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THE EFFECT OF VA-MYCORRHIZA, PHOSPHORUS, RHIZOBIUM AND NITROGEN ON GROWTH AND N_2 FIXATION IN LENTIL

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John Gehrer

A Thesis
Submitted to the Faculty of Graduate Studies
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ABSTRACT

The legume crop, lentil, is becoming more important in Canada, which has made Canada a major lentil exporter. However, yields are variable, and not much is known about its association with Rhizobium, except that N_2 fixation is lower than in other legumes.

Growth chamber experiments, using low P soil, were carried out to study how dry matter production and symbiotic N_2 fixation of lentil are affected by: 1) the application of different rates of phosphorus; 2) the inoculation with Glomus intraradices, a vesicular arbuscular mycorrhizal fungi (VAM); 3) the inoculation with Rhizobium leguminosarum and its interaction with G. intraradices; and 4) the application of two rates of nitrogen and its interaction with R. leguminosarum and G. intraradices. The addition of P (0-175 μg P g^{-1} soil) resulted in a significant shoot dry matter response at the pod stage. The lentil plants of the non P added treatment died, whereas oilseed rape was able to grow. Inoculation with G. intraradices caused increases in dry matter production and N and P uptake over the uninoculated uninfected treatments. Contamination by indigenous VAM in the uninoculated treatments eliminated any beneficial effect of inoculation with G. intraradices. Inoculation with Rhizobium resulted in an increase in dry matter production and N_2 fixation when the low rate of N (40 μg g^{-1}) was applied, however at the high rate of applied N (250 μg g^{-1}), while N_2 fixation was eliminated, dry matter production was very high. Inoculation with both symbionts caused a significant increase in dry matter production and N_2 fixation as compared to single inoculation when the low rate of N was applied.

This research showed that lentil is dependent on VAM or high levels of added P. However, the degree of VAM infection necessary was not determined.

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1. INTRODUCTION

The growth of plants in a native or agricultural environment is very complex. Many factors, including the following affect plant growth; the water and nutrient status of soil, plant species, the climatic environment, and the soil microbial populations. The roots of most plants can form symbiotic associations with mycorrhizal fungi. Mycorrhizal hyphae extending into the soil improve the uptake of immobile nutrients such as phosphorus (P). In return for the P, the plant host supplies energy in the form of fixed carbon to the mycorrhiza. A specific group of plant species, legumes, will also form symbiotic associations with bacteria of the genera Rhizobium. Rhizobium contained within the nodules on the roots of these plants can fix N_2 and supply the product to the plant host, thus affecting nitrogen (N) nutrition. As most legumes also contain vesicular arbuscular-mycorrhizae (VAM), this may affect nodulation by Rhizobium.

Lentil, a legume and high protein seed crop is of economic importance in Western Canada. The lentil's Rhizobium was found to fix less N_2 than some other legumes (Rodd, 1986; Rennie, 1987), thus improvements in N_2 fixation could lead to higher yields.

The effect of P on N_2 fixation has been studied extensively on many legumes, but to a lesser degree on lentil. The objective of this thesis research was to determine the effect of VAM, P, Rhizobium, and N on growth, and N_2 fixation of lentil.

2. LITERATURE REVIEW

2.1 Phosphorus nutrition

Native P exists in many forms in the soil, most of which are not readily available to the plant. In calcareous soil, fertilizer becomes less plant available as it precipitates as dicalcium phosphate dihydrate (DCPD) (pKsp=6.6), which in turn hydrolyses to octacalcium phosphate (OCP) (pKsp=93.8) within 4 to 44 weeks. OCP in turn changes to hydroxyapatite (HA) (pKsp=111.5). Concentrations of dissolved P in soil solution continue to decrease until an equilibrium is achieved (Barrow et al., 1977). The decreasing solubility of these P containing compounds has been termed P fixation or retention (Sample et al., 1980). The low solubility, and therefore low mobility, and mass flow of P in the soil creates special challenges in plant P uptake.

The concentration of nutrients in solution or intensity should indicate the availability of soluble nutrients for the plant at any one point in time. However, as P is very insoluble, 70% of all agricultural soils contain less than 0.155 mg P L^{-1} of soil solution, which is the lowest solution concentration, of the macronutrients (Marschner, 1986; Wild, 1988). Since this low concentration or intensity of P is readily depleted by the plants, the buffer power or exchange capacity of the soil is very important in P nutrition.

The P-nutrient capacity of a soil is described by how readily the fixed P is released into the solution (Marschner, 1986; Wild, 1988). Intensity and capacity determine the amount of P available at the root surface. The importance of a high capacity of the soil is highlighted in a calculation by Marschner (1986). During rapid growth of a wheat crop, between 300 and 500 g of P is taken up per hectare each day. If P in solution is 75 g per hectare, then the soil solution has to be

replenished with P several times each day, to ensure that P stress does not occur.

Due to the slow solubilization of P into the soil solution, P is often a limiting factor to crop production in most agricultural soils in (Ukrainetz et al., 1975; Mosse, 1977; Bala and Singh, 1985; Strong et al. 1988; Harinikumar and Bagyaraj, 1989). Phosphorus is needed in photosynthesis and respiration for ATP production (Milthorpe and Moorby, 1979). Therefore P deficiency causes poor energy transfer and plant growth. Symptoms of P deficiency include dark green colour of the older leaves and reddish stems.

The critical P level in the plant varies with age. Young plants contain higher concentrations than older plants, and at maturity, the seeds have a higher P concentration than straw. Critical P concentrations in shoots ranging from 0.1% to 0.25% have been reported by many researchers (Ozanne, 1980; Mengel and Kirkby, 1982; Ikombo et al., 1988). The variability could be due to the different physiological stages during which the plants were sampled.

Usually only a small fraction of the applied P is recovered by crop plants in the first year (Brady, 1984; Morden, 1986). Powell (1977) reported that ryegrass grown at 100 seeds per pot took up 14.3% of the added P, while pots with one plant took up only 7.6%. This suggests that root density or root proliferation has an effect on P recovery. Strong and Soper (1974a) found that rapeseed and buckwheat had a larger proportion of their roots in the fertilizer reaction zone of a non calcarious low P soil, than in the bulk soil. This preferential root growth was not as pronounced for flax and wheat. Therefore, the ability of certain plants' roots to grow into a fertilizer reaction zone can increase P utilization. Species differ in

the ability to take up P fertilizer (Barea and Azcon-Aguilar, 1983) and the variability in soil P levels make predicting yield responses to applied P in the field unreliable. Soil testing, attempts to predict the level of P concentration in soil solutions above which plants will not respond if more P is added. However, actual yield responses to recommended rates of applied P are variable, thereby indicating that factors other than soil solution concentration are involved in P nutrition.

Since P has low mobility in the soil, P concentration in the soil solution is higher than in the solution of the rhizosphere from which P is taken up into the root. In an attempt to determine the critical P concentration of the rhizosphere below which P uptake ceases, many experiments were conducted in solution culture. In a solution culture, root-soil contact is not a variable, since roots are bathed in a constant nutrient concentration. Barber (1984) found that the roots of many species no longer take up P if the solution concentration is below 0.006 mg P L^{-1}. Itoh and Barber (1983) found very similar results where wheat, lettuce, russian thistle, tomato, onion, and carrot could not take up P if the solution concentrations were below 0.004 to 0.02 mg P L^{-1}. However, Edwards (1988) found that cassava required 0.87 to 2.42 mg P L^{-1}, and cassava is very dependent on VAM (Howeler et al., 1982). Using a nutrient solution ranging from 0.031 to 31 mg P L^{-1}, Strong and Soper (1973) found that excised roots of flax took up much less P than wheat, rapeseed, and buckwheat per unit of dry mass of root. These experiments show that the critical P concentration varies considerably among species.

It was found that mass flow is not responsible for the majority of the P uptake, but that the diffusion rate of P is very slow in a P

adsorbing soil, so that the diffusion rate of P is limiting to P uptake in soil grown plants (Lewis and Quirk, 1966; Bole, 1973; Nye, 1977; Sanders et al., 1977). Therefore, the solution concentration at the root surface within the rhizosphere is lower than in the bulk soil, and the critical P concentration in a solution should not be taken as the critical concentration in a soil (Mackay and Barber, 1984).

In a study comparing roots grown in soil and in solution culture, Mackay and Barber (1984) found that corn roots and root hair growth varied inversely to soil P concentration. Thus, the root growth responded to a low P soil, by increasing growth of roots and root hairs. Root hair growth in solution culture was less than in soil, and it did not respond to solution P concentration changes.

As the root elongates into unexplored areas of soil containing higher nutrient concentrations, new root hairs form at the root tip. At the same time, the root hairs in the nutrient depleted zones are sloughed off. This could be an adaptation of the plant by which the plant grows into more fertile soil zones. Root hairs generally live only for a few days (Mengel and Kirkby, 1982) and then are no longer needed, because new P does not diffuse into the root hair depleted zone at any appreciable rate (Nye, 1977). An exception was found by Volkmar (1981), and Mengel and Kirkby (1982) who reported that P uptake can occur in the mature section of excised roots. This most likely is due to active (symplastic) uptake of P across the (suberized) Casperian strip. However in soil, diffusion is still the limiting factor in P uptake.

The slow diffusion rate of P leads to P depletion around the roots forming concentration gradients. Thus, responses to fertilizer P which increase solution P concentration are often observed in soils, although

the soil may contain large amounts of adsorbed P. Powell (1974) reported that dry matter yield of sedge increased exponentially due to P supplementation in a sterilized sandy loam. Sedge, a plant with a finely branched fibrous root system and long root hairs is able to explore the soil volume, thus it is not as dependent on diffusion, and responds well to P fertilizer, as was observed by Powell (1974).

To study P uptake kinetics in soil, Hendriks et al. (1981) used autoradiographs of roots. They found that the P concentration at the root surface decreased by 42% in rapeseed and 50-65% in corn within six days and formed depletion zones of 2.6 mm and 2.0 mm respectively. This difference was attributed to the difference in root hair length of rapeseed (1.3 mm) and corn (0.7 mm). The P concentration at the root surface after six days was 0.03 mg P L^{-1}, a large decrease from 0.8 mg P L^{-1}, but still five times higher than the critical level in nutrient solution of 0.006 mg P L^{-1}, reported by Barber (1984). The soil P concentration within the P depletion zone stayed fairly uniform over the entire period, when P was absorbed by the plant (Hendriks et al., 1981). This was attributed to the close spacing of root hairs which also took up P from the soil in between the root hairs. Bole (1973) found a relationship of root hair density and soil P uptake of wheat only when less than 50 root hair mm^{-1} of root were present. Rapeseed and flax, which had fewer root hairs took up more P, but root hair length was not considered in this experiment. Caradus (1981) pointed out that the root hairs, in the experiment by Bole (1973), were crowded together, and not allowed to extend into new soil to absorb more P. Caradus (1981) found that white clover with long root hairs had a higher shoot dry weight, than plants with short root hairs, when grown in sterilized soil.

If organisms or substances are able to solubilize or make P more available, the effects probably occur in the rhizosphere, which according to Burns (1985) is much less than one mm wide in young annuals, thus creating a very steep concentration gradient towards the root. Another complicating factor in P uptake is that the root can adapt and induce changes in the rhizosphere, if P deficiency occurs. Responses to low P levels such as an increase in root growth, root hair length, and increased root exudation, have been observed (Marschner, 1986). In the experiment by Strong and Soper (1974b) rapeseed and buckwheat reduced the water-soluble P concentration in the reaction zone to a concentration similar to a hydroxy apatite reaction zone concentration when octa calcium phosphorus was applied. Wheat and flax did not decrease the concentration to the same level. This could be attributed to the root characteristics and their ability to proliferate in the fertilizer application zone. Given the low solubility and diffusivity of P, it is obvious that root hairs are very important in P uptake (Barber, 1984).

According to Baylis (1975) "The length and frequency of root hairs is clearly the best single index of a plant's capacity for non-mycotrophic growth". Other factors which are also important in uptake of immobile nutrients such as P are, the root surface area, root zone proliferation, and the size of the nutrient depletion zone. Because these properties are related, plant species can be organized into overlapping groups (as is the case with most biological groupings) by compiling observed root characteristics and growth responses to the application of immobile nutrients (Table 1). The groups that emerge are; (1) plants that have very fine root hairs, and often contain no VAM, such as chenopodiaceae, cruciferae, rushes, and sedges, (2) the

graminoid group (also includes some other species) which has intermediate but highly variable root, and root hair growth, (3) the legumes which often have a coarser root system and less root hair than grasses (Crush, 1974), and (4) plants of different families, including some legume species with very coarse root systems and very short and few or no root hairs.

Crops which have a less than optimum root system for P uptake often form associations with VAM to improve nutrient uptake and survival.

2.2 VAM

Mykorrhizen was the term introduced by A.B. Frank in 1885 to describe a fungus that lives in symbiotic association with roots (Powell and Bagyaraj, 1984). Mycorrhizae are poor competitors for available carbon in the soil and thus depend on a host plant for carbon. Vesicular Arbuscular Mycorrhizae (VAM) is the most common fungus in the roots of agronomically important plants. These Zygomycetes form aseptate hyphae which extend into the soil for several centimetres and take up nutrients for themselves and the host (Kendrick, 1985; Paul and Clark, 1989). Although most plants form associations with VAM, different plant species and families benefit to a various degree. Only a few families, such as Cruciferae and Chenopodeaceae, do not form VAM associations (Barber, 1984), although under special circumstances, rapeseed has had some infection (Ocampo *et al.*, 1980).

Table 13. Properties of several plant families and species which influence P nutrition

property code#	Root property and P nutrition: high P uptake large root property					low P uptake small root property		reference
3+4	Rapeseed Buckwheat			Wheat			Flax	Strong and Soper, 1974
1+5	Rapeseed 1.3mm			Maize 0.7mm				Hendriks et al., 1981
1		Russian Thistle 0.6mm	Tomato 0.43mm	Lettuce 0.30	Wheat 0.29mm		Carrot Onion 0.04mm	Itoh and Barber, 1983
1		Young wheat 1mm						Lewis and Quirk, 1966
6		Russian Thistle	Tomato	Lettuce	Wheat		Carrot Onion	Itoh and Barber, 1983
2			grasses			legumes		Mosse et al., 1981
7	Proteaceae Lupins				*Trifolium subterranium* Red Clover			Trinick, 1977
2					*Lotus Trifolium*		*Centrosema Stylosanthes*	Crush, 1974
1	Rushes Sedges 2mm	Graminoid 1mm	*Solanum nigrum* soft shrub 0.8mm	Tomato	0.3mm		Magnolioi none	Baylis, 1975

1 root hair length (mm)
2 root surface area
3 root zone proliferation
4 P uptake
5 size of depletion zone
6 Root hair surface·Root surface^{-1}
7 specialized clusters of rootlets

At the same time, plants such as Leuceanea (Manjunath et al., 1984) and flax (Thompson et al., 1988) are almost totally dependent on VAM. Since VAM only grows in association with a root, it is very difficult and costly to produce VAM inoculum. Therefore field experiments where large areas are inoculated would be impractical, especially since the VAM is present in most soils.

Soil, if inoculated with VAM, or naturally present, aid many crop plants in the uptake of P as well as Zn, Cu (Bolan et al., 1983; Kucey and Paul, 1983; Thompson et al., 1988), and other nutrients such as N (Smith, 1980a), and K (Lambert et al., 1979). VAM are also found to increase drought resistance (Busse and Ellis, 1985), disease resistance (Kendrick, 1985), and N_2 fixation of legumes (Daft and El-Giahmi, 1974). Sometimes a decrease in plant growth during the early establishment phase of infection is observed, presumably due to nutrient removal from the host (Baylis, 1975; Smith, 1980a; Volkmar, 1981), but this quickly reverts to an overall positive effect. A review by Mosse et al. (1981) discusses the effect of VAM on plant growth.

When studying sustainable or organic agriculture, VAM are often discussed (Cook, 1984; Mosse, 1986). Mosse (1986) warns of the unknown consequences of not having mycorrhiza in soils, if current agriculture destroys it. Burns and Davies (1986) indicate that soil microorganisms including VAM improve the soil structure by forming aggregates.

During the 1970's, research was conducted to assess the effect of VAM on solubilizing unavailable P sources such as OCP and hydroxy apatite (HA) (Mosse, 1973). The hyphae of the VAM fungi grow into the soil as do roots and root hairs, thereby exploring soil volumes not yet depleted. It was concluded that benefits of VAM on P uptake were due to this increased root-hyphae-soil contact. The theory of increased

solubilization due to VAM is generally no longer accepted (Barrow et al., 1977; Sanders et al., 1977; Tinker and Gildon, 1983; Barber, 1984),and the main benefit of VAM is as extended roots. Barber (1984) suggests that VAM hyphae can take up P from a solution with a lower concentration than can roots. This is however very difficult to determine, since it is difficult to get a measurement of the soil solution concentration at the root surface, as discussed previously.

Species with many and long root hairs are better able to take up P, than plants with few and short root hairs. Conversely, species with fewer and shorter root hairs respond more to the inoculation with VAM fungi, especially in low nutrient soil.

The plant species affects VAM infection. Plenchette et al. (1983) ranked carrot, leek, tomato, wheat, and cabbage in decreasing order of VAM dependency, where all plants except the cabbage had at least 60% of their total roots infected with VAM. Harinikumar and Bagyaraj (1989) found that finger millet (grass) decreased the spore number in soil over that of cowpea and groundnut. The last two crops have a coarser root than millet, thus are more dependent on VAM. Lentil has a coarse root system (and possibly short root hairs), therefore it too may be dependent on VAM infection for growth.

