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# Nitrogenous and phosphorous nutrition of soybean in symbiotic association with mycorrhizae and rhizobia.

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# NITROGENOUS AND PHOSPHOROUS NUTRITION OF SOYBEAN IN SYMBIOTIC ASSOCIATION WITH MYCORRHIZAE AND RHIZOBIA

A Dissertation Presented

by

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Dedicated to My Children

Wideha and Nomaalie

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ABSTRACT

Nitrogenous and Phosphorous Nutrition of Soybean in Symbiotic Association with Mycorrhizae and Rhizobia

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Soybean forms a symbiotic association with rhizobial bacteria, and vesicular-arbuscular-mycorrhizal (VAM) fungi. This tripartite association of soybean-Rhizobium-VAM fungi could enhance crop production by increasing the efficiency of plant nutrition.

A series of sand culture experiments were carried out in a greenhouse at the University of Massachusetts at Amherst to determine the influence of P and N nutrition on VAM infection and VAM-induced growth benefits. Nodulating and nonnodulating isolines of soybeans (Glycine max Merr. cv Clay) were supplied with hydroxyapatite or dicalcium phosphate with N nutrition from 100% $NO_3^-$, 25% $NH_4^+$ and 75% $NO_3^-$, or 50% urea and 50% $NO_3^-$. Treatments containing $NH_4^+$ and urea will be referred to as the ammonium and urea regimes.

Plants treated with the urea regime exhibited the highest percentage of VAM infection, and the infection increased the tissue N content of these plants relative to the nonmycorrhizal plants. Enhancement of tissue P accumulation through VAM was greater with hydroxyapatite than with dicalcium phosphate. Since they received

adequate N, nonnodulating plants benefitted more from mycorrhizal infection than nodulating plants. Nodulating soybeans exhibited mycorrhizal benefits when the following factors were met: (1) greater than 70% infection with VAM; (2) availability of a reduced N form (3) a root N/P ratio approaching 15 and (4) a soil pH approaching 7. These conditions were met best by urea nutrition. For the nonnodulating isoline, a neutral pH was not essential for determining VAM benefits. Ammonium or urea nutrition met the first 3 conditions for these plants.

Effects of foliar or soil applications of P on the establishment and progression of mycorrhizal infection were investigated. High levels of P, either in soil or foliarly applied, inhibited VAM infection. The action of soil P in inhibiting VAM infection was limited to the of establishment of infection.

Kinetic parameters of phosphate uptake were determined. Mycorrhizally infected roots depleted culture solution P faster than nonmycorrhizal roots and were characterised by lower P efflux values than nonmycorrhizal roots. Mycorrhizal roots had a higher $V_{max}$ value than nonmycorrhizal roots but did not exhibit a higher affinity for phosphate than nonmycorrhizal roots.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

# INTRODUCTION

Soybean (Glycine max Merr. cv Clay) is a major protein supplying grain legume. Approximetely 40 to 45 percent and 18 to 20 percent of the soybean seed is made up of proteins and oils respectively. In spite of its low oil content, the output of oil per acre of soybean is often higher than that of other oilseeds. In the U.S. over 56 million acres are presently under soybean cultivation, and the current average yields are 1610 kg/ha (Boyer, 1982). Production of soybeans on low fertility soils is limited by inefficient recovery of nutrients of which P is of particular importance.

Soybeans usually form symbiotic associations with nodulating bacteria and mycorrhizal fungi. The symbiotic association with Rhizobium species allows the plant access to atmospheric nitrogen. The association with vesicular-arbuscular mycorrhizal fungi (VAM) allows the plant an increased access to limited nutrients and water. The P nutrition of the plant is of special significance in this regard. Phosphorous (Daft and Nicolson, 1966; Mosse, 1973), zinc (Gilmore, 1971), and copper (Ross and Harper, 1970; Daft and Hacskaylo, 1975) are found in higher concentrations in mycorrhizal than in non-mycorrhizal plants. These elements influence nodulation and $N_2$ fixation of leguminous plants (Van Schreven, 1958).

In addition to an enhanced access to phosphate and other nutrients, VAM fungi are believed to make a crop more tolerant to Al or Mn toxicity (Harley and Smith, 1983). These factors bear a special

relevance to the naturally acidic soils of the northeastern U.S., including those in Massachusetts (Brady, 1974; Van Wambeke, 1976). Some of the unfavorable conditions arising from soil acidity includes low availability of P along with excessive Al and or Mn cocentrations (Baker, 1976; Jackson, 1967). Studies on VAM would benefit developing and underdeveloped countries where soils are commonly deficient in phosphate and the cost and the rapid fixation of soluble P fertilizers preclude their use. Mycorrhizae also enable an increased utilization of cheaper sources of P such as rock phosphate, and a maximum growth response to be obtained with smaller applications of P.

The tripartite association of soybean-Rhizobium and VAM fungi, when properly exploited could help increase crop production. Maximum benefits from the fungus is obtained at a reasonably low level of P supply. At supra optimal levels the mycorrhizal fungus may behave as a carbon drain, instead of a P enriching source. Combined nitrogen in general has an inhibitoty effect on nodule initiation, nodule development and $N_2$ fixation. However, small amounts of combined N stimulate the symbiosis.

Strategies for achieving an efficient functioning of the symbionts therefore require a knowledge of the adequate amounts of N and P fertilizers that would enhance the symbiont's efforts without inhibiting them. This in fact would alleviate problems of further acidification resulting from higher N fertilizer applications to already acidic soils.

This work is aimed at determining the influence of P and N

nutrition on VAM infection and VAM induced growth benefits, in nodulating and nonnodulating soybeans. Effects of foliar and soil applications of P fertilizer on the establishment of mycorrhizal infection were investigated. In addition kinetic parameters of phosphate uptake were determined in mycorrhizal plants.

# CHAPTER I

# LITERATURE REVIEW

## Structure and Occurrence of Mycorrhizal Fungi

Mycorrhizae belong in two major categories of fungal, root associations, the ectomycorrhizae and the endomycorrhizae. A third but minor category, ectendomycorrhizae, which is intermediate in type, is present on certain tree species and under specific ecological conditions. The fungal associate of ecto-or the sheathing type of mycorrhizae penetrates the root intercellularly and partially replaces the middle lamella between the cortical cells. The hyphal arrangement formed is called the Hartig net (Bjorkman, 1970). The infected roots appear enlarged due to the formation of an external fungal sheath. The dichotomous branching of these mycorrhizal roots makes them easily identifiable with the naked eye. The majority of ectomycorrhizal fungi are classified among the higher Basidiomycetes (Marx, 1972). A few species belong among the Ascomycetes. Ectendomycorrhizae resemble ectomycorrhizae in forming a Hartig net and a fungal mantle. They resemble endomycorrhizae in that cortical tissues are intracellularly penetrated. Very little is known about the fungi responsible, and these are currently unclassified (Marx, 1972). Endomycorrhizas

include three distinct groups that do not form a fungal sheath around roots, and their presence is determined only microscopically. One, formed mainly by a group of cup fungi, is known only in the Ericales. Another confined to orchid protocorms is formed by certain Basidiomycetes. The third is the vesicular-arbuscular type formed by phycomycetous fungi of the family Endogonaceae. Briefly the vesicular-arbuscular mycorrhizae (VAM) have three components, the host root cells, the endophytic fungal hyphae, and hyphae growing outside the root. Arbuscules are profusely branched organs forming dense clumps of fine hyphae which fill cortical cells of the root early in the infection. Arbuscules become subjected to degeneration after a period of 4 to 15 days. The same cortical cells may be reinvaded by newly forming arbuscules (Cox and Tinker, 1976). Vesicles develop later than arbuscules and may be found as oil-filled, terminal swellings on both external and intercellular hyphae.

Vesicular-arbuscular mycorrhizal fungi are found in all climatic zones. While ecctomycorrhizae occur more frequently on tree species in temperate climates, the VAM occur on a far greater variety of plants including tropical tree species. Certain host species may have both endo-and ectomycorrhizae at the same time. Some families such as Cruciferae and Chenopodiaceae are rarely infected by VAM fungi (Kruckelmann, 1975).

## Functions of Mycorrhizae

### Phosphorous Nutrition

A large proportion of soil phosphate is in forms (organic or inorganic) not readily available to plants. Phosphate is very immobile in soil and tends to be adsorbed readily on to soil colloids. In addition, levels of available phosphate are subject to considerable fluctuations in some soils. These factors contribute to the complexity of P availability to roots.

Phosphorous application gives rise to profitable yield responses when the soil is low in available P and when other nutrients are adequately supplied (Miller et al., 1964). Phosphorous has been seen to increase nodule and soybean seed weight. This response depends on variety, seasonal conditions, and stage of development (de Mooy and Pesek, 1966). Soybeans absorb P predominantly during flowering (Hanway,1962).

### Vesicular-Arbuscular Mycorrhizae and Phosphorous Nutrition

Mycorrhizae are of benefit to the P nutrition of plants, under conditions of limited P availability. The mycorrhizal situation is essentially similar to the possession of extra root hairs by the plant (Baylis, 1972). Mycorrhizae increase the root soil contact and hence

the volume of soil exploited by the mycorrhizal root. Since the root absorbs phosphate ions more rapidly than the ions can be replenished by diffusion, phosphate depletion zones constantly arise around the root. By extending several centimeters into the soil VAM fungi gain access to P beyond these depleted zones. Therefore the fungi offer a means of overcoming the diffusive impedence around the roots, in a manner similar to root hairs. It is believed that responses to VAM infection are greater in species with unbranched roots and few root hairs (e.g. Allium, Coprosma, Citrus). This phenomenon is described as 'mycotrophy' (Baylis, 1972,1975).

High P availability precludes mycorrhizal benefits. Externally applied inorganic phosphate, whether soil applied (Mosse, 1973; Hayman, 1975; Jasper, Robson and Abbott, 1979) or applied as a liquid feed (Daft and Nicolson, 1969) decreases the level of VAM infection in many host species. Effects of P on fungal growth may be mediated via the root or directly on fungal growth in the soil. Mosse (1973) discounted the second possibility on the basis that in many cases hyphae growing on the surface of the root looked normal. Sanders (1975) injected a phosphate solution into the hollow leaves of onion plants and observed a drop in VAM infection. Amount of external mycelium produced per cm of infected root was reduced from 3.5 to 2 mg. Changes in the anatomy of infections similar to those seen by Mosse (1973) were observed. Based on these data, Sanders concluded that the effects of soil P in reducing infection were root-mediated. Split-root techniques and transplanting experiments suggest that high

tissue P reduced the fungal infection. Menge, Steirle et al., (1978) supplied high P to one-half of a split-root system (750 mg per kg soil) and found a reduction in mycorrhizal infection in the other half, even though it grew in a separate container of relatively low P soil. When plants were transplanted from sterilized soil low in P to a soil high in P, infection was reduced (Jasper et al., 1969), but a similar effect was not observed when plants from sterilized soil high in P were transferred to soil low in phosphorous cabable of infection. Increasing the P content of the soils into which plants were transferred reduced the percentage of VAM infection (Azcon et al., 1978). Results of transplanting experiments are hard to interpret. Increased growth in length of roots when tissue P is high (whether soil applied or foliarly applied) might be the only contributing factor in the reduction of percentage infection.

The host endophyte balance changes from mutualism to that of parasitism, depending on the nutritional status of the soil. Mycorrhizae promoted the growth of some grasses when the P content of soil was 4 mg P per kg, but became parasitic on the same grass in a soil having 8 mg P per kg (Crush, 1973). An unfavourable effect of some endophytes was observed on pasture legumes at high levels of added superphosphate (Crush, 1976). In nature, symbiotic imbalances would be less likely as the system tends to be self regulatory. Jensen and Jakobson (1980) did not observe significant differences in shoot P of plants grown in widely varying soluble P. Differerences in soil P, apparently were offset by adjustmnts of mycorrhizal infection.

Low P nutrition increases root membrane permeability leading to a net loss of root metabolites. The latter sustains the germination and growth of the mycorrhizal fungus during pre-and post-host infection. The subsequent mycorrhizal infection brings about an improvement of root P nutrition, and a reduction in membrane-mediated loss of root metabolites (Graham, Leonard and Menge, 1981).

## Insoluble Sources Of Phosphorous

A great deal of research has been done on the uptake of P from low solubility sources by VAM plants (Hayman and Mosse, 1972; Mosse et al., 1973, Powell, 1975; Pichot and Binh, 1976; Sanders and Tinker, 1979; Swaminathan, 1979; Gianinazzi-Pearson et al; 1981). The maximum growth reached with rock phosphate is less than that with superphosphate. Rock phosphate may be deleterious to plants on account of contaminants present in it (Pairunam et al., 1980). Different combinations of crops and fungi appear to respond differently to the supply of insoluble phosphorous(Jackson, 1966; Graw, 1979). It is now confirmed that the VAM plants do not render wholy unavailable sources useful, but they can accelerate the uptake from low-solubility sources (Hayman and Mosse 1972; Barrow et al., 1977). Rock phosphate became available to a plant species in acid soils, but it did not improve the growth of the same plant species in

neutral or alkaline soils (Sims, 1959; Jackson, 1966). In acid soils, adding rock phosphate increased P uptake, and in addition, utilization of the added phosphate was increasd greatly by mycorrhizal infection (Mosse, Powell, and Hayman, 1976). In neutral and alkaline soils, the rock phosphate remained unavailable to mycorrhizal or non-mycorrhizal plants. Mycorrhizal infection greatly increasd both P uptake and plant growth in three alkaline and one neutral soil, but in such soils, a more soluble source of phosphate was needed to maintain soil fertility. A detailed study of the relationship of soil pH and the availability of various phosphates to mycorrhizal and nonmycorrhizal plants was done using two species of the Compositae (Graw, 1979). Complex results were obtained with the two species giving quite different results, in spite of inoculation with the same strain of Glomus macrocarpus. At a pH of 4.3, VAM did not help absorption of P by Guizotia abyssinica from several compounds of P, with resultant inhibition of its growth, whereas mycorrhizal Tagetes minuta did well at this pH in the presence of monocalcium phosphate, hydroxyapatite, or aluminum phosphate. Phosphorous uptake and growth of mycorrhizal Guizotia was improved at pH 5.6 and surpassed the performance of non-mycorrhizal plants at pH 6.6. Growth of mycorrhizal Tagetes was reduced at pH 5.6 in the presence of monocalcium phosphate or hydroxyapatite but was improved at pH 6.6 in all treatments with the exception of hydroxyapatite as P source. Mosse (1971) obtained infection with Coprosma robusta in two soils with pH 5.6 and 7.0 but not in acid soils of pH 3.3 to 4.6. Strzemska (1973) found strong

development of VAM in the roots of fenugreek and soybean at pH 4.5. Nyabenda (1977) found that the mycorrhizal effect varied with soil temperature and with form of phosphate. The relative value of infection was greatest with insoluble phosphates, but this effect was also highly temperature dependent. In experiments carried out to test the ability of tomatoes and maize to utilize tricalcum phosphate, hydroxyapatite, and rock phosphate, the benefits gained from VAM was greatest with tricalcium phosphate, lesser with phosphate rock, and least with soluble phosphate additions (Daft and Nicolson, 1966; Murdoch et al., 1967). In contrast to this result, soybeans did not respond to additions of $Ca_3(PO_4)_2$ whether mycorrhizal or not (Gianinazzi-Pearson et al., 1981). There was no increase in phosphate inflow into mycorrhizal or non mycorrhizal Trifolium subterraneum in short-term experiments using $Ca_3(PO_4)_2$. A slight growth response was detected in long-term experiments (Smith et al., 1984). Soybeans have shown significant growth responses to a number of other P compounds, including Fe and Al phosphates (Ross and Gilliam, 1973). In pot experiments carried out on a range of soils to test the effect of phosphate rock on legume crops with or without mycorrhizae, it was found that VAM could improve but not confer, the ability to use phosphate rock efficiently (Mosse et al., 1976; Mosse, 1977).

## Kinetics Of Phosphorous Uptake

Phosphorous uptake exhibits complex kinetics (Bieleski, 1973) in an extended concentration range (0 to 10 mM). In cultivated soils, the available P is in uM range (Arnon et al., 1942; Barber, 1984) and in these uptake kinetics follow a simple hyperbolic pattern (Bieleski, 1973) with a $K_m$ of about 5 uM. In the lower concentration range, the phosphate transport system exhibits an intrinsic sensitivity to pH (Sentenac and Grignon, 1985). The transport system conforms to a low activity state around pH 6 and is switched to a state of high activity approaching pH 4. The relatively high concentration of $H_2PO_4^-$ around pH 4 provides further evidence that the phosphate uptake involves the $H_2PO_4^-$ ion (Bieleski, 1973).

Phosphorous influx into plant roots conforms to Michaelis-Menten Kinetics (Epstein, 1972). Edwards and Barber (1976) reported soybean to have the highest $V_{max}$ value approaching pod-setting stage, the value declining thereafter.

## Uptake by Mycorrhizal Fungi

A number of practical problems are involved in obtaining accurate measurements of ion uptake by VAM fungi. The uptake mechanisms of the

fungi closely follow those of higher plants (Harley and Smith, 1983). The data of Sanders and Tinker (1973) indicates a P influx of up to $10^{-12}$ mol. $cm^{-2}$ $s^{-1}$ for hyphae of around 8um diameter. Gray and Gerdemann (1967) observed a more rapid uptake of P by mycorrhizal roots than by uninfected ones in solution culture. The amounts of P taken up by excised roots of VA mycorrhizal plants are not very different from non-infected excised roots (Bowen et al., 1974). Using classical Epstein-Hagen kinetic analysis Cress et al., (1979) measured the uptake of P by excised tomato roots with or without VAM. An increase in the maximum rate of uptake ($V_{max}$) was not noticed for mycorrhizal roots. The affinity constant $K_m$ showed an increase of 2 to 3 fold for mycorrhizal roots implying a more efficient uptake mechanism in the fungus than in the root at low concentrations. Uptake measurements by mycorrhizal roots has some inherent weaknesses due to uncertainty in quantity of external mycelium remaining on the root. The extent to which stirring ensures that there are no gradients in the static layer of solution surrounding root can only be speculated (Walker et al., 1979; Tinker and Gildon, 1983). The internal fungal structures could act as a strong local sink or a source of P, confounding the results. Phosphorous uptake by roots in P-deficient soils shows that it is the diffusion impedance in the soil and not the physiology of the root which limits the rate of P uptake by the root (Nye and Tinker, 1977)

Kinetic analysis shows that two mechanisms may be important in P absorption by VAM. Phosphate uptake by tomato roots was studied at

concentrations between 1 and 100 uM $KH_2PO_4$. The concentrations upto 30 uM correspond to the levels of P in soil and are important in interpreting the mycorrhizal effects on plant growth in the soil. At both high and low P concentrations the Michaelis constant ($K_m$) for phosphate uptake is lower for mycorrhizal plants. This result indicates that a higher affinity for phosphate ions may exist in mycorrhizal roots. Mycorrhizal plants also exhibit a higher $V_{max}$ than non- mycorrhizal plants at higher P concentrations (Cress et al; 1979). This observation implies that in situations where P availibility is plentiful, the increase in the number of uptake sites provided by the fungus could contribute substantially to the total uptake, thereby lowering the threshold for effective phosphate absorption from soil (Mosse, Hayman and Arnold, 1973).

## Transloction Of Phosphorous

Reportedly Ca, P, S, and Zn are translocated by VAM hyphae(Rhodes and Gerdemann, 1978a; 1978b, Cooper and Tinker, 1978). Maximum fluxes of P through VAM hyphae have been calculated to be of the order of $10^{-8}$ mol. cm $^{-2}$ $s^{-1}$. Buildup of inorganic phosphates during periods of rapid uptake are prevented due to synthesis of polyphosphates (Cox and Tinker, 1976)), which are concentrated in granules of less than lu in diameter within the small vacuoles. Cytochalasin, the inhibitor of

protoplasmic streaming, stops translocation of P in hyphae (Cooper and Tinker, 1981). Translocation is highly-temperature sensitive and is dependent on plant transpiration. A combined bulk flow and protoplasmic streaming mechanism, operating on P concentrations aided by the formation of polyphosphate granules account for the high rates of P tanslocation in VAM. Maintenance of concentration gradients along the hyphal path could occur through mediation of "sources" and "sinks" by conversion of polyphosphates to soluble phosphates. Bidirectional translocation of nutrients in hyphae is explained if cytoplasmic streaming acts as a stirring mechanism. The stirring would lead to increased rates of translocation down concentration gradients between loading and unloading sites in the hyphae.

Arbuscules are believed to be the sites of breakdown of the polyphosphate granules (White and Brown, 1979). The enzyme alkaline phosphatase is responsible in breakdown of polyphosphate granules. Alkaline phosphatase, which is of fungal origin reaches peak activity in young arbuscular infections. Presence of active arbuscules and alkaline phosphatase activity are correlated (Gianinazzi-Pearson and Gianinazzi, 1978).

## Carbohydrate Physiology of Vesicular-Arbuscular Mycorrhizal Fungi

Bevege and Bowen (1975) found $^{14}C$-labelled photosynthate to appear in external hyphae after $^{14}C$-carbon dioxide was supplied to the leaves of the plant. Unlike ectomycorrhizae, VAM do not convert host photosynthate to the fungal carbohydrates, trehalose and mannitol, of which mannitol is not readily usable by the heterotroph (Hayman, 1974). A "carbohdrate-sink" that prevents the reciprocal flow of carbohydrates between autotroph and heterotrph does not exist in the VAM situation, nor do they possess a fungal sheath that may prove a substantial carbon sink. However, alternative sinks may operate in VAM. Lipids are major storage compounds in VAM (Cox et al., 1975) and mycorrhizal roots contain significantly more lipid than uninfected roots (Cooper and Losel, 1979). The lipid component of mycorrhizal roots become labelled when plants photosynthesize using $^{14}CO_2$ (Losel and Cooper, 1979). The concept of VAM fungi creating a significant carbon drain on the host remains a controversial issue. Mycorrhizal infection raises cytokinin levels (Allen et al., 1980) which in turn would raise the photosynthetic activity (Herold, 1980). Thus the small carbon drain that does exist could be offset by increased photosynthesis (Kucey and Paul, 1982). Cooper (1975) did not consider the fungal biomass associated with VAM roots as sufficient in terms of a substantial carbon drain.

Mycorrhizal Effects On Growth and Distribution Of Dry Matter.

Vesicular arbuscular mycorrhizae could have beneficial or deleterious effects on the growth of the host depending on environmental conditions. Mycorrhizal plants sometimes show a lower root/shoot ratio than similar non-mycorrhizal plants (Hunt et al., 1975). The low root/shoot ratio may partly be interpreted as a response to improved mineral nutrition. Increased soil P (Bowen and Cartwright, 1977) or N (Jenkinson et al., 1972) reduce the root/shoot ratio regardless of mycorrhizal infection. The dry matter increases in shoots and roots of VAM plants together with changes in the distribution of dry matter between root and shoot may be viewed as a feed-back response to nutrient uptake by the VAM (Smith, 1980).

Transitory depressions of growth occur in the early stage of the VAM symbiosis (Baylis, 1971; Furlan and Fortin, 1973; Cooper, 1975). This depression is attributed to the fact that the fungus and autotroph are competing for a limited supply of food at this stage. The mycorrhizal stimulus to plant growth is depressed or reversed by low light intensity. This effect probably is due to the reduction in the photosynthate available for destruction. Crush (1976) attributed the transitory growth depressions that is seen under low soil P as due to competition between fungus and host for the soil P, rather than the supply of photosynthate. Competition for P has been shown to be

important in other situations where P is in short supply (Gabrielson and Masden, 1960).

## Soybean and Nitrogen Requirements

The soybean plant cannot meet its total N requirement from $N_2$ fixation alone (Harper, 1974). Low amounts of N fertilizer given during the early growth of soybean enhances its growth and $N_2$ fixation (Harper, 1974). Beyond an optimum value the N applied brings about depressions in $N_2$ fixation (Sinclair and de Wit, 1976). The stimulatory effect of N on the symbiotic response, at low levels of applied N, varies depending on the form and amount of N, species, and cultivar (Gibson and Nutman, 1960; Dart and Wildon, 1970). Greenhouse experiments have demonstrated that applied N in the range of 30 to 180 mg N per plant are optimal for soybean $N_2$ fixation (Eaglesham et al., 1980). The strongest synergism occured in soybean with urea at 30 mg per plant; for every milligram of urea-N absorbed, an additional 15 mg of N were fixed symbiotically. Clearly these pot studies bear a relevance to field conditions only where soils are extremely deficient in available N. Fertilization with N under normal field conditions, is more likely to inhibit $N_2$ fixation than to stimulate it. When N was applied at 56 kg N per ha as $NH_4NO_3$ it had no effect on $N_2$ fixation; when the applied rates exceeded 244 kg per ha, $N_2$ fixation

declined to zero.

There is a direct relationship between photosynthetic flow to nodules and $N_2$ fixation in soybeans (Latimore et al., 1976). Nitrogen in $NH_4^+$ or $NO_3^-$ forms decreased nodular photosynthetic import and capacity of soybeans to fix $N_2$. Both sources are thought to be equal in reducing the energy flow to nodule bacteria. Ammonia appears to decrease $N_2$ fixation in part at least by decreasing the synthesis of nitrogenase of the bacteria (Bishop et al., 1975). Nitrate-N brings about inhibitory effects by interfering with root curling, which is an initial step in rhizobium infection (Thornton, 1936). Nitrate in the external medium also catalyzed the destruction of indoleacetic acid (IAA), whereas $NH_4^+$-N decreased the amount of trytophan converted to IAA (Tanner and Anderson, 1963). Both ions interfere with root hair curling. Inorganic N may exercise its effects in decreasing $N_2$ fixation in other ways (Stewart, 1966).

Conflicting reports exist as to the stage at which maximum $N_2$ fixation occurs in soybean. Harper and Hageman (1972) showed $N_2$ fixation to be maximum during pod filling, declining rapidly during the final three weeks of growth. Hardy et al., (1976) found $N_2$ fixation to reach a maximum at pod-filling, and remain constant until pod filling was complete. The seasonal $N_2$ fixation profile of soybean was described by Brun (1978) as having three phases; the first occurs during the vegetative period, when rate of fixation is low; the second occurs sometime after flowering, when fixation rate increases rapidly and reaches a peak during the vegetative period, and the third sets in

at the early seed filling period when fixation rate declines rapidly. Peat et al, (1981) emphasized the importance of young reproductive structures in promoting $N_2$ fixation around the time of flowering. However, the cause of this marked increase in the rate of fixation around flowering is not known despite the fact that it is of wide occurrence. This increase is reported in pea (Pisum sativum), cowpea (Vigna unguiculata), soybean, peanut (Arachis hypogea), broad bean (Vicia faba) and clover (Trifolium repens) (Hardy et al., 1971; Lawn and Brun, 1974; Masterson and Murphy, 1976; Bethlenfalvay and Phillips, 1977). Ability of the soybean plant to use nitrate diminishes at pod-filling. This decrease is indicated by a sharp drop in nitrate reductase activity within 2 to 3 weeks after flowering, and this activity continues to decrease through mid-pod filling (Thibodeau and Jaworski, 1975).

The drop in the efficiency at obtaining sufficient N is critical for the soybean plant, since it also occurs at a time of greatest demand for N (Harper, 1971; Harper and Hageman, 1972). In order to compensate for this loss of ability in fulfilling its N requirements, the plant resorts to a process of internal remobilization of N compounds. Important enzymes become subjected to hydrolysis at this stage, and the resultant amino acids are used to meet the demands of reproductive organs (Sinclair and de Wit, 1976). The plant suffers a loss of photosythetic capacity especially as a result of the breakdown of ribulose bisphosphate carboxylase. A premature senescence of the leaves occurs as a result of these events. The soybean plant has come

to be known as a "self destuctive" crop due to the nature of events that leads to its early death (Sinclair and de Wit, 1976).

## Soybean and Ureide Metabolism

The ureides allantoin and allantoic acid are the major nitrogenous compounds in soybean and several other legumes growing symbiotically (McClure and Israel, 1979; Herridge et al., 1978). Ureides become synthesized rapidly from the early products of $N_2$ fixation, ammonia and glutamine, via purine synthesis and degradation (Ohyama & Kumazawa, 1978; Triplett et al., 1980). Several enzymes involved in the assimilation of ammonia as well as key enzymes of purine synthesis and degradation increase severalfold in the nodule cytosol in parallel with increases in nitrogenase activity in the bacteroid (Reynolds et al., 1982). The use of ureides rather than amides for N-transport from root to shoot results in an improved carbon economy of the plant. Improved economy results from the low C:N ratio of the ureides.

Storage and assimilation of ureides occur mainly in the shoot. For soybeans relying entirely on $N_2$ fixation for its N supply, 86 percent of the total sap N is in the form of allantoin and allantoic acid (Israel and McClure, 1982). In a nonnodulating legume, the percentage contribution of ureides to the total sap N varied from 13

to 42 percent depending on the inorganic N-source fed to the plant (Thomas et al., 1980). It is hypothesized (Ishizuka, 1977) that the application of fertilizer N results in an increased amount of vegetative growth but in a decrease in the number of pods retained on the plant, whereas the ureides arising from $N_2$ fixation are stored in stems and leaves as materials for use later in pod formation. The hypothesis calls for further investigation since it is not known how vegetative growth is influenced by the composition of the plant N.

Ureides may play a key role in the detoxification of ammonia in root tissues (Reinbothe and Moth, 1962). Many of the legumes which have a high nitrogen requirement for seed production per g of available photosynthate (Sinclair and de Wit, 1971) e.g. soybean, cowpea, and Phaseolus sp., also use ureides as major transport forms of nitrogen. This phenomenon suggests a role for ureides as being particularly important in ammonia detoxification and conservation of carbon.

## Nitrogen Nutrition and Vesicular-Arbuscular Mycorrhizal Fungi.

Reports on the effect of combined N on VAM-host plant relationships are scarce. Only a few of these reports distingish between effects of $NH_4^+$ and $NO_3^-$ ions. These ions differ in their

mobility in soil (Fried and Broeshart, 1967; Haynes and Goh, 1978) as well as in their assimilatory pathways in plants (Huber and Watson, 1974). These different assimilatory pathways bear different implications on the organic and inorganic composition of the plant tissues (Wadleigh and Shive, 1939), which include production or consumption of $H^+$ ions and biochemical events associated with pH regulation, (Dijkshoorn, 1969; 1971; 1973; Barker and Jackson, 1966a; Barker et al., 1966b, Raven and Smith, 1976; Raven et al; 1978). Plants grown on $NH_4^+$ extrude $H^+$ ions from their roots, with a consequent drop in the pH of the culture solutions (Kirkby and Mengel, 1967), $NO_3^-$ assimilation produces an alkaline medium (Dijkshoorn, 1962). Acid pH is more inhibitory than alkaline pH for the development of certain VAM species (Green et al; 1976). The low rhizosphere pH, resulting from $NH_4^+$ assimilation, results in a reduction in the number of entry points and subsequent colonization of the root by these VAM species. Rhizobium prefers a pH of around seven (Vincent, 1965; de Mooy and Pesek, 1966). Amino acids and amides especially glutamate, glutamine, aspartate and asparagine predominate in the exudates of $NH_4^+$-fed plants, while carboxylic acids predominate in the exudates of $NO_3^-$fed plants (Raven and Smith, 1976). Information on how VAM might utilize such compounds is lacking in the literature. The lower mobility of $NH_4^+$ in soil in comparison to that of $NO_3^-$ implies that its uptake could be enhanced by VAM in a manner similar to that of P. The ease of mobilty of $NO_3^-$ in soil makes it unlikely that zones of depletion of $NO_3^-$ would form around roots.

## Nitrogen and Phosphorous Interactions

Increases in N concentrations were observed in roots and shoots of legumes infected by VAM (Mosse et al., 1976; Smith and Daft, 1977). Increases in N were attributed to enhanced nodulation and nitrogenase activity, rather than to increased uptake of $NO_3^-$ or $NH_4^+$ from soil (Mosse et al., 1976). Increased $N_2$ fixing ability has been attributed mainly to improved P nutrition and plant growth (Smith et al., 1979). The stimulatory effects of VAM on nodulation of the legumes lucerne and clover precede visible growth effects. This result suggested that the nodules acted as stronger sinks for phosphate, than the remaining plant organs (Smith and Daft, 1977; Smith et al; 1979). The importance between N and P interactions in legumes is emphasized by results with soybeans, in which VAM improved growth of a nodulating but not of a nonnodulating isoline (Schenck and Hinson, 1973). Mycorrhizal effects on legume nodulation, $N_2$ fixation, and plant growth are usually similar to the effects of adding phosphate (Munns and Mosse, 1980). Certain efficient endophytes have been seen to enhance growth much more than additions of 40 kg superphosphate per hectare (Hayman and Page, 1981).

Hepper (1983) observed that mycorrhizal infection shows parallel increases with increases in N supply, at all P levels. Increasing amounts of applied P generally depresses infection. However, the

extent to which mycorrhizal infection increases depends on the nitrogen to phosphorous balance. When high levels of N were provided, increased P concentratons did not harm the growth of the fungus within the root (Mosse, 1973; Hepper, 1983).

