a band containing 8 kg P ha^{-1} placed 2.5 cm below the seed) at the

1985 sites, or available P present after the previous years experiment at the 1986 sites, had an effect on lentil growth, total N uptake or N_2 fixation.

To investigate the effect of P and/or VAM on growth and N_2 fixation by lentil, a growth chamber experiment was conducted along with a field lysimeter experiment.

The growth chamber experiment showed lentil to respond to P application by an increase in dry matter accumulation and N_2 fixation in two of the three soil treatments, non-irradiated and irradiated +VAM. For all parameters measured, the lentil grown in the irradiated +VAM soil treatment performed better than those in the non-irradiated soil treatment, showing that microbial associations aided the plant in nutrient uptake. In the non-irradiated soil treatment, the relationship between P application and N_2 fixation was best described by linear regression ($r= 0.89$). Dinitrogen fixation was stimulated when lentil shoot P concentration reached about 0.24%. For the irradiated +VAM soil treatment the relationship was again described using linear regression ($r= 0.83$). However, for this soil treatment no threshold value of shoot P concentration needed to stimulate fixation was observed. In the third soil treatment for this experiment, irradiated -VAM soil, P applied at any of the rates (0 to 120 $\mu g\ g^{-1}$ soil) failed to stimulated growth. Once again, the nutrients released through irradiation were not available to the lentil, probably because the lentil root system was not able to utilize the nutrients without the aid of microbial populations.

In the lysimeter experiment, the application of both P and/or VAM inoculant increased the concentration of P in the lentil. This increase

in P concentration however, did not result in increases in dry matter accumulation. Dinitrogen fixation was not detected in either the non-inoculated or the inoculated soil treatments. Interestingly, a decrease in both Cu and Zn concentration in the lentil tissue with increasing rates of P applied occurred in both soil treatments, indicating that P was not a limiting factor in the lentil growth in this experiment.

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APPENDIX A

The data and statistical analysis for the observations not presented in the Results and Discussion section are reported in this appendix.

Table 1. The effect of phosphorus application on K and S uptake by lentil in the non-irradiated soil treatment (growth chamber experiment, 1985).†

Phosphorus applied	K uptake	S uptake
$\mu g\ g^{-1}$ soil	---------- $\mu g\ pot^{-1}$ ----------	
0	115.7 B	23.0 C
10	102.1 B	23.9 C
20	117.1 B	29.4 BC
40	144.0 B	31.5 BC
80	196.4 A	43.5 A
100	209.8 A	40.9 AB

† Means followed by the same letter are not significantly different at P=0.05 (Tukey's Studentized Range Test).

Table 2. The effect of phosphorus application on dry matter yield and P uptake by lentil roots in the non-irradiated soil treatment (growth chamber experiment, 1985).†

Phosphorus applied	Dry matter yield	P uptake
$\mu g\ g^{-1}$ soil	------ $g\ pot^{-1}$ ------	------ $mg\ pot^{-1}$ ------
0	1.56 BC	2.9 AB
10	1.37 C	2.5 B
20	1.95 ABC	3.6 AB
40	2.21 ABC	3.0 AB
80	2.49 AB	5.3 A
100	2.54 A	5.2 A

† Means followed by the same letter are not significantly different at P=0.05 (Tukey's Studentized Range Test).

Table 3. The effect of phosphorus application on dry matter yield, and phosphorus and total nitrogen uptake by winter rape roots (growth chamber experiment, 1985).

Phosphorus applied	Dry Matter yield	Phosphorus uptake	Total N uptake
$\mu g\ g^{-1}$ soil	g pot^{-1}	mg pot^{-1}	
Non-irradiated soil treatment			
0	1.71	2.33	n.d.
100	2.79	4.55	n.d.
Irradiated soil treatment			
0	4.16	7.61	n.d.
100	3.71	5.82	n.d.

Table 4. Effect of applied P on total dry matter yield of lentil at various growth stages, at the two field sites (1985).