VAM infection is often decreased by high soil P concentrations and by fertilizer additions by decreasing the number of spores in soil (Harinikumar and Bagyaraj, 1989), and VAM infection on lentil roots (Badr El-Din and Moawad, 1988).

VAM Inoculum density also affects %VAM infection. When fifty instead of five spores per pot were used as inoculum for French bean, the %VAM infection increased from 27% to 46% (Daft and El-Giahmi, 1974).

As the period of time from inoculation to sampling increased, it was found that %VAM infection increased (Daft and El-Giahmi, 1974; Lu and Miller, 1989; Walley and Germida, 1991). Smith (1980b) found the highest spore number during the host's reproductive phase. The spore number was also higher in a pasture situation, rather than a wheat field. Summerfallow decreased the VAM infection of the subsequent crop as compared to a continuous wheat rotation (Walley and Germida, 1991).

Studying ten unsterilized soils, Stribley et al. (1980) found the relationship between $NaHCO_3$-extractable P and yield to be very poor, such that a distinct quadratic yield response curve was observed for each of several soils. When VAM status (either sterilized soil or sterilized soil but inoculated with VAM as compared to unsterilized uninoculated soil) was included in the quadratic curve regression analysis, the coefficient of determination increased, so that all soils fitted one curve. Thus, VAM has a large effect on extending the explored soil volume and P uptake. Soil $NaHCO_3$ extractable P level was able to predict plant yield.

Although P uptake is the main benefit of VAM infection, it is also accepted that VAM plays a role in Zn and Cu uptake (Tinker and Gildon, 1983). Wellings et al. (1991) found that VAM inoculation increased P and Zn concentration and uptake of pigeonpea in a Vertisol. The plant Zn concentration of most treatments was below a 20 $\mu g\ g^{-1}$ soil critical level. The P/Zn ratio also increased due to VAM inoculation, indicating that P nutrition was enhanced more than Zn nutrition. In a field experiment, the soybean shoot yield, K, P, and N_2 fixation increased due to Glomus intraradices inoculation. However, the N and Zn concentrations decreased, while the N and Zn uptake still increased due to G. intraradices inoculation (Hamel et al., 1991). It is possible,

that high P levels inhibited VAM formation, which in turn inhibited Zn uptake. Although VAM is known to increase the uptake of certain micronutrients in deficient soils, Dueck et al. (1986) found that VAM alleviated the negative effect of Zn on growth of grasses growing in Zn polluted soil.

In a few cases, VAM has improved the N status of the host plant. With legumes, however, it is Rhizobium which have the greatest effect on plant N content by means of N_2 fixation.

Tinker and Gildon (1983) reported that boron may help a rapid VAM establishment on the host, thus it may be involved in Zn and Cu nutrition indirectly.

2.3 Rhizobium and N_2 Fixation

Nitrogen is taken up by roots usually as the readily soluble NO_3 ion, (Jones and Davis, 1983) but NH_4 ions can be taken up also. Nitrate is reduced to ammonium in the plant, where it functions mainly as a component of proteins.

Leguminosaeous crops often form symbiotic associations with Rhizobium bacteria which convert atmospheric N_2 into plant usable ammonia. Rhizobium bacteria can survive in the soil for several years without a plant host. They can also be cultured in the absence of plants, and therefore are readily available from manufacturers as an inoculum. When this research was started in 1987, only a combined pea and lentil inoculum was available, but in 1991 a special lentil inoculum became commercially available. Rhizobia bacteria are short to medium long Gram-negative rods which can form nodules on the host's roots within seven days after the plant germinates. N_2 fixation starts about one week after nodule formation, and the rate of fixation increases with

time. Thus a large part of N_2 fixation occurs late in the growing season (Richards and Soper, 1979; Jensen, 1987).

Most legumes including lentil form symbiotic associations with VAM and with Rhizobium.

The Rhizobium on a legume receives its energy and nutrients other than N from the host plant. Thus a nutrient deficient soil, which causes poor plant growth, can also inhibit Rhizobium development and N_2 fixation.

The costs of N_2 fixation are high and include the energy required to reduce N_2 to ammonium and energy for nodule growth and maintenance. This cost is higher than the energy needed for the reduction of NO_3 to NH_4. This high energy requirement could lead to high respiration rates. Nodulated roots respired twice as fast as non-legume roots, and used 14% more of the photosynthetic assimilates (Jones and Davis, 1983). Thus if soil N is available, then N_2 fixation is reduced.

Of all non-mobile essential nutrients used by plants, P is required in the largest amounts (Frieden, 1985). Amongst other uses, it is required in the nitrogenase enzyme to supply energy for the reduction of N_2 to ammonia (Eisbrenner and Evans, 1983). According to Freire (1984), P deficiency is the most limiting factor to N_2 fixation and legume production in the tropics and subtropics. In calcareous soils, P is fixed and thus it is not readily available (Cho et al., 1970; Sample et al., 1980).

To determine the effect of added P on nodulation, and on plant growth separately, non-inoculated plants with large amounts of added mineral N can be used as the control. Israel (1987) observed greater growth responses of soybean to added P in the treatments with added mineral N than in the Bradyrhizobium inoculated treatments to which no N

was added. These findings coincide with the law of the minimum, where plant growth is limited by the most limiting nutrient, in that when one growth factor is limiting (N in N_2 fixing plants), then no growth increase occurs due to other growth factors (added mineral P). Analysis of the nodules revealed that the P concentration in nodules increased by 40% from the lowest to highest P treatments. Over the same P treatments the whole plant P concentration increased by over 300%. This is evidence that nodules have a higher affinity for P when this nutrient is limiting, since they contain a relatively high P concentration at low levels of added P. Singleton et al. (1985) used five different strains of R. japonicum, two levels of added N and from 0 to 400 μg P g^{-1} soil to determine the amount of N_2 fixed by soybean. The shoot yield, and the N and P concentrations were always higher in the plants with added N than in the plants with the most efficient strain of Rhizobium. Nodule P concentration was not given, however, the higher yields of the N supplied plants at the low P treatments imply that nodules accumulate P up to a critical concentration and only then can they fix N_2. As more P was added, yield differences between the different Rhizobium strains became larger. Singleton et al. (1985) explained that this was due to the limiting nutrient P. Therefore yield differences became more pronounced as P became less limiting and the superior bacterial strains were able to fix N_2 up to their potential. Barber (1984) suggested that the critical P concentration of a solution culture below which N_2 fixation ceases is 0.006 mg P L^{-1}.

Using several legumes, P application increased nodulation, N_2 fixation and plant growth (Lüdecke, 1941; Mooy and Pesek, 1966; Hamdi, 1976; Malavolta et al., 1982; Smith, 1982; Bullen et al., 1983; Singleton et al., 1985; Bailey and Grant, 1988). In general, by

improving the P supply to a legume, the plant will grow larger, due to better P nutrition of the plant and nodules. If N_2 fixation functions efficiently at an early stage, no fertilizer N has to be applied in order to get good growth. This means that plants should not show a yield response to added N. Contrary to this theory, Israel (1987) found that inoculated soybean responded in yield to the addition of NO_3 N in an outdoor pot study. Malavolta et al. (1982) in a solution culture experiment, concluded that growth of French bean (Phaseolis vulgaris L.) was promoted when combined N was applied as compared to plants that were dependent on N_2 fixation. Breeze and Hopper (1987) found that white clover with added NO_3, grew larger than plants which depended on N_2 fixation. They also found an interaction between the source of N, and the P concentration of the roots. Roots of the plants which depended solely on N_2 fixation had a higher P concentration.

In a pot study by Richards and Soper (1979), the seed yield of fababean increased only slightly, when 300 μg N g^{-1} soil were applied, while in treatments with less applied N there was no response to added N. N_2 fixation was able to supply the plant with N almost equal to an application of 300 μg N g^{-1} soil. The N uptake and concentration of the fababean were not affected by N application. Ukrainetz et al. (1975) reported variable yield results due to N addition, and concluded that unless adverse environmental or soil conditions are known to be present, N should not be added to legumes.

Other than affecting yield, the addition of mineral N usually inhibits nodulation and N_2 fixation (Munns, 1977; Richards and Soper, 1979; Curl and Truelove, 1986; Walley, 1986). Increasing NO_3 concentrations inhibit root elongation and root hair production, reduce root hair curling, and prevent the normal development of infection

threads. However, Dadson and Acquaah (1984) found that low rates of added N increased the number, dry mass, and leghemoglobin content of nodules, as well as protein content in the seeds of soybean. Waterer et al. (1992) found that low levels of NH_4 stimulated nodulation of solution grown peas, whereas NO_3, even at low levels inhibited nodulation. This is known as the starter effect, where mineral N helps establish plants before N_2 fixation begins. Results from field experiments conducted in Saskatchewan support this theory (Bremer et al., 1988b). A significant increase in lentil seed yield due to added N (0 to 80 kg N ha^{-1}) was observed at two of three sites. As a starter dose, applied N overcomes the initial N deficiency before nodulation starts. Therefore, it is possible that lentil, which has a smaller seed than most other grain legumes and hence a small N reserve, would benefit significantly from this added N.

In addition to energy and P, the Rhizobium also requires many other nutrients which it obtains from the plant host. If these nutrients are not available to the bacteria in sufficient amounts, then N_2 fixation can be inhibited, and the plant will show N deficiency symptoms. The addition of N to these plants should overcome these symptoms since N usually acts quickly.

Rai and Prasad (1983) grew lentil in soils with a pH ranging from 4.8 to 7.8. No nodulation occurred below a soil pH of 6.5 when a commercial strain of Rhizobium was used, but mutant strains caused nodulation at a pH of 4.8. According to Munns (1977) the critical pH range of Rhizobium for Medicago and Pisum is 4.5 to 5.5. Soil acidity can affect the nutrient supply, and the survival and function of the Rhizobium (Ukrianetz et al., 1975; Munns, 1977; Curl and Truelove, 1986). This can be caused by acidity itself, acid induced Ca deficiency

or by a toxicity of Mn and Al (Smith, 1982). In addition, traces of heavy metals in acid fertilizers can cause poor nodulation (Curl and Truelove, 1986).

The Fe concentration of lentil nodules has been found to increase with increasing soil pH and decreasing Mn concentrations (Rai and Prasad, 1983). This was thought to be due to an antagonistic effect of Mn on Fe, at low soil pH values. The Fe concentration of the nodules correlated with the nodule number, nodule weight, relative N_2 fixation (acetylene reduction), but not to total plant N content. These results imply that nodule growth and N_2 fixation are affected more by Fe deficiency than is lentil growth, since there was more plant N than one could expect from N_2 fixation, at low soil pH values.

Molybdenum and iron are also important in N_2 fixation, as they are part of nitrogenase enzyme complex (Burgmayer and Stiefel, 1985). A Mo deficiency can lead to N deficiency in legumes due to its requirement in the N_2 fixation complex (Smith, 1982). Hamdi (1976) reported increased N_2 fixation of cowpea grown in Nile silt when Mo was added.

Boron deficiency resulted in the first symptoms being N deficiency in Vicia and Pisum grown in solution culture, and peas grown in soil. These symptoms are similar to Mn deficiency symptoms. Munns (1977) reported that boron deficiency severely reduced nodulation, and Hamdi (1976) found increased N_2 fixation when boron was applied.

Zinc is important in RNA synthesis, and a deficiency prevents the normal development of chloroplast grana (Mengel and Kirkby, 1982). Marsh and Waters (1985) studied the effects of adding Zn to cowpea in field and greenhouse experiments. The nodule number, dry weight, seed yield, and acetylene reduction increased significantly when Zn at levels of 2.5 - 5 $\mu g\ g^{-1}$ soil were added. As well, Mengel and Kirkby (1982)

report that when Zn is deficient, fewer buds are formed and thus crop yields are reduced. This may not be related to direct effects of Zn deficiency causing decreased fixation, but rather to a Zn deficiency in the plant (Marsh and Waters, 1985).

Although precise biochemical roles of copper are unclear, Munns (1977) summarized some effects of low Cu levels. Copper deficient nodules slowly incorporate ^{14}C into amino acids and protein. They also had fewer, smaller bacteroids containing more starch.

Freire (1984) reported on positive responses to the application of S on yield of alfalfa and soybean in the tropics.

The main effect of potassium is on plant growth, rather than nodulation, therefore no interaction between K and Rhizobium has been observed (Freire, 1984).

Based on these results, it is critical that the basal nutrients are applied at the correct concentrations in an experiment, so that no further complications other than the VAM-P-N_2fixation interaction occurs.

2.4 VAM and Rhizobium

Legumes contain Rhizobium bacteria in nodules on their roots, and most but not all legumes also form associations with VAM (Asai, 1944; Mosse, 1977; Barea and Azcon-Aguilar, 1983; Singh and Singh, 1986; Kucey and Bonetti, 1988). Therefore double inoculation of legumes with VAM and Rhizobium as compared to inoculation with either symbiont is expected to be beneficial.

Lambert et al. (1979) found an increase in soybean yield due to P application or inoculation with VAM. Mosse et al. (1976) determined that clover, and the tropical legumes Stylosanthes and Centrosema did

not form nodules in a P deficient soil unless VAM was added. Kucey and Paul (1983) concluded that VAM appeared to increase plant P uptake and N_2 fixation but did not interact directly with the nodules, since the nitrogenase activity per unit mass of nodule did not change when VAM was added.

Singh and Singh (1986) found positive effects of VAM and or P addition on lentil yields. Overall, there is overwhelming evidence, that VAM and or P application increase lentil growth and N accumulation. It is unclear however, whether this is caused through the plant, through the nodule bacteria or through both (Barea and Azcon-Aguilar, 1983).

Manjunath et al. (1984) found dual inoculation with VAM and Rhizobium to be beneficial to the fodder tree legume Leucaena. Inoculation with VAM alone improved nodulation by native rhizobia. Inoculation with Rhizobium increased root colonization of native VAM fungi. However, dual inoculation with both the organisms improved nodulation, VAM colonization, dry mass, N and P content of the plants compared to single inoculation with either organism. Daft and El-Giahmi (1974) found double inoculation increased growth, nodule number, nodule mass per gram of root, acetylene reduction, shoot %P, shoot %N, and shoot yield, but decreased the shoot/root ratio of French bean grown in a growth chamber. Cluett and Boucher (1983) reviewed work on the effect of double inoculation with VAM and Rhizobium; Inoculation with VAM usually increased nodulation. However, when nodulation was corrected for the increase in plant size due to VAM infection, a positive effect was no longer apparent. All other increases from VAM could be explained by increased P nutrition. Thus, it is not clear if a mutualistic symbiotic relationship between the two microbes exists.

Smith (1980a) stated that VAM associations can be important factors influencing N nutrition of plants, particularly when the comparatively immobile NH_4 ion rather than NO_3 is major source of N. Stribley and Read (1980) found that young evergreens could use amino acids as a N source only if they contained VAM. Barea and Azcon-Aguilar (1983) suggested that VAM increases root cell permeability, which could increase nodulation. This suggests VAM could affect N-nutrition other than through N_2 fixation alone. Lentil, a crop which does not fix as much N_2 as do other legumes could benefit from double inoculation.

2.5 Lentil

Lentil, a high protein seed crop, used primarily for human consumption, is part of the Leguminosae family. It was grown on 1.84 million ha worldwide in 1980 with an average seed yield of 655 kg ha^{-1} (Witcombe and Erskine, 1984). This 1.2 million tonnes production increased to about 2.7 million tonnes by 1990 (FAO). Canada is a major lentil grower, and Statistics Canada estimated the 1991 production to be 0.3 million tonnes.

Little research has been conducted on lentil and only a few improved varieties exist. There is, however, great variability within the existing germplasm collection which can be exploited. By selecting superior yielding plants from a seedrow, yield was almost doubled over the average. Variation in size between pea plants was found because of genetic differences, lodging, and seed size (Jones and Davis, 1983). Petr *et al*. (1988) reported that lentil yield variability was greater than that of pea, due mainly to high genetic variability, and lodging, and seed shattering problems.

Hamdi (1976) in Egypt found a seed yield response in lentil due to the addition of N as well as to Rhizobium inoculation, in the presence of large amounts of P. Since the plants responded to fertilizer N although they were inoculated, they were not able to accumulate all the needed N by N_2 fixation (Hamdi, 1976). Walley (1986) found that small amounts of applied N increased seed yield. It was concluded that lentil was not efficient in fixing sufficient N_2 to meet optimum plant growth requirements in a field experiment. In a field trial in Chile, where the soil contained inorganic N at 21 $\mu g\ g^{-1}$ soil, nodulation, plant mass, yield, and % protein of lentil increased by over 100% due to inoculation with Rhizobium (Herrera and Longeri, 1984). The indigenous Rhizobium and the soil were able to supply all the N required for a 1200 kg seed ha^{-1} yield, as no yield response to added N (65 kg ha^{-1}) was observed, while average N_2 fixation yielded 39 kg N ha^{-1}. Factors other than N supply must have limited the yield and the fixed N_2 to this level. Lentil fixed less N_2 than other legumes, such as fababean, pea and soybean, when grown under comparable conditions in growth chamber experiments (Rodd, 1986). Tsukomoto et al. (1976) in a field study at Brandon Manitoba found that the seed yield (kg ha^{-1}) of lentil (1418) was lower than that of field pea (2146) and fababean (1845). On the other hand, under irrigated field conditions in southern Alberta, Rennie and Dubetz (1986) reported a lentil seed yield of 5511 kg ha^{-1}, which was higher than that of fababean, pea and chickpea. It appears therefore, that lentil yields are often low, but there may be a very high yield potential under optimum conditions.