## Plant N Nutritional Status versus VAM

The influence of the nitrogen nutritional status of the host on VAM infection has received less attention. Ammonium nitrate applied at 100mg N per kg soil depressed nodulation as well as mycorrhizal infection in Pisum sativum (Lanowska, 1966). Initial applications of $(NH_4)_2SO_4$ or $NaNO_3$ in excess of 2 meq per pot (460 or 480 g of soil) reduced the level of infection in nodulating clover plants. This reduction was attributed partially to inhibition of the preinfection phase of the fungus (Chambers et al., 1980). In contrast, Wang and Hayman (1982) found that $NH_4NO_3$ did not affect mycorrhizal infection in Trifolium repens although it did reduce infection levels in onion roots. Applications of $NH_4NO_3$ or $NaNO_3$ decreased the level of mycorrhizal infection in soil-grown Lolium perenne (Buwalda and Goh, 1982). Infection levels of lettuce roots fell with increasing applications of $NaNO_3$, $NH_4NO_3$, or $(NH_4)_2SO_4$ (Owusu-Bennoh and Mosse, 1979). Calcium nitrate has been observed to reduce VAM infection (Mosse and Clark, 1978). Application of urea to field plots resulted in a decrease in VAM infection of Araucaria, when soil P levels were high (Bevege, 1971). At intermediate P levels, however, the infection

increased with increased urea application. In a comparison of the effects of foliar or soil applied nitrate on VAM infection, the plants with soil-applied nitrate always developed much less infection (Azcon et al., 1982). Thus soil nitrate may be inhibiting the establishment of infection rather than its spread within the root. In contrast to these results Brown, Schultz, and Kormanick (1981) found that application of nitrate or ammonium resulted in higher levels of mycorrhizal infection in sweet gum seedlings compared to minus N controls. Bowen and Smith (1981) examined the subject of uptake of N by mycorrhizal plants. Other review papers have suggested that either mycorrhizal fungi have a potentially important role in acquisition of N by plants (Raven et al, 1978; Smith, 1980) or that VAM fungi are not involved in uptake and transport of N (Rhodes and Gerdemann, 1980; Hayman, 1982).

These conflicting views have resulted from considerations of the form and availability of N in agronomic conditions or native plant communities. In cultivated soils where the readily mobile $NO_3^-$ ion predominates, VAM hyphal transport of N is of little importance (Rhodes and Gerdemann, 1980). In a climax community, however, the major portion of N occurs in bound organic form, and $NH_4^+$ constitutes the primary source of N available to plants (Clark and Paul, 1970; Raven et al., 1978; Bowen and Smith, 1981). Ion transport through VAM hyphae could be more important for the plant, where the only source of N is the relatively immobile $NH_4^+$ ion.

The N content of mycorrhizal plants, relative to controls, are

reported to be higher (Wallace, Mcnaughton, and Coughenour, 1982), lower (Nemec and Meredith, 1981; Hays et al., 1982) or of no difference (Schenck and Hinson, 1973; Carling et al., 1978). These diverse results could have resulted from the levels and forms of N supplied to the plants. Certain crops though well-infected with VAM fungi may not benefit from the mycorrhizae because N is limiting (Hall et al., 1984). Crush (1973), Cooper (1975) and Hall (1978) detected little response to VAM in ryegrass, when N was limiting. Powell (1974; 1977) and Powell and Daniel (1978) found a marked response to VAM in situations where soil was supplemented with nitrogen.

Nitrogen application stimulates growth and raises the shoot N/P ratios. This elevation probably accompanies lowering of plant P status and could be the reason why mycorrhizal fungi stimulate growth, when N was supplied. Ectomycorrhizae (France and Reid, 1979) and VAM (Bowen and Smith, 1981) are believed to have a preference for $NH^{+}$-N over $NO_3$-N. Ames et al. (1983) used the $^{15}N$ isotope of N in examining the source of N acquired by mycorrhizal and non-mycorrhizal celery (Apium graveolens L.). The labelled N in mycorrhizal plants was correlated significantly with percent mycorrhizal fungal colonization and total length of hyphae per gram of soil. Mycorrhizae did not help in the uptake of the organic N source.

## Conclusions

It is amply documented that VA mycorrhizae can improve plant growth through increased uptake of phosphorous (Mosse et al., 1973; Gerdemann, 1975). The effects of combined nitrogen on mycorrhizal infection and consequent nutrition of the host however, is not well known (Chambers et al., 1979; Brown, Schultz, and Kormanik, 1981). Preferential uptake of $NH_4^+$ over $NO_3^-$ by roots, hyphae of VA symbiont, or both may be partly responsible for the superior growth obtained with $NH_4^+$-N. Although $NH_4^+$-N is known to be a major source of N for some mycorrhizal fungi, it is not known whether Glomus mosseae in association with soybean exhibits preference for different forms of N. Since mycorrhizal root systems are able to meet phosphorous needs of plants sufficiently even at low soil P concentrations, nitrogen availability is probably a more critical factor than phosphorous availability in achieving optimum growth of mycorrhizal soybean.

Nitrogen source, level of N application, and their interaction have highly significant effects on mycorrhizal development and arbuscule formation (Brown, Schultz, and Kormanick, 1981). Seedlings grown at N supplies optimum for plant growth have the highest infection intensities in their roots. $NH_4^+$ has a less severe effect upon nodulation and acetylene reduction (Chambers et al., 1971) than $NO_3^-$, when applied N levels were low. Inhibition of mycorrhiza formation by $NH_4^+$, $NO_3^-$ or other ions may have a significant impact

upon nutrient uptake by plants from nutritionally poor soils. Sanders (1975) showed that reduction in mycorrhizal infection, induced by foliarly applied phosphate, results in decreased phosphate uptake from the soil. Reduction in mycorrhizal infection by other mechanisms (e.g., application of combined N to the soil) would have the same effect. It is often overlooked that $NH_4^+$ may be an important source of N in natural or cultivated soils (Rice and Poncholy, 1974; Haynes and Goh, 1978). There is increasing interest in the use of $NH_4NO_3$ fertilizers and in reducing nitrification (Barker, 1982), which could raise soil $NH_4^+$ to levels which might affect mycorrhiza formation. Reportedly $NH_4^+$ nourished plants have reduced root systems (Kirkby, 1969; Chambers et al., 1979). Indeed, in such situations mycorrhiza may compensate by extending the absorbing surface in the soil. Interactions between $NH_4^+$ and P nutrition (Riley and Barber, 1971) and changes in activity of N assimilating enzymes may have a bearing on the extent of mycorrhizal infection. A depressed rhizosphere pH is a consequence of $NH_4^+$ nutrition. Lowered rhizosphere pH can be important in the control of root infecting fungi which are sensitive to pH (Smiley, 1975; 1978). Glomus mosseae may prefer a neutral pH (Mosse, 1972). Plants fed on urea do not face the problem of root zone acidity and yet have access to $NH_4^+$ ions. The nitrogen-form preference of the soybean-Glomus mossea-rhizobial system is worthwhile investigating, since data gathered may help to optimize mycorrhizal benefits to the soybean plant.

In addition an understanding of the P uptake characteristics of

mycorrhizal soybean roots is important for developing practices that improve VAM efficiency.

# C H A P T E R  II

## EFFECTS OF N AND P INTERACTIONS ON ESTABLISHMENT OF MYCORRHIZAL INFECTION

### Materials and Methods

A greenhouse experiment was conducted to compare the effects of $NH_4NO_3$ or urea on establishment of mycorrhizal infection in nonnodulating soybean (Glycine max Merr. cv Clay) over a range of phosphate levels. Both N sources were supplied with a 50% $NO_3^-$ background.

Pot cultures of mycorrhizal inoculum were established and maintained as follows. A 3:1 soil:sand mixture was passed through a 2 mm sieve and then steam sterilized in bulk for an hour on each of three consecutive days. This mixture was placed in 1.8-liter sterilized plastic pots and then brought to 12% moisture content w/w by addition of water and stored for 6 days in the greenhouse. Prewetting ensures prompt mycorrhizal infection of seedlings. Paper towels were placed over the pot drainage holes to prevent soil loss. Mycorrhizal inoculum (Glomus mosseae) obtained from the Department of Agronomy, University of California at Davis, California (air-dried, sealed in plastic bags and stored at 5°C) was added to the pots 1/2 of the way up from the bottom of the pot. Sudan grass seeds were surface-sterilized in 0.26% sodium

hypochlorite for 30 min, rinsed in deionized water and were planted 6 seeds per pot. Because of its rapidly growing, fibrous root system, sudan grass makes an ideal "trap plant". However, the roots often grow out of pot drainage holes onto the bench itself, which could cause contamination of the culture. Roots were confined to the pots by placing a loose aluminum foil barrier around drainage holes. Beginning three to four months after planting, cultures were sampled periodically to determine maturity of new spores.

Soybean seeds were surface-sterilized in 75% ethyl alcohol, and rinsed in several changes of deionized water. These were then germinated on filter paper soaked in 0.2 mM $CaCl_2$ solution at 28°C in the dark. After two days, seedlings were selected for uniformity and a seedling was planted in each pot. The growth medium consisted of 2.6 liter of a perlite sand mixture (2:1, v/v) covered by a 2.5-cm layer of perlite. The medium was washed throughly with a solution of 0.2 mM $CaCl_2$ and 0.2 mM KCl. Upon drying, hydroxyapatite was added to the pots at 70, 140, 210, 280, or 350 mg per pot and mixed well. Mycorrhizal inoculum was introduced from a Glomus mosseae pot culture by a funnel inoculation technique (Gerdemann, 1955), which ensures that the roots contact the inoculum. The inoculum which consisted of soil, roots, and spores was added at a rate of 20 g per pot. Plants were watered with a nutrient solution (pH 5.8) which was equivalent to 1/4-strength Johnson's solution (Johnson et al., 1957). Phosphorous was excluded from the nutrient solution. The experiment was started in mid-March, flower initiation occurred in the middle of April. The plants were

harvested in early May, at the stage of pod set. The greenhouse temperature varied diurnally, with a mean day temperature of 30°C, and a mean night temperaure of 20°C. Most of the days of the experimental period were sunny. Daylength was extended to 16 hours by sodium lamps mounted overhead in parabolic reflectors and arranged to provide uniform supplementary light. Nutrient solution was provided 5 days a week, and the medium was flushed with deionized water on the remaining two days. Each treatment was replicated four times. Mycorrhizal infection was determined as described below. Data were analyzed for homogeniety of variance, and one way analysis of variance was carried out. If the F test was significant at $P< 0.01$ or $P< 0.05$, pairwise comparisons among treatment means were made.

Rating of Mycorrhizal Infection.

Approximately 1 to 2 g (fresh weight) of the feeder roots were placed in reusable Tissue-Tek capsules (Fisher Scientific Co., Pittsburg, Pa.). The plastic capsules enable a considerable reduction in handling of the individual root samples, once when the samples are placed in the capsule and once when they are placed in the destaining solution. These were cleared and stained by modifications of the methods of Kormanik, Bryan, and Schultz (1980) and Phillips and Hayman (1970). The capsules were placed in 10% KOH and heated at 90°C for 30 min. The KOH solution clears the host cytoplasm and nuclei

facilitating stain penetration. The KOH solution was then poured off, and the capsules were rinsed in a beaker using 3 complete changes of tap water or until no brown color appeared in the rinse water. Roots were then bleached in freshly prepared alkaline $H_2O_2$ (3 ml $NH_4OH$, 30 ml 10% $H_2O_2$, and 567 ml tap water) at room temperature for 10 min and rinsed 3 times in tap water to remove the $H_2O_2$ and acidified with 1% HCl for 3 to 4 min. The acid was then poured off, and the roots were stained for 20 min at 90$^{o}$C with 0.05% Trypan blue in lactic acid solution (875 ml lactic acid, 63 ml glycerin, and 62 ml tap water). Excess stain was removed by soaking the segments in pure lactoglycerin overnight. One hundred root pieces were rated for mycorrhizal infection. Each infected root piece was given a "+" rating. The number of ratings per 100 counts was determined to be the % infection.

## Results and Discussion

Roots of $NH_4NO_3$-or urea-treated plants exhibited a high degree of mycorrhizal infection (Fig. 2.1), at the lowest level of P supplied. Roots of urea-treated plants maintained this high level of infection throughout the range of P supplied. Mycorrhizal infection of the roots of $NH_4NO_3$-treated plants exhibited a sharp decline, when P supply was increased beyond 75mg/pot, and infection was virtually

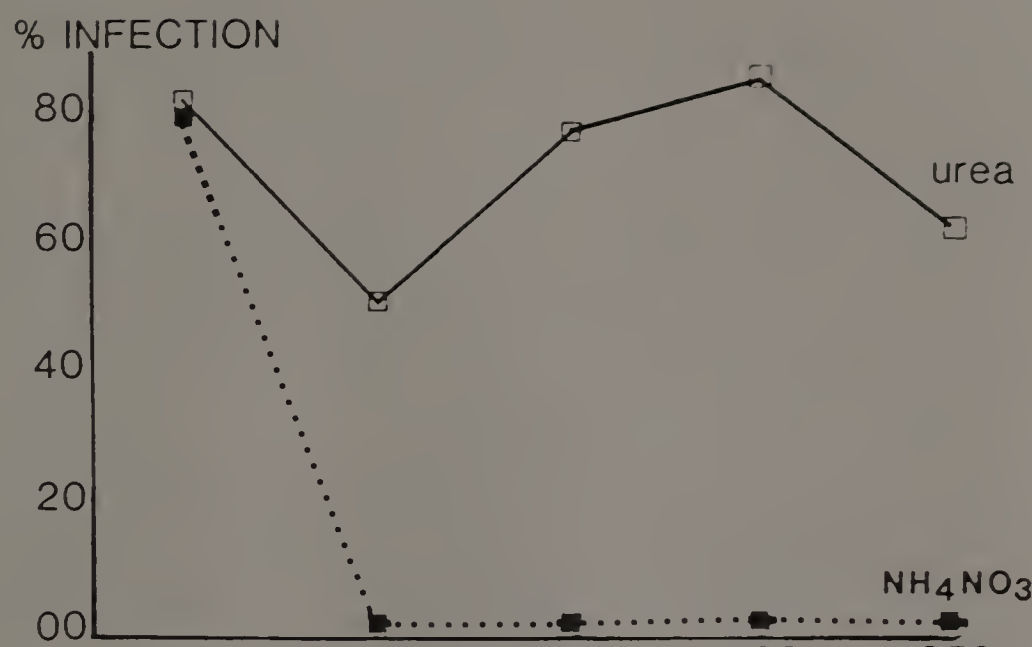


Figure 2.1 Percentage of mycorrhizal infection as affected by a N source and P regime. $Lsd_{(.01)} = 6.$

absent in roots of $NH_4NO_3$ treated plants, at the higher levels of P supply. Lanowska, (1966) reported depressed mycorrhizal infection in Phaseolus vulgaris treated with $NH_4NO_3$. Alternately, application of $NH_4^+$ salts produced improved response to mycorrhizal infection in sweetgum seedlings, than application of a non-reduced-N source. $NH_4NO_3$ was the better source of $NH_4^+$ than $NH_4SO_4$ since treatment with the latter lead to enhanced soil acidity (Brown, Schultz and Kormanick, 1981). Where improvements of growth of mycorrhizal plants were observed with ammonium fertilizers previously, the P supply may not have exceeded the limits that are inhibitory to infection. As P supply was increased, the concomitant increase in root P accumulation could have prevented the establishment of VAM infection in $NH_4^+$ treated plants in this experiment. An abundance of heavily staining large globular vesicles characterized the root infection in urea-treated plants (Fig. 3.39). Determination of arbuscular presence was difficult in urea-treated plants, since arbuscules stain very lightly and are easily obscured by overlapping vesicles. Frequent degeneration of arbuscules make their detection difficult in macerated root sections. Arbuscules are thought to be the exchange sites, and vesicles the organs of storage of VA mycorrhizae (Harley and Smith, 1983). An abundance of vesicles are speculated (though not proved) to express a parasitic tendency by the symbiont (Harley and Smith, 1983). Bevege, (1971) observed a high percentage of infection in urea treated plants at intermediate levels of P, the infection diminishing at the higher levels of P he used. The higher level of infection in urea

treated plants in this experiment may be attributed to the low root P percentage of these plants (Chapter III) that are concomitantly associated with high root N/P ratios (Chapter III).

On the basis of these observations, the experiment designed in Chapter III was performed to determine the effectiveness of N-sources in conferring VAM growth benefits.

# C H A P T E R III

## GROWTH AND NUTRITION OF MYCORRHIZAL SOYBEANS ARE INFLUENCED BY N SOURCE AND N AND P INTERACTION

### Materials and Methods

A greenhouse experiment was designed to investigate the interactive effects of N source and P regime on growth of mycorrhizal or nonmycorrhizal and nodulating or nonnodulating soybean (Glycine max Merr. cv. Clay) isolines. The isolines are genetically alike in characters other than their ability or inability to nodulate. Treatments involved 2 sources of P and 3 regimes of N; 100% $NO_3^-$, 25% $NH_4^+$ and 75% $NO_3^-$ or 50% urea and 50% $NO_3^-$.

The plants were grown in 1.8 litre plastic pots filled with a 1:2 mixture of fine and coarse quartz sand. The sand was washed several times with a solution of 0.2mM $CaCl_2$ and 0.2mM KCl. Upon drying, 75 mg of hydroxyapatite or tricalcium phosphate were added to the pots and mixed well. Mycorrhizal inoculum was introduced in a manner, similar to that described in Chapter II, except that in this experiment the inoculum was spread in a layer two third of the way up from the bottom of the pot. The funnel inoculation technique (Gerdemann, 1955) was not used in this experiment as it makes root

recovery laborious due to adhering filter paper. The controls were supplied with an equal amount of soil from pots in which sudan grass was grown in sterilized soil without mycorrhizal inoculum. At the start, these pots of sudan grass had received a microbial suspension prepared by mixing pot culture material (500 ml) and sterile water (1:2 v/v), incubating this suspension for 12 hours at room temperature and then using a Buchner funnel passing it through a millipore filter paper (5um).

Soybean seeds were surface sterilized for 5 minutes using 75% ethanol and then washed for 15 minutes in deionized water. The seeds were inoculated with a peat slurry of commercial Rhizobium japonicum inoculant at planting (Nitragin Co., Milwauke, WI). Six seeds were planted in each container, irrigated with water until 2 healthy cotyledons and a growing point was present and then were thinned to 1 plant per pot. Nutrient treatments were commenced approximately 2 weeks after seedling emergence. The composition of the nutrient solutions were as in Table 3.1. Concentration of N received by nodulating plants was approximately 1/3 of that received by the nonnodulating plants.

The experiment was started in early Spring. Temperature varied diurnally, with a mean day temperature of 30$^{o}$C and a mean night temperature of 15$^{o}$C. About 1/4 of the days during the experimental period was overcast or partly overcast. Daylength was extended to 16 hours by sodium lamps mounted overhead in parabolic reflectors and arranged to provide uniform supplementary light. Nutrient solution

Table 3.1
Composition of the nutrient solutions.

| | K | Ca | Mg | $NH_4$ | $SO_4$ | $NO_3$ | Cl | Urea |
|---|---|---|---|---|---|---|---|---|
| | | | | | mM/L | | | |
| Nonnodulating | | | | | | | | |
| N1* | 4 | 3 | 1 | | 1 | 10 | | |
| N2 | 4 | 4 | 1 | 5 | 1 | 5 | 14 | |
| N3 | 4 | 2.5 | 1 | | 1 | 5 | | 5 |
| Nodulating | | | | | | | | |
| N1 | 5 | 2.5 | 1 | | 1 | 3 | 7 | |
| N2 | 5 | 2.5 | 1 | 1.5 | 1 | 1.5 | | |
| N3 | 6 | 1 | 1 | | 1 | 1.5 | | 1.5 |

*N1,N2 and N3 stand for 100% $NO_3$, 50%$NO_3$+50%$NH_4$ and 50%$NO_3$ and 50% urea respectively.

MICRONUTRIENT CONCENTRATIONS.

| B | Mn | Zn | Cu | Mo | Co | Fe | Ni |
|---|---|---|---|---|---|---|---|
| | | | | uM | | | |
| 50 | 10 | 2 | 1 | .5 | .322 | 100 | 2.7 |

was provided 5 days a week, and the medium was flushed with deionized water on the remaining two days. Five replications were used, and positional differences in the greenhouse were minimized by daily rotation of pots. Plants were harvested on initiation of pod set. Leachates were collected by the Pour-through method (Yeager et al., 1983) 2 days prior to harvest and were analysed for P colorimetrically with ammonium molybdate-ascorbic acid (Zandstra, 1968). The pH of the leachates was determined. Roots and shoots were separated and dried at 75$^{o}$C for 1 day in a forced-draft oven and ground in a Wiley mill to pass a 40-mesh stainless steel screen. Total N was measured using 200-mg subsamples by wet digestion with sulfuric acid and a $K_2SO_4$-$CuSO_4$ catalyst mixture followed by steam distillation (Gewelling, 1976). Phosphorous content was determined by a colorimetric assay employing molybdovanadophosphoric acid (Kittson and Mellon, 1944) after 100 mg subsamples of the ground material were dry-ashed in a muffle furnace. VAM infection was rated as described in Chapter II.

A completely randomized design was used. Data were analysed for homogeneity of variance. Treatment effects were analysed by Analysis of Variance. If the F test was significant at $P< 0.01$ or $P< 0.05$, pairwise comparisons among treatment means were undertaken. Where more than, two means had to be compared, the means were ranked and analysed by the Duncans Multiple Range Test. A linear correlation analysis was run to determine the association between growth benefits due to mycorrhizal infection and shoot N/P ratios.

## Results and Disscussion

### Nodulating Isoline

The nodulating plants treated with $KNO_3$ were stunted, spindly and showed interveinal chlorosis (Fig. 3.1). The $NH_4NO_3$-treated plants showed cupping and chlorosis of leaves accompanied by incipient necrosis (Fig. 3.2). In contrast, plants treated with urea appeared healthy and did not show symptoms of nutritional disorders (Fig. 3.3). The plants treated with $KNO_3$ or $NH_4NO_3$ also had lower shoot dry weights and accumulated less N (Fig. 3.4 and 3.5) than those treated with urea (Fig. 3.4 and 3.5 ). Root dry weights did not depend on N source (Fig. 3.6). All nodulating soybean plants were nitrogen deficient, as indicated by the N percentages (Fig. 3.7 and Fig. 3.8) that were below sufficiency levels i.e. 4.5 to 5.2% (Small and Ohlrogge, 1977). Roots of all nodulating plants had nodules that were apparently healthy (Fig. 3.9), and the nodules were most abundant on the roots of urea-treated plants (Fig. 3.10 ).

Shoot N accumulation was highest for urea-treated plants and least for $KNO_3$-treated plants (Fig. 3.5).

One or more of the following factors could have contributed to the poor growth and N accumulation associated with nitrate nutrition of nodulating plants. Nodule growth and $N_2$ fixation are inhibited by the $NO_3^-$ ion (Tanner and Anderson, 1963; Sinclair and de Wit, 1976;

Figure 3.1 Chlorotic, stunted, and pale yellowish leaves of the nodulating, mycorrhizal plants treated with $KNO_3$.

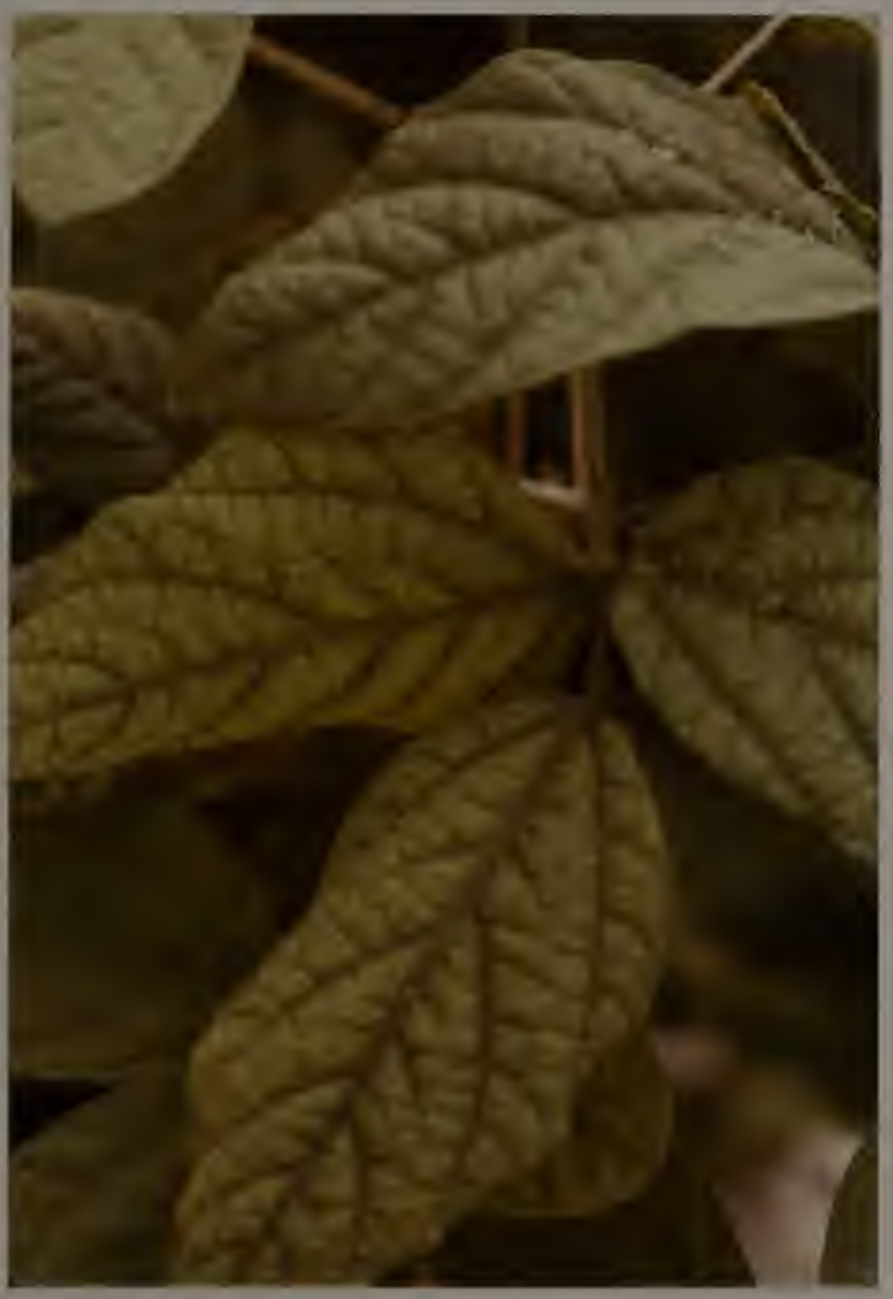

Figure 3.2 Cupping and chlorosis of the leaves of mycorrhizal nodulating soybean plants treated with $NH_4NO_3$.

Figure 3.3 Urea treated nodulating mycorrhizal soybean plants.

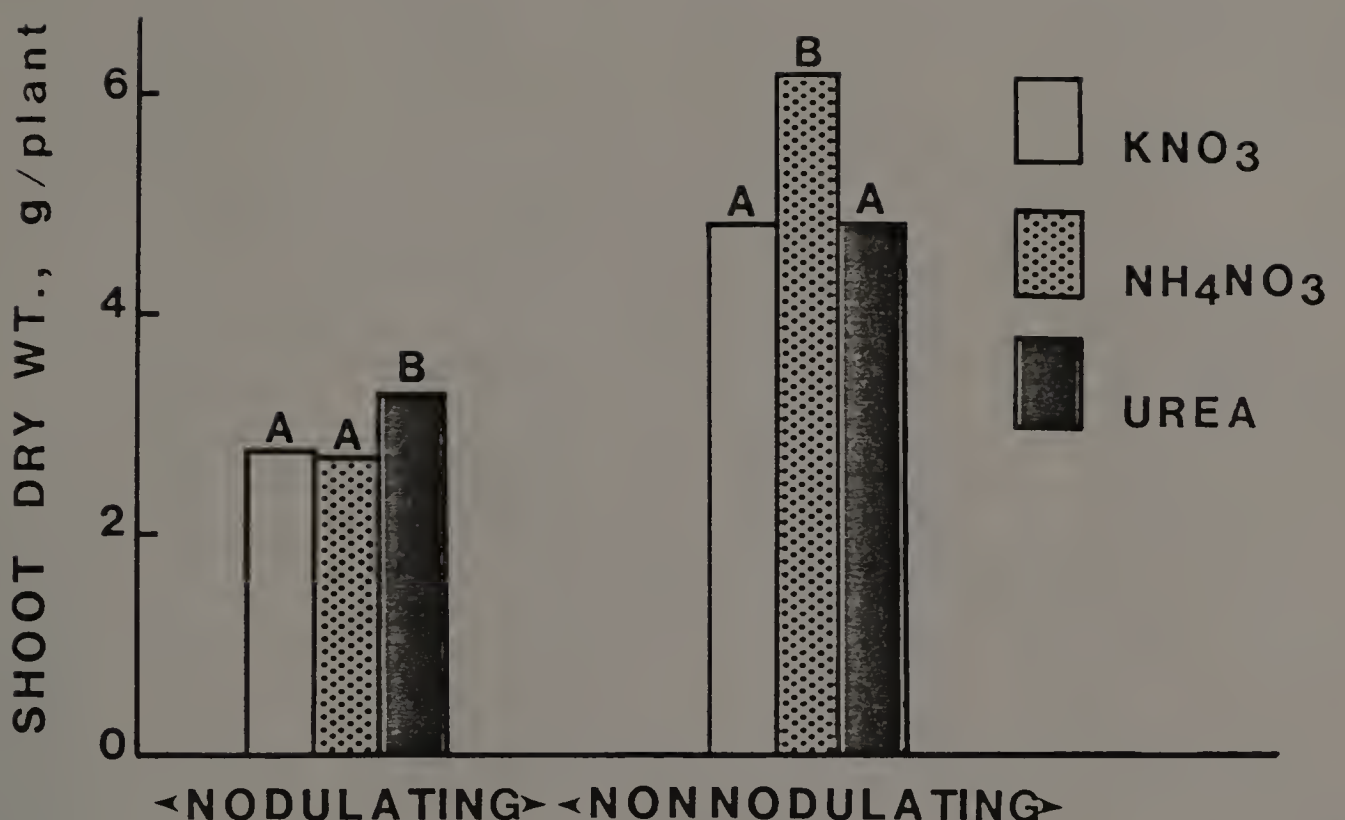


Figure 3.4 Shoot dry weight as affected by the nitrogen source within soybean isoline. Mean separation within isolines by Duncan's New Multiple Range test, 1% level. Each bar represents the mean of 20 observations.

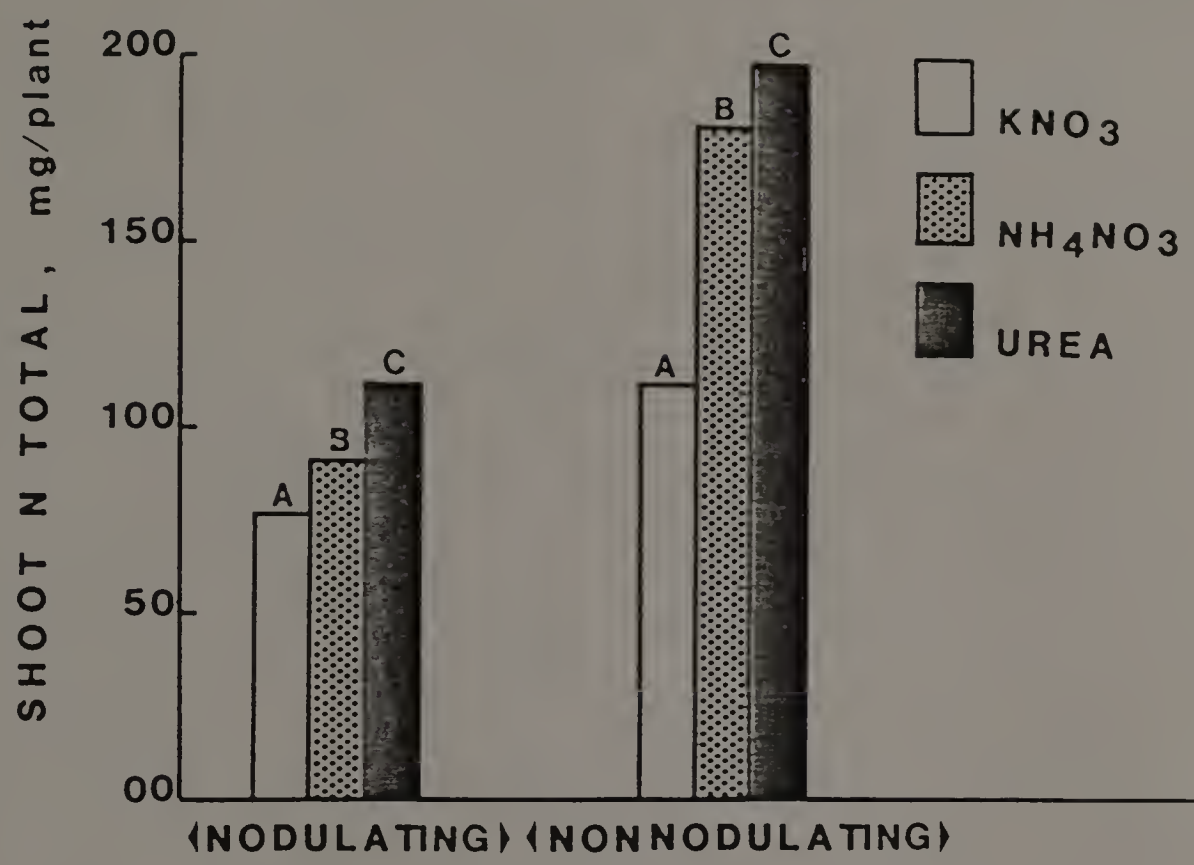


Figure 3.5 Shoot nitrogen accumulation as affected by nitrogen source and soybean isoline. Means separation by Duncan's New Multiple Range test, 1% level. Each bar represents the mean of 20 observations.