Phosphorus applied	Growth stage: Vegetative development	Bud to early bloom	Full bloom	Pod
kg ha^{-1}	---------- Mg ha^{-1} ----------			
Haywood site				
Broadcast treatment				
0	0.057 AB†	0.334 B	0.900 A	1.716 B
50	0.037 B	0.316 B	1.087 A	1.840 AB
100	0.049 AB	0.319 B	1.135 A	2.098 A
200	0.063 A	0.500 A	1.208 A	2.060 A
Broadcast plus banded treatment				
18	0.035 A	0.332 A	0.875 A	1.785 A
68	0.052 A	0.351 A	1.018 A	2.193 A
118	0.056 A	0.344 A	1.123 A	1.798 A
218	0.064 A	0.442 A	1.160 A	2.060 A
St. Claude site				
Broadcast treatment				
0	0.085 A	0.543 B	1.240 B	1.990 A
50	0.126 A	0.616 B	1.750 AB	2.333 A
100	0.102 A	0.964 A	1.923 A	2.408 A
200	0.122 A	0.880 A	1.895 A	2.673 A
Broadcast plus banded treatment				
18	0.089 A	0.669 A	1.565 A	2.375 A
68	0.105 A	0.694 A	1.889 A	2.305 A
118	0.111 A	0.925 A	1.802 A	2.498 A
218	0.117 A	0.893 A	1.795 A	2.640 A

† Means followed by the same letter for each site, within treatments and for each growth stage, are not significantly different at P=0.05 (Tukey's Studentized Range Test).

Table 5. Effect of applied P on total P uptake by lentil at various growth stages, at the two field sites (1985).

Phosphorus applied	Growth stage: Vegetative development	Bud to early bloom	Full bloom	Pod
kg ha^{-1}	---------- mg m^{-2} ----------			
Haywood site				
Broadcast treatment				
0	19 AB†	86 B	175 B	211 C
50	15 B	115 B	298 A	365 BC
100	19 AB	111 B	314 A	447 AB
200	22 A	192 A	380 A	553 A
Broadcast plus banded treatment				
18	13 A	100 B	208 B	257 B
68	18 A	118 B	281 AB	424 A
118	17 A	131 AB	311 AB	427 A
218	22 A	167 A	370 A	509 A
St. Claude site				
Broadcast treatment				
0	19 B	140 B	237 B	253 B
50	50 A	192 B	436 AB	508 A
100	41 AB	346 A	549 A	494 A
200	58 A	344 A	689 A	688 A
Broadcast plus banded treatment				
18	32 A	190 B	393 B	487 C
68	39 A	225 B	527 AB	514 BC
118	52 A	363 A	580 A	654 A
218	55 A	347 A	612 A	612 AB

† Means followed by the same letter for each site, within treatments and for each growth stage, are not significantly different at P=0.05 (Tukey's Studentized Range Test).

Table 6. Effect of applied P on total N uptake by lentil at various growth stages, at the two field sites (1985).

Phosphorus applied	Growth stage: Vegetative development	Bud to early bloom	Full bloom	Pod
kg ha^{-1}	---------- g m^{-2} ----------			
Haywood site				
Broadcast treatment				
0	0.24 AB†	1.14 AB	2.65 A	3.35 B
50	0.16 B	0.95 B	2.96 A	4.05 AB
100	0.21 AB	1.04 AB	3.24 A	4.33 AB
200	0.27 A	1.66 A	3.45 A	4.94 A
Broadcast plus banded treatment				
18	0.14 A	1.15 A	2.47 A	3.02 B
68	0.22 A	1.05 A	2.88 A	4.34 A
118	0.23 A	1.11 A	2.99 A	3.96 AB
218	0.27 A	1.37 A	3.49 A	4.83 A
St. Claude site				
Broadcast treatment				
0	0.35 B	1.90 B	2.78 B	3.74 A
50	0.56 A	1.98 B	4.34 AB	4.09 A
100	0.47 AB	2.90 A	4.24 AB	4.07 A
200	0.56 A	2.57 AB	4.56 A	5.71 A
Broadcast plus banded treatment				
18	0.39 A	1.98 A	3.53 A	4.05 A
68	0.48 A	2.23 A	4.77 A	4.68 A
118	0.51 A	2.85 A	4.34 A	4.70 A
218	0.55 A	2.76 A	4.34 A	5.58 A

† Means followed by the same letter for each site, within treatments and for each growth stage, are not significantly different at P=0.05 (Tukey's Studentized Range Test).

Table 7. Effect of applied P on Cu uptake by lentil at various growth stages, at the two field sites (1985).