The effect of P on yield and N_2 fixation of lentil in a growth chamber in sterilized and unsterilized soils has been studied (DeBeer, 1990). The lentil did not grow in the sterilized soil, however when the

soil was inoculated with G. intraradices, yield, P uptake and N_2 fixation increased. As well, increases in P additions increased yields further. Bala and Singh (1985) observed increased lentil growth, dry matter production, nodulation and N_2 fixation following VAM inoculation in unsterile, low and medium P soils.

A side effect of soil sterilization is the introduction of chemical and biological changes (Strong et al., 1988) which can lead to the production of toxins (Rovira and Bowen, 1966), as well decreases or increases of $NaHCO_3$-extractable P have been reported (Stribley et al., 1980). In unsterilized soil, Ross (1980) found a nondiffusible, heat labile factor, which reduced infection of VAM. It was assumed that this factor is similar to the factors that control a plant's susceptibility to certain fungal diseases. Heat treatment of the soil (15 min at 65°C) inactivated this factor, and VAM infection was increased. To overcome these complications in VAM research, the following experiments were conducted in unsterilized subsoil, so that lentil growth and N_2 fixation responses are observed due to the G. intraradices inoculation and P addition. It was expected that subsoil would be low in available P and organic matter and free of native VAM and Rhizobium.

3. MATERIALS AND METHODS

Four growth chamber experiments were conducted using an Almasippi subsoil (Michalyna et al., 1988) collected from the C horizon at a depth of about 1.5 m. Subsoils were collected prior to the start of each experiment from the same location in a summerfallow field. Subsoils were used primarily because they contained little or no indigenous VAM (Mosse et al., (1981). Thus eliminating the need for soil sterilization, so that the effect of inoculated VAM could be observed without the side effects of sterilization. Even though the subsoil in this study was not sterilized, it was assumed to have a lower microbial population than topsoil, based on its very low organic matter content, and the depth from which it was collected.

After conducting three experiments using the subsoil, the fourth experiment was conducted using the Almasippi subsoil blended with a Willowcrest topsoil (20:80 topsoil:subsoil) (Michalyna et al., 1988). This was done to introduce the microflora of a summerfallowed topsoil into the subsoil (Rovira and Bowen, 1966). However, it was assumed that small numbers of indigenous mycorrhizal fungi were introduced when this small proportion of summerfallowed topsoil was added to the subsoil (Kucey and Paul, 1983; Hunter and McCosker, 1988; Thompson et al., 1988).

After collection of the soils, they were air dried, sieved (2 mm), mixed, and analyzed for physical and chemical characteristics (Table 2). Texture was determined by hand texturing (Shaykewich, 1986); the pH and conductivity were measured in a water soil paste (1:1) (McLean, 1982); the field capacity was determined according to a method by Viehmeyer and Hendrickson (1949). Nutrient analyses of the soil were conducted as follows:

1) NO_3-N by $NaHCO_3$ soil extraction, and using a modification of the automated colorimetric procedure of Kamphake et al. (1967) on a Technicon Auto Analyzer.

2) Available P using a modification (2.5g soil) of the method of Olsen and Sommers (1982) with colour development by the acid molybdate ascorbic acid method (Murphy and Riley, 1962).

3) Exchangeable K by flame photometry (Knudsen et al., 1982).

4) SO_4-S after extraction with $CaCl_2$ on an Auto Analyzer II system (Hamm et al., 1973).

5) Exchangeable Ca and Mg was determined by the method of Lanyon and Heald (1982) with the following exceptions: soil (2.5g) was mixed with 25mL of 1.0N NH_4OAc, shaken for 30 minutes, and then filtered through a #1 paper. An 8 mL aliquot of the filtrate was taken, and 2 mL of a 5 % La solution added to it. This mixture was then analyzed using an atomic absorption spectrophotometer (Isaac and Kerber, 1980).

6) DTPA extractable Zn, Cu, Fe and Mn were determined (Lindsay and Norvell, 1969), and the filtrate was analyzed by using atomic absorption spectrophotometer (Follett and Lindsay, 1971).

Although the designs of the four experiments were different, the methods of seed sterilization and Glomus intraradices and Rhizobium leguminosarum inoculation were the same.

Seeds were surface sterilized by immersion in 95% ethanol for ten seconds and then placed in a two percent sodium hypochlorite solution for two minutes. The seeds were then rinsed five times with distilled water and allowed to dry. The R. leguminosarum inoculated treatments were inoculated by mixing the seeds in a slurry of 5 g of Nitragin 'C'

Table 2. Summary of physical and chemical properties of soils used in the experiments.

Experiment	Soil Series	Texture	pH	Ec[§] dS m^{-1}	NO_3-N	P	K	SO_4-S	Zn	Cu	Fe	Mn
					Soil properties							
					----------μg g^{-1} soil----------							
1	Almassippi	v.f. sand	8.4	0.1	3.2	3.0	47	6.2	0.24	0.40	6.2	1.6
2	Almassippi	v.f. sand	8.0	0.2	5.0	1.2	99	20+	0.48	0.58	13	1.1
3	Almassippi	f. sand	8.2	0.1	1.4	0.4	25	2.1	0.14	0.52	10	1.2
4	Almassippi	f. sand	8.2	0.2	2.0	1.0	23	1.6	0.12	0.26	10	1.2
	Willowcrest	l.f. sand	7.2	0.3	7.6	7.0	94	5.0	0.97	0.92	16	12

§ Electrical Conductivity

culture (Nitragin Company, Milwaukee, WI 53219) and 10 mL distilled water.

One of the difficulties when working with VAM is that the inoculum is not available commercially. Initial inoculum has to be obtained from another researcher, or it has to be isolated from the roots of field grown plants. For these experiment, corn or wheat were planted in calcined montmorillonite clay (Zorb-all, a commercial floor absorbent) in sterilized pails. Each pail was inoculated with eight, one-gram moist root-mats which were infected with G. intraradices, placed at a depth of four cm into the montmorillonite, and sterilized seeds were placed directly above the infected roots to a depth of 2.5 cm. Zorb-all was kept at maximum moisture and twice a week the plants received a Hoagland (1950) nutrient solution modified to contain less P (10 mg P L^{-1}). The pails were kept on a growth bench at 21°C ± 4°C and with a day/night cycle of 10/14 hours, which provided 310 μmoles of photons cm^{-2} sec^{-1} at canopy height. At about 50 days the shoots were removed. The roots were washed with distilled water, drip dried, cut into 3 to 5 cm long pieces and mixed together from all the pails. After verifying VAM infection (Ocampo et al., 1980; Sutton, 1973) inoculum was either used directly in an experiment, kept in a plastic bag at 4°C, or propagated into more inoculum grown alternately on corn or wheat, to prevent the occurrence of root disease which could occur in a continuous monoculture. Uninfected corn roots were grown using the same method, but without being inoculated with G. intraradices. The uninfected corn roots were used in the control treatment, so that all pots had the same amount of C containing material, as well as the same microorganisms which are associated with root growth.

3.1 Experiment 1

The purpose of this experiment was to determine the effect of G. intraradices inoculation and or P application on dry matter production, and P, and N accumulation in lentil (Lens esculenta var. Eston). An experiment was conducted in a growth chamber where large amounts of N were supplied to the pots, so that N_2 fixation was not limiting to the growth of the plants. Oilseed rape (Brassica napus var. Westar) was used as the control crop to determine potential growth under the given uninoculated conditions.

A completely randomized experiment (Little and Hills, 1978) with lentil was conducted with nine levels of P (0, 20, 40 60, 80, 100, 125, 150 and 175 μg P g^{-1} soil), with and without G. intraradices. Each treatment was replicated three times. Oilseed rape, the control, had two levels of P (0 and 100 μg g^{-1} soil) and was not inoculated with G. intraradices. The -G. intraradices treatments of lentil and oilseed rape received uninoculated corn root mats to supply similar amounts of C to each pot.

Prior to seeding, the P treatments were applied by spraying a 25 mL solution of diluted phosphoric acid onto 2.5 kg of soil. Hand mixing followed, to apply the P uniformly throughout the soil to simulate soil P conditions.

In the +G. intraradices treatments, a one-gram moist corn root-mat infected with G. intraradices was pushed 4 cm into the soil at four locations. The uninoculated lentil and the oilseed rape received a similar but uninfected corn root-mat.

The lentil seeds were not inoculated with a Rhizobium. One sterilized lentil seed or two sterilized oilseed rape seeds were placed directly over each corn root-mat to a depth of 2cm into the soil. The

subsoil was then brought to 75% of field capacity by mass with distilled water. After emergence, the soil was brought to field capacity by watering with distilled water on a daily basis.

Basal nutrients were applied to all pots to help ensure sufficient nutrient supply as well as to limit N_2 fixation of lentil. This was done by applying 10 equal increments of nutrients, dissolved in distilled water, to the pots, every 4 days, starting at six days after seeding. The final concentrations (μg of nutrient g^{-1} soil) was as follows: N as NH_4NO_3 at 250, S as K_2SO_4 at 50, K as K_2SO_4 at 122, K as KCl at 128, Zn as $ZnSO_4 \cdot 7H_2O$ at 5, Cu as $CuSO_4 \cdot 5H_2O$ at 2.5, Fe as $FeSO_4 \cdot 7H_2O$ at 5, Mn as $MnSO_4 \cdot H_2O$ at 2.5, B as H_3BO_3 at 0.15, and Mo as $NaMoO_4 \cdot 2H_2O$ at 0.05. Watering followed every addition of basal nutrients to move the nutrients into the soil. The NH_4NO_3 was selected as a N source, because urea was found to have deleterious effects on seed germination and early plant growth in this soil (Toews and Soper, 1978).

All pots were placed in a growth chamber in which the photoperiod was adjusted to 16 hours per day with a photosynthetically active radiation of 630 μmoles of photons m^{-2} sec^{-1} at plant canopy height. The day/night temperature and relative humidity was 22°C/18°C and 50%/90%, respectively. Observations of plant growth were recorded throughout the period. The pots were thinned, at 16 days after seeding, to two plants per pot.

The shoots were harvested in the early pod stage, 62 days after seeding, when flowering was approximately 80% complete. It is difficult to define a growth stage in lentil, due to its indeterminate growth habit. The roots were harvested by washing the soil root mass on a fly screen. The roots were then rinsed with distilled water.

3.2 Experiment 2

The purpose of this experiment was to determine the effect of G. intraradices inoculation and or P application on dry matter production, P and N accumulation, and N_2 fixation in lentil. To promote N_2 fixation, only small amounts of fertilizer N were supplied (Rodd, 1986; Walley, 1986).

Experiment 2 was repeated (Experiment 2b) due to low VAM infection of the lentil roots in Experiment 2a.

The experiment was a completely randomized factorial (Little and Hills, 1978) with 6 levels of applied P (Experiment 2a: 40, 60, 80, 100, 125, and 175 μg P g^{-1} soil, Experiment 2b: 0, 40, 60, 80, 125, and 175 μg P g^{-1} soil) with and without G. intraradices inoculation, for a total of 12 lentil treatments. Each treatment had 3 replications. In Experiment 2b, the 40 and 125 μg P g^{-1} soil treatments were duplicated, so that analysis could be made at two harvest dates.

All treatments received small amounts of labelled fertilizer N (40 μg g^{-1} soil) as a starter source of N, and were inoculated with R. leguminosarum to supply the plant with N.

Two treatments of oilseed rape were used as a control crop to determine potential growth and N_2 fixation of lentil using the isotope dilution method. There were two levels of added N (40 or 250 μg g^{-1} soil) with P at 100 μg g^{-1} soil added to both treatments.

Prior to seeding all nutrients except N were incorporated at one time into the 2.5 kg of soil per pot in experiment 2a and 5 kg of soil per pot in experiment 2b (as described for P in Experiment 1). The rates of nutrients were the same as in Experiment 1, except the amount of Zn, Cu, and Mo were doubled to help prevent deficiencies. The N was

added as NH_4NO_3 doubly labelled with 2.50 (Experiment 2a) and 3.05 (Experiment 2b) atom% excess ^{15}N.

For Experiment 2a, to achieve uniform plant growth, the seeds were pregerminated for three days. One sprout with a radicle of approximately eight mm was then transplanted directly over one of the four corn root-mats in each of the prepared pots in a similar manner as in Experiment 1.

Since G. intraradices infection was low in Experiment 2a, inoculum that was harvested just prior to the start of Experiment 2b was used to help insure a high degree of infection. Uninfected corn roots were not available at the start of the experiment, therefore 220 g of infected roots were placed in a sterilized vacuum filter apparatus on a Whatman #4 filter paper to prepare uninfected corn roots. The roots were rinsed with 800 mL of distilled water. The roots were then autoclaved for 30 min. (at 141 kPa, 121 °C). The autoclaved roots were added to uninoculated treatments in the same manner as the infected roots. The filtrate (10 mL per pot) was added to all treatments including oilseed rape. The unsterilized filtrate and autoclaved roots were intended to simulate the uninfected corn roots (Sanders et al., 1977; Bethlenfalvay et al., 1985; Piccini et al., 1988).

In Experiment 2b, two lentil or two oilseed rape seeds was placed over each of the four corn root-mats. Watering was carried out as in Experiment 1.

All pots were kept in a growth chamber in which the photoperiod was adjusted to 16 hours per day with a photosynthetically active radiation of 350 (Experiment 2a) and 750 (Experiment 2b) μmoles of photons m^{-2} sec^{-1} at plant canopy height. The day/night temperature and relative humidity was 24°C/16°C and 50%/85% respectively, in Experiment

2a; and 24°C/15°C, and 55%/95%, respectively, in Experiment 2b. Observations of the plants were recorded throughout the growth period. To select for uniform plants within each pot, the plants were thinned at 18 days after planting, to two per pot in Experiment 2a, and four plants per pot in Experiment 2b.

The shoots were harvested in the early pod stage (61 days). All other harvest procedures and sample preparation for analysis were as described in Experiment 1, except in Experiment 2b, where the roots were washed further by placing them in a two litre pail containing one litre of water. With the lid closed, the roots were shaken for one hour on a wrist action shaker. Thereafter, the pail was allowed to sit for 40 minutes to allow the sand and silt settle out (Viehmeyer and Hendrickson, 1949). The roots were screened off (0.8 mm screen), and rinsed with distilled water. Harvest of mature plants (Experiment 2b, 40 and 125 μg P g^{-1} soil) was carried out at 80 days after seeding by cutting the shoots just above the soil level. The plants were then hot air dried, and hand thrashed.

3.3 Experiment 3

The purpose of this experiment was to determine the interaction effect of inoculation with *G. intraradices* and *R. leguminosarum* at two rates of applied N, on the dry matter production, P and N accumulation, and N_2 fixation in lentil.

Analysis of Experiment 3 revealed low VAM infection, and the experiment was repeated as Experiment 3b. All materials and methods of Experiments 3a and 3b were as in Experiments 2a and 2b respectively. All treatments received 175 μg P g^{-1} soil. The experiment was a completely randomized factorial (Little and Hills, 1978) with two rates

of applied N (40 or 250 $\mu g\ g^{-1}$ soil), with or without G. intraradices and or R. leguminosarum inoculation for a total of eight lentil treatments. Each treatment had three replicates. The oilseed rape from Experiment 2 were used as the control.

All nutrients, seeding, and watering, were applied as in Experiment 2. Growth chamber conditions were also as in Experiment 2. Observations of the plants were recorded throughout the growth period. Harvesting was also as in Experiment 2.

3.4 Experiment 4

The purpose of Experiment 4 was to combine the treatments of the previous 3 experiments to allow for determination of the interaction effects of P and or N fertilization, with or without G. intraradices and or R. leguminosarum inoculation on lentil growth and N_2 fixation.

The treatments were similar as in Experiment 3a and 3b, with the exception of introducing two rates of applied P. To determine the plant growth stages where the treatment effects occur, six plants in the 5 kg soil per pot were included to allow for three harvest dates, by harvesting two plants each time. To introduce soil conditions associated with a topsoil, the subsoil was amended with a Willowcrest topsoil (20% by mass). The blended soil had a field capacity of 12.4% and a $NaHCO_3$-extractable P level of 2.4 $\mu g\ g^{-1}$ soil. The blended soil was incubated at field capacity in a growth chamber for four days prior to seeding.

Experiment 4 was a completely randomized factorial (Little and Hills, 1978) with 2 rates each of applied P (30 and 100 $\mu g\ P\ g^{-1}$ soil) and N (40 and 250 $\mu g\ N\ g^{-1}$ soil), with and without G. intraradices and R. leguminosarum inoculations, totalling 16 lentil treatments. Each

treatment was replicated three times. There were also two treatments of the control crop oilseed rape, which received 100 μg P g^{-1} soil at the rates of 40 and 250 μg N g^{-1} soil.

To minimize variability among experiments, all nutrients were applied at the same rate and method as in Experiment 2b and 3b. The NH_4NO_3 was doubly labelled with 3.05% atom % ^{15}N excess.

The R. leguminosarum treatments were inoculated. Corn root-mats for G. intraradices inoculum were placed at six locations in each pot. Seeding was carried out as described in Experiment 2b and 3b, with the exception of two lentil seeds being placed over each of the six corn root-mats. Watering was carried out as described in the previous three experiments.