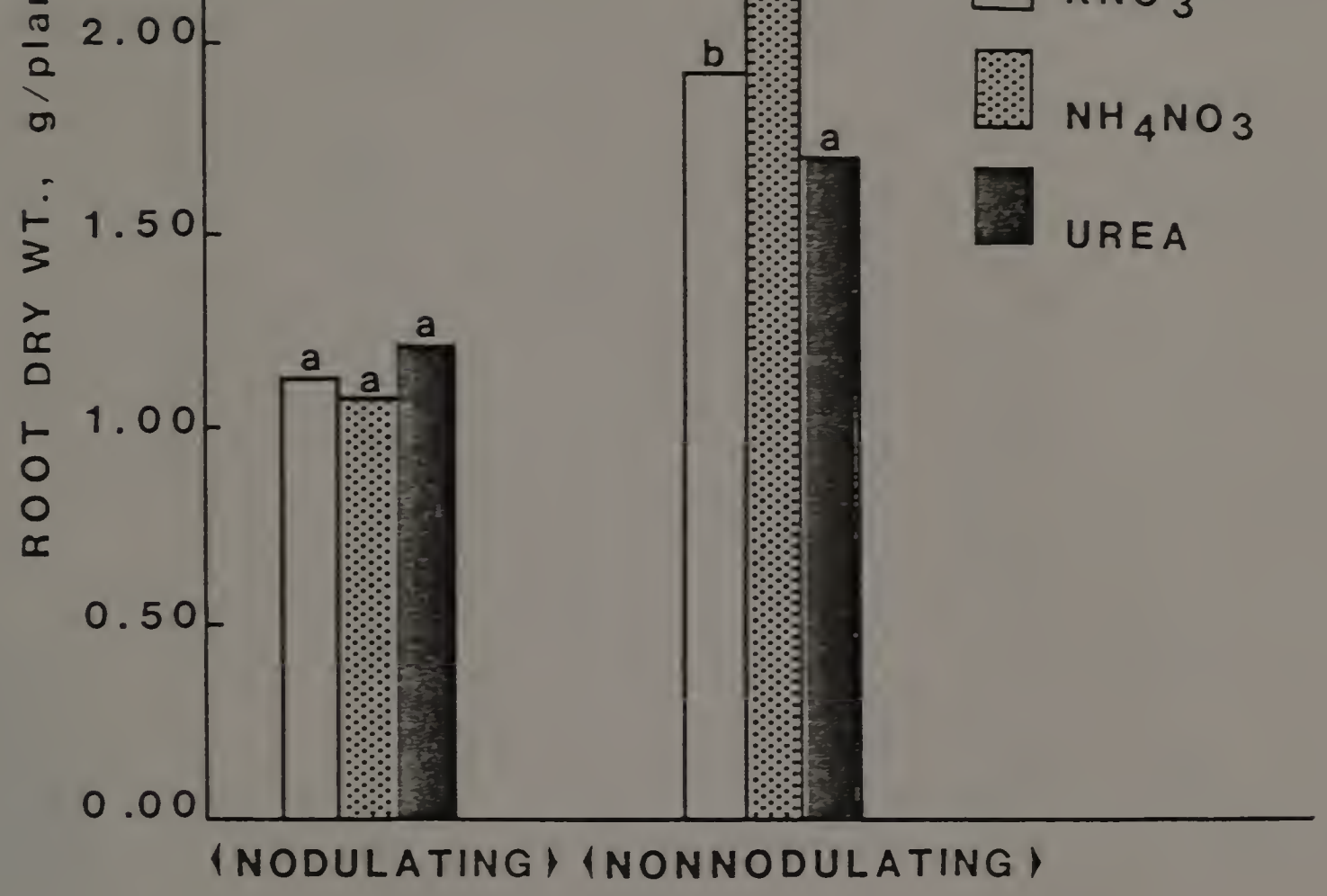


Figure 3.6 Root dry weights as affected by nitrogen source within soybean isoline. Mean separation within isoline by Duncan's New Multiple Range test, 5% level. Each bar represents the mean of 20 observations.

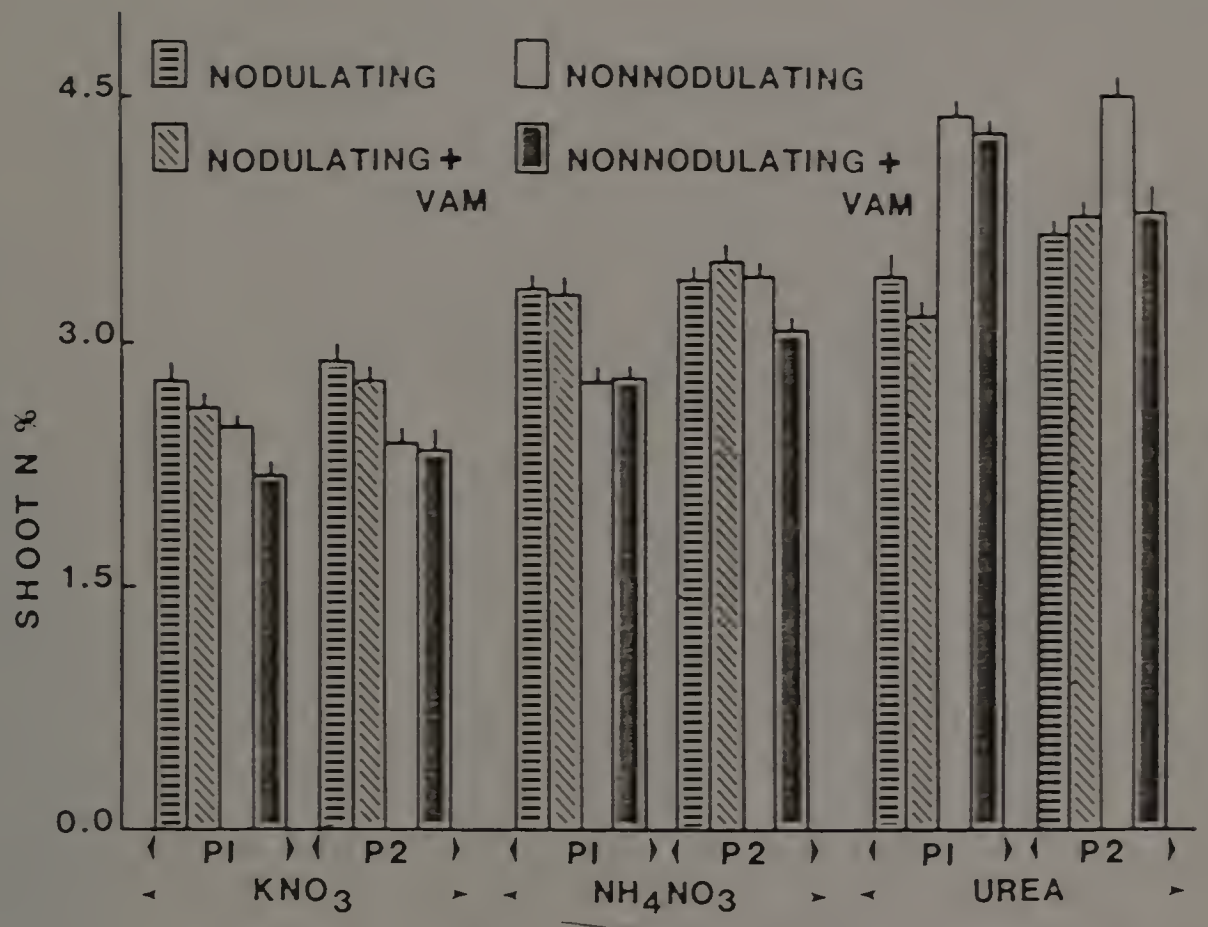


Figure 3.7 Shoot N percentage of mycorrhizal or nonmycorrhizal plants as affected by phosphorous regime and nitrogen source. One SE shown at top of each bar. Each bar represents the mean of 5 observations. P1 and P2 denote hydroxyapatite and dicalcium phosphate, respectively. VAM denotes vesicular- arbuscular mycorrhizae.

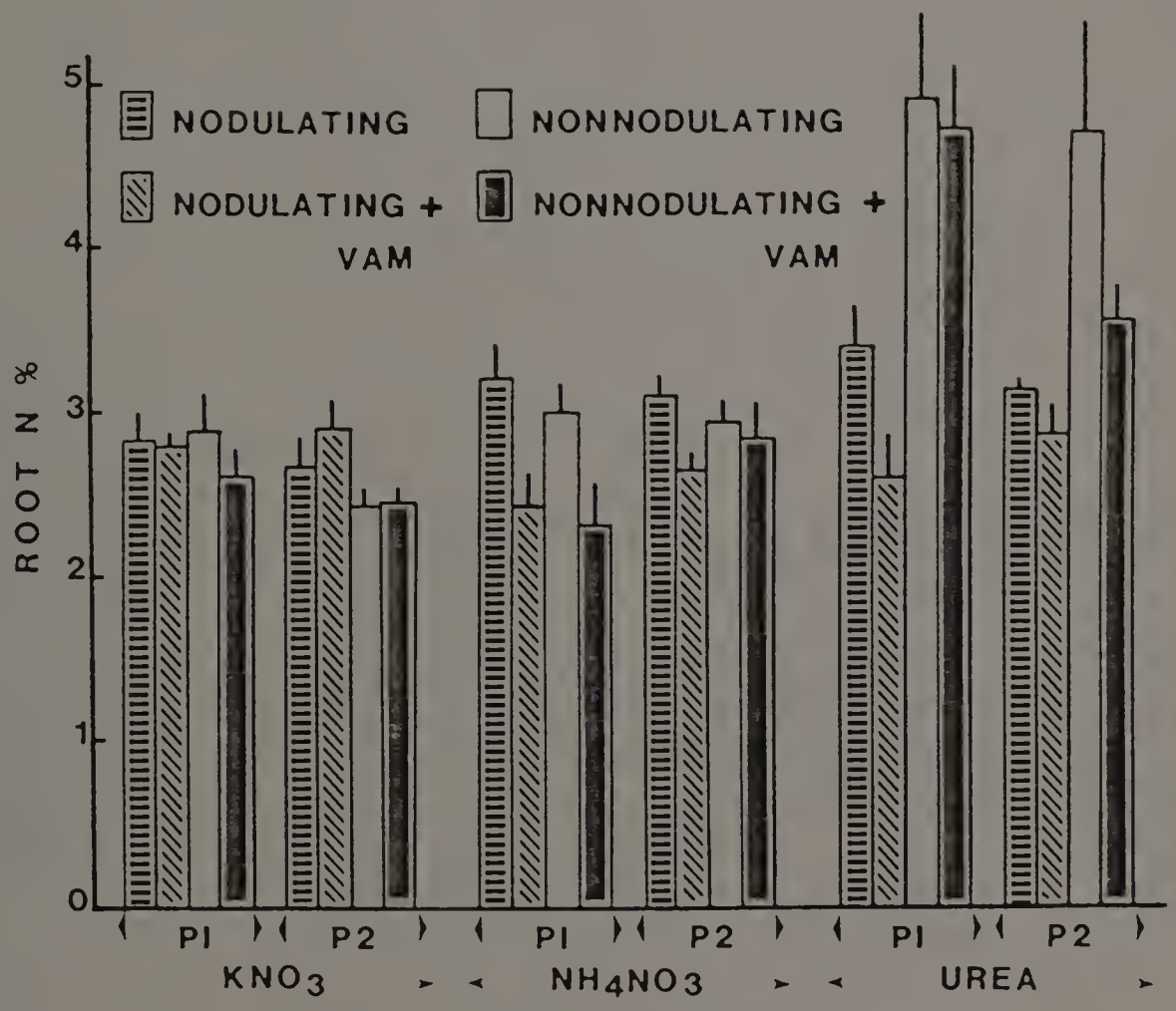


Figure 3.8 Root N% of mycorrhizal or nonmycorrhizal nodulating and nonnodulating plants as affected by phosphorous regime and nitrogen source. One SE shown at top of each bar. Each bar represents the mean of 5 obsevations. P1 and P2 denote hydroxyapatite and dicalcium phosphate, respectively. VAM denotes vesicular-arbuscular mycorrhizae.

Figure 3.9 Roots of the nodulating and nonnodulating mycorrhizal plants treated with the different N sources.

Figure 3.10 Urea-treated, nodulating plants exhibit abundant nodulation.

Latimore et al., 1977; Streeter, 1985a; 1985b). Reliance on $NO_3^-$ as the sole N source is not conducive to effective induction of nitrate reductase activity, because the induction of nitrate reductase requires a reduced N source. Nitrate reductase activity can proceed only at a suboptimal level when this condition is not fulfilled (Polacco, 1976). Alternately, nodulating soybean is a ureide-producing plant. Such plants transport the N products of root nodules as allantoin or allantoate (Tajima and Yamamoto, 1977; Thomas et al., 1980; Thomas et al., 1981; Reynolds et al., 1982)). The C:N ratio of ureides is lower than glutamine or asparagine-forms of N commonly transported by plants (McClure and Israel, 1979), which gives the plant an advantage in the carbon economy of N transport. Metabolism of ureides however depends on availability of methionine, an amino acid critical for the synthesis of urease. Feeding plants on nitrate alone results in impaired methionine synthesis. Impaired methionine synthesis would lead to an inadequate supply of urease, which is not conducive to effective nitrogen metabolism of nodulating soybean (Polacco, 1976; 1977; Tajima and Yamamoto, 1977). Furthermore, nitrate reduction has a high energy requirement. When $NO_3^-$ reduction is associated with $N_2$ fixation, demands on carbohydrate reserves are doubled (Oghoghorie and Pate, 1971; Latimore et al., 1976). Because C is being used in the $NO_3^-$ assimilatory pathway (Oghoghorie and Pate, 1971) "photosynthetic deprivation" of nodules occurs. Reportedly, reliance on $NO_3^-$ as the sole N source leads to a phenomenon called $NO_3$ induced Fe deficiency (Haynes and Goh, 1978). The high level of

organic acids accumulating under nitrate nutrition could chelate the Fe, making Fe unavailable to the plant.

The reduced shoot dry weights and N accumulation of nodulating plants treated with $NH_4NO_3$ (Fig. 3.4 and 3.5) also could be attributed to one or more factors. The decreased pH accompanying $NH_4^+$ nutrition (Fig. 3.11) would be expected to inhibit rhizobial activity (Loneragan and Dowling, 1958; Vincent, 1965). Acid inhibition of rhizobial activity is aggravated when P is limiting (Jensen, 1944). Cupping of the leaves and the incipient necrosis shown by the $NH_4NO_3$-treated plants (Fig. 3.2) resembles $NH_4^+$ toxicity (Bennett, 1974). The demand for energy and carbon skeletons of $NH_4^+$ detoxification and $N_2$ fixation could exhaust the energy inputs of photosynthesis, a situation which is further aggravated by a low level of P supply to the plants. A low level of P was supplied in this experiment because a higher level, especially when combined with $NH_4^+$ nutrition inhibits VAM infection (Chapter II). Alternately, supplies of barely adequate carbohydrate reserves or phosphorous lead to $NH_4^+$ toxicity, as a consequence of protein breakdown (Rabe and Lovatt, 1984; 1985). The nonprotein fraction of arginine rises when a plant is either carbohydrate or phosphorous stressed (Rabe and Lovatt, 1985). Accumulation of arginine is coupled with a reduction of other amino acids present in the protein fraction. These phenomena may have caused the symptoms of $NH_4^+$ toxicity and chlorosis of the nodulating plants treated with $NH_4NO_3$.

Unlike the other two nitrogen sources urea, does not inhibit $N_2$

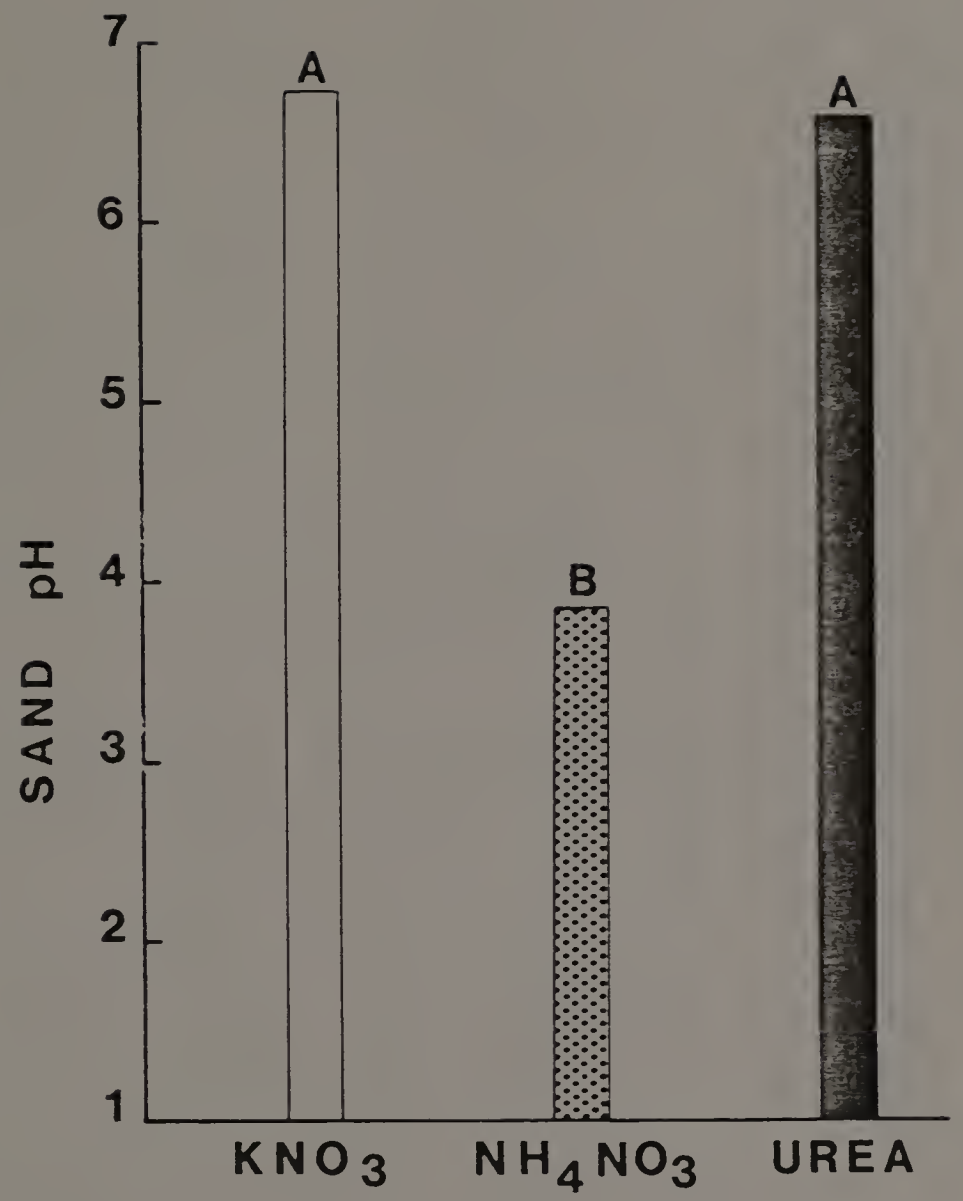


Figure 3.11 pH of the sand leachate as affected by nitrogen source. Mean separation by Duncan's New Multiple Range test, 1% level. Each bar represents the mean of 40 observations.

fixation (Vigue et al., 1977; Eaglesham et al., 1983). Indeed urea has been reported to promote nitrogen fixation in nodulating soybean (Vigue et al., 1977). The benefits of urea to nodulating plants may have a basis in the fact that it provides the plant with a reduced N source, without causing a reduction in pH as is the situation with a $NH_4NO_3$ fed plant (Fig. 3.11). Urea can be taken up as a neutral molecule and gradually degraded to $NH_4^+$. Also a plant under urea nutrition does not have to expend energy for charge balance in ion uptake, as is the case with an $NH_4^+$ or $NO_3^-$ fed plant (Raven and Smith, 1976).

Within the nodulating isoline, P accumulation was greatest for urea-treated plants, and $KNO_3$ and $NH_4NO_3$-treated plants showed similarity in P accumulation (Fig. 3.12). Nodulating plants receiving dicalcium phosphate (Fig. 3.13) had higher shoot P totals than the plants that received hydroxyapatite. Nodulating plants treated with dicalcium phosphate and urea had higher shoot dry weights than nodulating plants that were treated with dicalcium phosphate and $NH_4NO_3$ (Fig. 3.14). Treatment of nodulating plants with urea and dicalcium phosphate may provide the plant with a better P:N balance than treatment with $NH_4NO_3$ and dicalcium phosphate.

Nonnodulating Isoline

Symptoms of mineral disorders were absent in the nonnodulating plants treated with $KNO_3$ or $NH_4NO_3$ and their appearance was similar to

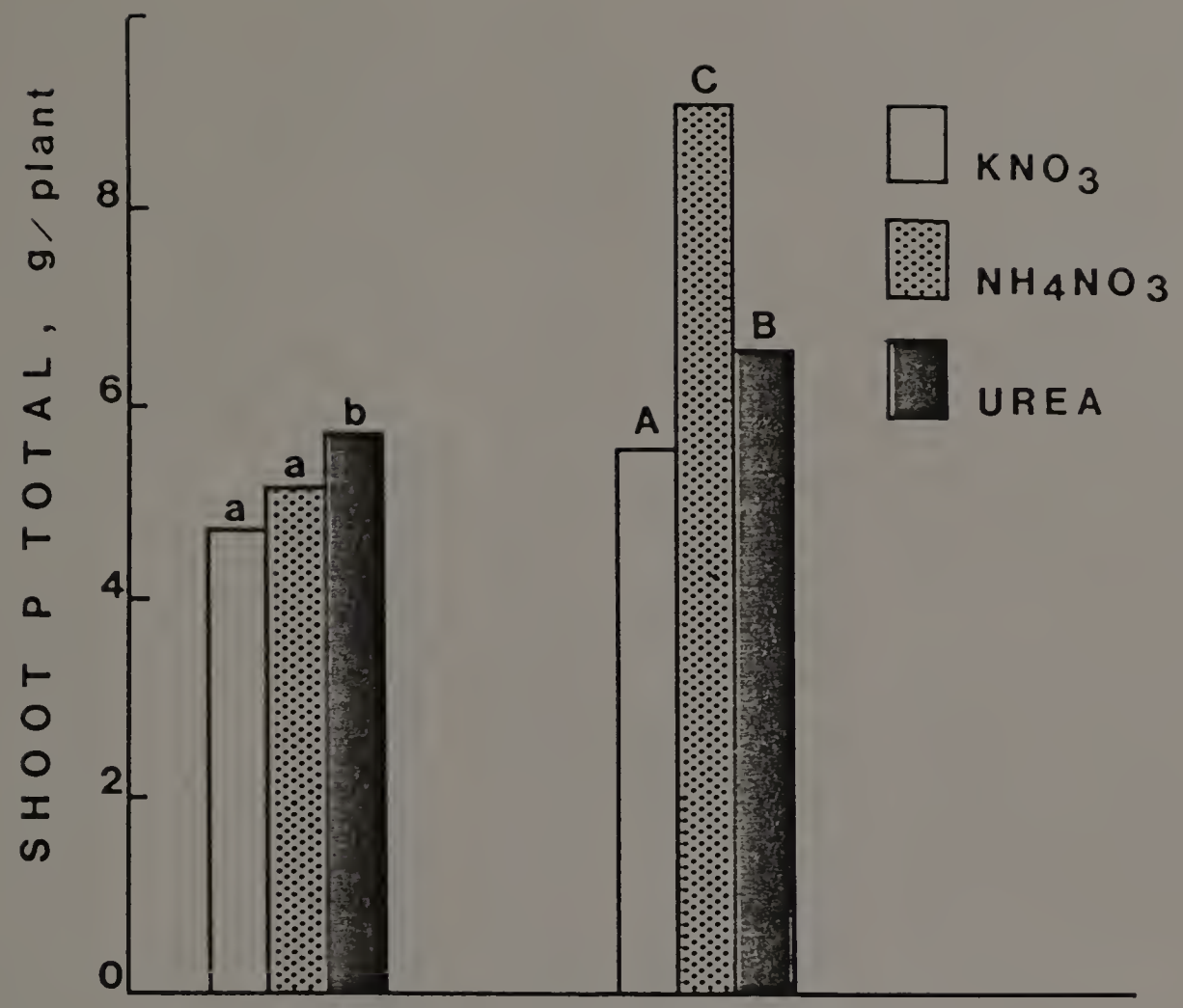


Figure 3.12 Shoot phosphorous accumulation as affected by the nitrogen source within soybean isoline. Mean separation within isoline by Duncan's New Multiple Range test, 5% (lower case letters) level or 1% (uppercase letters) level. Each bar represents the mean of 20 observations.

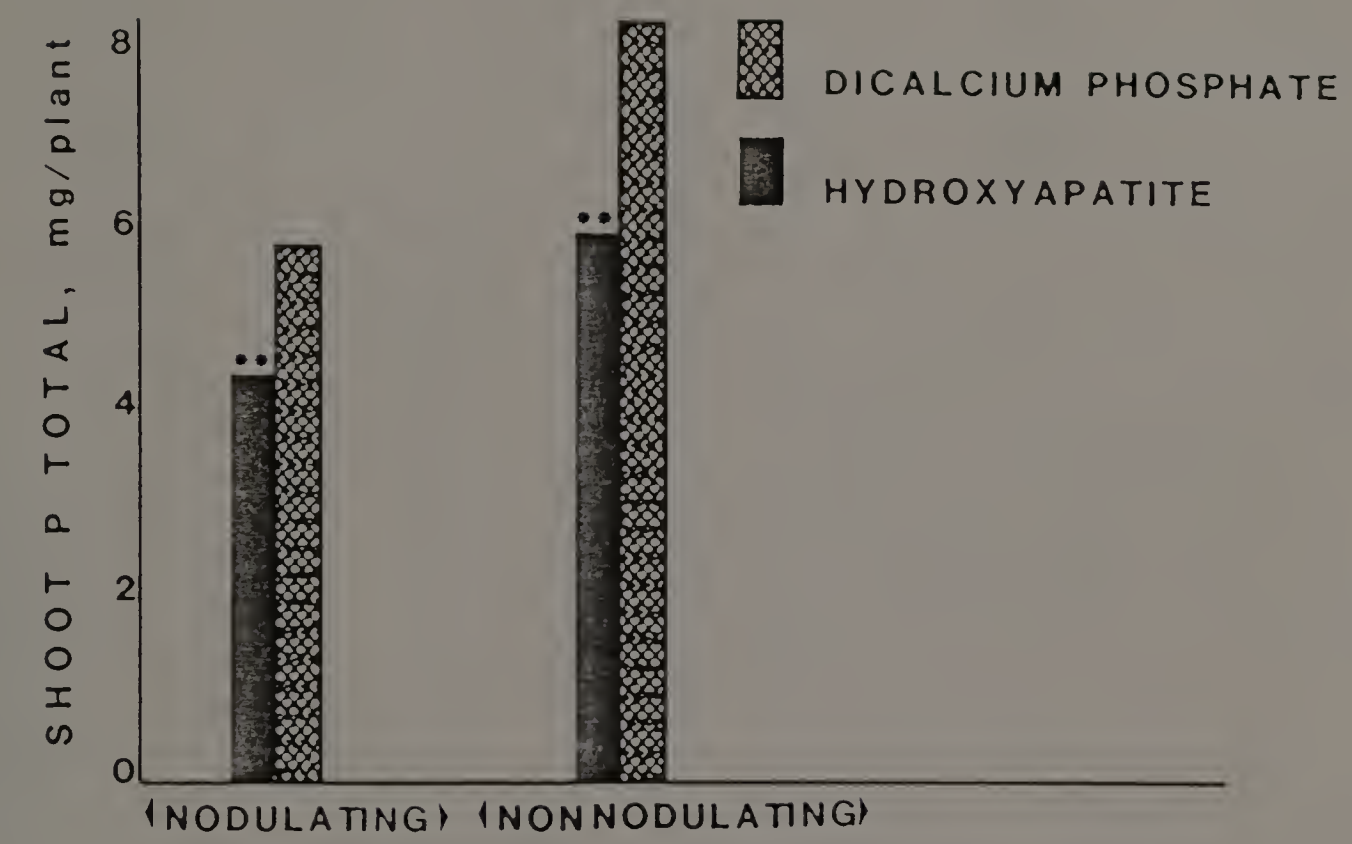


Figure 3.13 Shoot phosphorous accumulation as affected by phosphorous source within isoline. **Significant at 1%(**) level. Each bar represents the mean of 30 observations.

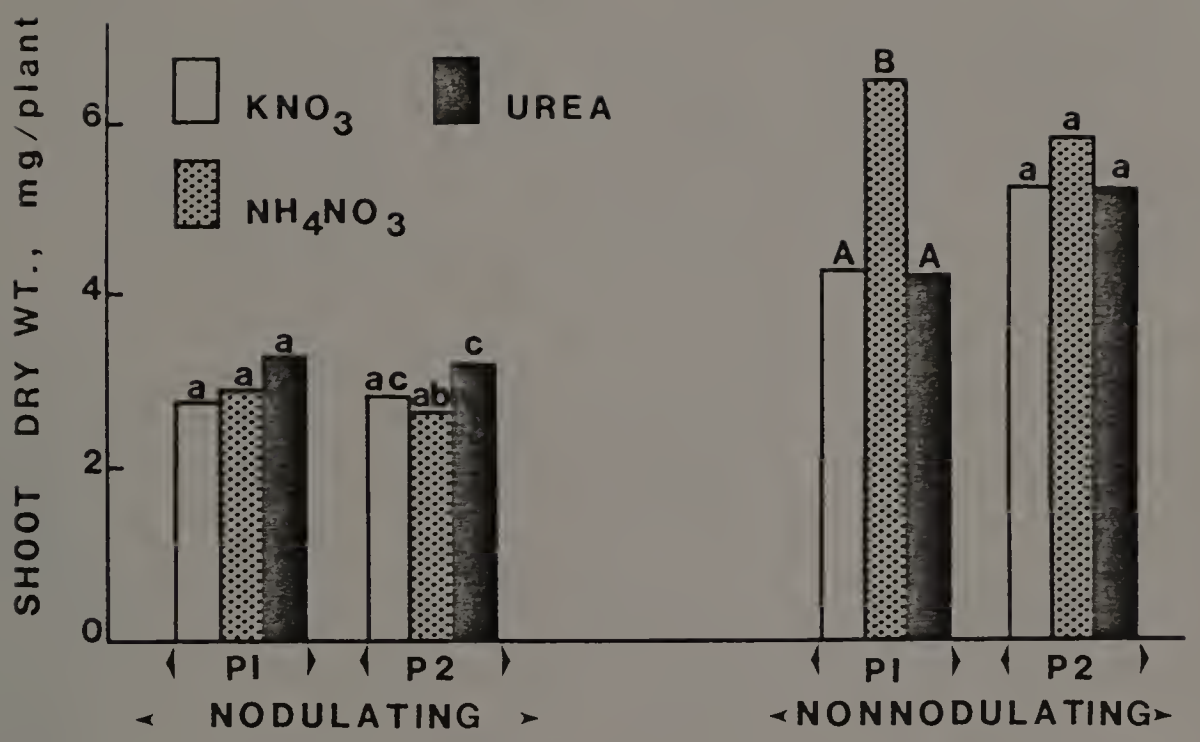


Figure 3.14 The shoot dry weights as affected by nitrogen source, within phosphorous regime, and soybean isoline. Mean separation within phosphorous regime, and isoline, by Duncan's New Multiple Range test, 5% (lower case letters) level or 1% (uppercase letters) level. Each bar represents the mean of 10 observations. P1 and P2 denote hydroxyapatite and dicalcium phosphate, respectively..

Figure 3.15 $KNO_3$-treated nonnodulating mycorrhizal plants.