Phosphorus applied	Growth stage: Vegetative development	Bud to early bloom	Full bloom	Pod
kg ha^{-1}	---------- $\mu g\ m^{-2}$ ----------			
Haywood site				
Broadcast treatment				
0	68 A†	n.d.	1102 A	1343 A
50	38 B	n.d.	1086 A	1368 A
100	52 AB	n.d.	1169 A	1325 A
200	55 AB	n.d.	1248 A	1248 A
Broadcast plus banded treatment				
18	35 A	n.d.	978 A	897 A
68	49 A	n.d.	1078 A	1262 A
118	53 A	n.d.	1181 A	1053 A
218	46 A	n.d.	1126 A	1203 A
St. Claude site				
Broadcast treatment				
0	214 A	n.d.	1342 A	n.d.
50	254 A	n.d.	1607 A	n.d.
100	164 A	n.d.	1011 A	n.d.
200	173 A	n.d.	937 A	n.d.
Broadcast plus banded treatment				
18	263 A	n.d.	1521 A	n.d.
68	182 AB	n.d.	1401 A	n.d.
118	163 AB	n.d.	977 A	n.d.
218	73 B	n.d.	1081 A	n.d.

† Means followed by the same letter for each site, within treatments and for each growth stage, are not significantly different at P=0.05 (Tukey's Studentized Range Test).

Table 8. Effect of applied P on Zn uptake by lentil at various growth stages, at the two field sites (1985).

Phosphorus applied	Growth stage: Vegetative development	Bud to early bloom	Full bloom	Pod
kg ha^{-1}	---------- $\mu g\ m^{-2}$ ----------			
Haywood site				
Broadcast treatment				
0	178 A†	1111 A	2856 A	4796 A
50	93 B	933 A	2598 A	4310 A
100	122 AB	932 A	2598 A	3911 A
200	130 AB	1183 A	2294 A	4702 A
Broadcast plus banded treatment				
18	93 A	1021 A	2391 A	3430 A
68	118 A	911 A	2795 A	4147 A
118	117 A	843 A	2857 A	3824 A
218	115 A	1031 A	2336 A	3701 A
St. Claude site				
Broadcast treatment				
0	396 A	2220 A	4881 A	7038 A
50	376 AB	1626 A	4732 A	5135 B
100	273 AB	2100 A	4891 A	4670 B
200	246 B	1782 A	4090 A	4371 B
Broadcast plus banded treatment				
18	327 A	2036 A	5392 A	6423 B
68	282 A	1669 A	4830 A	4917 AB
118	258 A	2162 A	4198 A	5151 AB
218	223 A	1627 A	4760 A	3918 B

† Means followed by the same letter for each site, within treatments and for each growth stage, are not significantly different at P=0.05 (Tukey's Studentized Range Test).

Table 9. Effect of available P on total dry matter yield of lentil at various growth stages, at the two field sites (1986).

Available phosphorus	Growth stage			
	Vegetative development	Bud to early bloom	Full bloom	Pod
μg g-1 soil	----------	Mg ha^{-1}	----------	
Haywood site				
0	n.d.	1.173 A†	2.530 A	3.478 A
9	n.d.	1.140 A	2.275 A	4.088 A
29	n.d.	1.130 A	2.683 A	4.183 A
55	n.d.	1.139 A	2.668 A	3.603 A
St. Claude site				
5	0.855 A	1.390 A	3.300 A	4.268 A
12	0.715 A	1.760 A	3.518 A	5.215 A
20	0.740 A	1.658 A	3.253 A	5.213 A
29	0.683 A	1.753 A	2.858 A	4.370 A

† Means followed by the same letter for each site, and for each growth stage, are not significantly different at P=0.05 (Tukey's Studentized Range Test).

Table 10. Effect of available P on total P uptake by lentil at various growth stages, at the two field sites (1986).

Available phosphorus	Growth stage: Vegetative development	Bud to early bloom	Full bloom	Pod
$\mu g\ g^{-1}$ soil	----------	mg m^{-2}	----------	
Haywood site				
0	n.d.	310 A†	474 B	703 A
9	n.d.	399 A	862 A	1330 A
29	n.d.	396 A	967 A	1421 A
55	n.d.	444 A	1023 A	1421 A
St. Claude site				
5	308 A	467 A	1139 A	1378 A
12	265 A	598 A	1186 A	1680 A
20	298 A	613 A	1073 A	1704 A
29	315 A	731 A	1058 A	1871 A

† Means followed by the same letter for each site, and for each growth stage, are not significantly different at P=0.05 (Tukey's Studentized Range Test).

Table 11. Effect of available P on total N uptake by lentil at various growth stages, at the two field sites (1986).