All pots were kept in a growth chamber where the photoperiod was adjusted to 16 hours per day with a photosynthetically active radiation of 710 μmoles of photons m^{-2} sec^{-1} at plant canopy height. The day/night temperature and relative humidity was 25°C/15°C and 55%/90% respectively. Observations of the plants were recorded throughout the growth period. To help to assure uniform plants within the pots, the plants were thinned at 19 days after seeding, to six per pot.

Two of six lentil plants in each pot were harvested during the following harvest periods, vegetative (30 days), early pod (61 days), and seed maturity (89-99 days). Roots were removed after the final harvest and washed in the same manner as in Experiment 2b and 3b. Sample preparation and analysis were as in the previous experiments.

3.5 Post experiment determinations

After harvest, the shoots and roots were oven dried (60°C for 72 hours) and their mass recorded. Roots and shoots were then ground

(<2 mm) in a Wiley mill. The prepared soil and plants were analyzed, and the roots were inspected for VAM infection. Kjeldahl N of plants was determined by a modified Kjeldahl-Gunning method Jackson (1958). In the experiments, where N_2 fixation was measured, the ^{15}N isotope ratio was determined (Bremner, 1965), and N_2 fixation was calculated (IAEA, 1983). Other nutrients were determined after digestion of plant samples in a nitric-perchloric acid mixture (Chapman and Pratt, 1961; Bowen, 1967). The concentration of total P was determined by the molybdate-blue method (Murphy and Rily, 1962). Zinc, copper, iron, and manganese concentrations were determined by atomic adsorption (Isaac and Kerber, 1980).

VAM infection on the roots was analyzed to determine the effectiveness of the inoculum. In order to make fungal structures visible, randomly selected root pieces from every pot were cleared and stained (Phillips and Hayman, 1970). Infection of VAM fungi was scored according to a method as described by Ocampo et al. (1980) and Sutton (1973) and critiqued by Clewett et al. (1988). This vesicle counting method of assessing the degree of VAM infection has been questioned by Australian researchers (Abbott et al., 1988), but in Manitoba, the hyphae freeze during the winter, thus it is the spores that cause infection the following spring. Presence of the genus Glomus (Trappe, 1982) were verified using a compound microscope (1000x magnification).

The data were subjected to analysis of variance using "Proc GLM" (Goodnight et al., 1988) in the Statistical Analysis System on a microcomputer. Mean comparison tests were carried out using Duncans Multiple Range Test, and line fitting programs "Proc Nlin" (Goodnight and Ihnen, 1988), and "Proc Reg" (Ihnen et al., 1988) were used to calculate coefficients of determination.

4. RESULTS AND DISCUSSION

4.1 Experiment 1: RESPONSE OF LENTIL AND OILSEED RAPE TO P AND *G. intraradices* INOCULATION

The purpose of Experiment 1 was to determine if addition of P and or inoculation with *Glomus intraradices* increased growth of lentil. Large amounts of N were added to the soil, so that the lentil did not depend on N_2 fixation as a source of N. Therefore, there was no interaction between *Rhizobium leguminosarum* and *G. intraradices*.

The lentil plants without added P showed P deficiency symptoms (chlorosis, leafdrop, and purple stems) starting at 18 days after seeding. No branches developed, and most plants wilted and died by day 40.

Shoot and root dry matter production of lentil showed a response to added P up to the rate of 80 μg P g^{-1} soil, after which the changes in production were nonsignificant (Table 3 and Figure 1a). As the level of added P increased, plants were darker in colour, had more branches, and lost less leaves. Since poor growth was overcome by the addition of P, the P status of the soil (3.0 μg P g^{-1} soil, $NaHCO_3$-extractable) was too low for lentil growth.

A lentil production response to P addition in soils low in P has been reported by Saxena (1981) and DeBeer (1990). In the present experiment, the lentil dry matter production was several times higher than the production observed by Singh and Singh (1986), and Badr El-Din and Moawad (1988). However these researchers applied no basal nutrients, as well as lower rates of P and, different strains of lentil were used. Therefore, it is difficult to compare the observed production responses of these researchers to the present research. The

Table 3. Effect of added P and inoculation with G. intraradices on dry matter production and the root/shoot ratio of lentil and the effect of added P on oilseed rape (Experiment 1)

P application ($\mu g\ g^{-1}$ soil)	Shoot dry matter production ($g\ plant^{-1}$)				Root dry matter production ($g\ plant^{-1}$)				Root/Shoot ratio	
	Inoculation with G. intraradices									
	-		+		-		+		-	+
Lentil										
0	0.08	C¶	0.1	E	0.04	C	0.04	E	0.50	0.40
20	0.4	C	3.5	D	0.4	C	1.2	D	1.00	0.34
40	4.0	BC	6.4	C	1.4	BC	2.0	C	0.35	0.31
60	4.9	ABC	9.9	B	1.6	ABC	3.4	B	0.33	0.34
80	8.8	AB	11.5	A	2.7	AB	4.6	A	0.31	0.40
100	10.0	A	11.3	AB	3.3	A	3.3	B	0.33	0.29
125	8.5	AB	11.5	A	2.7	AB	3.6	B	0.32	0.31
150	8.0	AB	12.6	A	2.8	AB	3.7	B	0.35	0.29
175	9.5	A	12.2	A	2.7	AB	3.5	B	0.28	0.29
mean	6.0	b§	8.8	a	2.0	b	2.8	a	0.33	0.32
Oilseed rape										
0	9.6	B	-		15.0	A	-		1.56	-
100	12.8	A	-		17.1	A	-		1.34	-

¶ Duncans Multiple Range Test, means followed by the same uppercase letter in each column are not significantly different at p = 0.05

§ Duncans Multiple Range Test, means followed by the same lowercase letter in each row are not significantly different at p = 0.05

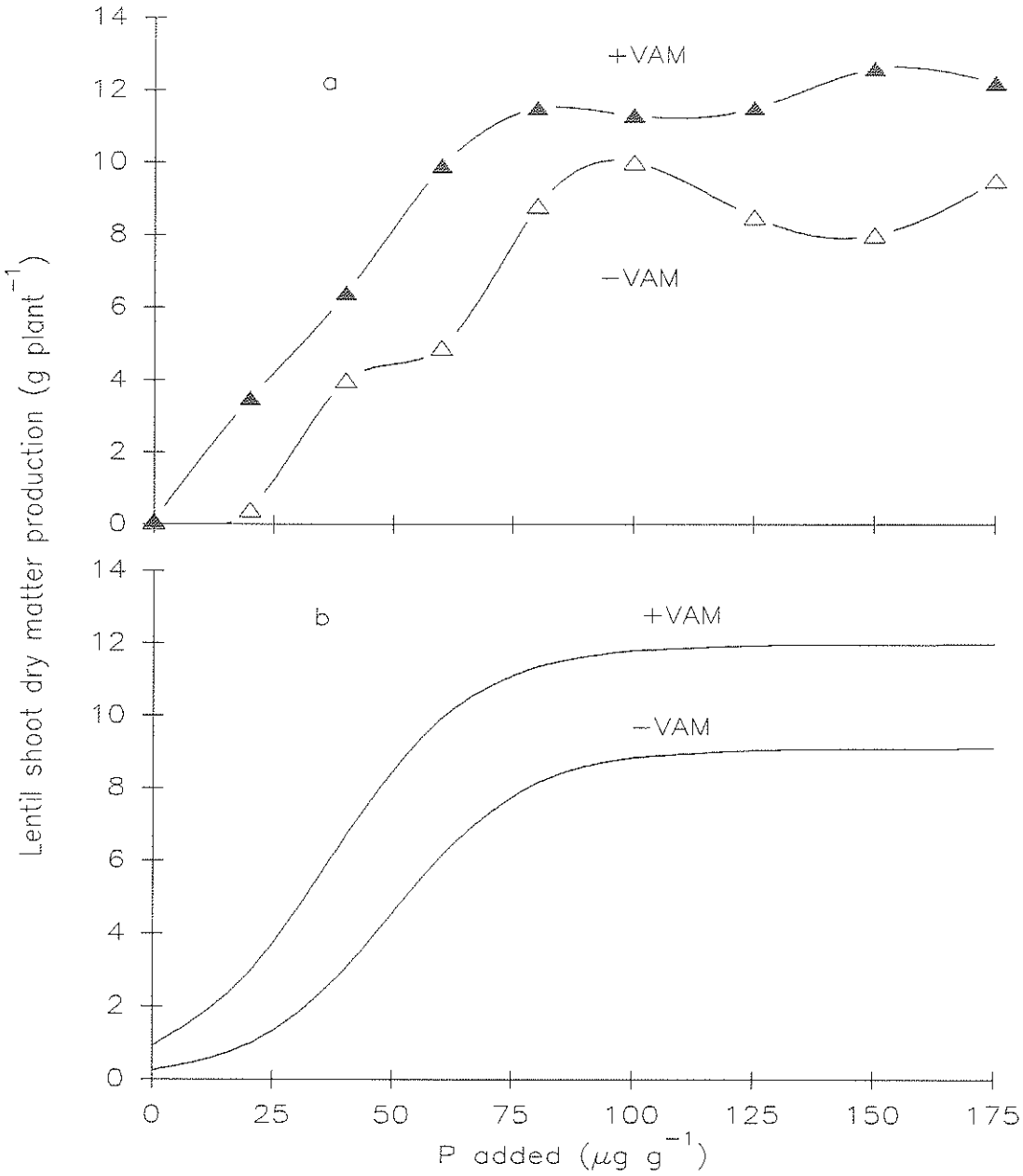

Figure 1. Lentil shoot dry matter production versus P added a) actual b) logistic curve (Experiment 1)

+VAM Y=11.97/(1+11.93 exp (-0.067*X)) CD = 0.96
-VAM Y= 9.10/(1+34.85 exp (-0.071*X)) CD = 0.69 or 0.90¶
¶ the CD was increased when the means of the replicates were analyzed.

dry matter production in this experiment was similar to the lentil growth observed by DeBeer (1990).

Shoot dry matter production of oilseed rape responded significantly to the addition of 100 μg P g^{-1} soil, while the increase in root dry matter production was not significant (Table 3).

Oilseed rape, without added P, had a shoot dry matter production of 9.6 g $plant^{-1}$, whereas the lentil without added P died. Therefore, it appears that oilseed rape is much better at accumulating (scavenging) P than lentil, in low P soils. This observation can be partially explained by the high root to shoot ratio and root mass of oilseed rape as compared to lentil (Table 3) as observed here, and by other researchers (Strong and Soper, 1973; Paul and Clark, 1989). Measuring other root characteristics that affect nutrient uptake, Hendriks et al. (1981) showed a larger P depletion zone around roots of rapeseed than of corn, due to their longer root hair lengths. Crush (1974) and Marschner (1986) reported that grasses have a wider root hair cylinder than legumes. Lentil, a legume, has an even coarser root system and less root hairs, than does corn with a grass type root. It follows, that lentil would take up less P than rapeseed due to the shorter root hairs and smaller predicted depletion zone. Strong and Soper (1974a) ranked flax, wheat, buckwheat and rapeseed in increasing order of root zone proliferation in the fertilizer applied zone. The ranking of Strong and Soper (1974a) and the findings of Hendriks et al. (1981) coincide, since wheat, a grass, has a coarser root system than does rapeseed (Plenchette et al., 1983). Strong and Soper (1973) suggest that both the ability of a root to absorb P at low P soil solution concentrations, and the ability to proliferate roots around a fertilizer application zone, determine the response of plants to P uptake.

The relatively low dry matter production response of oilseed rape, due to added P, is due to the high production of the 0P treatments. Brewster et al. (1976) also found poor yield response of rapeseed to applied P as compared to onion, a species that has few root hairs. This indicates the importance of root hairs to P uptake in low P soil conditions.

Inoculation with G. intraradices caused a visible improvement in lentil growth and branching, and also increased dry matter production for all except the 0P treatment, which died. Mosse et al. (1976) also found that VAM was unable to extract P and improve plant growth in very P deficient soils.

The lentil -G. intraradices plants had no shoot dry matter production response to 20 μg P g^{-1} soil, but responded when larger amounts of P were added. The +G. intraradices plants however, did respond when 20 μg P g^{-1} soil were added. Thus, it appears that G. intraradices inoculation lowered the soil P threshold level for lentil growth. Similar growth responses were reported from Australia for subterranean clover when grown on subsoil (Bolan et al., 1983), for cassava, which has a coarse root system (Howeler et al., 1982), and lentil grown in sterilized soil (DeBeer, 1990). The large increase in growth due to G. intraradices inoculation, at the low P treatments, show that lentil depends on VAM in low P soils. However, there is still a positive effect due to inoculation, when P is adequate (Table 3). This was also observed by Singh and Singh (1986).

These findings suggest a sigmoidal (S-shaped) growth response, of the -G. intraradices plants, to added P, with the threshold between 20 and 40 μg P g^{-1} soil (Figure 1b). Based on this, lentil shoot dry matter production response curves were fitted, using a logistic equation

Y=a/(1+b*exp(-c*x)). In this equation with a, b, and c are constants with a= asymptote, b= measure of the starting size, and c= rate constant. The exp= 2.71828, x= applied P, and Y= yield (Milthorpe and Moorby, 1979; Hunt, 1982). This was feasible because there were several treatments in the low P range in Experiment 1, and a full range of responses from acute deficiency to saturation levels were analyzed. The logistic function is an asymptotic function which approaches a maximum. This type of function was therefore more suitable for the obtained data than a quadratic polynomial function which decreases in Y as X increases greatly (Hunt, 1982).

The logistic curve of the -G. intraradices treatment showed a sigmoidal (S-shaped) dry matter production response, which resulted in a coefficient of determination (CD) of 0.69. The CD of the +G. intraradices treatment was 0.97, but the curve was not S-shaped. The lack of a S-shape indicates that a reasonable production can be obtained at low levels of added P. The same responses in soybean to VAM and added P, were found by Lambert et al. (1979). Growth response curves using the quadratic polynomial regressions resulted in lower CD's, also indicating that a logistic curve describes the obtained data better. The relatively low CD (0.69) of the -G. intraradices treatment was due to high variability within the replicates as compared to the inoculated treatment. The CD was increased from 0.69 to 0.90 when the means of the replicates were analyzed. There was very little increase of the CD when the +G. intraradices treatments were analyzed in the same manner.

Many researchers have found that when large amounts of P are added, the production of the ±VAM treatments are not significantly different (Lambert et al., 1979; Bolan et al., 1983; DeBeer, 1990). In this experiment this was not the case, as the effect of inoculation with

G. intraradices was not inhibited at very high P levels. The beneficial effect of G. intraradices cannot be completely explained by its positive effect on P supply, suggesting that other factors are involved.

The root to shoot ratio of lentil had a slight decreasing trend, when large amounts of P were added (Table 3). Also, the low P treatments with the +G. intraradices treatment seemed to have a lower ratio. This increase in relative root mass might be an adaptation of plants in low P soils, without VAM, to increase nutrient uptake.

There was no infection by VAM (0%), as identified by vesicles and arbuscules, in the -G. intraradices treatments, although there was limited hyphal growth on some roots. Some of these hyphae were septate which usually is not a characteristic of G. intraradices and other species of VAM (Barber, 1984; Powell and Bagyaraj, 1984). Thus, it was concluded that the subsoil did not contain viable infectious VAM species.

Maximum VAM infection on the roots of the plants with the +G. intraradices treatments occurred between the 20 to 80P treatments (Table 4). A pre-experimentation incubation study determined that between 40 and 50% of applied P was recovered by $NaHCO_3$ extraction when less than 100 μg P g^{-1} soil were added[&]. This suggests that 40 μg g^{-1} soil of added P would correspond to approximately 18 μg P g^{-1} soil in the soil. Stribley et al. (1980) observed the highest %VAM infection of

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[&] In the pre-experiment, rates of applied P ranged from 0-350 μg g^{-1} soil and the soils were kept at field capacity for 21 days. $NaHCO_3$ extractable P was determined after the incubation period. When less than 80 μg g^{-1} soil of P was applied, then less than 50% of the applied P was recovered. The percent recovery increased up to 84% at the highest level of added P.