Figure 3.16 $NH_4NO_3$-treated nonnodulating mycorrhizal plants.

Figure 3.17 Urea-treated, nonnodulating mycorrhizal plants.

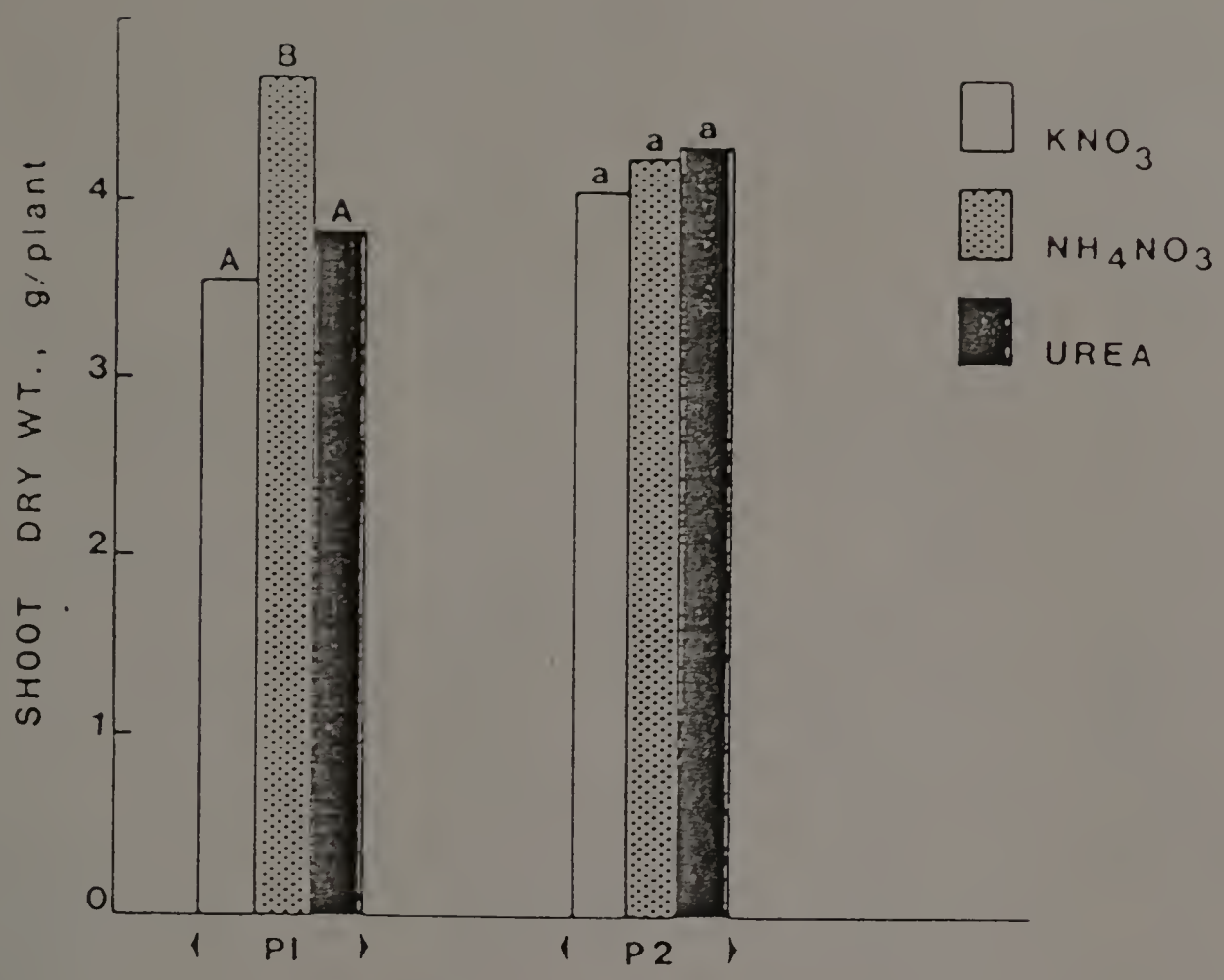


Figure 3.18 Shoot dry weights as affected by nitrogen source within phosphorous source. Mean separation within phosphorous source by Duncan's New Multiple Range test, 5% (lowercase letters) level or 1% (uppercase letters) level. Each bar represents the mean of 20 observations. P1 and P2 denote hydroxyapatite and dicalcium phosphate, respectively. VAM denotes vesicular-arbuscular mycorrhizae.

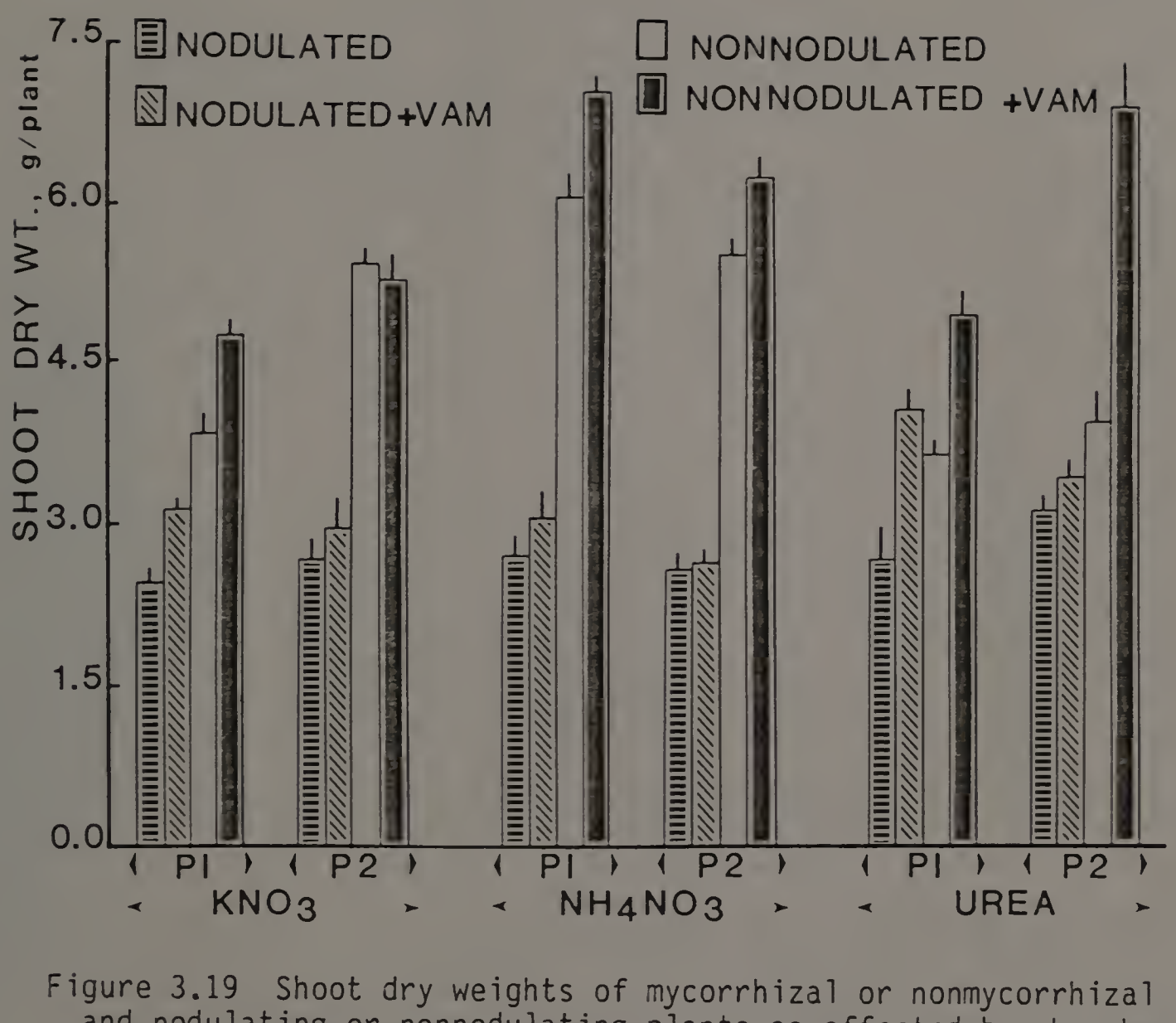


Figure 3.19 Shoot dry weights of mycorrhizal or nonmycorrhizal and nodulating or nonnodulating plants as affected by phosphorous regime and nitrogen source. One SE shown at top of each bar. Each bar represents the mean of 5 observations. P1 and P2 denote hydroxyapatite and dicalcium phosphate respectively. VAM denotes vesicular-arbuscular mycorrhizae.

the plants treated with urea (Fig. 3.15, 3.16, 3.17). Presumably this result was due to independance of the nonnodulating isoline of $N_2$ fixation and consequently a reduced demand for carbon reserves. Barely 10% of the N assimilation in nonnodulating soybean is dependent upon the metabolism of ureides (Thomas and Schrader, 1975). Because the dependence on ureide metabolism is low, $NO_3^-$ inhibition of ureide metabolism is less of a problem to the nonnodulating isoline.

Highest N totals were found in the urea-treated plants, and lowest N totals were found in the $KNO_3$-treated plants (Fig. 3.6). The nitrogen percentages of the urea treated plants fell within the N sufficiency range of 4.5- 5.2% (Small and Ohlrogge, 1977), while those of the nitrate treated plants (Fig 3.7 and Fig. 3.8) fell below the adequate range.

The $NH_4NO_3$-treated, nonnodulating plants had significantly greater shoot dry weights than those of the urea or $KNO_3$-treated, nonnodulating plants, when the source of P supply was hydroxyapatite (Fig. 3.14). Over the 3 N sources, however, dicalcium phosphate proved to be the most favorable fertilizer for the nonnodulating isoline (Fig. 3.18). The dramatic enhancement of P accumulation seen in the $NH_4NO_3$-treated, nonnodulating plants compared to the urea-treated, nonnodulating plants (Fig. 3.13 ) could be the reason why the nonnodulating isoline responded best to $NH_4^+$ nutrition. The increases in P accumulation usually associated with $NH_4^+$ nutrition (Grunes, 1959; Riley and Barber, 1971; Bieleski, 1973), are related to the low pH accompanying $NH_4^+$ nutrition. Depressions in pH favor P uptake

because abundance of the $H_2PO_4^-$ ion, the form of P more commonly taken up by plants, increases under low pH (Sentenac and Grignon, 1985) and the lowered pH would enhance the solubility of dicalcium phosphate and hydroxyapatite, especially the hydroxyapatite (Lindsay and Moreno, 1978).

Comparison of the growth of Nodulating and Nonnodulating Isolines

Growth of the nonnodulating plants was superior to the nodulating plants as reflected in the shoot dry weights, and in N, and P accumulation (Fig. 3.19, 3.20, 3.21). Limited P supply may have been a factor in the poor overall growth of the nodulating plants compared to the nonnodulating plants(Fig. 3.22 and 3.23). Nonnodulting plants may have had a lower P requirement since they did not fix $N_2$: hence, the restricted P supply apparently satisfied their demands. Superior overall growth of these plants may be evidence of this low P requirement.

Potassium nitrate was the least favorable fertilizer for nodulating or nonnodulating isolines, as reflected in the shoot dry weights, and in N, and P accumulation (Fig. 3.25, 3.26 and 3.27). Reliance on $NO_3^-$ as the sole N source leads to an imbalance in the amino acid composition of the plant (Domska, 1974). The high pH associated with $NO_3^-$ feeding also is not conducive to P uptake (Sentenac and Grignon, 1985). Urea was the most favorable fertilizer

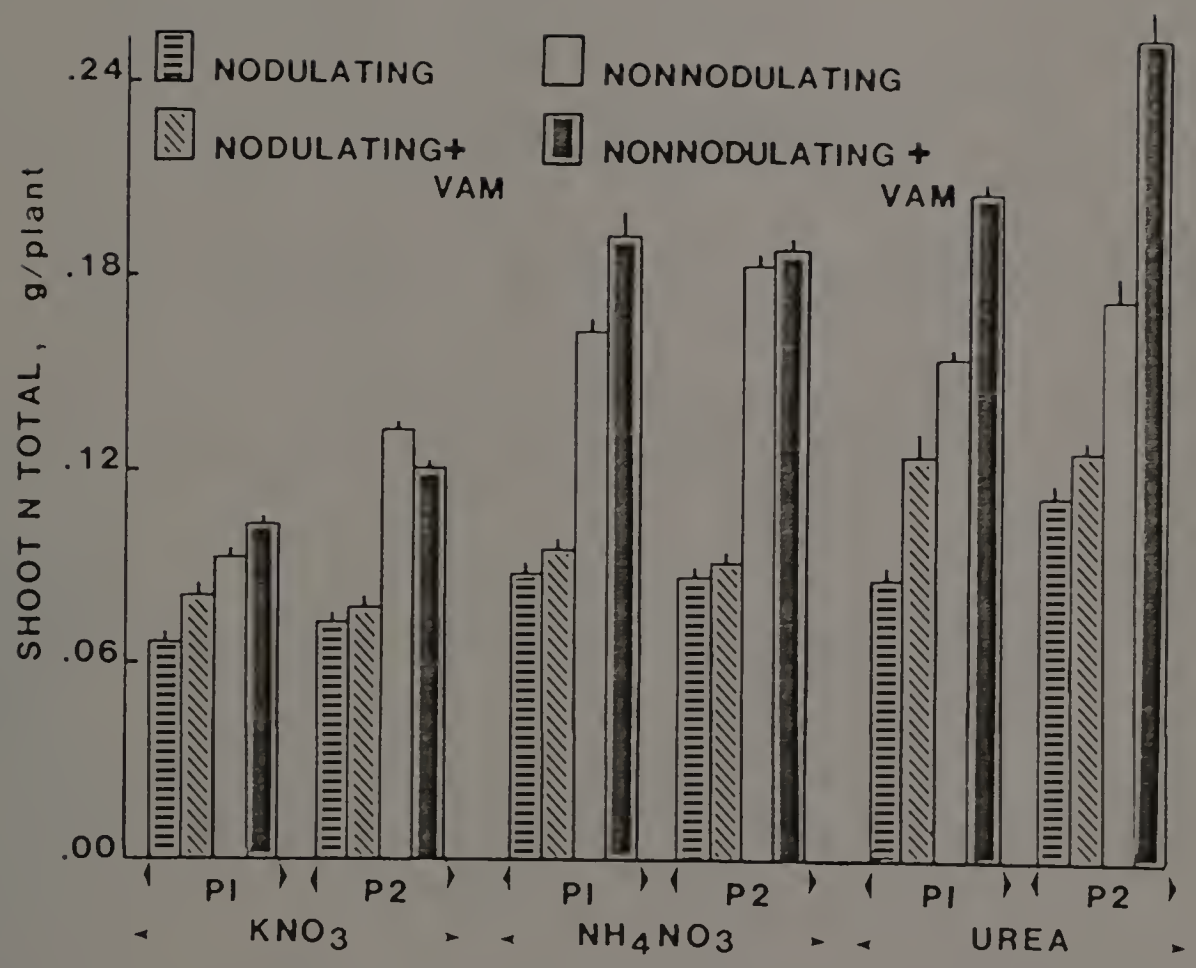


Figure 3.20 Shoot nitrogen accumulation of mycorrhizal and nonmycorrhizal, nodulating and nonnodulating soybean as affected by nitrogen source and phosphorous regime. One SE shown at top of each bar. Each bar represents the mean of 5 observations. P1 and P2 denote hydroxyapatite and dicalcium phosphate, respectively. VAM denotes vesicular-arbuscular mycorrhizae.

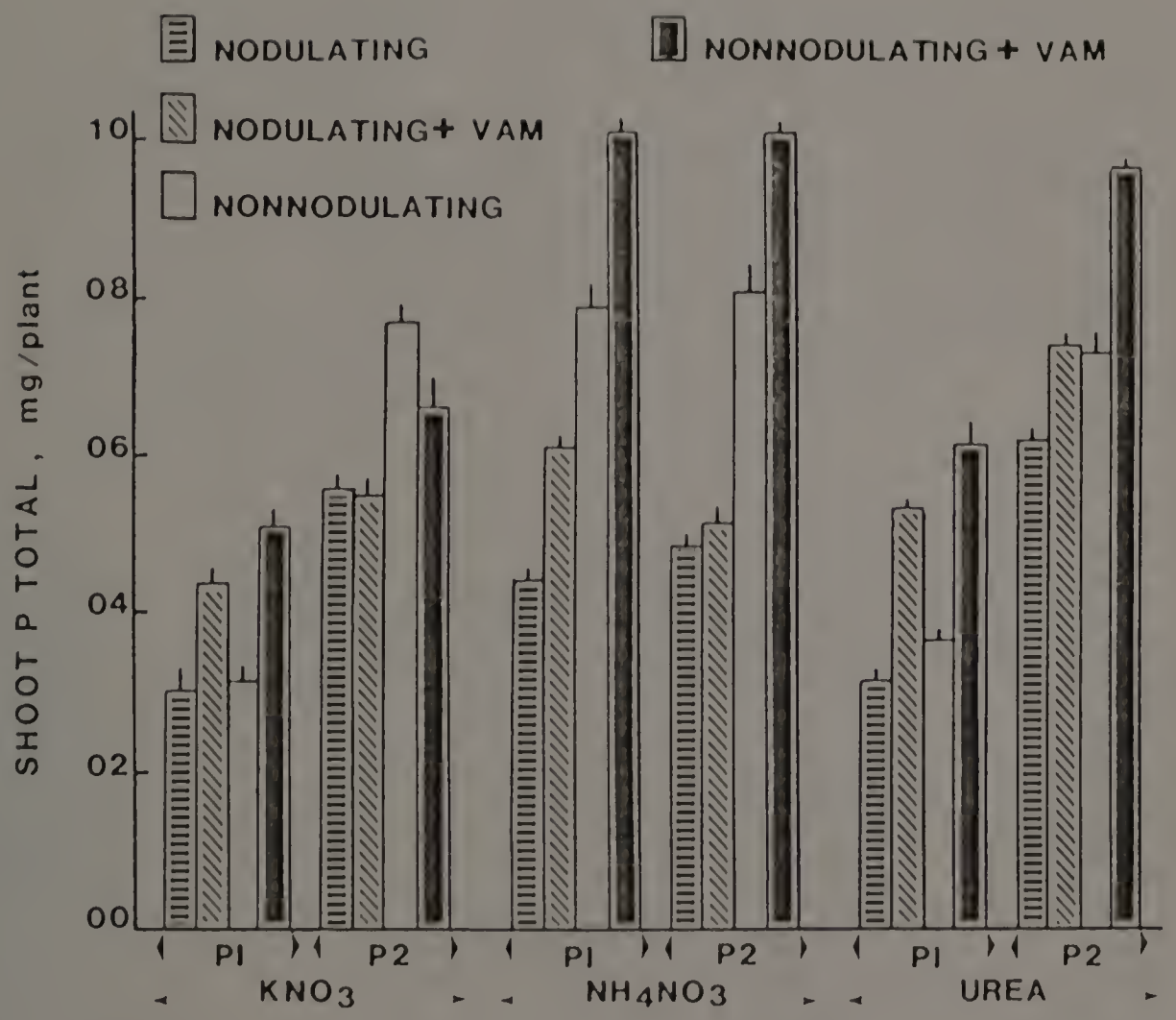


Figure 3.21 Shoot phosphorous totals as affected by phosphorous regime, nitrogen source, nodulation, and mycorrhizal infection. One SE shown at top of each bar. Each bar represents the mean of 5 observations. P1 and P2 denote hydroxyapatite and dicalcium phosphate, respectively. VAM denotes vesicular-arbuscular mycorrhizae.

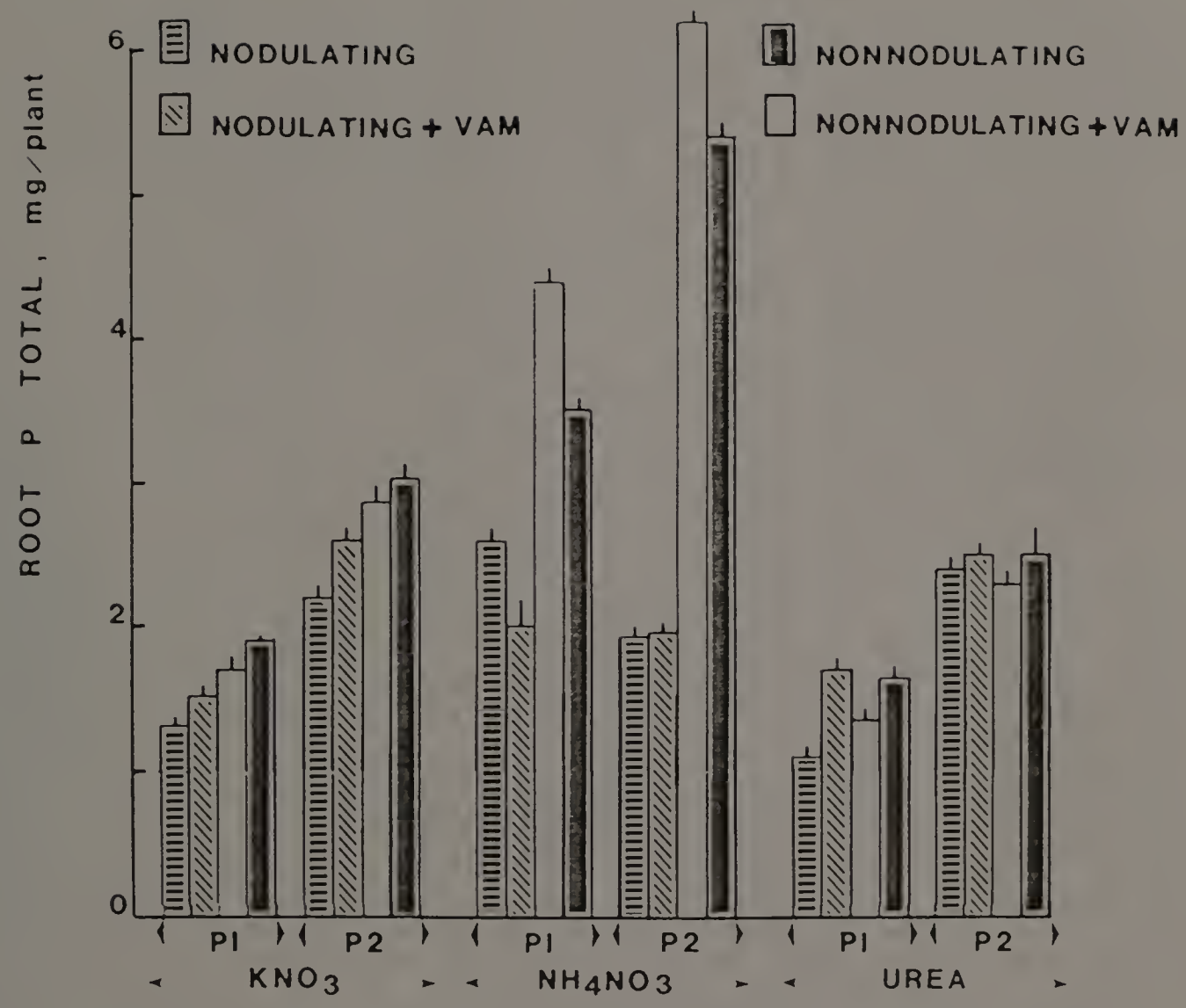


Figure 3.22 Root phosphorous totals of the nodulating and nonnodulating, mycorrhizal and nonmycorrhizal plants as affected by phosphorous source and nitrogen regime. One SE shown at top of each bar. Each bar represents the mean of 5 observations. P1 and P2 denote hydroxy-apatite, and dicalcium phosphate, respectively. VAM denotes vesicular-arbuscular mycorrhizae.

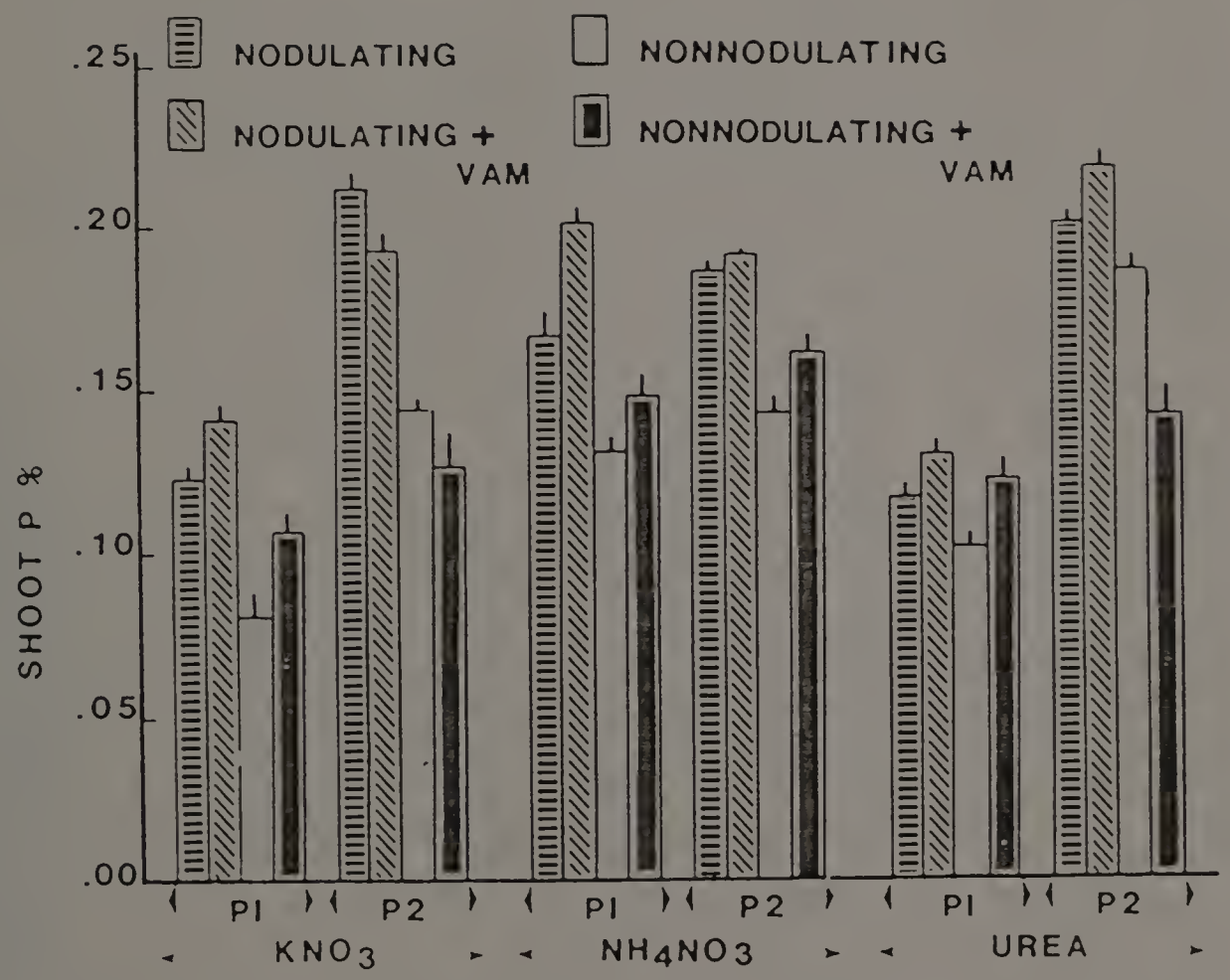


Figure 3.23 Shoot phosphorous percentage of the nodulating and nonnodulating mycorrhizal and nonmycorrhizal plants as affected by nitrogen source and phosphorous regime. One SE shown at top of each bar. Each bar represents the mean of 5 observations. P1 and P2 denote hydroxyapatite, and dicalcium phosphate, respectively. VAM denotes vesicular-arbuscular mycorrhizae.

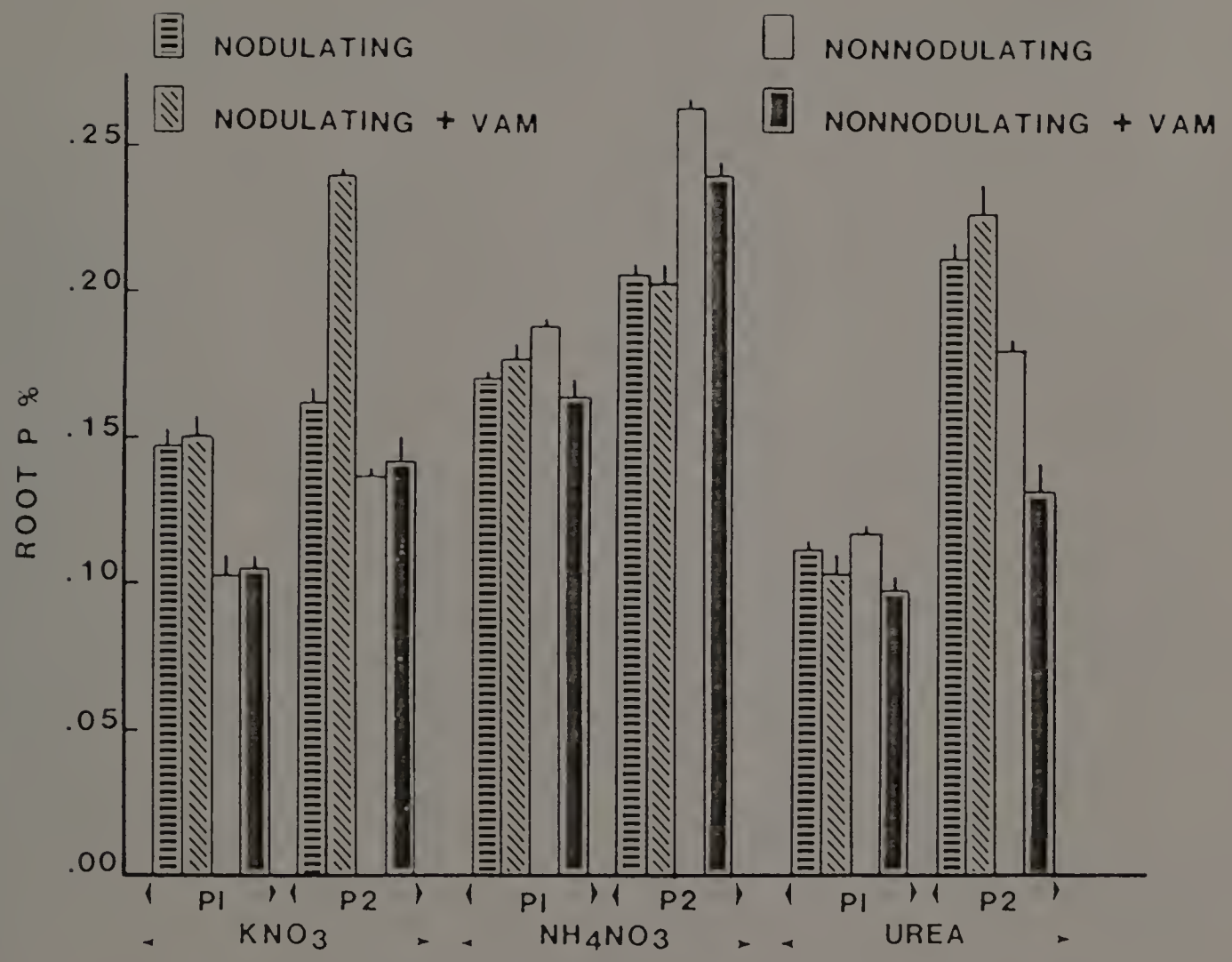


Figure 3.24 Root P% of the nodulating and nonnodulating mycorrhizal and nonmycorrhizal plants as effected by phosphorous regime and nitrogen source. One SE shown at top of each bar. Each bar represents the mean of 5 observations. P1 and P2 denote hydroxyapatite and dicalcium phosphate respectively. VAM denotes vesicular-arbuscular mycorrhizae.