Available phosphorus	Growth stage: Vegetative development	Bud to early bloom	Full bloom	Pod
$\mu g\ g^{-1}$ soil	----------	$g\ m^{-2}$	----------	
Haywood site				
0	n.d.	3.7 A†	6.3 A	8.5 A
9	n.d.	3.6 A	7.8 A	10.0 A
29	n.d.	3.3 A	7.0 A	10.8 A
55	n.d.	3.6 A	7.0 A	8.9 A
St. Claude site				
5	3.2 A	3.9 A	8.8 A	11.2 A
12	2.6 A	5.2 A	9.2 A	12.7 A
20	2.8 A	5.3 A	7.8 A	11.7 A
29	2.6 A	5.5 A	7.4 A	11.7 A

† Means followed by the same letter for each site, and for each growth stage, are not significantly different at P=0.05 (Tukey's Studentized Range Test).

Table 12. Effect of available P on N_2 fixation by lentil at various growth stages, at the two field sites (1986).

Available phosphorus	Growth stage: Vegetative development	Bud to early bloom	Full bloom	Pod
$\mu g\ g^{-1}$ soil	---------- kg ha^{-1} ----------			
Haywood site				
0	n.d.	8 A†	27 A	42 A
9	n.d.	6 A	43 A	56 A
29	n.d.	5 A	35 A	64 A
55	n.d.	6 A	34 A	46 A
St. Claude site				
5	0	0	23 A	38 A
12	0	0	27 A	46 A
20	0	0	14 A	36 A
29	0	0	12 A	37 A

† Means followed by the same letter for each site, and for each growth stage, are not significantly different at P=0.05 (Tukey's Studentized Range Test).

Table 13. Effect of available P on Cu uptake by lentil at various growth stages, at the two field sites (1986).

Available phosphorus	Growth stage: Vegetative development	Bud to early bloom	Full bloom	Pod
$\mu g\ g^{-1}$ soil	----------	$\mu g\ m^{-2}$	----------	
Haywood site				
0	n.d.	898 A†	2228 A	2873 A
9	n.d.	825 A	1718 A	3380 A
29	n.d.	682 A	1740 A	3031 A
55	n.d.	631 A	1515 A	2148 A
St. Claude site				
5	596 A	849 A	2813 A	3886 A
12	454 A	1258 A	3658 A	4403 A
20	468 A	872 A	4518 A	4871 A
29	389 A	829 A	3129 A	3740 A

† Means followed by the same letter for each site, and for each growth stage, are not significantly different at P=0.05 (Tukey's Studentized Range Test).

Table 14. Effect of available P on Zn uptake by lentil at various growth stages, at the two field sites (1986).

Available phosphorus	Growth stage: Vegetative development	Bud to early bloom	Full bloom	Pod
μg g-1 soil	----------	μg m^{-2}	----------	
Haywood site				
0	n.d.	3003 A	5206 A	11415 A
9	n.d.	3291 A	4554 A	8957 A
29	n.d.	3097 A	4532 A	12272 A
55	n.d.	2370 A	4186 A	7253 A
St. Claude site				
5	2632 A	3187 A	8426 A	8711 A
12	1834 AB	4155 A	8100 A	8321 A
20	1728 AB	3465 A	8043 A	11968 A
29	1408 B	2817 A	4984 A	5131 A

† Means followed by the same letter for each site, and for each growth stage, are not significantly different at P=0.05 (Tukey's Studentized Range Test).

Table 15. The effect of phosphorus application on root yield and root P uptake by lentil (growth chamber experiment, 1986).

Phosphorus applied	Root yield	Root P uptake
$\mu g\ g^{-1}$ soil	------ g pot^{-1} ------	------ mg pot^{-1} ------
Non-irradiated soil treatment		
0	0.88 C†	n.d.
20	1.91 BC	n.d.
60	2.23 AB	4.3 A
80	3.16 A	6.9 A
120	2.77 AB	6.9 A
Irradiated +VAM soil treatment		
0	2.69 A	4.0 B
20	4.27 A	7.2 AB
60	4.70 A	9.7 AB
80	5.03 A	9.9 AB
120	4.91 A	12.3 A

† Means followed by the same letter for each soil treatment are not significantly different at P=0.05 (Tukey's Studentized Range Test).

Table 16. The effect of phosphorus application on dry matter yield, and phosphorus and total nitrogen uptake by winter rape roots (growth chamber experiment, 1986).

Phosphorus applied	Dry Matter yield	Phosphorus uptake	Total N uptake
$\mu g\ g^{-1}$ soil	g pot^{-1}	mg pot^{-1}	
Non-irradiated soil treatment			
0	3.67	2.97	n.d.
120	3.62	8.22	n.d.
Irradiated +VAM soil treatment			
0	4.14	3.32	n.d.
120	4.53	7.16	n.d.