Table 4. Effect of added P and inoculation with G. intraradices on VAM infection, and shoot P concentration and uptake of lentil, and the effect of added P on shoot P concentration and uptake of oilseed rape (Experiment 1)

	Root VAM (% vesicle infection)		shoot P (%)		Shoot P uptake (mg plant^{-1})	
P application (μg g^{-1} soil)	Inoculation with G. intraradices					
	-	+	-	+	-	+
Lentil						
0	0 A¶ b§	36 C a	0.094 D a	0.088 F a	0.1 C a	0.1 G a
20	0 A b	79 A a	0.126 BC a	0.145 E a	0.6 C b	5.1 F a
40	0 A b	83 A a	0.118 CD b	0.161 CDE a	4.8 BC b	10.3 E a
60	0 A b	70 AB a	0.120 BCD b	0.154 DE a	5.9 BC b	15.3 D a
80	0 A b	72 AB a	0.150 ABC a	0.166 BCD a	13.1 AB b	19.1 C a
100	0 A a	53 BC a	0.151 AB a	0.176 BC a	15.7 A a	19.9 BC a
125	0 A b	57 B a	0.150 ABC b	0.186 AB a	13.0 AB a	21.2 B a
150	0 A b	58 B a	0.142 ABC b	0.197 A a	11.5 AB b	24.8 A a
175	0 A b	57 B a	0.169 A a	0.205 A a	17.1 A a	24.9 A a
mean	0 b	62 a	0.136 b	0.164 a	9.1 b	15.6 a
Oilseed rape						
0	-	-	0.202 B	-	19.3 B	-
100	-	-	0.296 A	-	37.7 A	-

¶ Duncans Multiple Range Test, means followed by the same uppercase letter in each column are not significantly different at p = 0.05

§ Duncans Multiple Range Test, means followed by the same lowercase letter in each row are not significantly different at p = 0.05

leek when the soil $NaHCO_3$-extractable P was between 10 and 20 $\mu g\ g^{-1}$ soil, which is similar to the results of the present experiment. When no P, or large amounts of P, were added to the soil, the VAM infection was lower (Table 4). This trend of decreasing VAM infection as available P increased has been observed for several crops (Mosse et al., 1981; Powell and Bagyaraj, 1984; Fairchild and Miller, 1988; Ikombo et al., 1988; Harinikumar and Bagyaraj, 1989; Walley and Germida, 1991). It has been suggested that at high soil P levels, VAM are not required for growth. This is favourable, as the plant does not have to expend energy to supply a symbiont. In contrast, Singh and Singh (1986) who grew lentil in a P deficient loamy sand soil, observed no decrease in VAM infection level when 80 μg P g^{-1} soil was added, although the shoot P concentration was very high. Thus, it appears that other variables are involved in controlling VAM infection levels. The low infection at the 0 μg P g^{-1} soil treatments in this experiment, was probably due to the very severe P deficiency, where the P deficient plants were unable to support VAM growth.

Lentil and oilseed rape shoot P concentration and uptake increased significantly with increased rates of added P (Table 4). Across all P treatments, inoculation with G. intraradices significantly increased the lentil shoot P concentration by 21% and the P uptake by 71% (Table 4). It is common that +VAM treatments have a higher shoot P concentration than do -VAM treatments (Stribley et al., 1980; Akthar et al., 1987; Badr El-Din and Moawad, 1988). However, for the individual P treatments, the inoculation with G. intraradices did not always increase shoot %P or P uptake (Table 4). G. intraradices inoculation increased the shoot production (Table 3) to a greater degree than the shoot P concentration (Table 4). This indicates that inoculation with G.

intraradices has other positive effects in addition to increasing dry matter production by means of increasing the P supply.

The -G. intraradices plants reached a shoot P concentration of 0.14% at the 80P treatment, and no treatments exceeded 0.17% P in the shoot. On the contrary, the +G. intraradices treatments contained less than 0.14% P only when P was not added. These P concentrations are close to values of lentil observed (0.175% P) by Badr El-Din and Moawad (1988). The critical P level of vegetative soybean shoots has been determined by Singleton et al. (1985) to be between 0.14 and 0.17%P. Little information on lentil is available, however, DeBeer (1990) did observe good lentil growth when the shoot P concentration was about 0.17% at the early pod stage. Other researchers reported P concentrations of lentil between 0.2 and 0.35% P in the shoot or leaves. In general, the P concentration decreases with age of the plant, once past the vegetative stage (Akhtar et al., 1987).

In order to determine if a plant nutrient was diluted or concentrated, Jarrel and Beverly (1981) suggested that plant nutrient uptake, dry matter production, and nutrient concentration have to be known. Only when the nutrient concentration decreases and production increases, will a dilution effect exist between the treatment and the measured response. A synergistic effect exists between treatments if all three variables increase. In this experiment, it appears that inoculation with G. intraradices had a direct positive or synergistic effect, because the P uptake, shoot production, and P concentration all increased. Kucey and Janzen (1987), growing field bean, found a synergistic effect between VAM inoculation and P, Zn, Cu, and Fe uptake.

The soil $NaHCO_3$-extractable P concentration was determined after

harvest. The P recovery from the soil was up to 36% of the applied P (Figure 2). Recovery was greater in the - G. intraradices treatments at the low levels of applied P (<100 μg P g^{-1} soil). However at the higher P rates, the recovery was greater in the + G. intraradices treatments. The lower recovery of added P from the soil at low levels of added P in the + G. intraradices treatments was due to the increased P uptake by the plants. When less than 80 μg P g^{-1} soil were added, the shoot P uptake was increased by over 100% with the G. intraradices inoculation. The maximum amount of P recovered from the soil and plant combined was 60% of the added P.

In an attempt to reduce N_2 fixation, seeds were sterilized and not inoculated with R. leguminosarum. As well, the subsoil used was collected from a depth of 1.5 m, and was assumed not to contain Rhizobium. In addition, large amounts (250 μg g^{-1} soil) of N were added to all treatments, and the addition of N has been found to decreases N_2 fixation (Galbraith, 1969; Summerfield, 1981; Rodd, 1986; Walley, 1986). Therefore, it was not expected that N_2 fixation contributed significantly to the N supply of the lentil. However, nodules were observed on the roots of lentil whose shoot contained at least 0.14% P (Table 5). The nodules were very small and located close to the primary root. In addition, the nodules were white inside, suggesting that they were not actively fixing N (Stanier et al., 1986).

Galbraith (1969) also found nodules on pea roots in a pot experiment, even though the seeds, sand, and water were sterilized. Ciafardini and Barbieri (1987) reported that Bradyrhizobium japonicum, although not very mobile in clay soils, was mobile in a silt clay loam such that inoculated irrigation water increased nodulation and seed

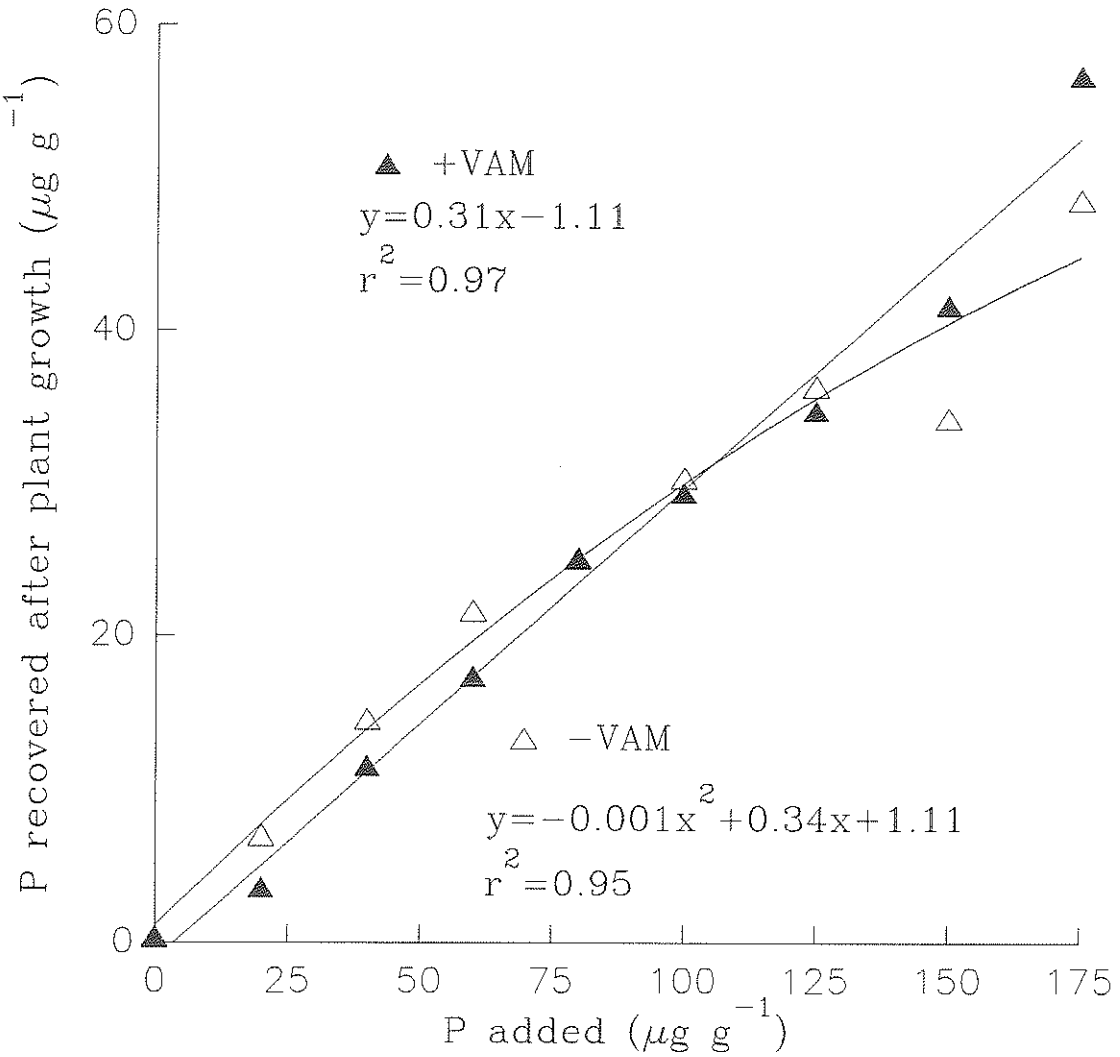

Figure 2. Soil $NaHCO_3$ extractable P after plant harvest (Experiment 1)

Table 5. Effect of added P and inoculation with G. intraradices on nodule number, shoot N uptake, percent N of lentil, and the effect of added P on N content of oilseed rape (Experiment 1)

	Nodules		Shoot % N		Shoot N uptake (mg plant^{-1})	
Rate of P (μg g^{-1})			Inoculation with G. intraradices			
	-	+	-	+	-	+
Lentil						
20	none	none		3.38 A		117.7 C
40	none	some	3.08 A¶a§	2.57 B b	122.5 B a	164.6 B a
60	none	yes	2.75 A a	2.23 C b	135.7 AB b	221.0 A a
80	yes	yes	2.36 A a	1.97 CD a	203.5 A a	226.1 A a
100	yes	yes	2.31 A a	2.20 CD a	208.2 A a	248.9 A a
125	yes	yes	2.43 A a	2.08 CD a	197.7 AB a	238.5 A a
150	yes	yes	2.37 A a	1.93 D a	184.4 AB a	244.5 A a
175	yes	yes	2.39 A a	2.19 CD a	206.1 A a	266.6 A a
mean			2.53 a	2.32 b	179.7 b	216.0 a
Oilseed rape						
0	-	-	2.53 A	-	239.8 A	-
100	-	-	2.06 A	-	263.3 A	-

¶ Duncans Multiple Range Test, means followed by the same uppercase letter in each column within each experiment are not significantly different at p = 0.05

§ Duncans Multiple Range Test, means followed by the same lowercase letter in each row are not significantly different at p = 0.05

yield. The Rhizobium which infected the lentil plants were either present in the subsoil, or were introduced during the term of the experiment in the growth chamber (Galbraith, 1969).

In the +G. intraradices treatments, lentil shoot %N decreased significantly, while N uptake increased significantly with increasing amounts of added P and inoculation with G. intraradices (Table 5). In oilseed rape, the N uptake, and % N were not affected by the addition of P. This type of effect, if the shoot production also increased, has been termed "classical dilution" (Jarrel and Beverly, 1981). The dilution was not severe, as the N uptake still increased, and thus plant growth was not limited by a lack of N. Many researchers reported increased N uptake as affected by P addition of several crops (Jarrel and Beverly, 1981; Badr El-Din and Moawad, 1988) including lentil (Bala and Singh, 1985).

Lentil concentration and uptake of Zn and Cu are reported in Table 6. In general the Zn and Cu concentrations did not change significantly when increasing rates of P were added. Zinc and Cu uptake increased with increased P applications, thus it appears that dilution occurred at the mid ranges of applied P. The dilution effect was not severe enough to inhibit growth, as micronutrient uptake still increased. Marschner (1986) lists a critical Cu concentration in vegetative growth of 3-5 $\mu g\ g^{-1}$. Based on this, the lentil plants approached the critical concentration, but were not severely deficient.

With respect to Zn, Saxena (1981) states that if the P:Zn ratio is greater than 400, a P induced Zn deficiency could occur. In this experiment, the P:Zn ratio was much lower. Bolan et al. (1983) states the critical Zn level of subterranean clover shoots to be at 20 $\mu g\ g^{-1}$, which is close to the observed values (Table 6). Lambert et al. (1979)

Table 6. Effect of added P and inoculation with G. intraradices on shoot Zn and Cu concentration and uptake of lentil (Experiment 1)

Rate of P ($\mu g\ g^{-1}$)	shoot Zn concentration ($\mu g\ g^{-1}$)		shoot Cu concentration ($\mu g\ g^{-1}$)	
	Inoculation with G. intraradices			
	-	+	-	+
0	14.8#	1.2#	7.6 A¶ b§	15.3 A a
20	15.2 ABC a	18.9 AB a	8.0 A a	8.2 B a
40	11.4 C a	17.4 AB a	6.4 A a	7.0 BC a
60	13.7 C a	16.9 B a	5.9 A a	5.4 BC a
80	14.4 BC b	17.9 AB a	5.4 A a	5.7 BC a
100	16.9 ABC a	17.3 B a	6.3 A a	5.5 C a
125	21.8 AB a	18.8 AB a	5.4 A b	7.5 BC a
150	19.2 ABC a	20.4 AB a	5.3 A a	7.0 BC a
175	22.5 A a	21.3 A a	6.1 A b	8.0 BC a
mean	17.3 b	18.6 a	6.3 b	7.7 a

	Zn uptake (μg shoot^{-1})		Cu uptake (μg shoot^{-1})	
0	1#	0#	0.6 B b	1.6 G a
20	6 C b	66 E a	3.6 B b	28.7 F a
40	48 BC b	111 D a	25.9 AB a	44.4 E a
60	64 BC b	168 C a	28.5 AB b	53.8 D a
80	128 AB b	206 BCa	48.6 A a	65.4 C a
100	166 AB a	195 BCa	64.3 A a	62.4 D a
125	193 A a	216 B a	47.2 A b	85.7 B a
150	159 AB a	256 A a	44.1 A b	87.9 AB a
175	212 A a	260 A a	60.6 A a	97.0 A a
mean	113 b	186 a	35.9 b	58.5 a

¶ Duncans Multiple Range Test, means followed by the same uppercase letter in each column are not significantly different at p = 0.05

§ Duncans Multiple Range Test, means followed by the same lowercase letter in each row are not significantly different at p = 0.05

Treatments were not included in statistical analysis, because values were obscure

observed decreasing Zn and Cu concentrations of soybean and maize when P was added, but only in +VAM treatments. In the current experiment both the + and -G. intraradices treatments showed this dilution effect, however, it was not significant (Appendix).

Inoculation with G. intraradices increased P uptake and growth, and thus a dilution of the Zn and Cu concentration was expected. However, the opposite was observed. The G. intraradices had a positive but not always significant effect on Zn and Cu uptake and concentration (Table 6). In a greenhouse experiment with field bean, Kucey and Janzen (1987) observed that VAM directly increased P, Zn, Cu, and Fe uptake and concentration. Lambert et al. (1979) also observed increased Zn, Cu, Fe, and Mn concentrations of soybean, inoculated with VAM.

Table 7 shows the lentil concentration and uptake of Fe and Mn. There was a nonsignificant increase in the Fe and Mn concentration of the +G. intraradices plants with increased rates of P added, but the -G. intraradices treatment did not show this (Table 7). Thus a synergistic effect due to P addition was not observed. The addition of increasing rates of P and inoculation with G. intraradices showed significant increases in mean Fe and Mn uptake.

Table 7. Effect of added P and inoculation with G. intraradices on shoot Fe and Mn concentration and uptake of lentil (Experiment 1)

Rate of P (μg g^{-1})	Shoot Fe concentration (μg g^{-1})		Shoot Mn concentration (μg g^{-1})	
	Inoculation with G. intraradices			
	-	+	-	+
0	104.0 A¶ a§	110.1 A a	62.1 A a	73.4 A a
20	69.3 B a	49.5 B a	47.8 AB a	26.0 B a
40	56.4 B a	49.5 B a	35.6 BC a	31.7 B a
60	44.0 B a	48.2 B a	28.7 C a	36.0 B a
80	55.4 B a	51.5 B a	36.8 BC a	39.2 B a
100	52.8 B a	60.8 B a	33.0 BC b	38.6 B a
125	44.6 B b	71.2 B a	32.2 BC a	42.5 B a
150	58.7 B a	61.1 B a	31.8 BC b	38.4 B a
175	51.0 B b	76.0 B a	38.3 BC a	44.3 B a
mean	59.6 a	63.4 a	38.5 a	41.1 a

	Fe uptake (μg shoot^{-1})		Mn uptake (μg shoot^{-1})	
0	9 C a	11 F a	5 C a	8 F a
20	30 C b	176 EF a	20 C b	91 E a
40	231 ABC a	320 DE a	143 BC a	204 D a
60	220 BC b	479 CD a	143 BC b	358 C a
80	480 AB a	589 BC a	328 AB a	449 B a
100	545 A a	680 AB a	327 AB a	435 B a
125	380 AB b	810 A a	277 AB b	487 AB a
150	470 AB a	776 A a	253 AB b	486 AB a
175	496 AB a	839 A a	375 A a	539 A a
mean	318 b	520 a	208 b	340 a

¶ Duncans Multiple Range Test, means followed by the same uppercase letter in each column are not significantly different at p = 0.05

§ Duncans Multiple Range Test, means followed by the same lowercase letter in each row are not significantly different at p = 0.05

4.2 Experiment 2: RESPONSE OF LENTIL TO P AND G. intraradices INOCULATION

The purpose of Experiment 2a and 2b was to determine if the addition of P and or inoculation with G. intraradices increased dry matter production, P and N accumulation, and N_2 fixation in lentil. To promote N_2 fixation, only small amounts of fertilizer N were supplied.