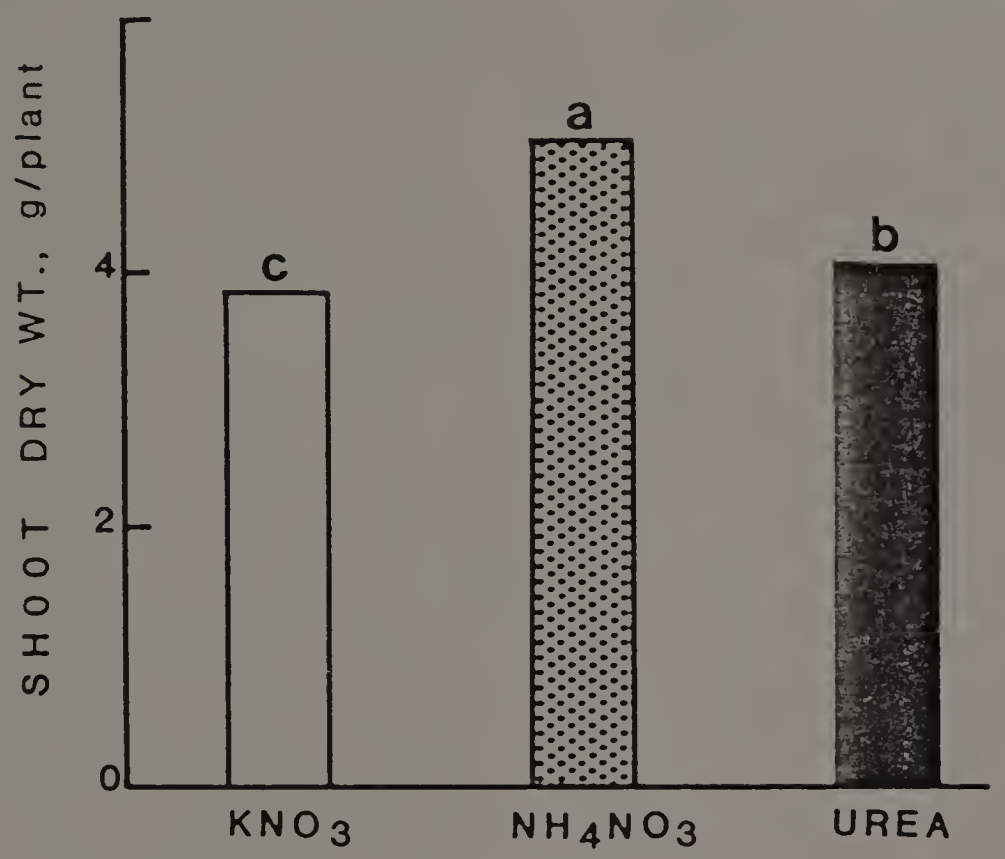


Figure 3.25 Effect of N sources on shoot dry weights. Each bar represents the mean of 40 observations. Mean separation by Duncan's New Multiple Range test, 1% level.

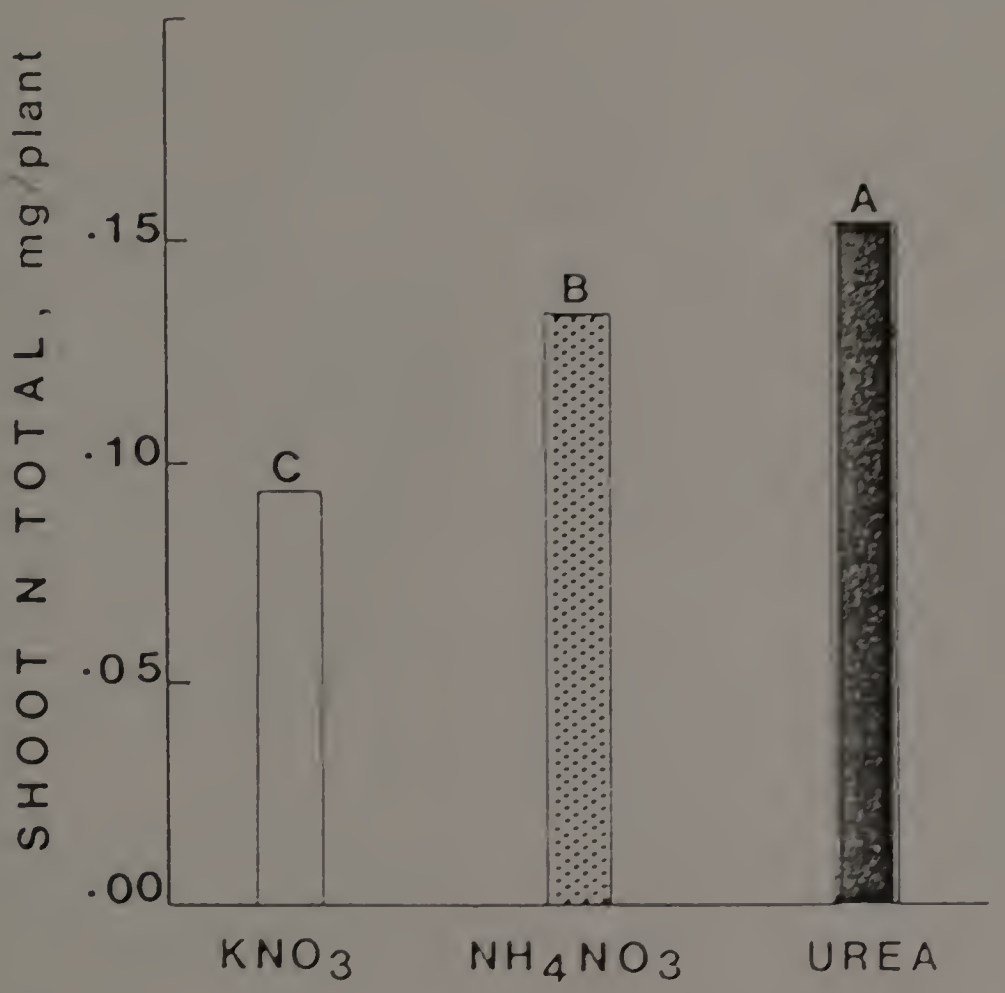


Figure 3.26 Shoot nitrogen accumulation as affected by nitrogen source. Each bar represents the mean of 40 observations. Mean separation by Duncan's New Multiple Range test, 1% level.

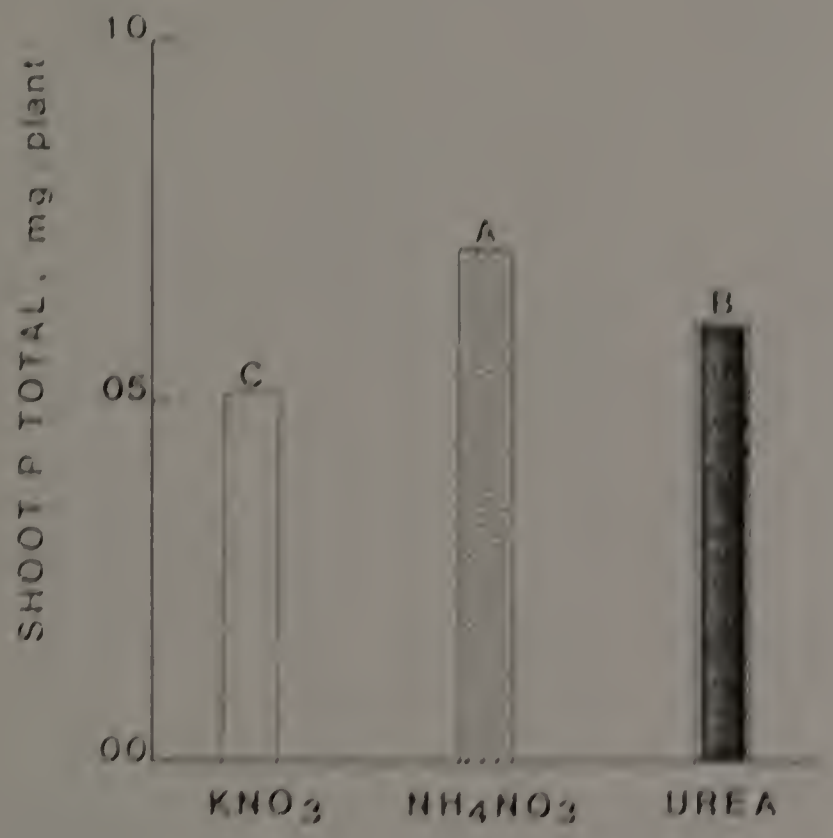


Figure 3.27 Shoot phosphorous accumulation as affected by nitrogen source. Mean separation by Duncan's New Multiple Range test, 1% level. Each bar represents the mean of 40 observations.

for the nodulating isoline. The higher shoot dry weights, and N and P accumulation being evidence of urea being the most favorable fertilizer (Fig. 3.4, 3.5 and 3.12). In contrast, $NH_4NO_3$ proved to be the most desirable fertilizer for the nonnodulating isoline (Fig. 3.4). In spite of the fact that nitrogen accumulation was maximal under urea nutrition (Fig. 3.5), growth of the nonnodulating plants on urea was restricted due to the inadequacy of P accumulation under urea nutrition (Fig. 3.12). The pH of 7 associated with urea nutrition again being unfavourable for P uptake (Centenac and Grignon, 1985).

Plants treated with $NH_4NO_3$ had the highest shoot and root P totals: urea-treated plants had an intermediate value (Fig. 3.22, 3.23, 3.42).

The absence of an enhancement in P accumulation in the $NH_4NO_3$-treated, nodulating plants compared to the $NH_4NO_3$-treated, nonnodulating plants (Fig. 3.14) may be attributed to the overriding harmful effects of low pH (Fig. 3.11) (created by $NH_4^+$ nutrition) on $N_2$ fixation. The resulting impaired $N_2$ fixation could have blocked the pH enhancement of P accumulation.

Within both isolines, the dicalcium phosphate-treated plants had higher P totals than the hydroxyapatite-treated plants (Fig. 3.13). Presumably, this observation was due to the fact that dicalcium phosphate was the more available P source (Lindsay and Moreno, 1960). The increase in P totals however was greater for the nonnodulating isoline than for the nodulating isoline, which may be explained as due to its better overall growth.

Percentage of mycorrhizal infection was lower in urea-treated nodulating plants than in the roots of urea-treated nonnodulating plants (Fig. 3.28). The decresed root N percentage (Fig. 3.8) of urea-treated nodulating plants relative to the urea treated nonnodulating plants could account for the decreased infection percentage of the urea-treated nodulating plants. Reportedly low root nitrogen levels are not conducive to mycorrhizal infection (Hepper, 1983).

Comparison of Mycorrhizal and Nonmycorrhizal Plants

Presence of VAM lead to increases in growth of nodulating or nonnodulating plants (Fig. 3.29). Percentage of VAM infection was highest for the urea-treated plants and least for the $KNO_3$-treated plants (Fig. 3.30). Degree of VAM benefits depended upon N source, P source and interactions among N source, P source, and soybean isoline (Fig. 3.31, 3.32, 3.33).

Within all nitrogen regimes, shoot dry weights of the mycorrhizal plants were higher than those of the nonmycorrhizal plants (Fig. 3.31). The increase for the $NH_4NO_3$ and urea-treated plants was significant at the 1% level while the increase for the $KNO_3$ treated plants was significant at the 5% level (Fig. 3.30).

Root dry weights of the urea-treated, mycorrhizal plants were higher than those of the nonmycorrhizal plants (Fig. 3.34). In contrast, root dry weights of the $KNO_3$-and $NH_4NO_3$-treated mycorrhizal

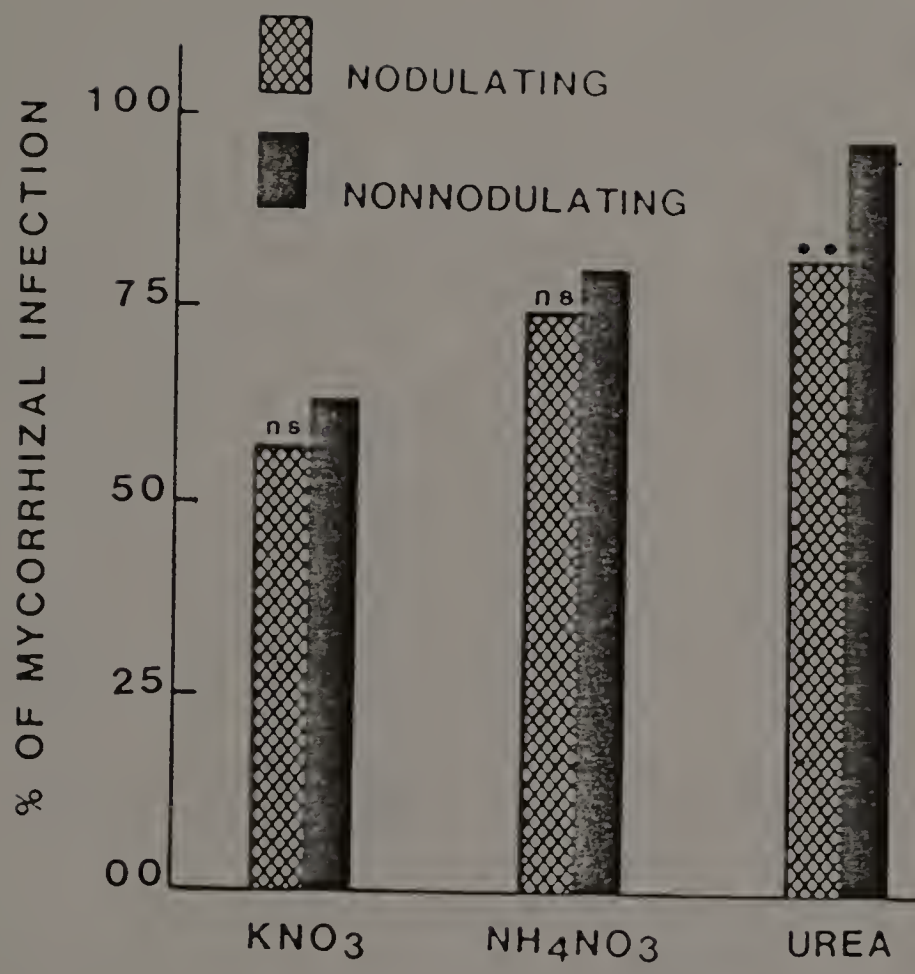


Figure 3.28 Percentage of mycorrhizal infection as affected by isoline, within nitrogen source. ns, ** nonsignificant(ns) or significant at 1%(**) level. Each bar represents the mean of 10 observations.

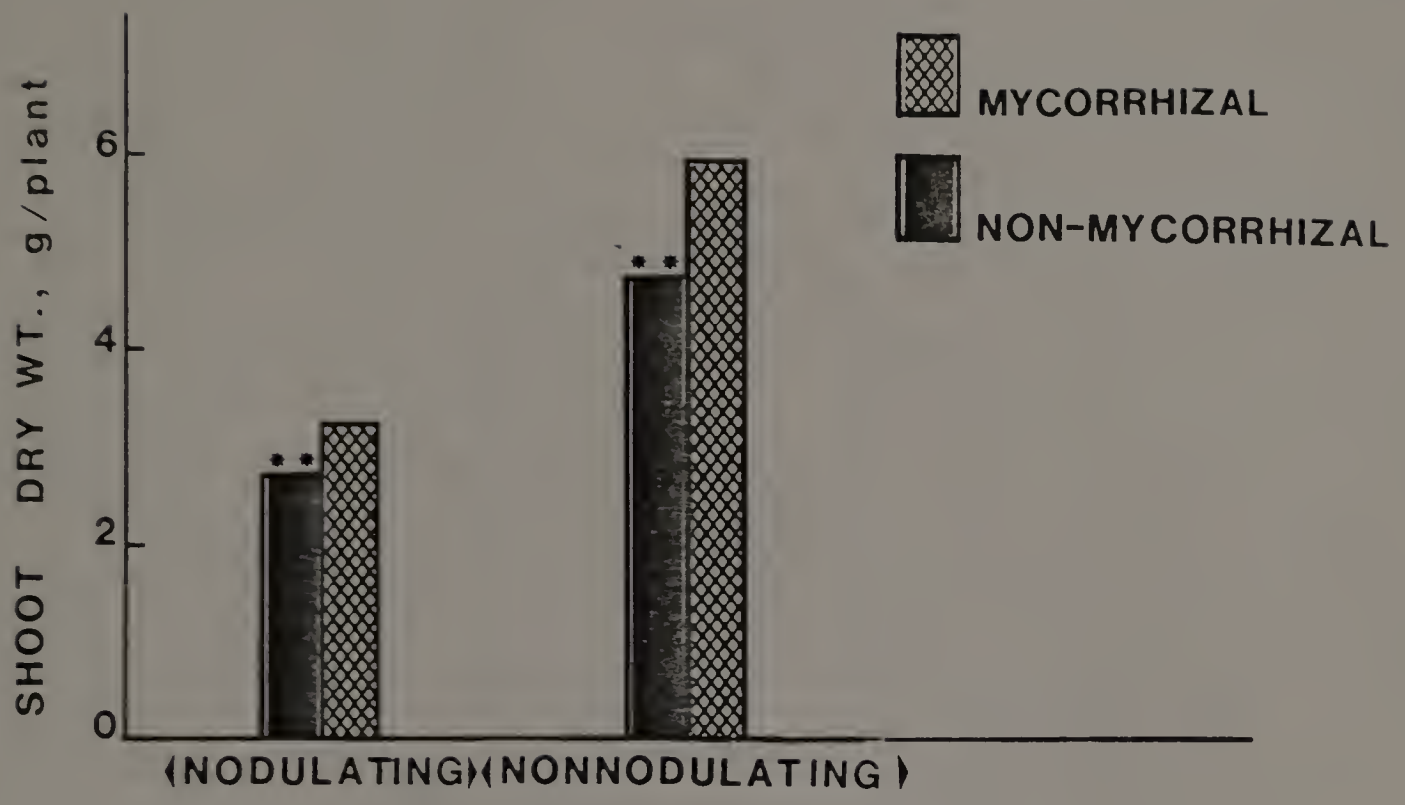


Figure 3.29 Shoot dry weight as affected by mycorrhizal infection within isoline. ** at 1% level. Each bar represents the mean of 30 observations.

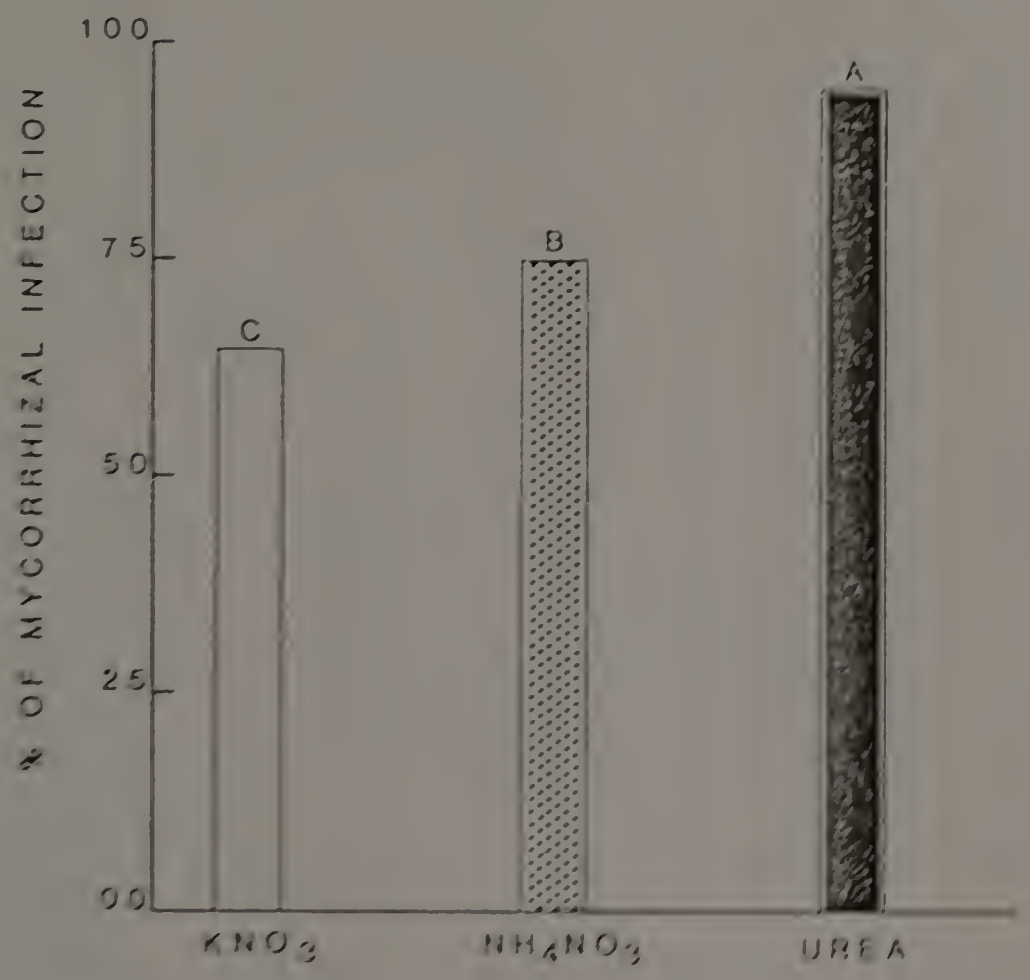


Figure 3.30 Percentage of mycorrhizal infection as affected by nitrogen source. Mean separation by Duncan's New Multiple Range test, 1% level. Each bar represents the mean of 20 observations.

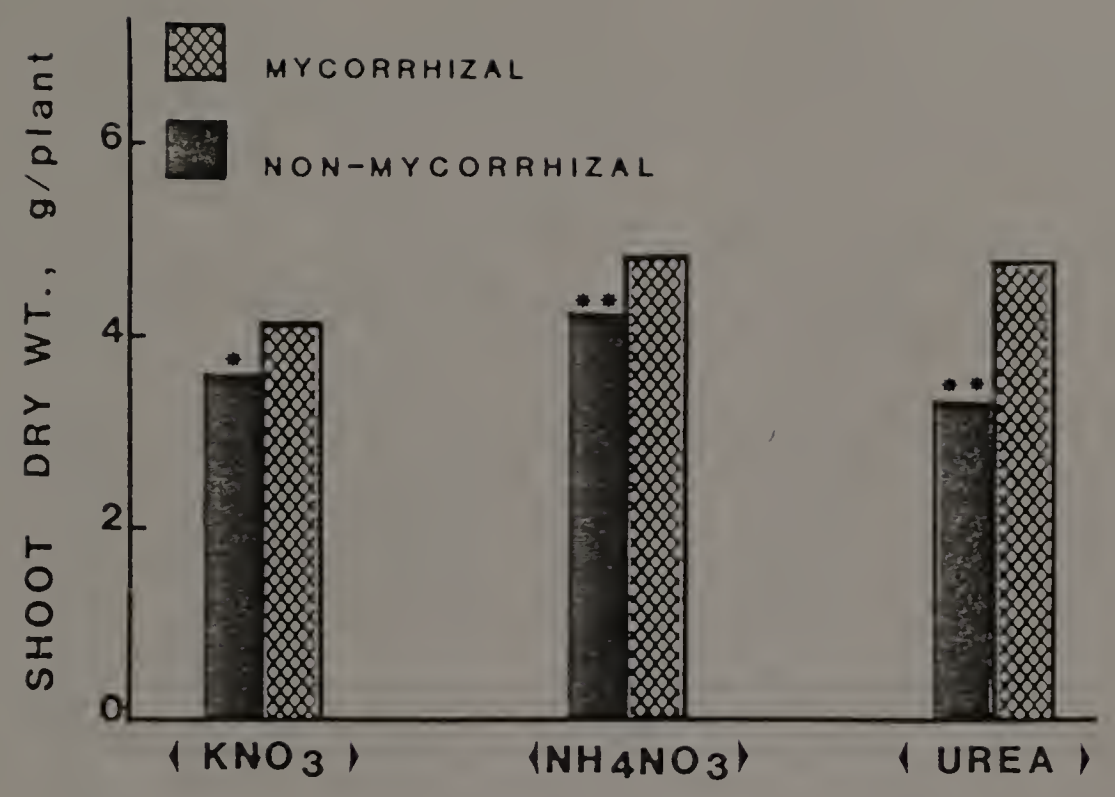


Figure 3.31 Shoot dry weight as affected by mycorrhizal infection within nitrogen source. *,**Signficant at at 5% (*) level, or 1% (**) level. Each bar represents the mean of 20 observations.

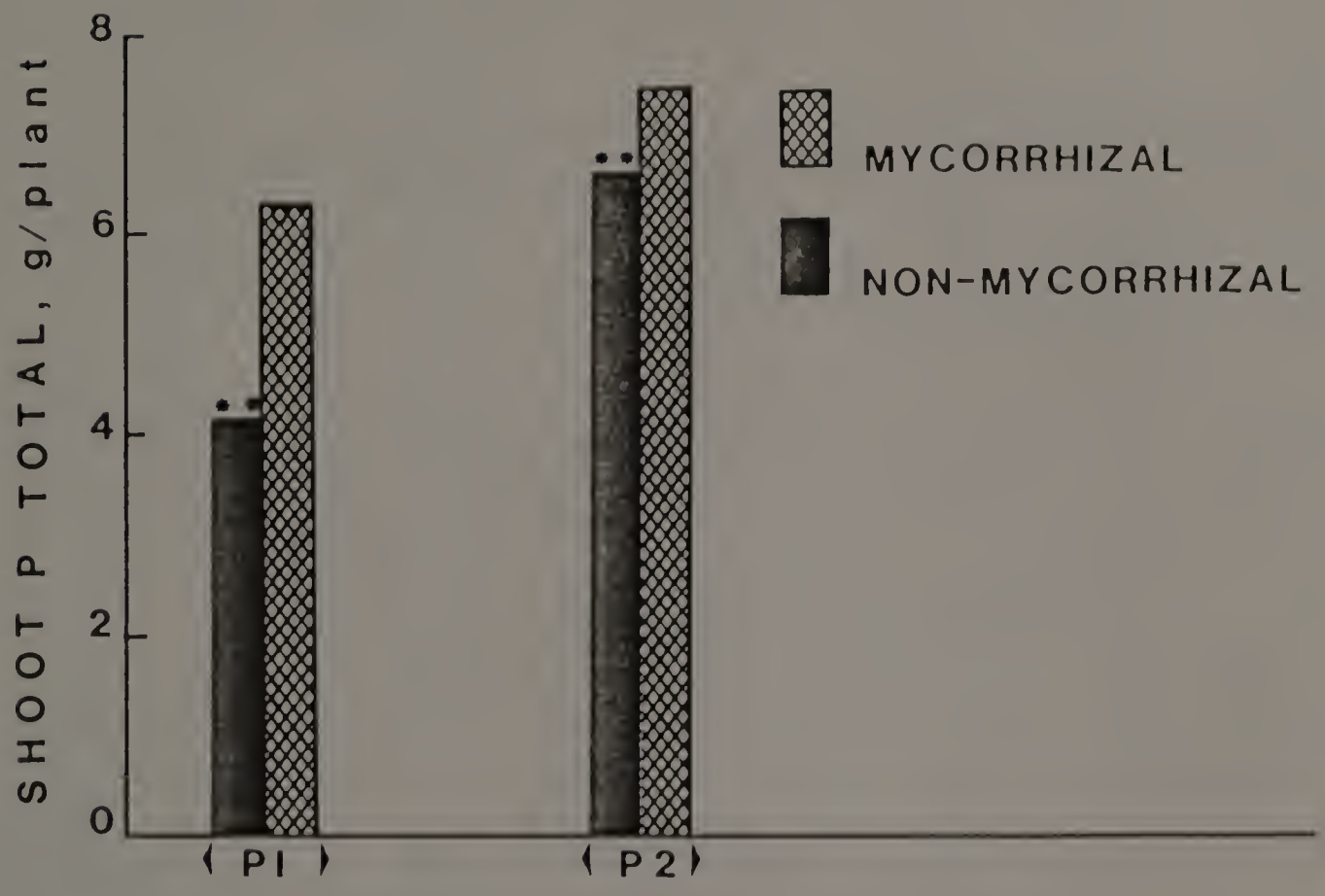


Figure 3.32 Shoot phosphorous accumulation as affected by mycorrhizal infection within phosphorous source. **Significant at 1% level. Each bar epresents the mean of 30 observations.

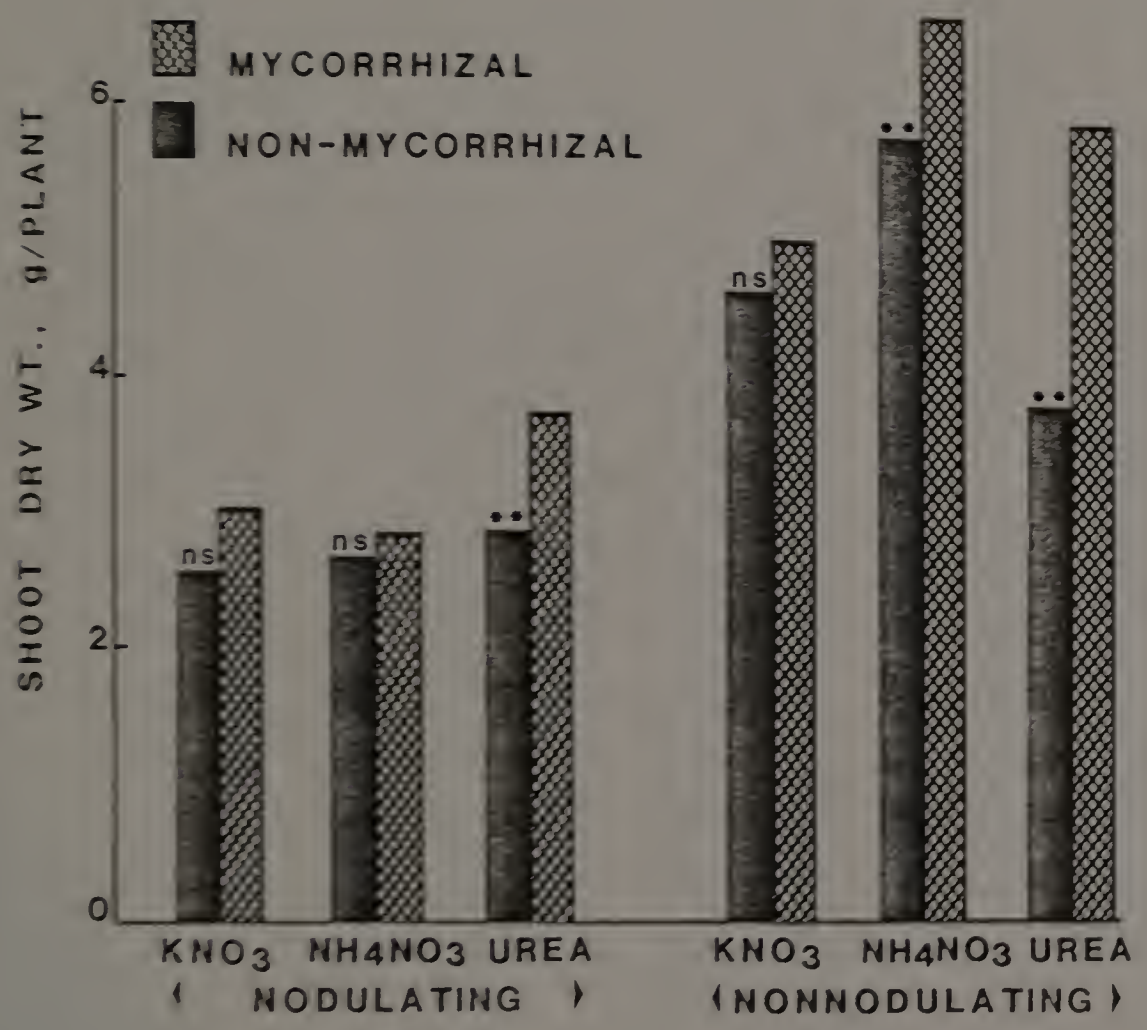


Figure 3.33 Shoot dry weight as affected by mycorrhizal infection within nitrogen source and isoline. ns, ** Nonsignificant (ns) or significant at 1%(**) level. Each bar represents the mean of 10 observations.

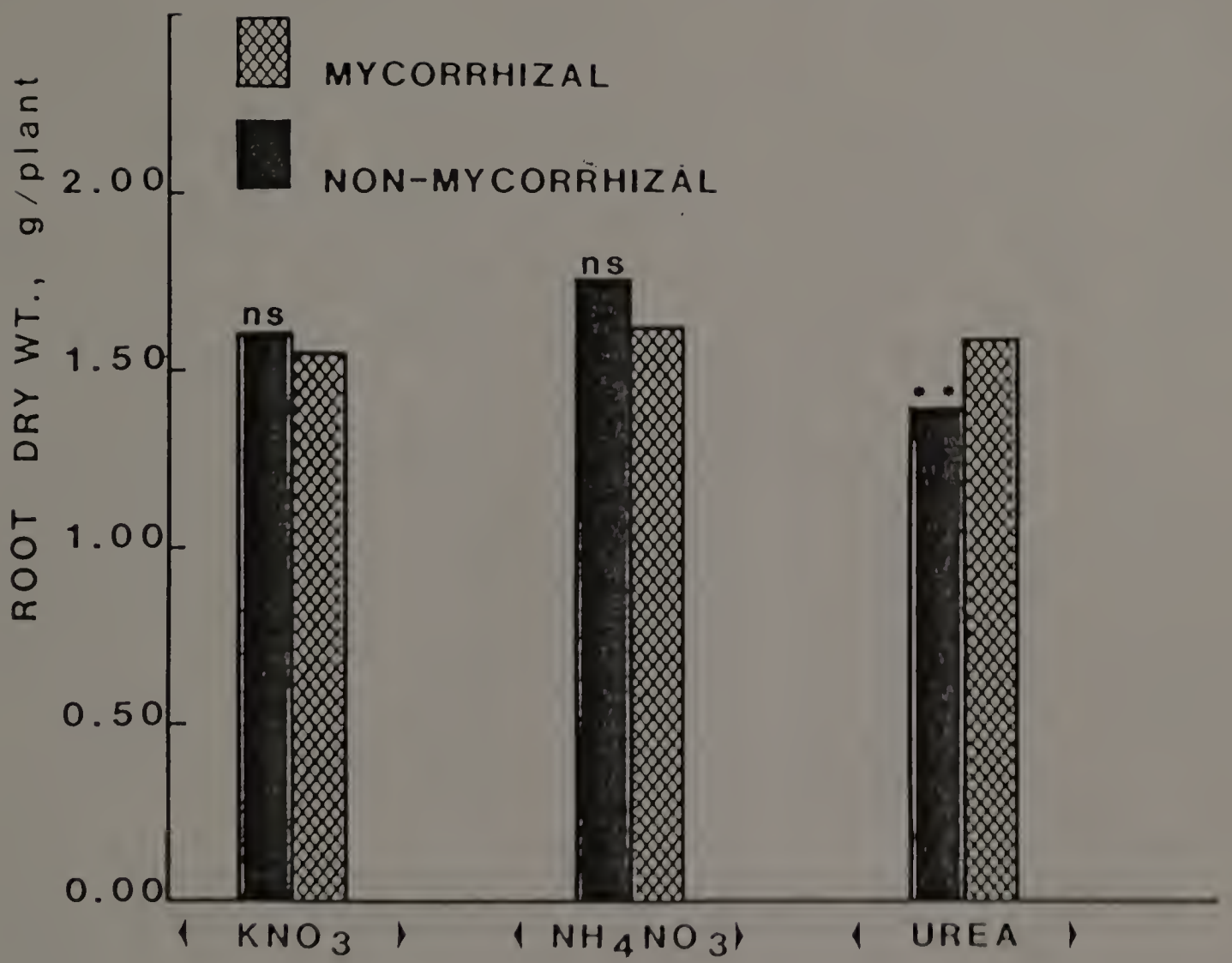


Figure 3.34 Root dry weight as affected by mycorrhizal infection within nitrogen source. ns,**Nonsignficant (ns), or significant at 1% (**) level. Each bar represents the mean of 20 observations.

plants did not differ from the root dry weights of the nonmycorrhizal plants (Fig. 3.34).

Within the nodulating isoline VAM growth benefits were limited to the urea-treated plants (Fig. 3.33). Both urea-and $NH_4NO_3$-treated plants exhibited VAM growth enhancements within the nonnodulating isoline (Fig. 3.33).

The N source interacted with VAM to affect the shoot and root N composition. Urea-treated, mycorrhizal plants had higher shoot and root N totals than those of the urea-treated, nonmycorrhizal plants (Fig. 3.35 and 3.36). Root N totals of the mycorrhizal, $NH_4NO_3$-treated plants (Fig. 3.35) were depressed significantly compared to the $NH_4NO_3$-treated nonmycorrhizal plants. Shoot P totals of both $NH_4NO_3$ and urea-treated, mycorrhizal plants were higher than those of the $NH_4NO_3$-and urea-treated nonmycorrhizal plants (Fig. 3.37).

Irrespective of nodulation, mycorrhizal plants had significantly depressed root N totals when treated with $NH_4NO_3$ and hydroxyapatite (Fig. 3.38). Supply of hydroxyapatite and urea to the mycorrhizal plants led to a significant enhancement of root N totals (fig. 3.38). The root P totals of the nonnodulating isoline treated with $NH_4NO_3$ was significantly increased under both P regimes (Fig. 3.39). The higher root P content of $NH_4NO_3$-treated plants would have partly contributed to the reduction in VAM infection associated with $NH_4^+$ nutrition (Fig. 3.29).

Nodulating or nonnodulating, mycorrhizal plants had higher shoot P totals than nonmycorrhizal plants, under both P regimes (Fig. 3.32).

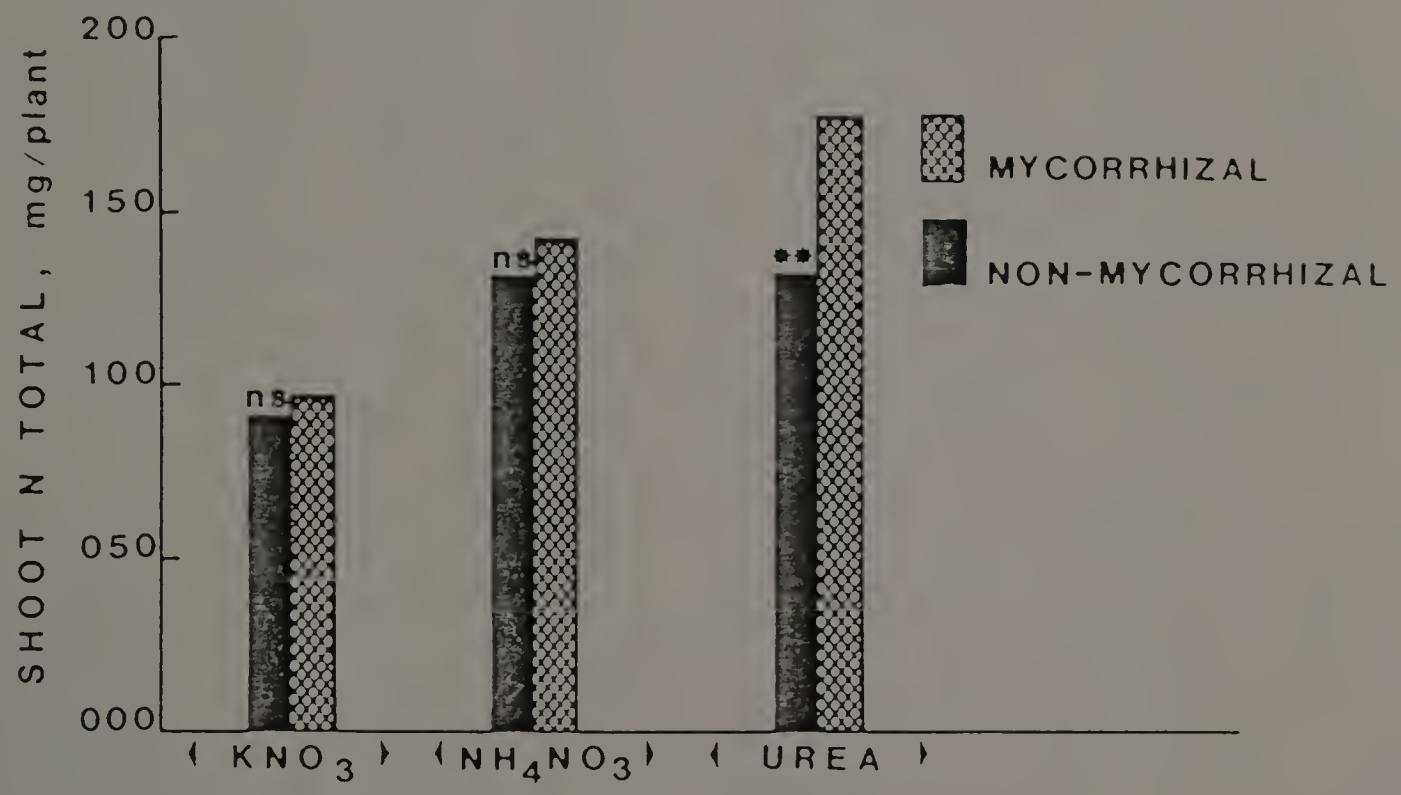


Figure 3.35 Shoot nitrogen accumulation as affected by mycorrhizal infection within nitrogen source. ns, **Nonsignficant (ns), or significant at 1% (**) level. Each bar represents the mean of 20 observations.

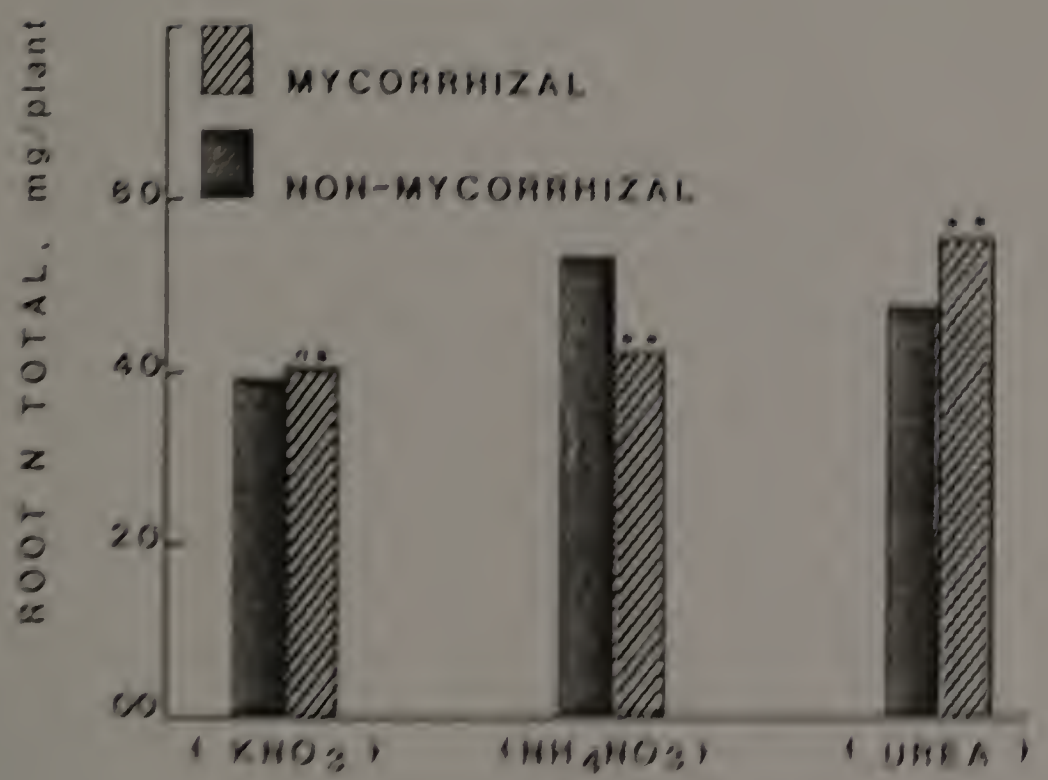


Figure 2.35 Root nitrogen accumulation as affected by mycorrhizal infection within nitrogen source. ns, **insignificant (ns), or significant at 1% (**) level. Each bar represents the mean of 20 observations.

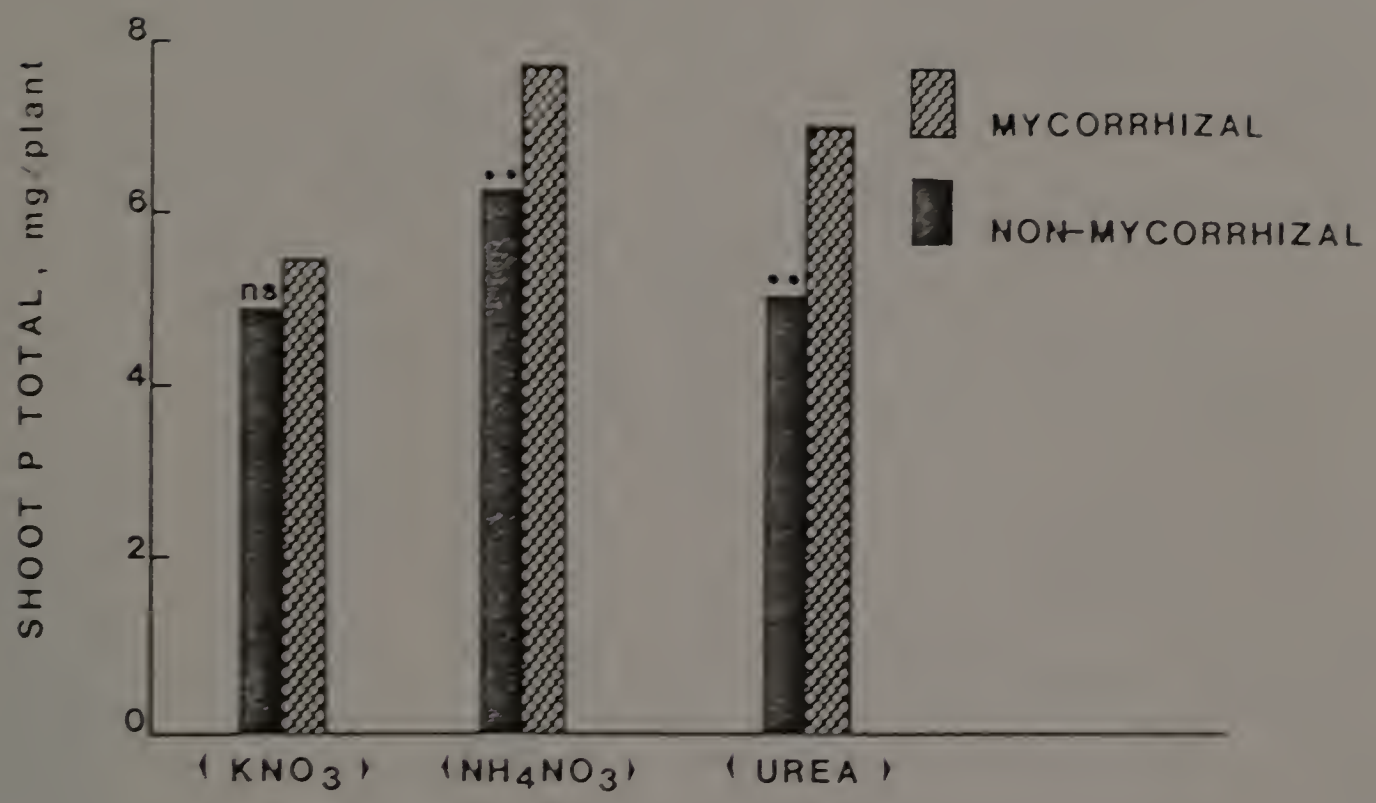


Figure 3.37 Shoot phosphorous accumulation as affected by mycorrhizal infection within nitrogen source. ns, **Nonsignficant (ns), or significant at 1% (**) level. Each bar represents the mean of 20 observations.

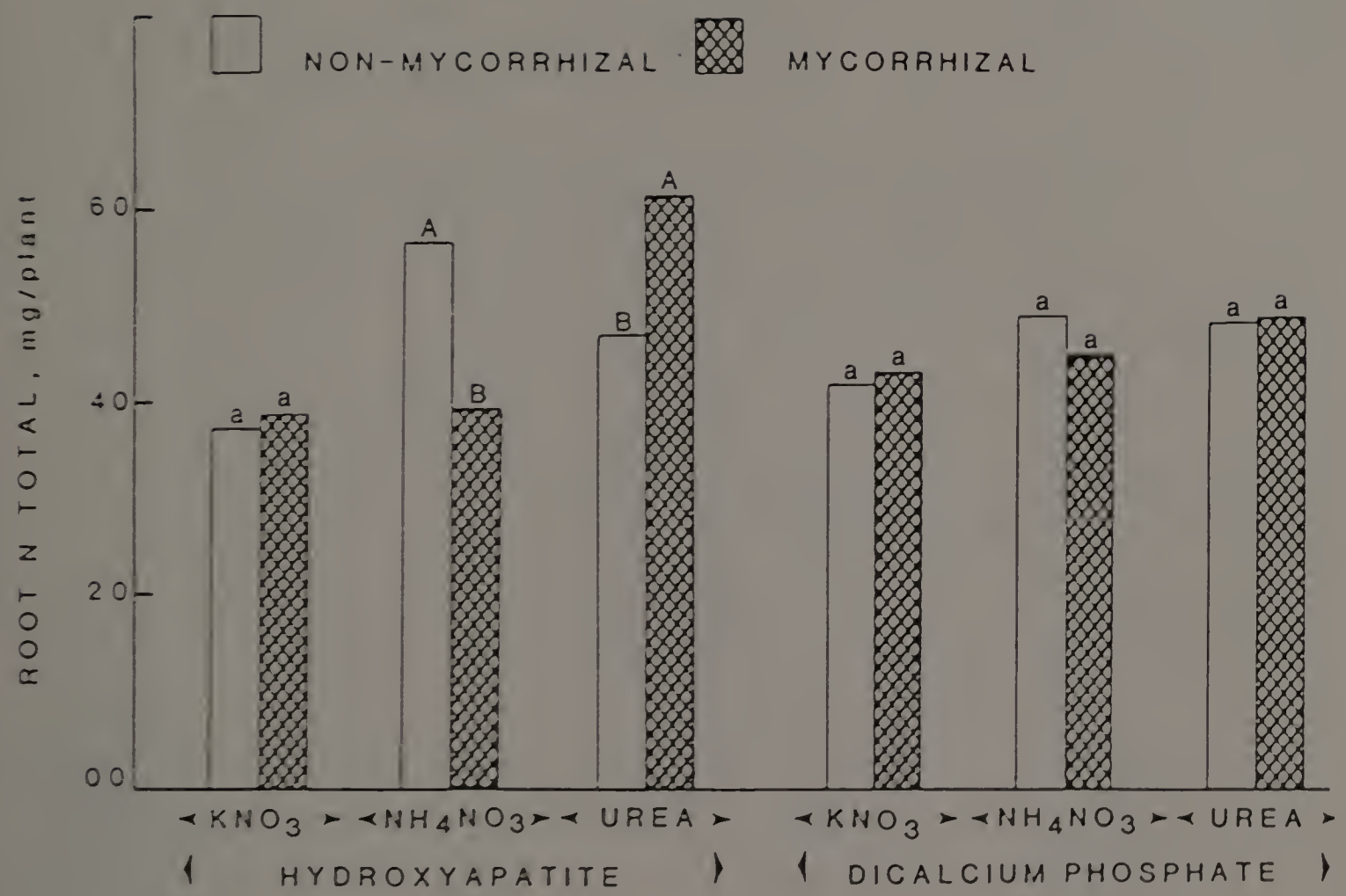


Figure 3.38 Root nitrogen accumulation as affected by mycorrhizal infection within nitrogen source and phosphorus regime. Mean separation within nitrogen source and phosphorous source by Duncan's New Multiple Range test, 5% (lowercase letters) level or 1% (uppercase letters) level. Each bar represents the mean of 10 observations.

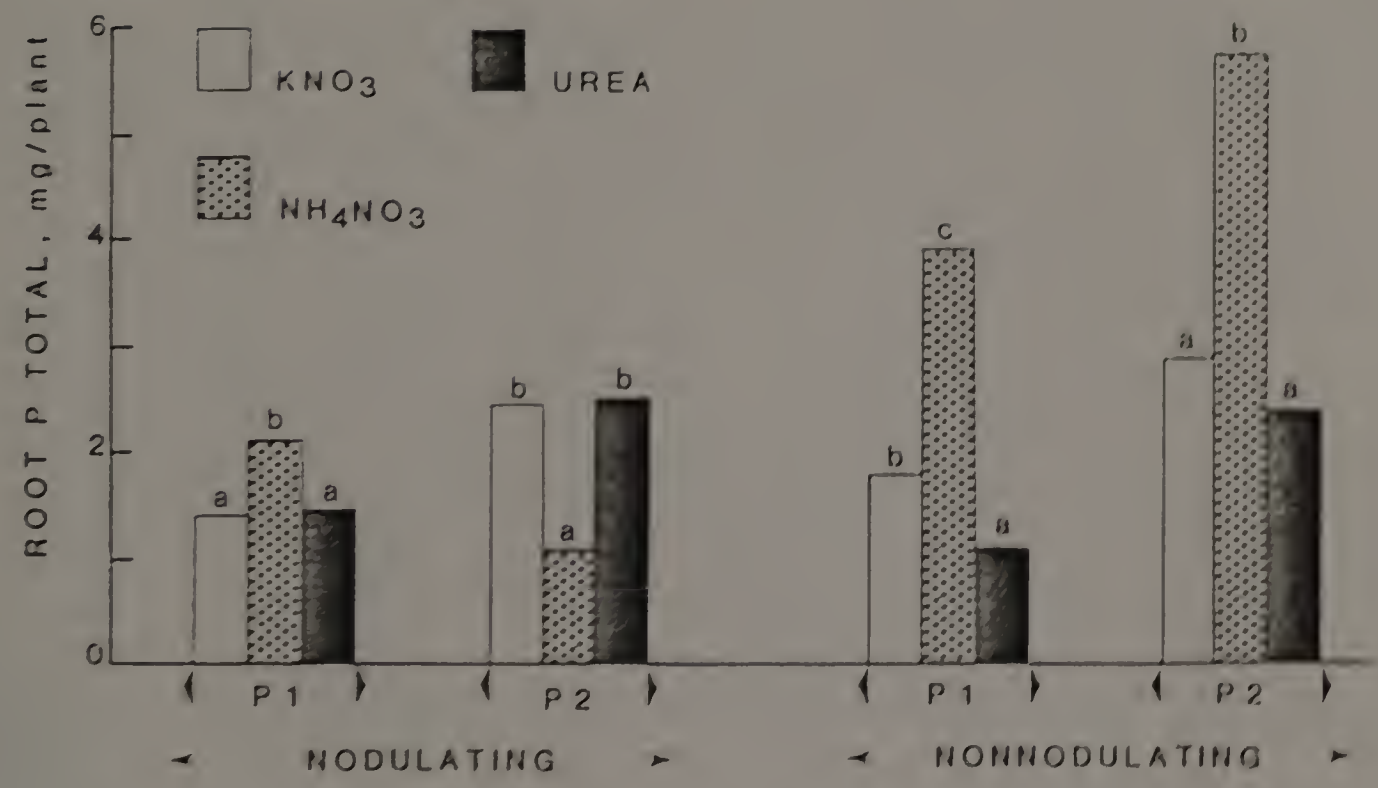


Figure 3.39 Root P accumulation as affected by nitrogen source, within phosphorous source and soybean isoline. Mean separation within phosphorous source and isoline by Duncan's New Multiple Range test, 1% level. Each bar represents the mean of 10 observations.

This VAM-induced tissue P enhancement was higher for the hydroxyapatite-treated plants than for the dicalcium phosphate-treated plants (Fig. 3.32), confirming the theory that mycorrhizal growth benefits are greater under situations of less available P (Harley and Smith, 1984).

The observation of enhanced growth response of the nonnodulating plants to mycorrhizal infection disagrees with previous reports. Schenck (1972) and Carling et al; (1978) found a positive growth response to mycorrhizal infection in nodulating soybean but did not find a similar response to mycorrhizal infection in nonnodulating soybean. Schenck and others (Asai, 1944; Schenck and Hinson, 1972; Abbott and Robson, 1977; Mosse, 1977; Redente and Reeves, 1981) suggested that a specific synergism existed between mycorrhizal fungi and $N_2$-fixing bacteria. This hypothesis was supported by reports that several legumes did not become established in sterilized soils, unless VAM was present (Asai, 1944). Sterilization of soil could lead to Mn or $NH_4^+$ toxicity (Russel and Russel, 1976). The role of VAM in these instances may have been an enhanced tolerance to Mn or $NH_4^+$ toxicity. High concentrations of zinc and copper are found in mycorrhizal plants (Gilmore, 1971; Ross, 1971). Perhaps mycorrhizal establishment led to a correction of either Cu or Zn deficiency in these plants. Both of these elements influence nodulation and nitrogen fixation (Van Schreven, 1958).

Nitrogen availability is more critical than P availability in achieving optimum growth of mycorrhizal plants (Koucheki and Read,

1975; Brown, Schultz, and Kormanik, 1981). This may partly be related to the fact that nitrogen deficient conditions lead to reduced root exudation (Bowen, 1969) as opposed to P deficient conditions which lead to enhanced root exudation (Rathnayake et al., 1978). If the amount of fungal colonization is regulated by root exudation, as suggested by Rathnayake et al. (1978), then N and P starvation have contrasting effects on the success of mycorrhizal infection. The response of nonnodulating plants to mycorrhizal infection in my experiment can be attributed to the supplement of inorganic nitrogen.

In other experiments comparing nodulating and nonnodulating plants, nitrogen supplements were withheld from the nodulating plants in order to standardize experimental conditions (Schenck and Hinson, 1972; Carling et al; 1978). Results of such experiments may be flawed (1); an accurate standardization is not possible in this situation, because the nodulating plants have access to atmospheric nitrogen and exhibit a superior overall metabolism whereas the metabolism of the nonnodulating plants was curtailed severely due to N starvation. It is not surprising that the nonnodulating plants lacked a positive growth response to mycorrhizal infection in this situation. The imposition of a symbiont (an additional carbon drain) on a N-stresed plant would increase the severity of its problems.

Nodulating plants treated with urea and hydroxyapatite exhibited the highest degree of mycorrhizal benefits (Fig. 3.19). Within the nonnodulating isoline maximum mycorrhizal benefits were derived by plants treated with a combination of urea and dicalcium phosphate

(Fig. 3.19). With low P, urea caused enhancements in root dry weights of the mycorrhizal nodulating and nonnodulating plants. With the higher P regime, only the urea-treated, nonnodulating plants exhibited an increase in root dry weights (Fig. 3.40).

According to the results of this experiment, the maximum advantage of Glycine-Glomus-Rhizobium symbiosis may be gained when urea is used as the N fertilizer with a 50% $NO_3$ background. Supplying N in the form of urea ensures a maximal tissue N level, and the mycorrhizal infection ensures a higher tissue P content. Urea was the most beneficial fertilizer for the nodulating isoline. Both $NH_4NO_3$ or urea proved to be highly beneficial to the nonnodulating isoline. Earliar reports that VAM did not help increase tissue N in the nonnodulating isoline (Schenck and Hinson, 1972; Carling et al., 1978), perhaps was a direct result of the inadequacy of N supply to these plants. This experiment shows that, when adequate N is available, VAM infection increases the tissue N levels of nonnodulating plants (Fig. 3.7 and Fig. 3.8).

The higher benefits of the reduced N sources in the expression of growth enhancements due to mycorrhizae may be explained partly as follows. A reduced N source, while providing nitrogen also is a means of energy economy to the plant. Because a symbiotic system imposes burdens on the energy budgeting of the plant, the efficiency of such a system would be facilitated by a reduced N source. Mycorrhizal fungi reportedly have a preference for $NH_4$-N over $NO_3$-N (Bowen and Smith, 1981). Nitrate fertilization of soil also diminishes mycorrhizal

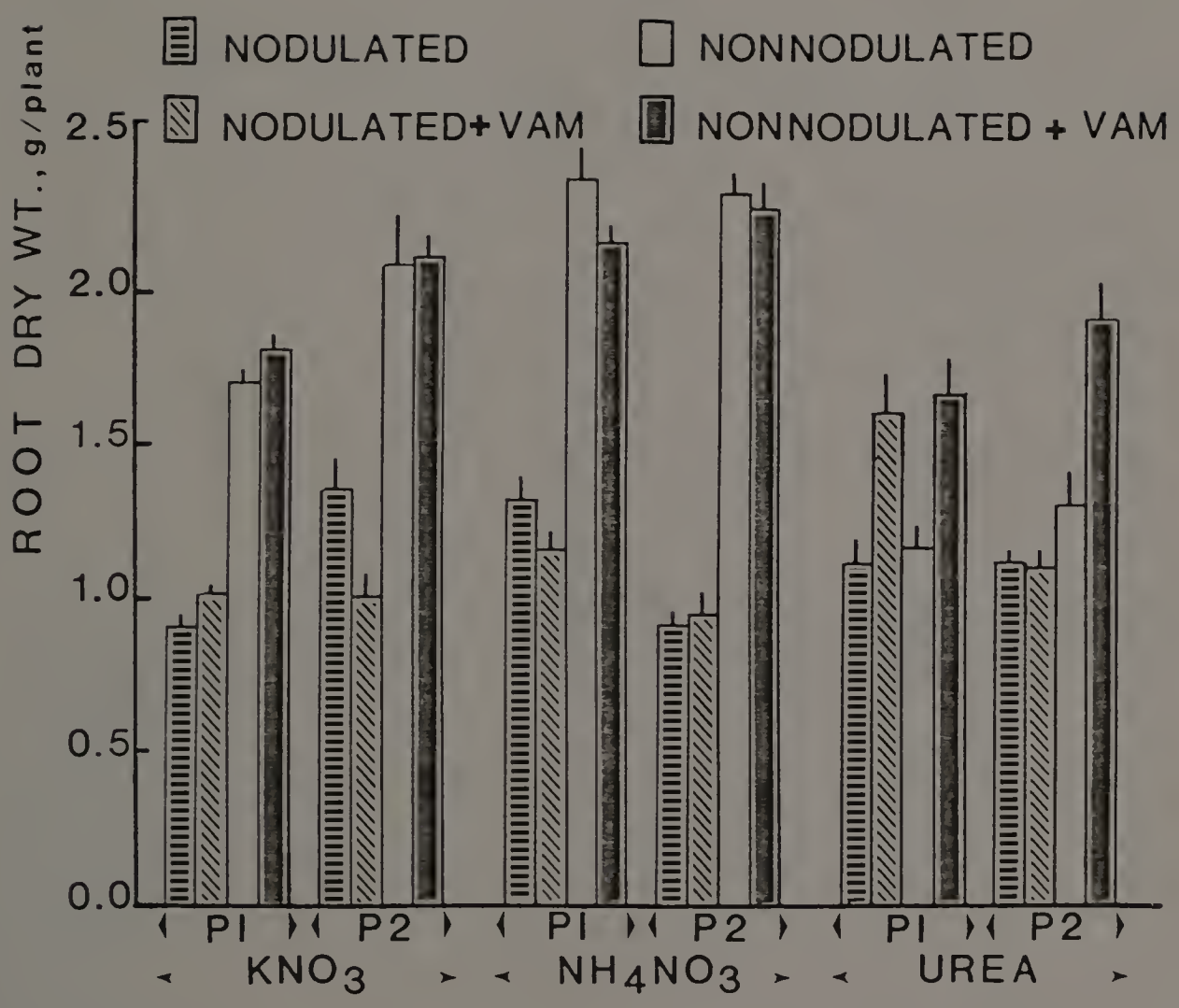


Figure 3.40 Root dry weights of mycorrhizal or nonmycorrhizal, and nodulating or nonnodulating plants as affected by phosphorous regime and nitrogen source. One SE shown at top of each bar. Each bar represents the mean of 5 observations. P1 and P2 denote hydroxyapatite and dicalcium phosphate, respectively. VAM denote vesicular-arbuscular mycorrhizae.

development (Richards, 1965). Nitrate may have an inhibitory effect on some mycorrhizal fungi (Harley and Smith, 1983). These studies are in agreement with my results in which the percentage of VAM infection and the growth enhancements due to mycorrhizae were least with nitrate nutrition. The mycelium of the mycorrhizae and the vesicles could serve as additional sites of ammonia detoxification, which in turn could make a supply of amino acids readily available to the plant. There is evidence for the transfer of glutamine from the fungal mycelium to the host (Lewis, 1976). The higher percentage of infection and the larger size of the vesicles (Fig. 3.41) associated with urea nutrition perhaps is important in this respect in explaining the superiority of urea fertilizer in obtaining VAM benefits.

The different N sources also led to differences in nitrogen and phosphorous partitioning between the root and the shoot of the plant. P concentrations were lower in the root systems of urea-treated plants than in the root systems of $NH_4NO_3$-treated plants (Fig. 3.42). Associated with the low root P concentration was a significant enhancement of root N totals of urea treated mycorrhizal plants (Fig. 3.36). A high root N/P ratio reportedly is conducive to VAM benefits (Hepper, 1983). The positive correlation of root N/P ratios to the growth enhancements due to mycorrhizae of this experiment (Fig. 3.44) supports this hypothesis. Plants treated with urea exhibited the highest root N/P ratios, compared to the plants treated with the other N sources (Table 3.2). The high root N/P ratios of urea-treated plants may explain to a great extent, the susceptibility of these

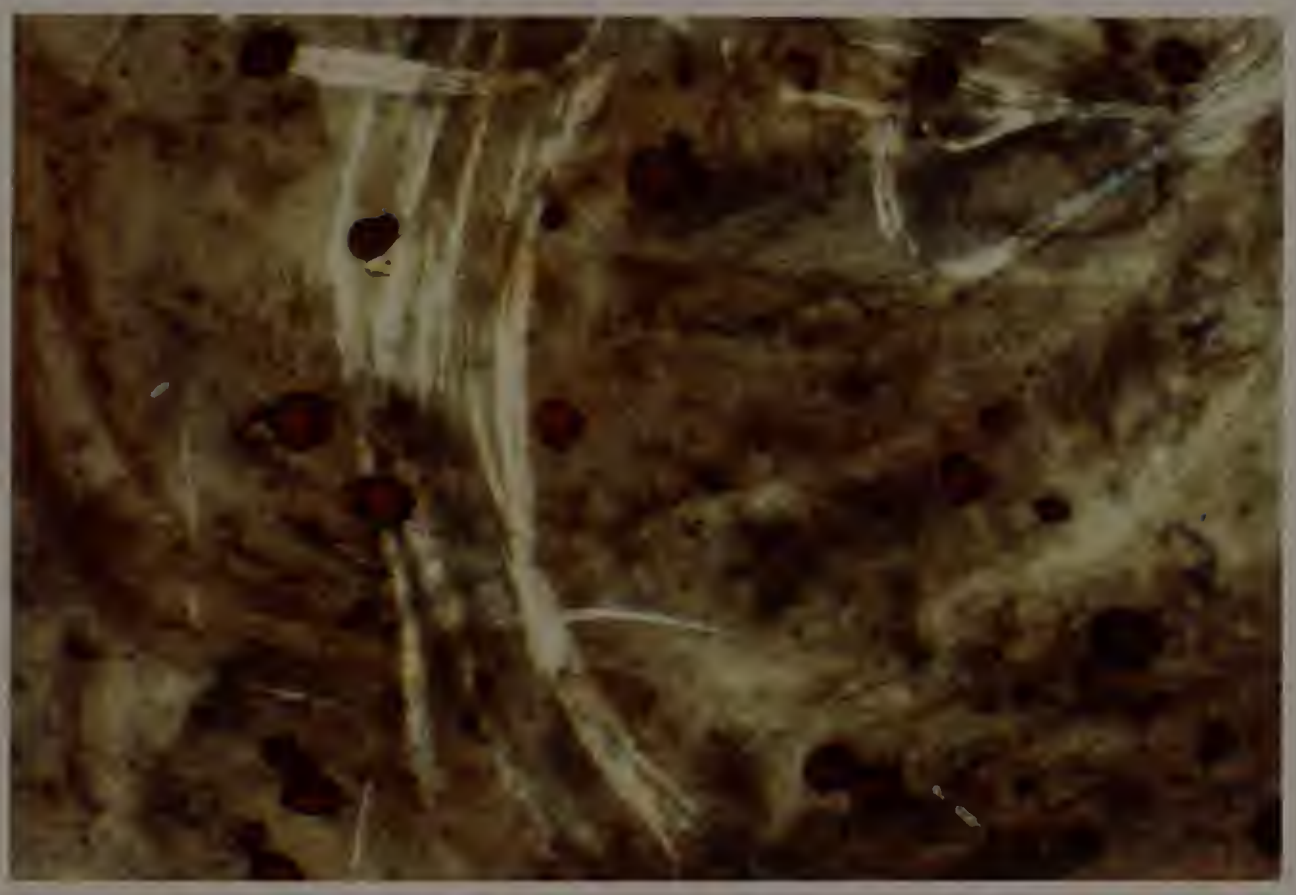

Figure 3.41 Large internal vesicles of the roots of urea-treated plants.