Analysis of Experiment 2a revealed low VAM infection of the lentil roots, possibly due to the use of old VAM inoculum. Thus, the experiment was repeated (Experiment 2b) using fresh inoculum.

In both experiments, the lentil plants without added P showed P deficiency symptoms (chlorosis, leafdrop, and purple stems) starting at 18 days after seeding. No branches developed, and most plants wilted and died by day 40. In treatments where at least 40 μg P g^{-1} soil were added, many plants showed N deficiency symptoms (chlorotic older leaves).

Addition of increasing rates of P increased shoot and root dry matter production of lentil, but the increases were generally not significant (Table 8). No trend and no significant effect of G. intraradices inoculation was observed. In both of the experiments, the shoot dry matter production of the -G. intraradices was the highest at about 125 μg g^{-1} soil of added P, and then decreased when 175 μg P g^{-1} soil were added, however, these noted differences were not significant (Table 8).

The oilseed rape plants looked N deficient, and the shoot and root dry matter production was similar to that in lentil.

The root dry matter production and root/shoot ratio in Experiment 2b was lower than in Experiment 2a. This is due to the fact that the

Table 8. Effect of added P and inoculation with G. intraradices on dry matter production and the root/shoot ratio of lentil and oilseed rape in the early pod stage

Rate of P ($\mu g\ g^{-1}$)	Shoot dry matter production ($g\ plant^{-1}$)		Root dry matter production ($g\ plant^{-1}$)		Root/Shoot ratio	
	Inoculation with G. intraradices					
	-	+	-	+	-	+
Lentil Experiment 2a						
40	1.9 B¶ a§	2.1 B a	1.0 A a	1.2 A a	0.52 A a	0.61 A a
60	2.3 B a	3.9 AB a	1.0 A a	1.4 A a	0.41 A a	0.40 A a
80	2.4 B a	5.2 AB a	1.3 A a	1.8 A a	0.52 A a	0.35 A a
100	3.9 AB a	4.2 AB a	2.3 A a	1.6 A a	0.54 A a	0.36 A a
125	6.4 A a	5.8 AB a	3.4 A a	2.1 A a	0.49 A a	0.35 A a
175	3.1 AB a	6.2 A a	1.3 A a	2.6 A a	0.42 A a	0.40 A a
mean	3.3 a	4.5 a	1.7 a	1.8 a	0.48 a	0.41 a
Lentil Experiment 2b						
0	0.04 B b	0.08C a	0.05C b	0.1 C a		
40	1.4 B a	2.4 B a	0.3 BC b	0.5 B a	0.19 A a	0.22 A a
60	2.9 AB a	2.0 BC a	0.5 AB a	0.4 B a	0.17 AB a	0.22 A a
80	5.7 A a	3.1 AB a	0.6 AB a	0.5 B a	0.13 AB a	0.16 A a
125	5.8 A a	3.0 AB a	0.7 A a	0.5 B a	0.12 B a	0.18 A a
175	2.9 AB a	5.2 A a	0.5 AB b	0.9 A a	0.17 AB a	0.18 A a
mean	3.1 a	2.6 a	0.4 a	0.5 a	0.16 b	0.19 a
Oilseed rape Experiment 2a						
100	3.1	-	1.3	-	0.42	-
Oilseed rape Experiment 2b						
80	3.6	-	1.1	-	0.31	-

¶ Duncans Multiple Range Test, means followed by the same uppercase letter in each column are not significantly different at p = 0.05

§ Duncans Multiple Range Test, means followed by the same lowercase letter in each row within each experiment are not significantly different at p = 0.05

method used to wash roots in Experiment 2b removed more soil from the roots of the plants.

Lentil shoot dry matter production response curves were fitted, using a logistic curve as described in Experiment 1 (Milthorpe and Moorby, 1979; Hunt, 1982). In Experiment 2a, curve fitting of lentil shoot productions with the logistic function did not yield sigmoidal shaped (S-shaped) curves (Figure 3a), as there were no low P added treatments. The CD's of the logistic (Figure 3a), linear, and quadratic polynomial functions (not shown) were quite low, indicating high variability within the replicates. In Experiment 2b, which had a 0P treatment, the lentil -G. intraradices plants had no shoot dry matter production response to 40 μg P g^{-1} soil, but responded when larger amounts of P were added, whereas the +G. intraradices plants responded when 40 μg P g^{-1} soil were added. Thus it appears that G. intraradices inoculation lowered the soil P threshold level for lentil growth. Similar results were observed in Experiment 1. These findings suggest a sigmoidal (S-shaped) growth response, of the -G. intraradices plants, to added P with a threshold above 40 μg P g^{-1} soil. The logistic curve of the -G. intraradices treatment in Experiment 2b showed a sigmoidal (S-shaped) production response (Figure 3b). The curve of the +G. intraradices treatment was not S-shaped. The lack of a S-shape indicates that a reasonable production can be obtained at low levels of added P. Lambert et al. (1979) also observed S-shaped growth curves when soybean were not inoculated with VAM. Growth response curves using the quadratic polynomial regressions resulted in lower CD's, also indicating that a logistic curve describes the obtained data better.

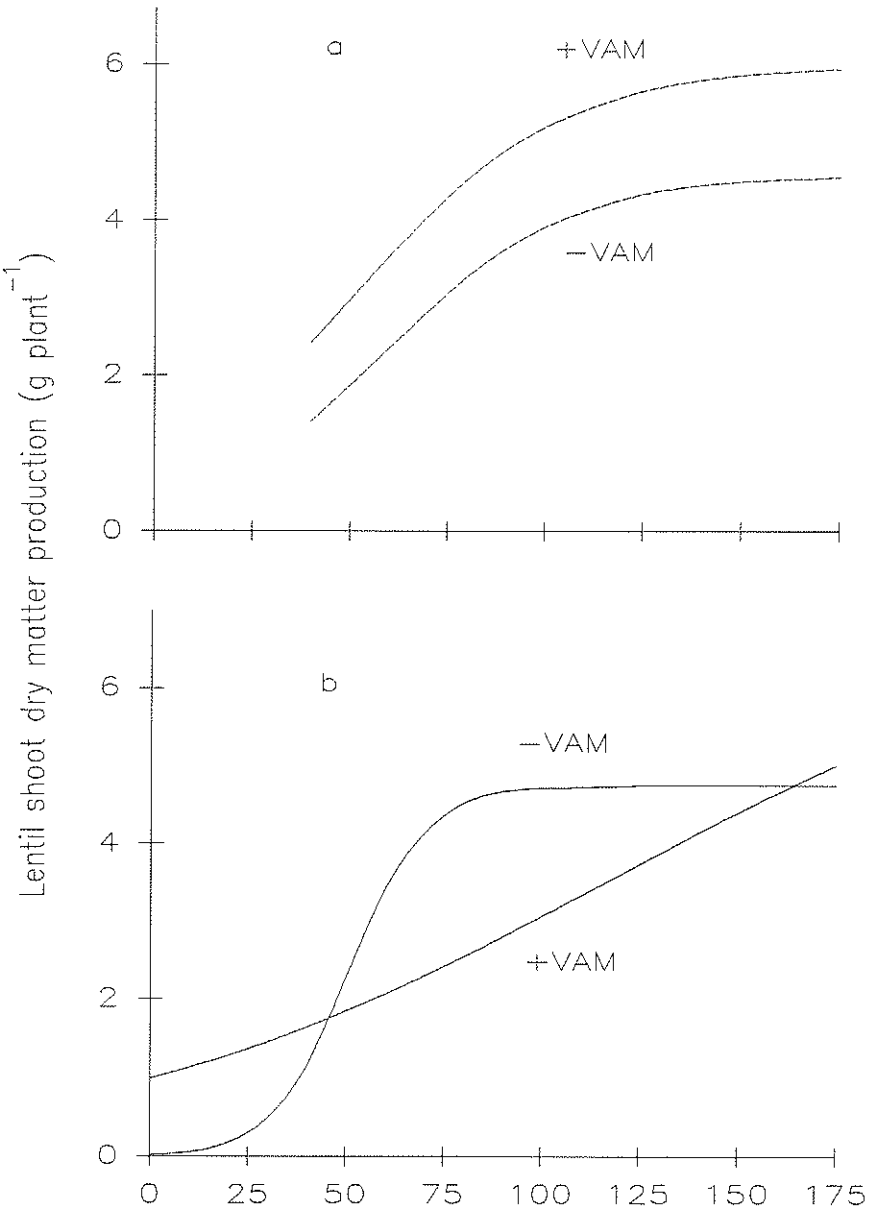

Figure 3. Logistic regression of the effect of added phosphorus and VAM inoculation on shoot dry matter accumulation of lentil a) Experiment 2a, b) Experiment 2b

Experiment 2a +VAM	Y= 5.99/(1+ 6.71 exp (-0.038*X))	CD=0.35
Experiment 2a -VAM	Y= 4.58/(1+12.72 exp (-0.043*X))	CD=0.25
Experiment 2b +VAM	Y= 7.09/(1+ 6.16 exp (-0.015*X))	CD=0.58
Experiment 2b -VAM	Y= 4.75/(1+196.13 exp (-0.104*X))	CD=0.45

In Experiment 2a, there was no infection by VAM, as identified by vesicles and arbuscules, in the -G. intraradices treatments (Table 9). However, there was limited growth of hyphae on some roots. The hyphae were septate, which is usually not a characteristic of G. intraradices and other species of VAM (Barber, 1984; Powell and Bagyaraj, 1984). In Experiment 2b, the -G. intraradices treatment lentil roots had a very low VAM infection (3%) throughout the range of applied P (Table 9). This contamination probably came from the autoclaved roots or the filtered root washings.

In Experiment 2a and 2b, maximum VAM infection on the roots of the +G. intraradices plants occurred between the 40P and 60P treatments, but the values were not significantly different from most other treatments (Table 9). Overall infection was lower in Experiment 2a, than in Experiment 2b. Low infection is sometimes observed, and it could be due to many influences. Sanders et al. (1977) observed a high rate of initial infection failures and slow infection, when four month old VAM inoculum was used. However, by final harvest, they found the infection to be equal between new and stored inoculum. In Experiment 2a, the plants were harvested in the pod stage, so it is not known if VAM infection would have increased at a later harvest date. Light intensity is also known to affect VAM infection (Schenk, 1984). In Experiment 2a the light intensity was 350 μmoles of photons m^{-2} sec^{-1} and in Experiment 2b it was 750 μmoles of photons m^{-2} sec^{-1}. Schenk (1984) recommends a photosynthetically active radiation of 500-700 μmoles of photons m^{-2} sec^{-1} to achieve good VAM infection.

It is unclear what percentage of infection is considered low. This also depends on the method used to measure infection. The effects of low VAM infection on plant growth are also unclear.

Table 9. Effect of added P and inoculation with G. intraradices on VAM infection, shoot P concentration and shoot P uptake of lentil and on oilseed rape

Rate of P ($\mu g\ g^{-1}$)	Root % VAM vesicle infection		shoot %P		Shoot P uptake (mg plant^{-1})	
	Inoculation with G. intraradices					
	-	+	-	+	-	+
Lentil Experiment 2a						
40	0 A¶b§	13 A a	0.14 B a	0.14 D a	2.8 B a	3.0 C a
60	0 A b	20 A a	0.15 B a	0.16 CD a	3.4 B a	6.3 BC a
80	0 A b	16 A a	0.19 A a	0.19 BC a	4.7 B a	9.8 ABC a
100	0 A b	10 A a	0.20 A a	0.20 B a	8.1 AB a	8.5 ABC a
125	0 A b	13 A a	0.22 A a	0.22 B a	14.4 A a	12.9 AB a
175	0 A b	4 A a	0.22 A b	0.26 A a	6.8 B a	16.3 A a
mean	0 b	13 a	0.19 a	0.20 a	6.7 a	9.5 a
Lentil Experiment 2b						
0	2 A b	67 A a	x#	x	x	x
40	6 A b	80 A a	0.15 D b	0.24 A a	2.2 B a	5.8 B a
60	3 A b	79 A a	0.19 C b	0.26 A a	5.4 AB a	5.0 B a
80	2 A b	72 A a	0.21 BC b	0.25 A a	11.6 AB a	7.7 AB a
125	2 A b	65 A a	0.24 A a	0.22 A a	14.3 A a	6.2 B a
175	1 A b	48 B a	0.23 AB a	0.26 A a	6.7 B a	13.6 A a
mean	3 b	69 a	0.20 b	0.26 a	8.0 a	7.7 a
Oilseed rape Experiment 2a						
100	-	-	0.26	-	8.3	-
Oilseed rape Experiment 2b						
80	-	-	0.34	-	12.4	-

¶ Duncans Multiple Range Test, means followed by the same uppercase letter in each column are not significantly different at p = 0.05

§ Duncans Multiple Range Test, means followed by the same lowercase letter in each row within each experiment are not significantly different at p = 0.05

Treatment was not analyzed due to lack of plant material

Piccini et al. (1988) observed low VAM infection (<40 %) on VAM inoculated lucerne roots, but the dry matter production responded due to VAM inoculation. In contrast, Stribley et al. (1980) found that if less than 20% of onion roots were infected with VAM, then there was no positive effect of the VAM on plant growth. It appears that low VAM infection of the +G. intraradices treatments in Experiment 2a, and the -G. intraradices treatments in Experiment 2b (Table 9) was adequate to supply the lentil with nutrients, as there was no significant effect on lentil dry matter production due to increased VAM infection in the +G. intraradices treatments of Experiment 2b (Table 8).

Lentil shoot P concentration generally increased with increasing rates of P addition, and the increase was significant with both inoculation treatments in Experiment 2a, but only with the -G. intraradices treatment in Experiment 2b (Table 9). This lack of increase in shoot % P in the +G. intraradices treatment of Experiment 2b, due to added P, was probably due to a very high P concentration of the lowest added P treatments.

Across all P treatments, inoculation with G. intraradices significantly increased the lentil shoot P concentration only in Experiment 2b, but it did not significantly increase shoot P uptake in either Experiment 2a or 2b (Table 9). All treatments had greater than 0.14 % P in the shoot, including the -G. intraradices plants.

The oilseed rape were taking up P, and had an adequate shoot P concentration, although the plants were N stressed.

In Experiments 2a and 2b, nodules were observed on the roots of all lentil plants (Table 10). The nodules were very small and located close to the primary root. In general, the nodule size and number increased, as the P application rate increased (Table 10). Across all P

treatments in Experiment 2a, the inoculation with G. intraradices significantly increased the number of nodules.

N_2 fixation occurred in all treatments, and the percent N derived from the atmosphere (%NdfA) generally increased with increasing P treatments (Table 10). In Experiment 2a, the inoculation with G. intraradices increased the %NdfA. The mean %NdfA across all treatments in both experiments was 60%, indicating a significant proportion of the N accumulated was from N_2 fixation. As plant size increases due to improved P nutrition by P addition or G. intraradices inoculation, the plant also needs more N. In Experiments 2a and 2b, a significant proportion comes from the nodules by N_2 fixation, thus more nodules are needed.

The mean shoot %N was higher due to G. intraradices inoculation, and in Experiment 2b, this increase was significant (Table 11). However, the shoot %N was not affected by P applications (Table 11). Nitrogen does not appear to be the limiting factor to growth, since there is no dilution of N, as the P treatment and production increased. Shoot N uptake also had an increasing, but not a significant trend with P addition, since the dry matter production and the N concentration increased (Table 11). N uptake was not significantly affected by G. intraradices inoculation (Table 11).

Table 10. Effect of added P and inoculation with G. intraradices on nodule number, percent N derived from atmosphere (%NdfA) in lentil shoots (Experiments 2a and 2b)

	Nodules (per plant)		% NdfA	
Rate of P ($\mu g\ g^{-1}$ soil)	Inoculation with G. intraradices			
	-	+	-	+
Lentil Experiment 2a				
40	50 D¶ a§	83 A a	31 B a	41 A a
60	53 CD a	87 A a	32 B a	52 A a
80	74 BCD a	132 A a	37 AB a	72 A a
100	98 BC a	149 A a	58 AB a	65 A a
125	157 A a	159 A a	72 A a	76 A a
175	101 B a	150 A a	58 AB a	69 A a
mean	89 b	127 a	48 b	62 a
Lentil Experiment 2b				
0	plants died			
40	22 ABC a	43 AB a	48 A a	66 AB a
60	14 BC a	24 AB a	60 A a	54 B a
80	62 AB a	69 A a	71 A a	69 AB a
125	69 A a	31 AB a	73 A a	67 AB a
175	50 ABC a	69 A a	57 A a	82 A a
mean	36 a	40 a	62 a	67 a

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§ Duncans Multiple Range Test, means followed by the same lowercase letter in each row are not significantly different at p = 0.05

Table 11. Effect of added P and inoculation with G. intraradices on shoot N percent and shoot N uptake in lentil shoots

	Shoot % N		N uptake (mg shoot^{-1})	
Rate of P (μg g^{-1} soil)	Inoculation with G. intraradices			
	-	+	-	+
Lentil Experiment 2a				
40	1.78 A¶a§	1.75 A a	33.9 B a	42.3 A a
60	1.42 A a	1.70 A a	31.7 B a	68.3 A a
80	1.53 A b	2.01 A a	36.9 B a	105.8 A a
100	1.75 A a	1.84 A a	74.5 AB a	78.3 A a
125	1.93 A a	1.93 A a	130.6 A a	118.1 A a
175	1.78 A a	1.90 A a	57.0 AB a	129.1 A a
mean	1.70 a	1.86 a	60.7 a	93.1 a
Lentil Experiment 2b				
0	plants died			
40	2.22 A b	2.58 A a	31.6 A a	62.1 AB a
60	2.16 A a	2.20 B a	64.6 A a	42.8 B a
80	2.21 A a	2.41 AB a	130.5 A a	74.8 AB a
125	2.22 A a	2.18 B a	137.2 A a	66.6 AB a
175	1.75 A b	2.39 AB a	51.9 A a	126.5 A a
mean	2.11 b	2.35 a	83.2	74.6 a
Oilseed rape Experiment 2a				
100	0.55	-	17.1	-
Oilseed rape Experiment 2b				
80	0.82	-	29.5	-

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§ Duncans Multiple Range Test, means followed by the same lowercase letter in each row are not significantly different at p = 0.05

In Experiments 2a and 2b, the Zn concentration significantly decreased with increasing rates of P. However, the concentration only went below the critical 20 μg Zn g^{-1} soil level described by Bolan et al. (1983) at the highest rate of added P. With respect to Zn, Saxena (1981) states that if the P:Zn ratio is greater than 400, a P induced Zn deficiency could occur. In Experiment 2a and 2b, the P:Zn ratio was much lower, and a Zn deficiency was not observed. The Cu concentrations generally decreased in Experiments 2a and 2b due to the addition of P, but it did not go below the critical Cu concentration of 3-5 μg Cu g^{-1} soil stated by Marshner (1986). Lambert et al. (1979) observed decreasing Zn and Cu concentrations of soybean and maize when P was added, but only in +VAM treatments.