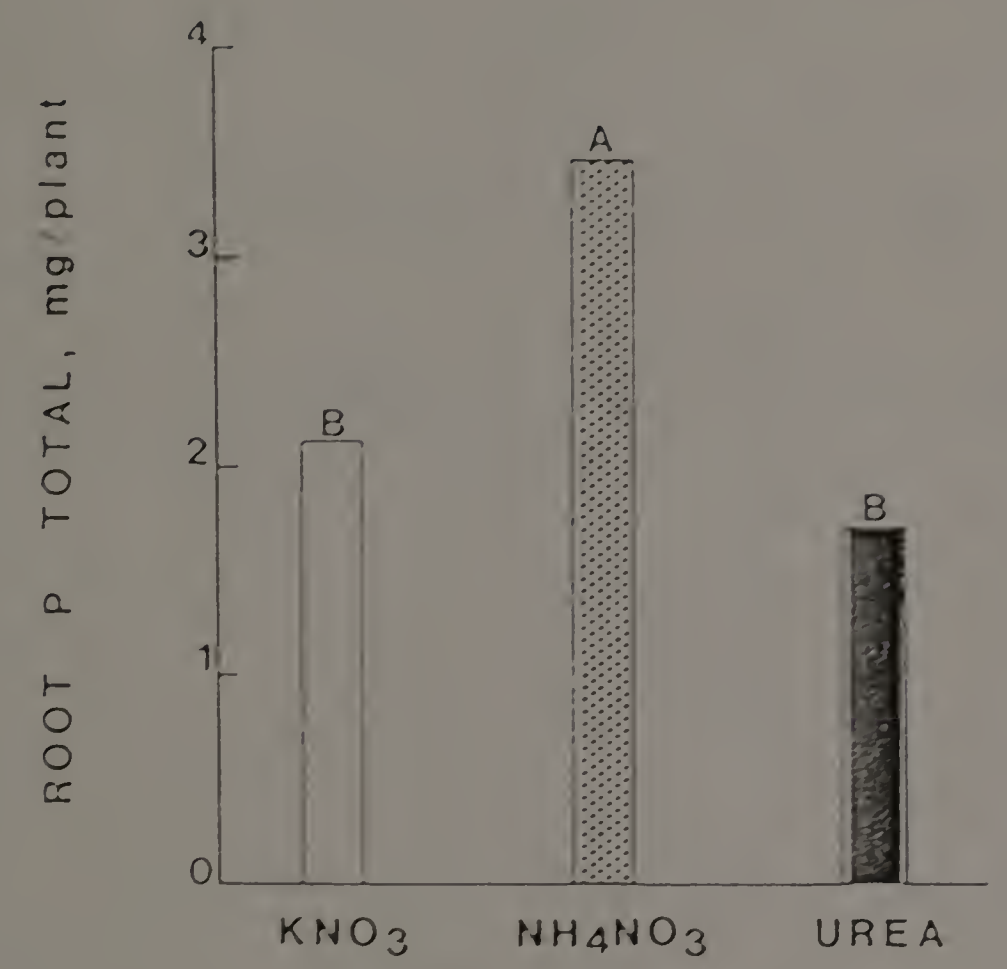


Figure 3.42 Root phosphorous accumulation as affected by nitrogen source. Mean separation by Duncan's New Multiple Range test, 1% level. Each bar represents the mean of 40 observations.

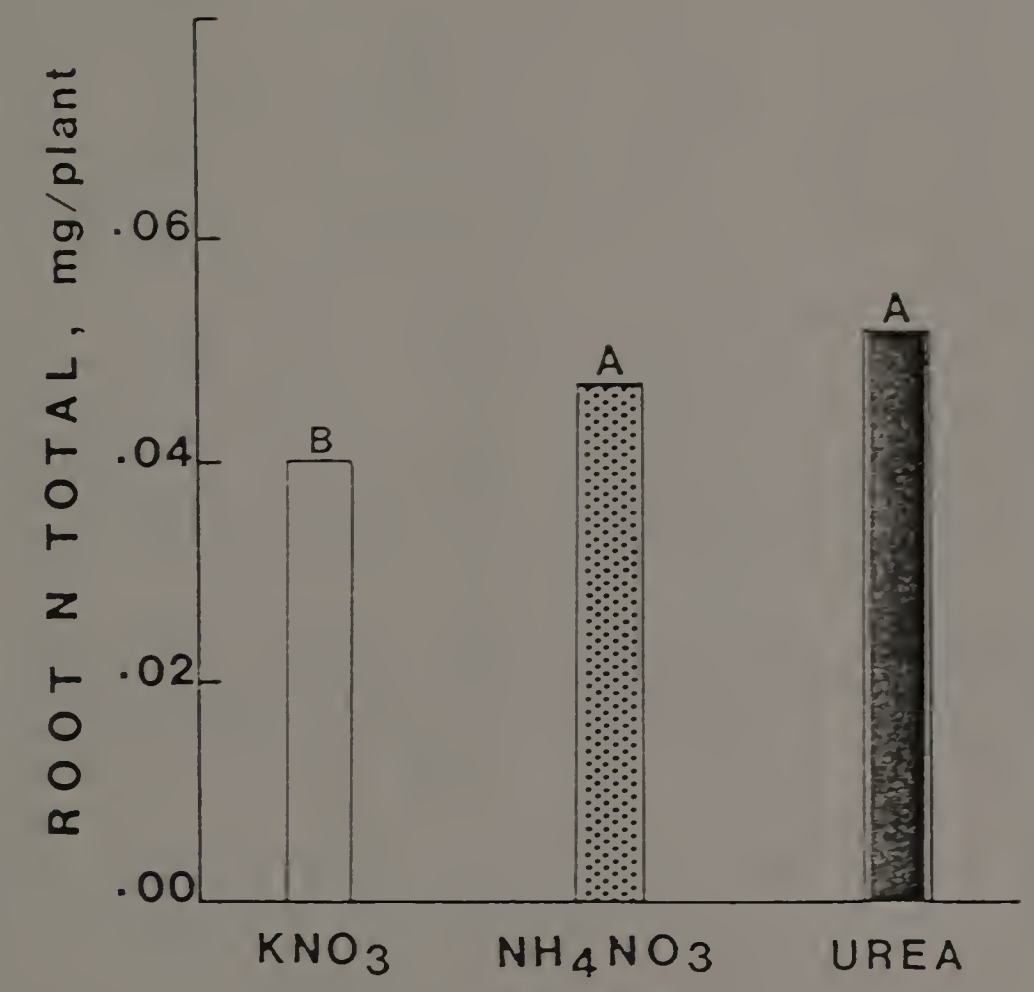


Figure 3.43 Root nitrogen accumulation as affected by nitrogen source. Mean separation by Duncan's New Multiple Range test, 1% level. Each bar represents the mean of 40 observations.

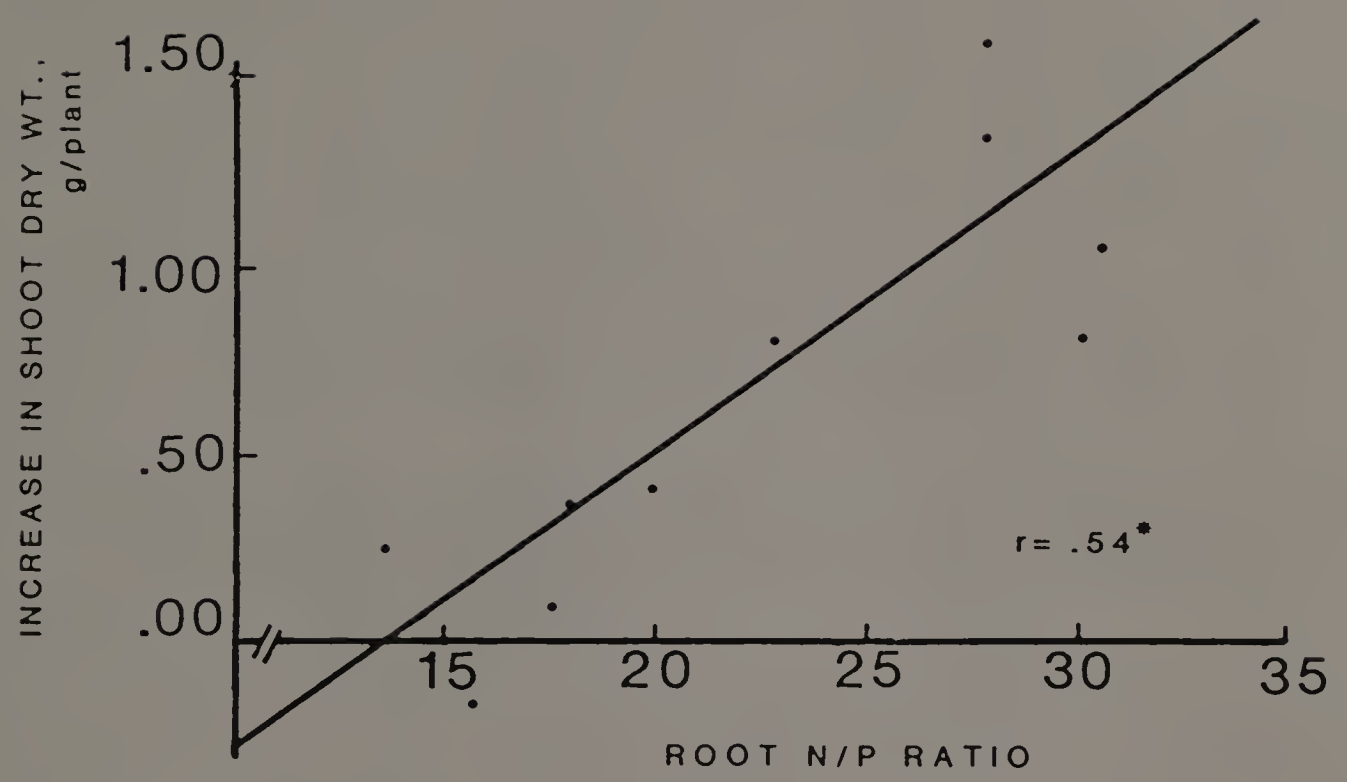


Figure 3.44 Relationship between mycorrhizal benefits (expressed as increase in shoot dry weight due to mycorrhizal infection) and root N/P ratio. * Siginificance of the correlation coefficient (r) at 5% level *.

Table 3.2 Effect of soybean isoline, and N source on root N/P ratio.

| Isoline | $KNO_3$ | $NH_4NO_3$ | UREA |
|---|---|---|---|
| Nodulating | 17.13A | 15.45A | 21.11B |
| Nonnodulating | 22.44B | 13.55A | 36.53C |

Row mean separation by Duncan's New Multiple Range test, 1% level.

Table 3.3 Effects of N source, and P regime on root N/P ratio.

| | HYDROXYAPATITE | DICALCIUM PHOSPHATE |
|---|---|---|
| $KNO_3$ | 23.48** | 16.08 |
| $NH_4NO_3$ | 16.07* | 12.93 |
| UREA | 37.14** | 20.51 |

*,**Signficant at 5% and 1% levels, rows.

plants to VAM infection. Within the nonnodulating isoline, $NH_4NO_3$-treated plants had lower root N/P ratios than $KNO_3$-treated plants. However, the VAM growth enhancements of $NH_4NO_3$-treated plants were higher than for the $KNO_3$-treated plants. The benefits of a high root N/P ratio may tend to be lost when the N source is non reducing. Higher root N/P ratios were obtained with the hydroxyapatite treated plants than with the dicalcium phosphate treated plants (Table 3.3) implying the potential for greater mycorrhizal benefits under the less available P source.

According to this experiment, the efficiency of the Glomus, Glycine, and Rhizobial tripartite symbiosis appear to depend upon the supply of (1). Reduced N (2). high root N/P ratio and (3). and a neutral root environment pH. Superiority of urea in enhancing VAM benefits may be because urea fulfills these 3 requirements best. Urea was also the only reduced N source under which VAM association led to enhanced shoot N totals (Fig. 3.35).

This experiment suggests that, under conditions of sufficient N availability and when P is not limiting, the N/P ratio of the roots may be a major determinant of the degree of susceptibility of soybean to VAM infection. The percentage of infection, by itself, does not reflect the potential VAM benefits if either N or P is severely limiting. A high percentage of VAM infection (Fig. 3.28) and the absence of VAM growth benefits in nodulating $NH_4NO_3$-treated plants (Fig. 3.33) are evidence of this. Alternately, mycorrhizal infection caused growth reductions in soybean (Glycine max "Kent"), and bean

(Phaseolus vulgaris L. cv. Dwarf), inoculated with Glomus Fasciculatus (Bethlenfalvay et al; 1982a; 1982b). Intersymbiont competition for P and photosynthate reportedly was responsible for these growth reductions (Bethlenfalvay et al; 1982a; 1982b). VAM benefits of the urea-treated, nodulating plants in this experiment may have been increased if the P availability was greater (through indirect enhancement of $N_2$-fixation). One of the possible restrictions of this experiment can be removed by increasing the P supply. However, in this case the options for using N fertilizers will be limited to urea and $KNO_3$, since VAM infection is inhibited in $NH_4NO_3$-treated plants, at high levels of P (Chapter II).

The available phosphorous in the growth medium ranged from 3 to 30ppm (Fig. 3.45). Reportedly mycorrhizal growth benefits occur best at corresponding levels of soil P (Harley and Smith, 1983). The upper limit of this range may have been more conducive to mycorrhizal benefits, as the highest response to VAM infection was obtained with urea treated plants.

A comparative study of how urea and $KNO_3$ affect the N/P ratios of roots grown with different P concentrations will help to determine what concentrations of these fertilizers are more appropriate in situations where VAM growth benefits are desired. The fertilizer combination that will give the highest root N/P ratio, in a situation in which the N supply is neither restricting nor excessive to plant growth, may be also the situation in which highest VAM growth growth benefits will be derived. Alternately, application of P has to be

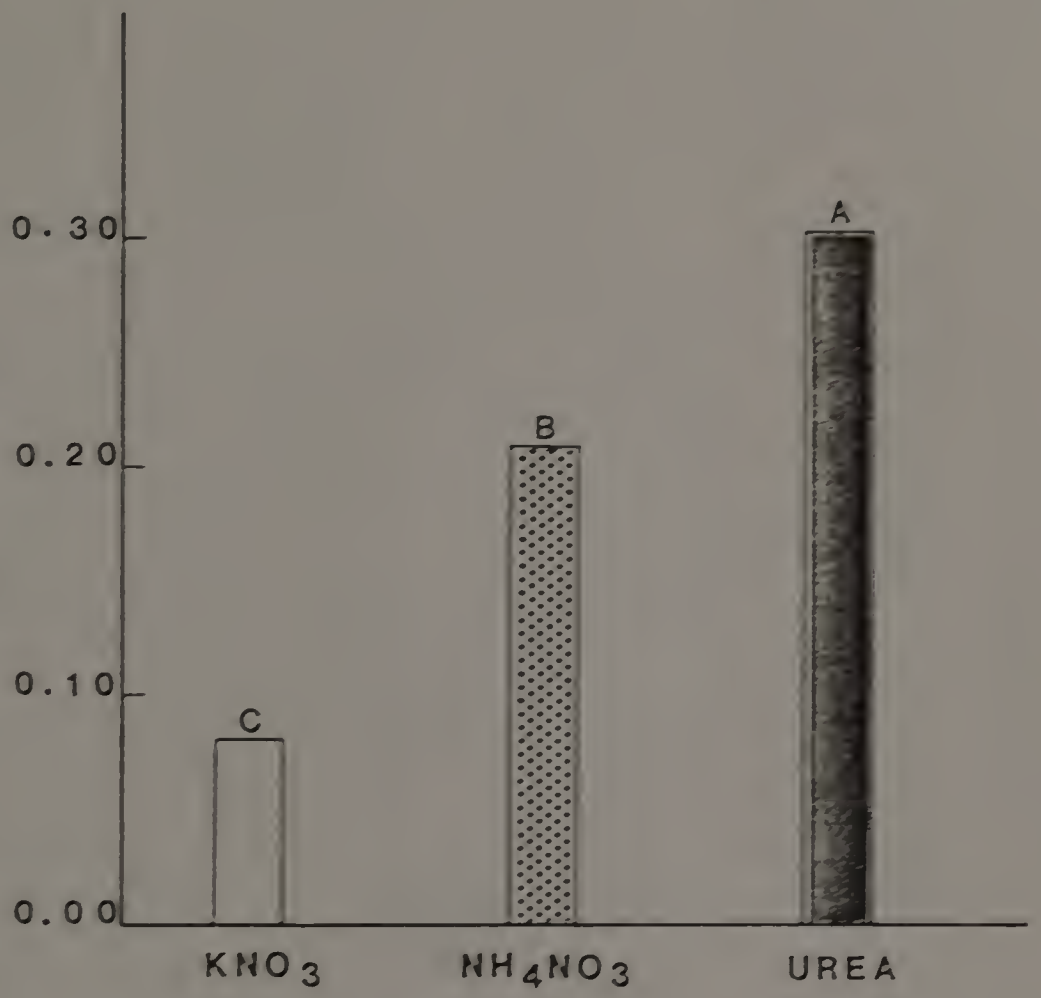


Figure 3.45 Phosphorous concentration in the sand leachate as affected by source of nitrogen. Mean separation by Duncan's New Multiple Range test, 1% level. Each bar represents the mean of 40 observations.

manipulated in order that P supply does not restrict plant growth or exceed the limits that prevent mycorrhizal benefits.

# CHAPTER IV

## EFFECTS OF N SOURCE AND N AND P INTERACTION ON THE N AND P COMPOSITION OF NODULATING AND NONNODULATING SOYBEANS

### Materials and Methods

A greenhouse experiment was designed to investigate the effects of low and high levels of 2 sources of N: 100% $NO_3^-$ or 50% urea with 50% $NO_3^-$, combined with a low and a high level of dicalcium phosphate on nodulating or nonnodulating soybean.

Preparation of growth medium was similar to that described in Chapter II. Dicalcium phosphate was added to the sand at the rate of 250 or 500 mg per pot. Seeds were surface-sterilized, and nodulating plants were inoculated with rhizobia (Chapter III). Four seeds were planted in each pot, watered until 2 healthy cotyledons and a growing point emerged, and then thinned to 1 plant per pot. Nutrient treatments were commenced 2 weeks after seedling emergence. Nutrients were provided as recommended by Johnson et al. (1957) except for N. Nodulating plants were supplied with 5mM or 10mM N, while nonnodulating plants received double this stength of N. The 2 sources of N applied will be referred to as nitrate or urea regimes.

The experiment was started in late October. Temperature varied

diurnally, mean day temperature was 25°C, and mean night temperature was 10°C. About half of the days during the experimental period were overcast. Supplementary light was provided throughout the experimental period (Chapter II). Nutrient solution was provided 5 days a week and the medium was leached with deionized water on the remaining two days. Plants were harvested in early-December at the onset of podset. Each treatment was replicated 5 times. Pots were rotated daily to avoid positional effects of the greenhouse. Statistical analyses was carried out similar to that described in Chapter III. After harvest tissues were dried, weighed and analysed for P and N according to the procedures described in Chapter III.

## Results and Discussion

### Nodulating Plants

Nodulating plants treated with urea exhibited more vigorous growth than those treated with nitrate (Fig. 4.1). Incipient chlorosis occurred in the older leaves of the nitrate-treated, nodulating plants, and these symptoms were more pronounced in the plants treated with the higher level of nitrate than those treated with the lower level of nitrate(Fig. 4.1). A nitrate-induced, Fe deficiency (Haynes and Goh, 1978) may be a possible reason for these

Figure 4.1 Nodulating plants treated with high and low levels of $KNO_3$ or urea. Older leaves of the $KNO_3$ treated plants (left) show chlorosis. Symptoms of mineral disorder are absent in the urea-treated plants (right). Low and high levels of N refer to 5mM and 10 mM N.

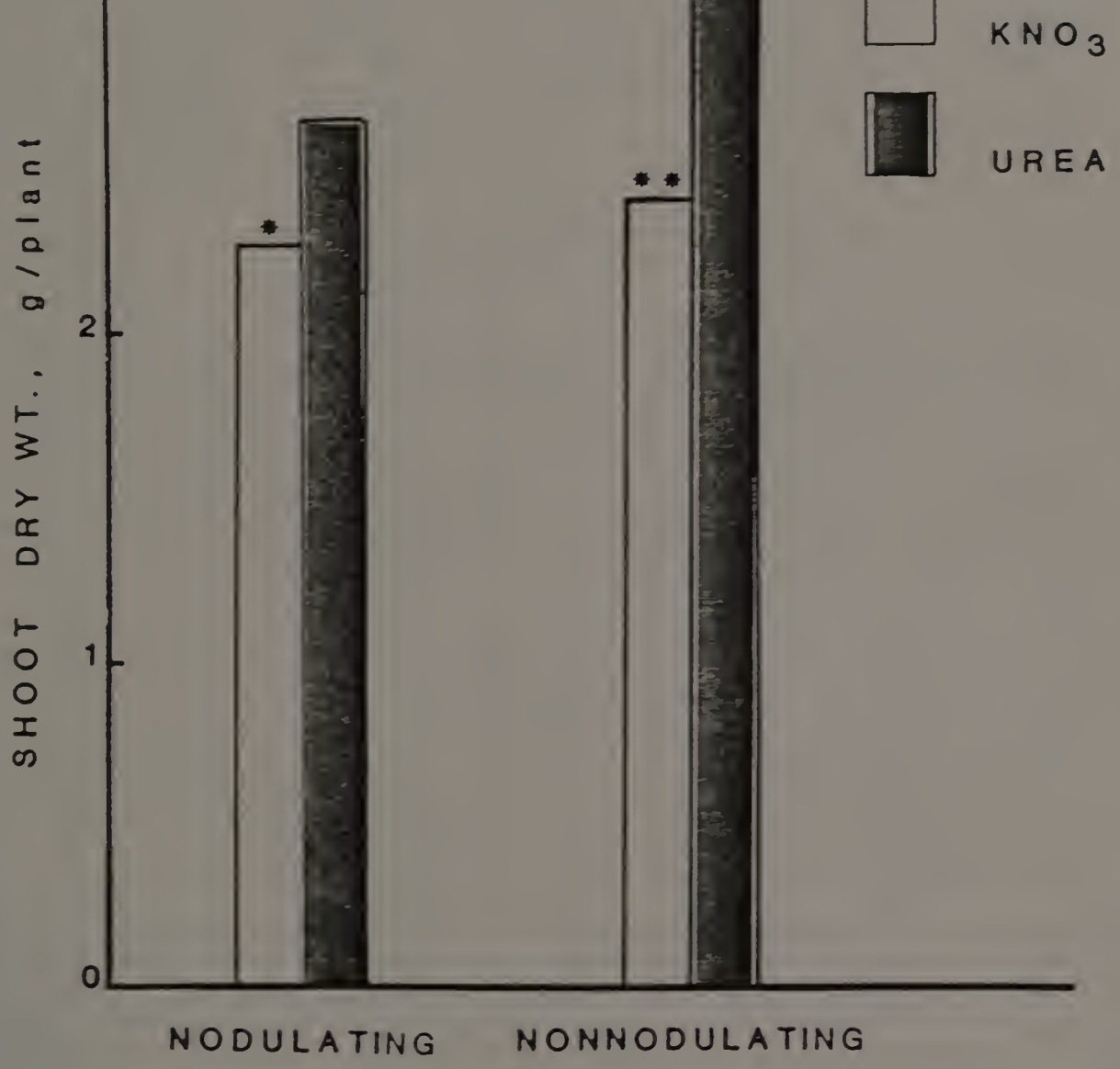


Figure 4.2 Shoot dry weights as affected by nitrogen source within soybean isoline. *, **Significant at 5% or 1% level.

symptoms. Urea-treated nodulating plants had higher shoot dry weights than the nitrate-treated nodulating plants (Fig. 4.2). Application of a higher level of urea led to increased shoot dry weights of the nodulating plants, but the application of a higher level of nitrate did not elicit a similar response (Fig. 4.3). Root dry weights were not increased by the higher level of nitrogen (Fig. 4.4).

Plant nitrogen composition. Nodulating plants treated with the higher level of urea showed higher shoot N totals than the plants treated with the lower level of urea (Fig. 4.5), but a similar response was not shown by the $KNO_3$-treated nodulating plants. Nodulating plants showed improved growth with the higher level of urea under both P regimes (Fig. 4.5). The favorable effect of urea compared to nitrate in N accumulation and in enhancing shoot dry weights may be because urea promotes $N_2$ fixation in nodulating soybean (Vigue et al., 1977). The poorer growth obtained with $NO_3^-$ nutrition (Fig. 4.2), can be attributed to the inhibitory effects of $NO_3^-$ ion on $N_2$ fixation. The lower shoot and root N totals of the $NO_3$-treated plants compared to the urea-treated plants is evidence of this nitrate inhibition of $N_2$ fixation (Table 4.1).

Plant phosphorous composition. Higher levels of N increased the shoot and root P totals (Fig. 4.6 and 4.7). Therefore availability of N must determine the efficiency of P accumulation. When the interaction of N source, N level, and P regime was considered, it was seen that the higher level of urea increased the shoot P totals, under both P regimes (Fig. 4.8). The higher level of nitrate increased the

Table 4.1 Shoot and root nitrogen and phosphorous percentages as affected by nitrogen source, nitrogen rate and phosphorous regime.

| N source | N rate mM | P rate ppm | shoot N% | root N% | shoot P% | root P% |
|---|---|---|---|---|---|---|
| Nodulating Isoline | | | | | | |
| $KNO_3$ | 5 | 100 | 3.16 | 2.52 | 0.41 | 0.26 |
| urea | 5 | 100 | 4.29 | 2.74 | 0.45 | 0.21 |
| $KNO_3$ | 5 | 200 | 3.19 | 2.66 | 0.47 | 0.28 |
| urea | 5 | 200 | 3.81 | 2.75 | 0.40 | 0.22 |
| $KNO_3$ | 10 | 100 | 3.75 | 2.90 | 0.54 | 0.44 |
| urea | 10 | 100 | 4.79 | 3.02 | 0.47 | 0.24 |
| $KNO_3$ | 10 | 200 | 3.29 | 3.10 | 0.44 | 0.33 |
| urea | 10 | 200 | 4.72 | 3.10 | 0.47 | 0.27 |
| Nonnodulating isoline | | | | | | |
| $KNO_3$ | 10 | 100 | 4.13 | 2.67 | 0.44 | 0.25 |
| urea | 10 | 100 | 4.65 | 3.13 | 0.32 | 0.22 |
| $KNO_3$ | 10 | 200 | 3.64 | 2.82 | 0.30 | 0.25 |
| urea | 10 | 200 | 4.73 | 3.11 | 0.33 | 0.24 |
| $KNO_3$ | 20 | 100 | 4.53 | 3.096 | 0.52 | 0.29 |
| urea | 20 | 100 | 6.40 | 5.20 | 0.38 | 0.26 |
| $KNO_3$ | 10 | 200 | 4.47 | 3.24 | 0.46 | 0.26 |
| urea | 10 | 200 | 6.50 | 5.02 | 0.32 | 0.20 |
| $LSD_{.05}$ | | | .32 | ns | .18 | .13 |
| $LSD_{.01}$ | | | .42 | ns | .24 | .17 |

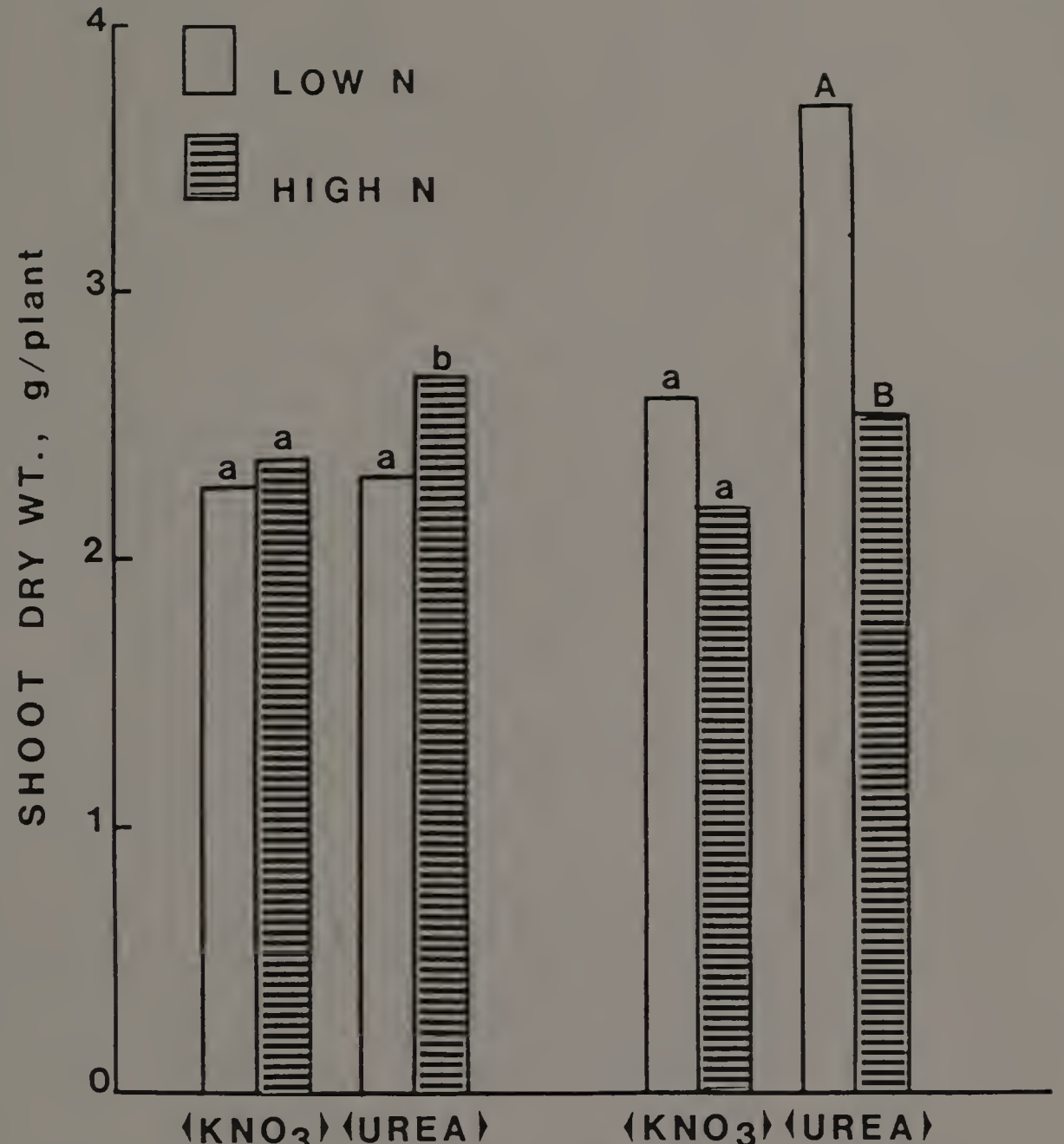


Figure 4.3 Shoot dry weights as affected by nitrogen rate, within nitrogen source and soybean isoline. Mean separation within nitrogen source and soybean isoline by Lsd , 5% (lowercase letters) level: 0.45, or 1% (uppercase letters) level: 0.59. Low and high levels of N correspond to 10mM and 5mM N for nodulating plants, and 10mM and 20 mM N for nonnodulating plants.