In Experiment 2a, G. intraradices inoculation increased the mean Zn and Cu concentration (Table 12). Thus, even a low VAM infection had a positive effect on Zn and Cu uptake (Table 14). Lambert et al. (1979) also observed increased Zn, and Cu concentrations of soybean, if inoculated with VAM.

Fe and Mn concentrations were generally not affected by the different rates of P, except in Experiment 2b where the Mn concentration decreased significantly as P application rates increased (Table 13). Increasing P rates had little influence on Fe and Mn uptake (Table 15). In addition, there generally was no significant effect of G. intraradices inoculation on Fe and Mn uptake (Table 15, and Appendix). Lambert et al. (1979) also observed increased Fe, and Mn concentrations of soybean, if inoculated with VAM.

Table 12. Effect of added P and inoculation with G. intraradices on shoot Zn and Cu concentration of lentil

P Rate ($\mu g\ g^{-1}$ soil)	Zn concentration ($\mu g\ g^{-1}$ soil)		Cu concentration ($\mu g\ g^{-1}$ soil)	
	Inoculation with G. intraradices			
	-	+	-	+
Lentil Experiment 2a				
40	50.5#A¶ a§	56.7 A a	6.0 A a	8.2 AB a
60	38.8 B a	41.7 B a	5.7 A a	5.7 B a
80	33.8 B b	42.7 B a	4.8 A b	9.3 A a
100	28.2 BC a	32.0 B a	5.0 A a	6.5 AB a
125	31.5 B a	34.0 B a	5.8 A a	6.2 AB a
175	19.2 C b	35.7 B a	5.0 A a	9.2 AB a
mean	32.7 b	40.4 a	5.4 b	7.5 a
Lentil Experiment 2b				
0	plants died			
40	59.0#A a	42.3 A a	9.5 A a	10.5 A a
60	42.0 B a	34.5 B a	8.6 AB a	8.5 AB a
80	31.5 C a	26.5 BC b	8.8 AB a	9.0 AB a
125	17.5 D a	23.7 BC a	7.0 BC a	7.0 BC a
175	13.3 D a	15.3 C a	5.5 C a	5.3 C a
mean	32.7 a	28.5 a	7.9 a	8.1 a

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§ Duncans Multiple Range Test, means followed by the same lowercase letter in each row are not significantly different at p = 0.05

Table 13. Effect of added P and inoculation with G. intraradices on shoot Fe and Mn concentration of lentil

P Rate (μg g^{-1} soil)	Fe concentration (μg g^{-1} soil)		Mn concentration (μg g^{-1} soil)	
	Inoculation with G. intraradices			
	-	+	-	+
Lentil Experiment 2a				
40	276.7 A¶a§	49.5 A b	28.0#A a	35.3 A a
60	112.8 B a	46.5 A a	27.7 A a	32.0 AB a
80	55.8 B a	63.0 A a	26.5 A a	31.2 AB a
100	61.0 B a	53.8 A a	26.5 A a	28.5 AB a
125	64.8 B a	54.8 A a	29.8 A a	30.0 AB a
175	61.2 B a	48.0 A a	25.2 A a	27.8 B a
mean	105.4$ a	52.5 b	27.2 b	30.8 a
Lentil Experiment 2b				
0	plants died			
40	52.5 A a	66.2 A a	41.7#A a	42.0 A a
60	61.5 A a	63.3 A a	40.3 A a	31.8 AB b
80	62.8 A a	57.2 A a	38.8 AB a	30.5 B b
125	57.0 A a	57.0 A a	30.5 BC a	32.2 AB a
175	52.7 A a	55.7 A a	28.5 C a	28.5 B a
mean	57.3 a	59.9 a	36.0 a	33.0 a

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§ Duncans Multiple Range Test, means followed by the same lowercase letter in each row are not significantly different at p = 0.05

$ the Fe concentration of the 40 and 60 P treatments in Experiment 2a are very high, and when omitted the mean concentration is 60.7 μg g^{-1} soil

Table 14. Effect of added P and inoculation with G. intraradices on shoot Zn and Cu uptake of lentil

P Rate (μg g^{-1} soil)	Zn uptake (μg plant^{-1})		Cu uptake (μg plant^{-1})	
	Inoculation with G. intraradices			
	-	+	-	+
Lentil Experiment 2a				
40	89#AB¶a§	120 A a	10.0#B a	17.3 B a
60	90 AB a	172 A a	13.0 AB a	22.3 B a
80	81 AB a	213 A a	11.7 AB b	44.0 AB a
100	118 AB a	136 A a	22.7 AB a	27.7 AB a
125	205 A a	202 A a	40.7 A a	35.7 AB a
175	62 B a	230 A a	15.7 AB a	62.0 A a
mean	108 b	179 a	19.5 b	34.8 a
Lentil Experiment 2b				
0	plants died			
40	86 AB a	75 A a	14.0 A a	25.2 A a
60	122 AB a	66 A a	25.3 A a	16.3 A a
80	176 A a	81 A a	46.8 A a	27.5 A a
125	104 Ab a	72 A a	42.4 A a	20.8 A a
175	39 B a	80 A a	16.3 A a	28.3 A a
mean	105 a	75 a	29.0 a	23.6 a

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§ Duncans Multiple Range Test, means followed by the same lowercase letter in each row are not significantly different at p = 0.05

One replicate was not included in statistical analysis, because values are obscure

Table 15. Effect of added P and inoculation with G. intraradices on shoot Fe and Mn uptake of lentil

P Rate (μg g^{-1})	Fe uptake (μg plant^{-1}) Inoculation with G. intraradices -	+	Mn uptake (μg plant^{-1}) Inoculation with G. intraradices -	+
Lentil Experiment 2a				
40	495 A¶ a§	102 A b	48#B a	73 A a
60	268 ABC a	189 A a	64 B a	129 A a
80	133 C a	346 A a	64 B a	167 A a
100	258 ABC a	219 A a	108 AB a	119 A a
125	420 AB a	322 A a	198 A a	179 A a
175	190 BC a	317 A a	80 AB a	178 A a
mean	294 a	249 a	96 b	141 a
Lentil Experiment 2b				
0	plants died			
40	75 A a	163 A a	61 A a	102 AB a
60	189 A a	121 A a	123 A a	62 B a
80	355 A a	175 A a	226 A a	93 AB a
125	334 A a	176 A a	191 A a	98 AB a
175	154 A a	313 A a	83 A a	146 A a
mean	222 a	189 a	137 a	100 a

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§ Duncans Multiple Range Test, means followed by the same lowercase letter in each row are not significantly different at p = 0.05

One replicate was not included in statistical analysis, because values are obscure

4.3 Experiment 3: RESPONSE OF LENTIL TO N AND G. intraradices AND R. leguminosarum INOCULATION

The purpose of Experiment 3 was to determine the interaction effect of inoculation with G. intraradices and R. leguminosarum at two rates of applied N (40 and 250 $\mu g\ g^{-1}$), on the dry matter production, P and N accumulation, and N_2 fixation in lentil, at one rate of P (175 μg P g^{-1} soil).

Analysis of Experiment 3a revealed low VAM infection of the lentil roots. This was possibly due to the use of old inoculum, and the experiment was repeated (Experiment 3b) using fresh inoculum.

The lentil plants did not show P deficiency symptoms, but N deficiency symptoms (chlorotic older leaves) were observed when 40 μg N g^{-1} soil was added. The high N plants were dark green in colour. No plants died during the growth period.

With the 40 μg N g^{-1} soil treatments, shoot dry matter production did not increase significantly due to R. leguminosarum inoculation (Table 16). G. intraradices also did not significantly increase shoot dry matter production. The inoculation with both microorganisms did increase shoot production significantly in Experiment 3a, but not in Experiment 3b. With the 250 μg N g^{-1} soil treatments, shoot dry matter production did not increase significantly due to inoculation with either organism, but a positive trend due to G. intraradices inoculation was observed in Experiment 3a. Gupta et al. (1985) also found increased lentil dry matter yield and nodule number due to inoculation with VAM or Rhizobium.

In Experiment 3a, when 40 μg N g^{-1} soil were added, the shoot %P, and root VAM infection increased due to G. intraradices inoculation

Table 16. The effect of G. intraradices (VAM) and R. leguminosarum (Rhiz) inoculation on lentil shoot dry matter production, shoot % P, % VAM infection, Shoot N uptake, and % N derived from Atmosphere (% NdfA)

Rate of N		Shoot yld g plant^{-1}	Shoot %P	%VAM inf.	Shoot N mg shoot^{-1}	%N fixed
Experiment 3a						
40μgN g^{-1}	-Rhiz -VAM	2.0 b¶	0.22 b	0 b	20 b	0 b
	-Rhiz +VAM	2.9 b	0.26 a	2 a	46 ab	42 ab
	+Rhiz -VAM	3.1 b	0.22 b	0 b	57 ab	58 a
	+Rhiz +VAM	6.2 a	0.26 a	4 a	129 a	69 a
250μgN g^{-1}	-Rhiz -VAM	5.6 a	0.10 a	0 a	134 a	0 a
	-Rhiz +VAM	9.4 a	0.13 a	0 a	184 a	0 a
	+Rhiz -VAM	6.1 a	0.11 a	0 a	141 a	0 a
	+Rhiz +VAM	9.5 a	0.13 a	1 a	198 a	1 a
Experiment 3b						
40 μg N g^{-1}	-Rhiz -VAM	4.2 a	0.27 a	2 b	100 a	66 ab
	-Rhiz +VAM	2.3 a	0.25 ab	59 a	40 a	39 b
	+Rhiz -VAM	2.9 a	0.23 b	1 b	50 a	57 ab
	+Rhiz +VAM	5.2 a	0.26 ab	48 a	130 a	82 a
250 μg N g^{-1}	-Rhiz -VAM	6.1 a	0.13 a	3 b	130 b	0 a
	-Rhiz +VAM	5.4 a	0.14 a	32 a	140 b	0 a
	+Rhiz -VAM	10.6 a	0.17 a	1 b	200 a	4 a
	+Rhiz +VAM	7.7 a	0.15 a	30 a	160 ab	2 a

¶ Duncans Multiple Range Test, means followed by the same letter are not significantly different at p = 0.05

(Table 16). This was not observed when 250 μg N g^{-1} soil were added, as there was no VAM infection on these roots. In Experiment 3b, the roots of the -G. intraradices treatments all had a low infection of VAM. G. intraradices inoculation significantly increased the infection. The increase in VAM infection did not cause an increase in shoot %P in Experiment 3b (Table 16). R. leguminosarum inoculation had no effect on shoot %P and VAM infection.

With the 40N treatments of Experiment 3a, N_2 fixation did not occur when the plants were not inoculated, but inoculation with either organism caused N_2 fixation. Although the VAM infection was very low, N_2 fixation increased due to G. intraradices inoculation, as was observed by Manjunath et al. (1984), Gupta et al. (1985), and Daft and El-Giahmi (1974). Shoot N content of the 40N treatments in Experiment 3a responded in a similar manner as the VAM infection. In Experiment 3b, the uninoculated 40N treatment had a very high shoot N content, and N_2 fixation. Inoculation with either G. intraradices or R. leguminosarum appears to have had a negative effect on the above parameters. Inoculation with both microorganisms increased the above parameters, but not beyond the uninoculated treatments.
When 250 μg N g^{-1} soil were added, N_2 fixation stopped, and there was no change in the shoot N content.

In Experiment 3a, G. intraradices inoculation increased the lentil shoot Zn, and Cu concentration and uptake independent of the R. leguminosarum inoculation, in the 40N treatments (Table 17). In Experiment 3b, the uninoculated 40N treatment had a very high Zn concentration and uptake, and Cu uptake. Inoculation with either of G. intraradices or R. leguminosarum appears to have had a negative effect on the above parameters. When 250 μg N g^{-1} soil were added, there was a

Table 17. The effect of G. intraradices (VAM) and R. leguminosarum (Rhiz) inoculation on lentil shoot Zn and Cu concentration and uptake

Rate of N		Shoot Zn $\mu g\ g^{-1}$	Shoot Zn $\mu g\ plant^{-1}$	Shoot Cu $\mu g\ g\text{-}1$	Shoot Cu $\mu g\ plant^{-1}$
Experiment 3a					
40 $\mu g\ N\ g^{-1}$	-Rhiz -VAM	49 b¶	80 b	6.0 b	12 b
	-Rhiz +VAM	64 a	186 ab	9.0 a	26 ab
	+Rhiz -VAM	19 c	62 b	5.0 b	16 b
	+Rhiz +VAM	36 b	230 a	9.2 a	62 a
250 $\mu g\ N\ g^{-1}$	-Rhiz -VAM	15 b	80 b	3.3 a	18 a
	-Rhiz +VAM	14 b	136 ab	5.2 a	51 a
	+Rhiz -VAM	19 ab	127 ab	3.5 a	24 a
	+Rhiz +VAM	22 a	217 a	5.0 a	49 a
Experiment 3b					
40 $\mu g\ N\ g^{-1}$	-Rhiz -VAM	32 a	138 a	6.5 ab	28 a
	-Rhiz +VAM	28 a	66 b	7.5 a	17 a
	+Rhiz -VAM	13 b	38 b	5.5 b	16 a
	+Rhiz +VAM	15 b	81 ab	5.3 b	28 a
250 $\mu g\ N\ g^{-1}$	-Rhiz -VAM	27 a	149 ab	4.8 a	29 a
	-Rhiz +VAM	19 a	83 b	4.5 a	24 a
	+Rhiz -VAM	33 a	339 a	7.8 a	85 a
	+Rhiz +VAM	19 a	156 ab	6.7 a	58 a

¶ Duncans Multiple Range Test, means followed by the same letter are not significantly different at p = 0.05

significant interaction response of Zn uptake and concentration in Experiment 3a and 3b (Table 17).

In Experiment 3a, there was no infection of VAM on the roots of the - G. intraradices lentil at both levels of added N (Table 16). The infection of the G. intraradices inoculated treatments was very low. However, inoculation did increase the shoot %P. Low VAM infection was also observed by Trinick (1977) when plants were grown in a low light environment as was the case in Experiment 3a. These results suggest, a large VAM infection is not needed for it to have a positive effect on P nutrition and lentil growth. It is possible, that vesicle count does not give a true picture of the VAM's ability to increase nutrient uptake. Possibly hyphal growth would be a better indicator of the increase in effective root surface area. Although VAM infection was low in the 40 μg N g^{-1} soil level in Experiment 3a, it decreased further when 250 μg N g^{-1} soil was added. As was the case here, Powell and Bagyaraj (1984) stated that in higher soil fertility, VAM infection is low. In Experiment 3a, virtually no VAM vesicles were found on the roots, but G. intraradices inoculation increased lentil shoot yield, %P, and Cu concentration and uptake of the low N treatment.

In Experiment 3a, R. leguminosarum inoculation had no effect on yield, %P, VAM infection, N uptake, but on $\%N_2$ fixation. In Experiment 3b, the - G. intraradices treatments had low VAM infection on the roots (Table 16). This was due to contamination or due to native VAM fungi. In Experiment 3b, the -G. intraradices treatment was prepared by adding filtered root washing to supply microorganisms and autoclaved infected roots to supply equal amounts of C, N and P as in the +G. intraradices treatment.

N_2 fixation occurred in all but one treatment when 40 μg N g^{-1} were added. When 250 μg N g^{-1} were added, the N eliminated the need for the plant to fix N_2, and virtually no N_2 fixation occurred in Experiment 3a and 3b.

The lentil seed yield in Experiment 3b increased when the treatments were inoculated with G. intraradices, and the high level of P was added. (Table 18). This seed yield increase was observed, although the inoculation of G. intraradices had a negative effect on the vegetative shoot yield (64 day harvest) in this experiment (Table 16). It is possible that G. intraradices inoculation and the VAM infection only had a positive effect later than 64 days after seeding, and thus the positive effect was only observed at final harvest. Thompson et al. (1988) has found significant seed yield increases of flax and wheat when VAM was present as compared to the non VAM treatment.

Table 18. The effect of P addition and G. intraradices inoculation on lentil seed yield (Experiment 3b)

	seed yield (g plant^{-1})	
Rate of P (μg g^{-1} soil)	inoculation with G. intraradices	
	-	+
40	0.6 b¶	1.7 ab
125	2.3 ab	3.1 a

all pots received N at 40 μg g^{-1} soil, and were inoculated with R. leguminosarum

¶ Duncans Multiple Range Test, means followed by the same letter are not significantly different at p = 0.05

4.4 Experiment 4: RESPONSE OF LENTIL TO N AND P AND G. intraradices AND R. leguminosarum INOCULATION

The treatments in Experiment 4 were designed to allow tests for interaction effects of N and P addition, as well as inoculation with G. intraradices and R. leguminosarum.

In Experiment 4 soil was used that consisted of 80% (by mass) subsoil and 20% topsoil from a summer fallowed field. A pre-experiment detected no VAM fungal infection on lentil roots in this soil mixture at a harvest of 30 day old plants.

During the growth period, most lentil plants were healthy, except in the high N low P treatments, which started to show symptoms 35 days after seeding. These plants were dark green in colour, had reduced branching, but lodged, and 5% of the plants died. Thus, N toxicity was suspected at low P levels. Walley (1986), and Rodd (1986) also reported of N toxicity in lentil when large amounts of N were added.

When the shoot and seed production interaction effects of the different treatments were analyzed, the addition of the high level of added P or N with the low rate of the other, did not increase shoot dry matter production except at the 30 day harvest due to P addition (Table 19). However at high rates of both nutrients, there was a significant increase in shoot and seed dry matter production. At the 61 day harvest, inoculation with R. leguminosarum had no significant effect, except a negative one in the high N and P treatments. The seed yield increased significantly due to R. leguminosarum inoculation in the low N, high P treatments. All other treatments were not affected by R. leguminosarum inoculation. G. intraradices inoculation had no interaction effect on lentil production at any harvest date.

Table 19. Interaction effects of added P and N and inoculation with *G. intraradices* and *R. leguminosarum* on lentil shoot and seed dry matter production (g plant^{-1}) at different harvest dates

		Rate of N (μg g^{-1} soil)			
		40		250	
		inoculation with *R. leguminosarum*			
Rate of P (μg g^{-1} soil)	inoculation with *G. intraradices*	-	+	-	+
30 day harvest	(shoot)	- - - - - - - - - - g plant^{-1} - - - - - - - - -			
30	-	0.2 b¶	0.2 b		
	+	0.2 b	0.2 b		
100	-	0.4 a	0.5 a		
	+	0.5 a	0.5 a		
61 day harvest	(shoot)				
30	-	1.6 d	2.4 cd	2.2 cd	2.5 cd
	+	1.6 d	2.1 cd	2.1 cd	2.2 cd
100	-	2.5 cd	3.2 c	6.9 a	5.7 b
	+	2.0 cd	3.0 c	7.0 a	5.7 b
seeds					
30	-	0.9 g	1.5 defg	1.5 defg	2.1 cdef
	+	0.9 fg	1.7 defg	1.4 defg	2.3 cde
100	-	1.2 efg	2.4 cd	5.2 ab	5.3 a
	+	1.1 fg	2.8 c	4.3 b	4.7 ab

¶ Duncans Multiple Range Test, means followed by the same letter are not significantly different at p = 0.05

Although there was some N toxicity in certain treatments, the lentil shoot dry matter production and seed yield showed a positive main effect due to the addition of the high rate of N (Table 20). Similarly, the high rate of added P had a positive main effect on lentil production at all three harvest dates. Inoculation with G. intraradices did not increase lentil production at any of the three harvest dates. Inoculation with R. leguminosarum had a positive main effect on seed production but not on shoot dry matter production (Table 20). This indicates that N_2 fixation has the most effect at a later stage in plant development, as no main response was found at the 30 and 61 day harvest. In soybean, the seed yield increased due to Rhizobium inoculation, but the shoot dry matter production was not recorded (Ciafardini and Barbieri; 1987). Dadson and Acquaah (1984) found an increase in shoot dry matter production and in seed yield, when the soybean was inoculated with Rhizobium. In lentil, Herrera and Longeri (1984) reported significant shoot dry matter productions and seed yields due to Rhizobium inoculation.

Inspection of the roots after final harvest revealed that + and - G. intraradices inoculated treatments, had equal VAM infection (Table 21). Lu and Miller (1989) concluded that VAM infection starts at about 14 days after seeding. Therefore, the finding of VAM vesicles was not expected, based on the results from the 30 day pre-experiment. Equal VAM infection of the two inoculation treatments explain the lack of a shoot and seed production response due to G. intraradices inoculation.

The, native VAM species possibly originated in the soils, or they were introduced with the corn root-mats. Several observations were made, and it appears, that some Gigaspora spores (Kendrick, 1985) were

Table 20. The effect of added P and N and inoculation with G. intraradices and R. leguminosarum on lentil shoot and seed dry matter production (g plant^{-1}) at different harvest dates (Experiment 4).

Treatments	30 day harvest shoot	61 day harvest shoot	seed
	- - - - - - - - -	g plant^{-1} - - - - - - -	
Rate of N (μg g^{-1} soil)			
40		2.30 b¶	1.57 b
250		4.30 a	3.34 a
Rate of P (μg g^{-1} soil)			
30	0.19 b	2.10 b	1.54 b
100	0.47 a	4.49 a	3.37 a
G. intraradices			
-	0.32 a	3.38 a	2.50 a
+	0.34 a	3.21 a	2.41 a
R. leguminosarum			
-	0.33 a	3.21 a	2.05 b
+	0.34 a	3.38 a	2.86 a

¶ Duncans Multiple Range Test, means followed by the same letter are not significantly different at p = 0.05 within each main effect

present on certain roots. This leads to the conclusion that the VAM originated from the soil, as no species of VAM have been found on this inoculum in the past. G. intraradices inoculation did not increase VAM infection, and VAM infection was below 25% in all treatments, although fresh inoculum was used, and the light intensity was high. Therefore, a higher infection rate of the G. intraradices was expected, but it could not reach its high potential infection in this experiment. It has been reported that VAM infection decreased greatly, when 5% unsterilized soil was added to sterilized soil (Ross, 1980). Although the subsoil in Experiment 4 was not sterilized, there could have been antagonism among the induced G. intraradices, and a native soil microorganisms including the native VAM. However, Powell and Bagyaraj (1984) reported the opposite effect. The low P low N treatments had the highest VAM infection (Table 21). Where N and or P was added, VAM infection decreased. It appeared that the need for VAM infection decreased not only when soil P increased, but also when the plant was less N deficient. R. leguminosarum inoculation did not affect VAM infection (Table 21).

Table 21. Effect of added P and N and inoculation with G. intraradices and R. leguminosarum on % VAM vesicle infection of lentil roots

Rate of P ($\mu g\ g^{-1}$ soil)	inoculation with G. intraradices	Rate of N ($\mu g\ g^{-1}$ soil) 40		250	
		inoculation with R. leguminosarum -	+	-	+
		% VAM			
30	-	17 a¶	19 a	3 b	2 b
	+	17 a	23 a	5 b	6 b
100	-	2 b	5 b	6 b	5 b
	+	2 b	2 b	2 b	3 b

¶ Duncans Multiple Range Test, means followed by the same letter are not significantly different at p = 0.05

5. CONCLUSION

Growth chamber experiments, using a low P subsoil, were conducted to determine the effect of nutrient addition (P and N), and inoculation with G. intraradices and R. leguminosarum, on lentil growth.

The most significant and consistent increase in lentil shoot and seed dry matter production was observed by the addition of the nutrients N and P. However, when either of these nutrients was at a low level, as in Experiments 2a and 2b, the increase in dry matter production due to the addition of the other nutrient was not predictable.

Inoculation with R. leguminosarum generally increased shoot dry matter production and N_2 fixation only when low amounts of N where added (Experiments 3a, 3b, and 4). The addition of large amounts of N had a more consistent effect on lentil production than did R. leguminosarum inoculation. Thus lentil does not appear to be able to fix enough N_2 to replace fertilizer N.

G. intraradices inoculation always increased VAM infection in the experiments where only subsoil was used. In Experiment 1, 2a, and 3a, where the - G. intraradices plants had no VAM infection, a positive shoot dry matter response due to G. intraradices inoculation (not significant in Experiment 2a, and 3a) was found. In Experiment 1, inoculation with G. intraradices caused a significant increase in shoot and root dry matter production, N uptake, and P, Zn, Cu, Fe and Mn uptake, and concentration (not significant for Fe, and Mn concentrations) of lentil shoots. In Experiment 1, the positive effect of inoculation with G. intraradices was not inhibited at very high P levels. The beneficial effect of G. intraradices cannot be completely explained by its positive effect on P supply, suggesting other factors were involved. G. intraradices decreased the amount of P needed to

achieve good lentil growth. When no P was added, the lentil plants died, whereas the oilseed rape which does not depend on VAM had a high shoot dry matter production. In the experiments where the uninoculated plants had as little as a 3 % VAM infection, no dry matter production response due to G. intraradices inoculation was found.

Although either G. intraradices or R. leguminosarum often increased growth, one could only be sure of an improvement when both symbionts were added (Experiment 2a, 3a, and 4). Thus it appears that agricultural practices which improve VAM survival would increase growth, P uptake, and N_2 fixation in lentil.

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7. APPENDIX

Curve analysis of shoot Zn and Cu concentrations

In Experiment 1, the Zn and Cu concentrations of low P treatment lentil shoots decreased in response to P addition up to the 60 μg P g^{-1} and 80 μg P g^{-1} treatments respectively. When more than 80 μg P g^{-1} was added, the Zn and Cu concentrations increased, and therefore a direct positive effect of P addition was observed. This type of a response is often referred to as a "C-shaped response" or the Piper- Stjenberg effect (Robson and Reuter; 1981). This is because, if one plots the dry matter yield against the nutrient concentration, a C-shaped curve is observed. In Experiment 1, the C-shaped response was most pronounced with the Cu (Figure 4) and Zn (Figure 5) concentrations.

Lambert et.al. (1979) observed decreasing Zn and Cu concentrations of soybean and maize when P was added, but only in +VAM treatments. In Experiment 1, both the

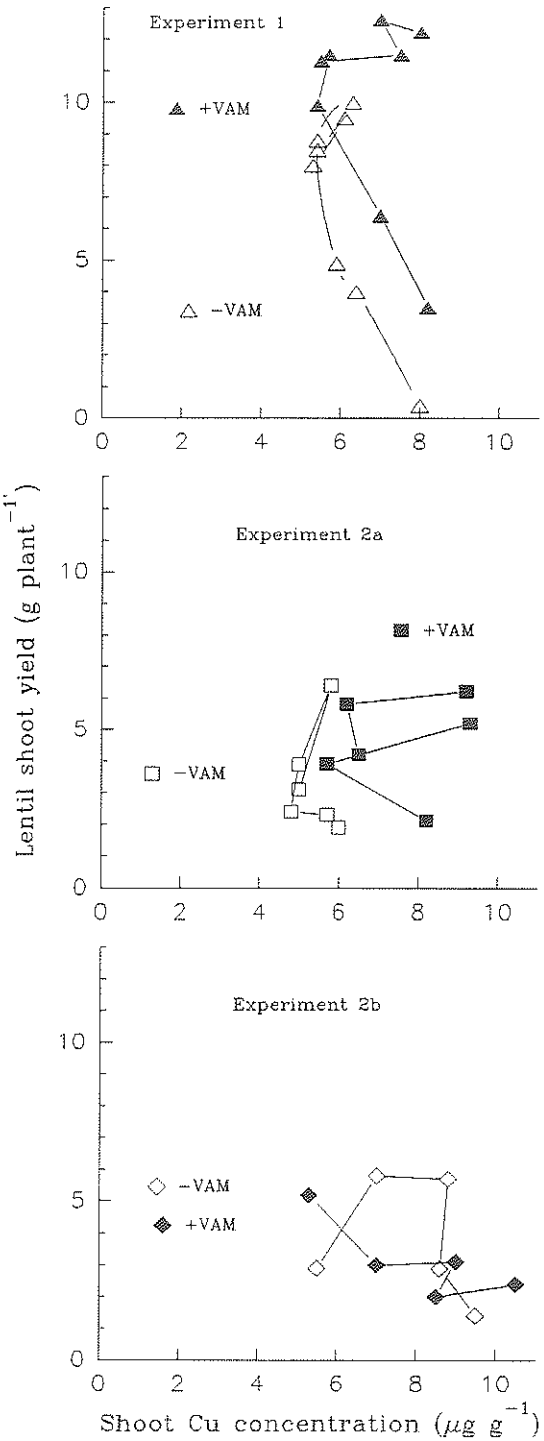

Figure 4. Lentil shoot yield versus shoot Cu concentration (μg g^{-1})

+ and - G. intraradices treatments showed this dilution effect and C-shaped curves. In Experiment 2a, a C-shaped curve was observed for Cu, whereas no curve was observed for Zn, which is consistent with the theory, that nutrient concentrations in the plant above a critical level do not cause C-shaped curves. Experiment 2b showed no curves, but it appears, that there is a dilution of Zn and Cu in response to higher shoot yields.

The Piper-Stjenberg effect has been shown to occur, in very Cu and Zn deficient soils. The soils in the current experiment were only marginally Zn and Cu deficient. The physiological circumstances, which cause a C-shaped curve are not fully understood, but a possible explanation is; a lack of Cu movement from young to old senecing leaves before they drop off (Robson and Reuter; 1981). Another hypothesis is that necrosis of the apical meristem with a corresponding

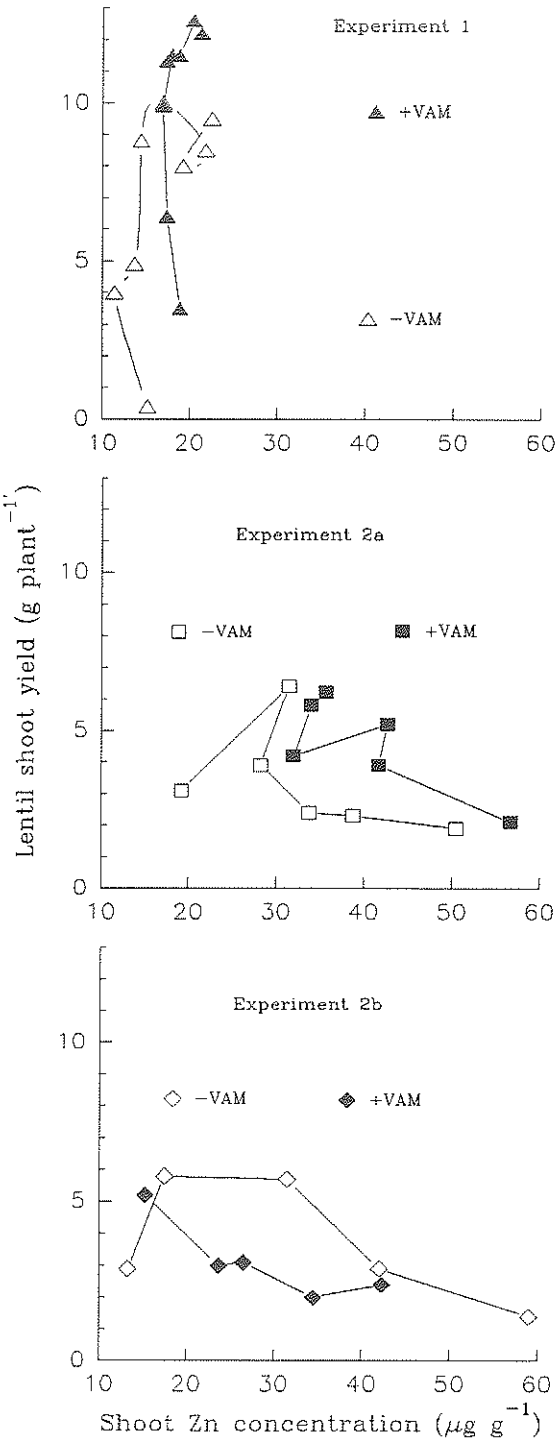

Figure 5 Lentil shoot yield versus shoot Zn concentration (μg g^{-1})

cessation of growth despite further uptake of small amounts of the mineral nutrient in extremely deficient plants (Marschner; 1986). Hiatt and Massey, (1958) suggested that under severe Zn stress, a plants potential for growth may be destroyed, and it stops to grow, while it still accumulates small amounts of nutrients. This explanation is plausible for the nutrients which are not part of the treatments as in the present experiments, where the micronutrient concentrations are high when little or no P was added, and the plant did not grow, probably due to a severe P deficiency. The upper part of the C-shaped curves could possibly be explained, because the yield no longer responded to high levels of added P. C-shaped curves for P were not observed, as P was part of the treatment. Jarrell and Beverly (1981) however, listed many experiments where C-shaped curves were observed for Zn, Cu, Mn, and P but not for Fe. Regardless of the cause, a C-shaped curve indicates a deficiency problem due to P addition. In Experiment 1, it was observed with Zn and Cu.

Inoculation with G. intraradices increases P uptake and growth, thus a dilution of the Zn and Cu concentration was expected, but the opposite was observed in Experiment 1 and 2a (significant), whereas no significant differences were found in Experiment 2b.