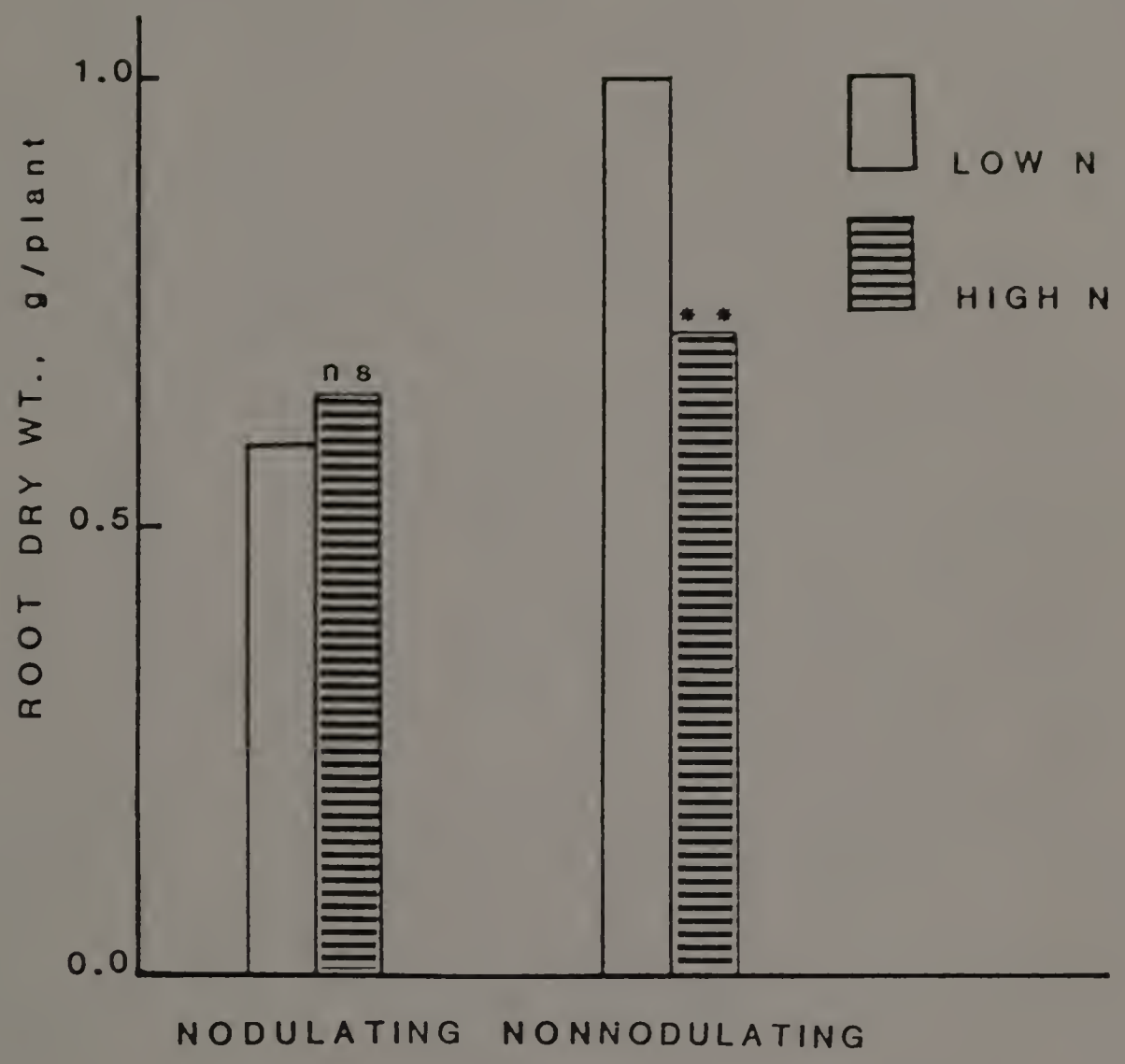


Figure 4.4 Root dry weights as affected by nitrogen rate within soybean isoline. Insert Lsd . Low and high levels of N refer to 5mM and 10mM N for nodulating plants and 10mM and 20mM N for nonnodulating plants.

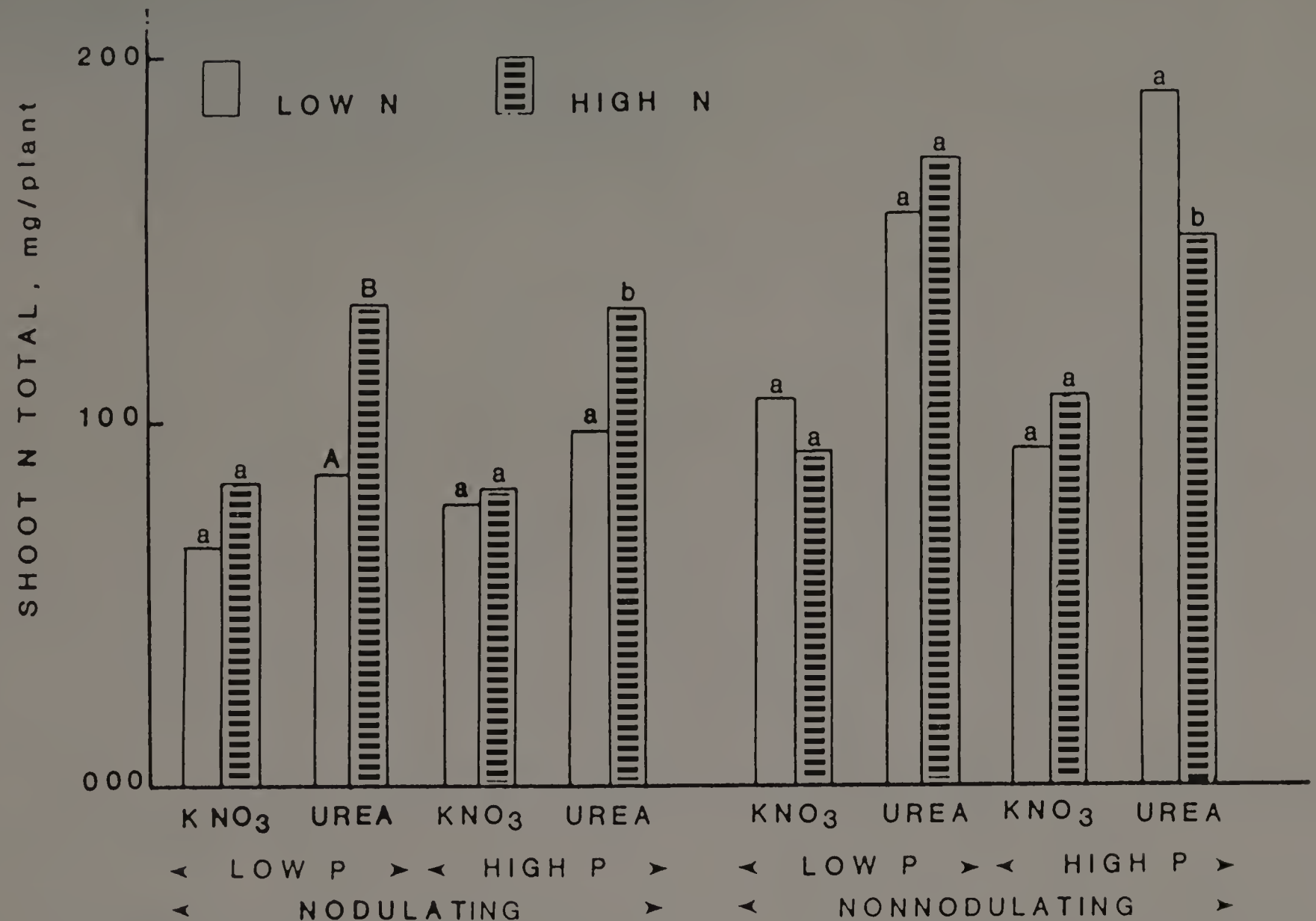


Figure 4.5 Effect of nitrogen source, N rate, and P regime, and soybean isoline on shoot N accumulation. Mean separation by Lsd, 5% (lowercase letters) level :57.7, or 1% (uppercase letters) level:76.2. Low and high levels of N refer to 5 and 10 mM N for nodulating plants and 10 and 20 mM N for nonnodulating plants.

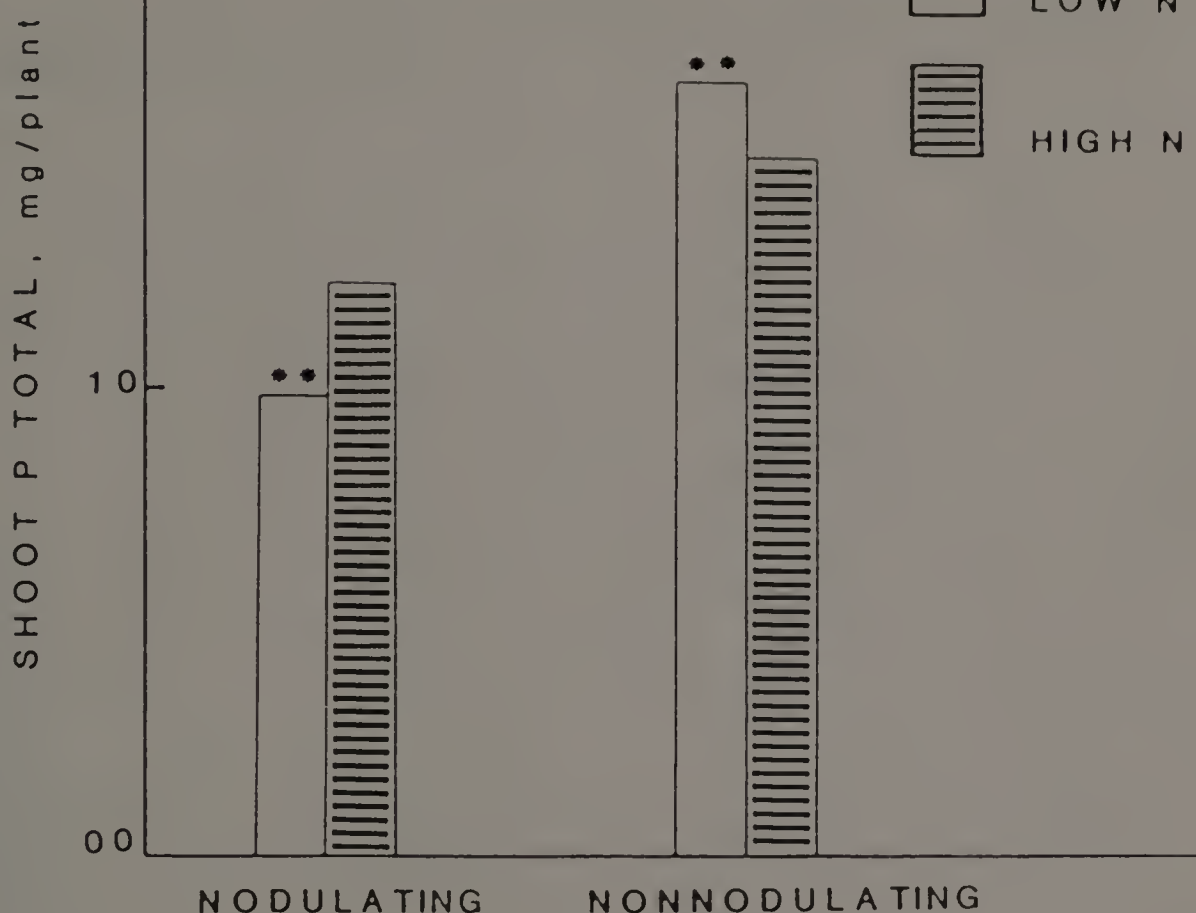


Figure 4.6 Shoot P accumulation as affected by N rate within soybean isoline. **Significant at 1% level.

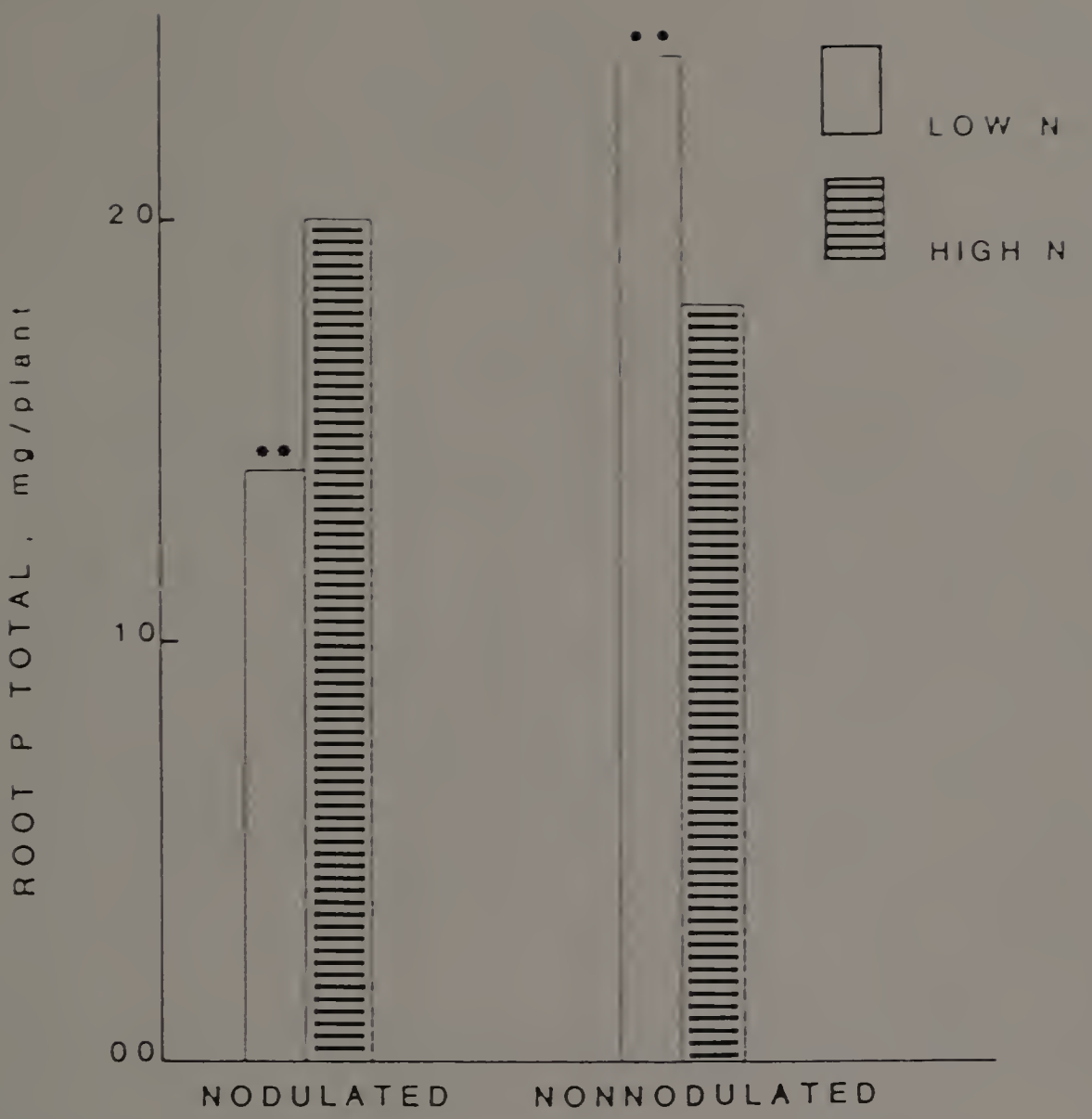


Figure 4.7 Root phosphorous accumulation as affected by nitrogen rate, within soybean isoline. **Significant at 1% level. Low and high levels of N refer to 5mM and 10mM N for nodulating plants and 10mM and 20mM N for nonnodulating plants.

shoot P totals, under the lower P regime (Fig. 4.8) but did not raise the shoot P totals under the higher P regime. Failure of shoot P totals to increase under the higher P regime may be attributed to an unfavorable shoot N/P ratio that arose when $KNO_3$ and P supply were raised at the same time.

Urea-treated, nodulating plants showed a significant reduction in root P totals, compared to the $NO_3$-treated plants (Fig. 4.9). N source appeared to play a major role in the partitioning of P between the shoot and the root-a result suggested by the first experiment as well and confirmed by the results of this experiment which was carried out under P sufficient-conditions. Reduction in root P content of plants subjected to urea nutrition (Fig. 4.9) may be of major importance in making these plants susceptible to VAM infection.

Nonnodulating Plants

Plants treated with the lower levels of nitrate or urea lacked symptoms of mineral disorder (Fig. 4.10). Symptoms of ammonium toxicity appeared in the plants treated with the higher level of urea (Fig. 4.11), and these exhibited reduced shoot dry weights. They also ceased height growth prematurely (Fig.4.3). Urea-treated, nonnodulating plants had higher shoot dry weights than the nitrate-treated, nonnodulating plants (Fig. 4.4).

Plant Nitrogen Composition. Urea-treated plants had higher shoot and root N totals than the $NO_3^-$-treated plants (Fig. 4.12 and Table

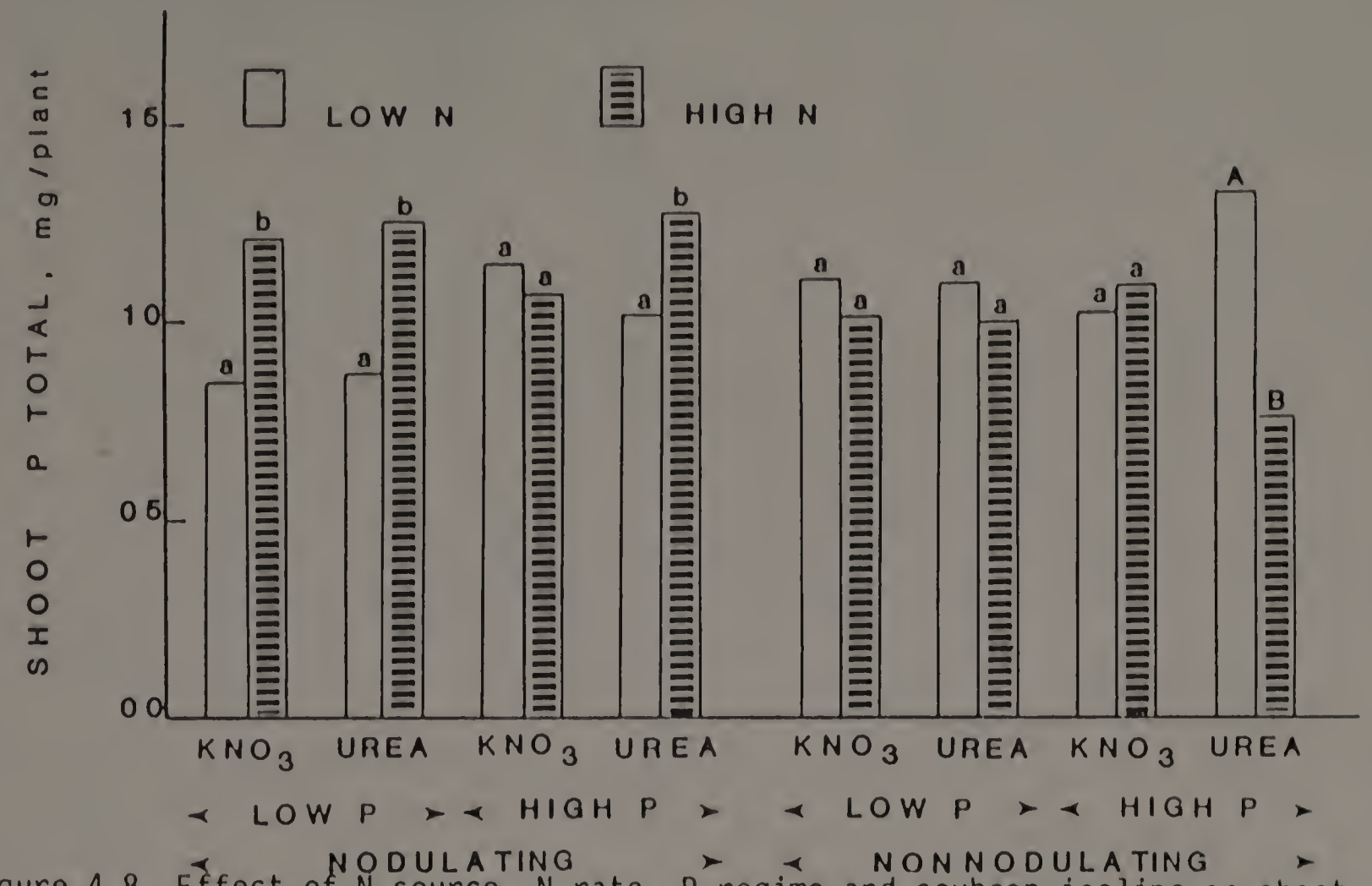


Figure 4.8 Effect of N source, N rate, P regime and soybean isoline on shoot P accumulation. Mean separation by LSD, 5% (lowercase letters) level, 6.40, or 1% (uppercase letters) level, 8.47. Low and high levels of N refer to 5mM and 10mM N for nodulating plants and 10mM and 20mM N for nonnodulating plants.

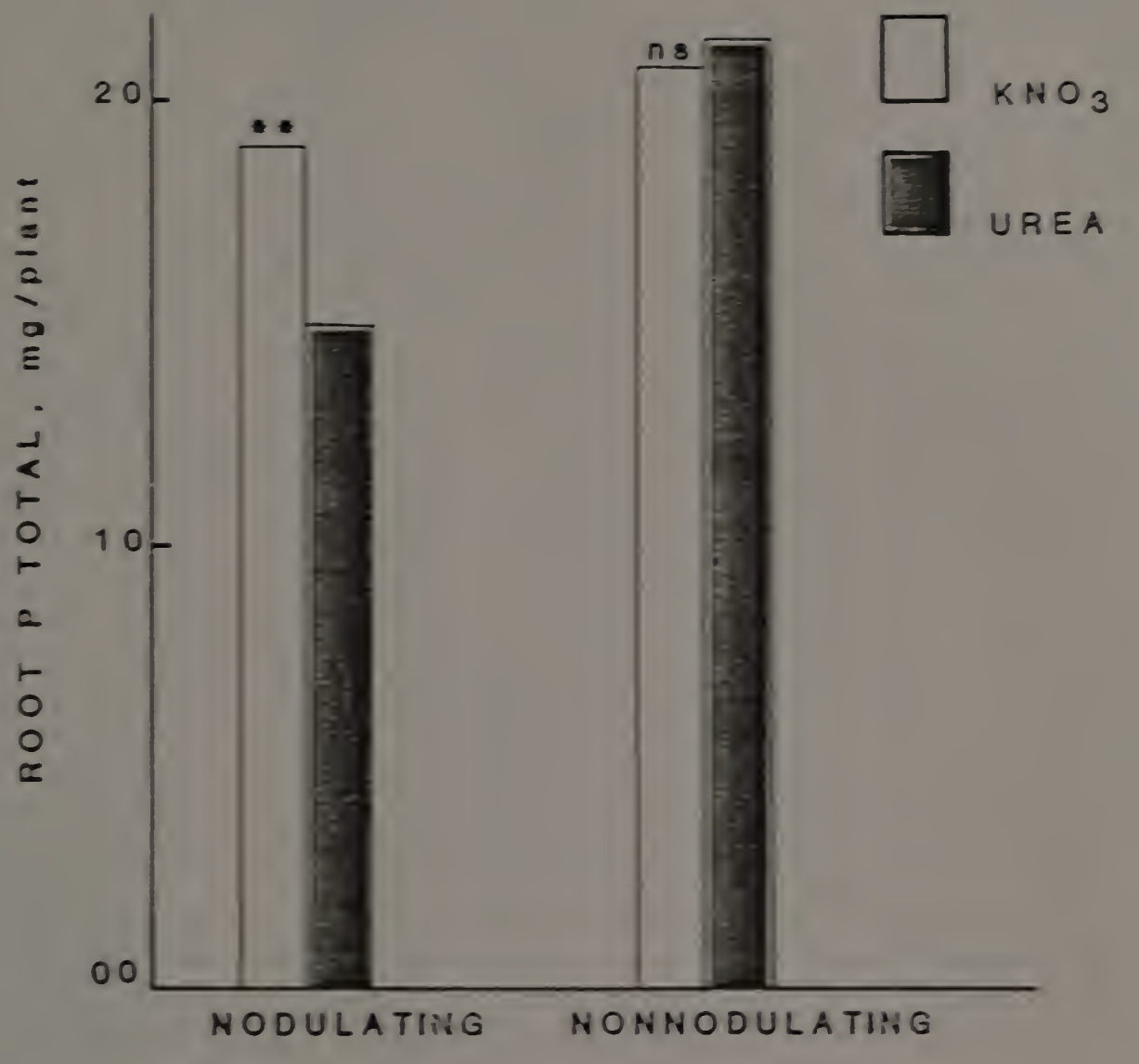


Figure 4.9 Effect of N source within soybean isoline on root P accumulation. ns, **Nonsignficant or significant at 1% level.

Figure 4.10 Nodulating (left) and nonnodulating (right) plants treated with high and low levels of nitrate. High and low levels of nitrate refer to 5mM and 10mM N for nodulating plants and 10mM and 20mM N for nonnodulating plants.

Figure 4.11 Younger leaves of the nonnodulating plants treated with the higher level of urea. Dark green color, cupping and malformation symptomatic of ammonia toxicity. Higher level of urea refer to 20mM of N.

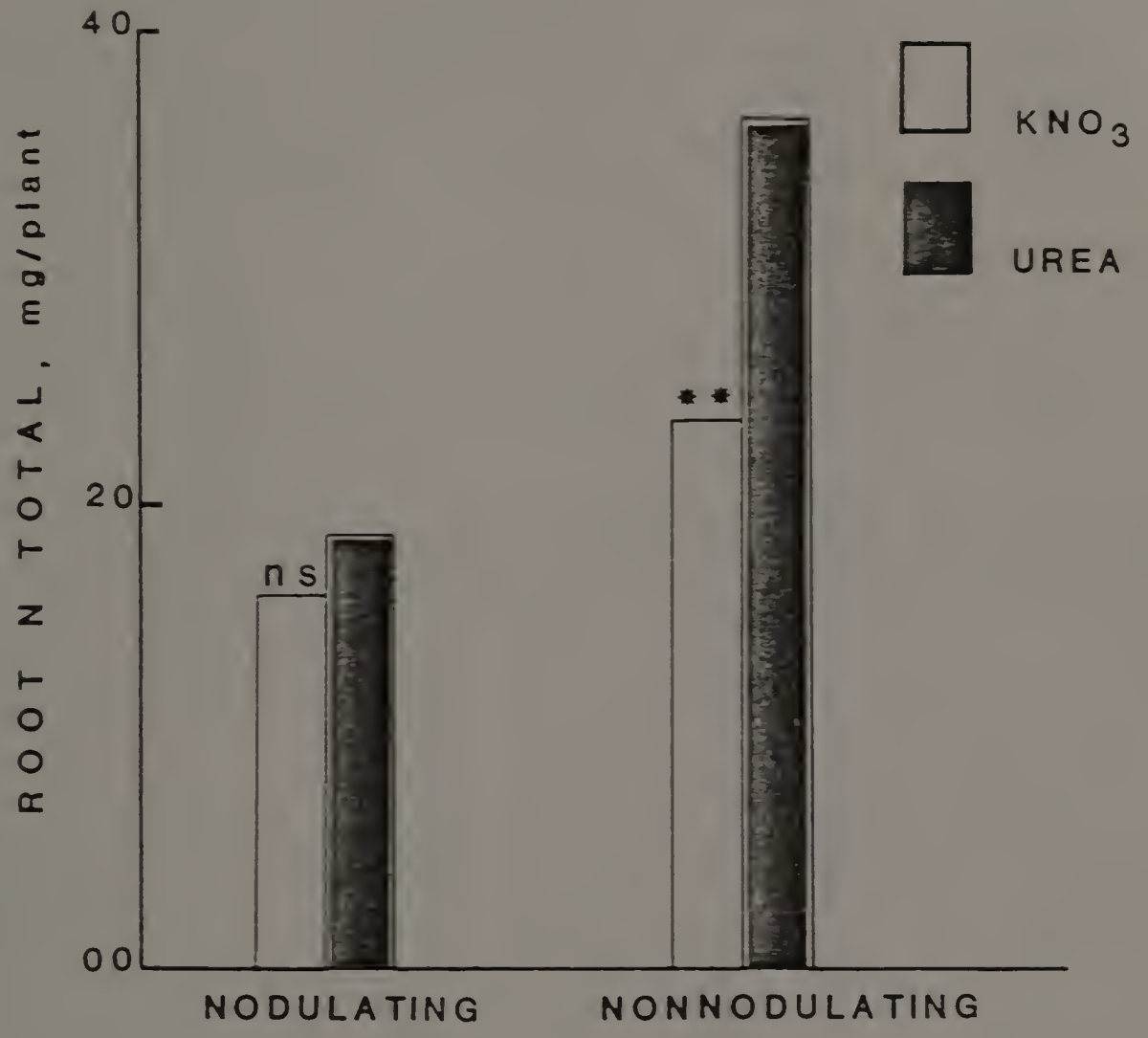


Figure 4.12. Root N totals as affected by nitogen source within soybean isoline. ns, **Nonsignficant or significant at 1% level.

4.1). Plants treated with a higher level of urea however had their root and shoot N percentages increased to a degree (Table 4.2) that exceeded the sufficiency levels of nitrogen 4.26 to 5.5% (Small and Ohlrogge, 1977) for normal growth. Increased tissue N content of plants treated with the higher level of urea (Table 4.2) was associated with a sharp drop in shoot dry weights and shoot N totals (Table. 4.1).

The N percentage of plants treated with the higher level of $NO_3$ did not show increases parallel to the increases in N percentage of the plants treated with the higher level of urea (Table 4.2). Neither did the plants treated with the higher level of $KNO_3$ show a depression in growth, as did the plants treated with the higher level of urea (Fig. 4.3 and Table 4.5). Apparently urea is the more beneficial form of N than $KNO_3$ to plants, when tissue N levels are not raised beyond the N sufficiency range. However, when the 2 nitrogen sources are provided at levels comparable to the higher levels of N supplied in this experiment, the benefits of urea tend to get lost due to ammonium toxicity. The less vigorous growth, flaccidity, dark green color, cupping and malformation of leaves (Bennett, 1974) of the nonnodulating plants treated with the higher level of urea were evidence of the ammonium toxicity. Higher P availability led to overall increases in shoot N totals (Fig. 4.13).

Plant phosphorous composition. The nitrogen source did not cause a difference in the root P totals of the nonnodulating isoline (Fig. 4.9). Plants treated with a higher urea level and a higher P supply,

Table 4.2 Shoot and root nitrogen and phosphorous percentages as affected by nitrogen source, nitrogen rate and phosphorous regime.

| N source | N rate mM | P rate ppm | Shoot dry wt. g/plant | Root dry wt. g/plant | Shoot N total mg/plant | Root N total mg/plant |
|---|---|---|---|---|---|---|
| Nodulating Isoline | | | | | | |
| $KNO_3$ | 5 | 100 | 2.07 | 0.61 | 65 | 13 |
| urea | 5 | 100 | 1.99 | 0.49 | 85 | 13 |
| $KNO_3$ | 5 | 200 | 2.24 | 0.60 | 78 | 16 |
| urea | 5 | 200 | 2.60 | 0.66 | 98 | 18 |
| $KNO_3$ | 10 | 100 | 2.47 | 0.55 | 84 | 14 |
| urea | 10 | 100 | 2.80 | 0.72 | 134 | 20 |
| $KNO_3$ | 10 | 200 | 2.48 | 0.64 | 82 | 18 |
| urea | 10 | 200 | 2.78 | 0.70 | 131 | 22 |
| Nonnodulating isoline | | | | | | |
| $KNO_3$ | 10 | 100 | 2.60 | 0.78 | 107 | 21 |
| urea | 10 | 100 | 3.43 | 0.92 | 159 | 29 |
| $KNO_3$ | 10 | 200 | 2.58 | 1.06 | 94 | 29 |
| urea | 10 | 200 | 4.04 | 1.28 | 190 | 39 |
| $KNO_3$ | 20 | 100 | 2.00 | 0.62 | 91 | 19 |
| urea | 20 | 100 | 2.71 | 0.74 | 174 | 39 |
| $KNO_3$ | 20 | 200 | 2.30 | 0.76 | 107 | 25 |
| urea | 20 | 200 | 2.34 | 0.76 | 152 | 39 |
| $LSD_{.05}$ | | | ns | ns | 57 | ns |

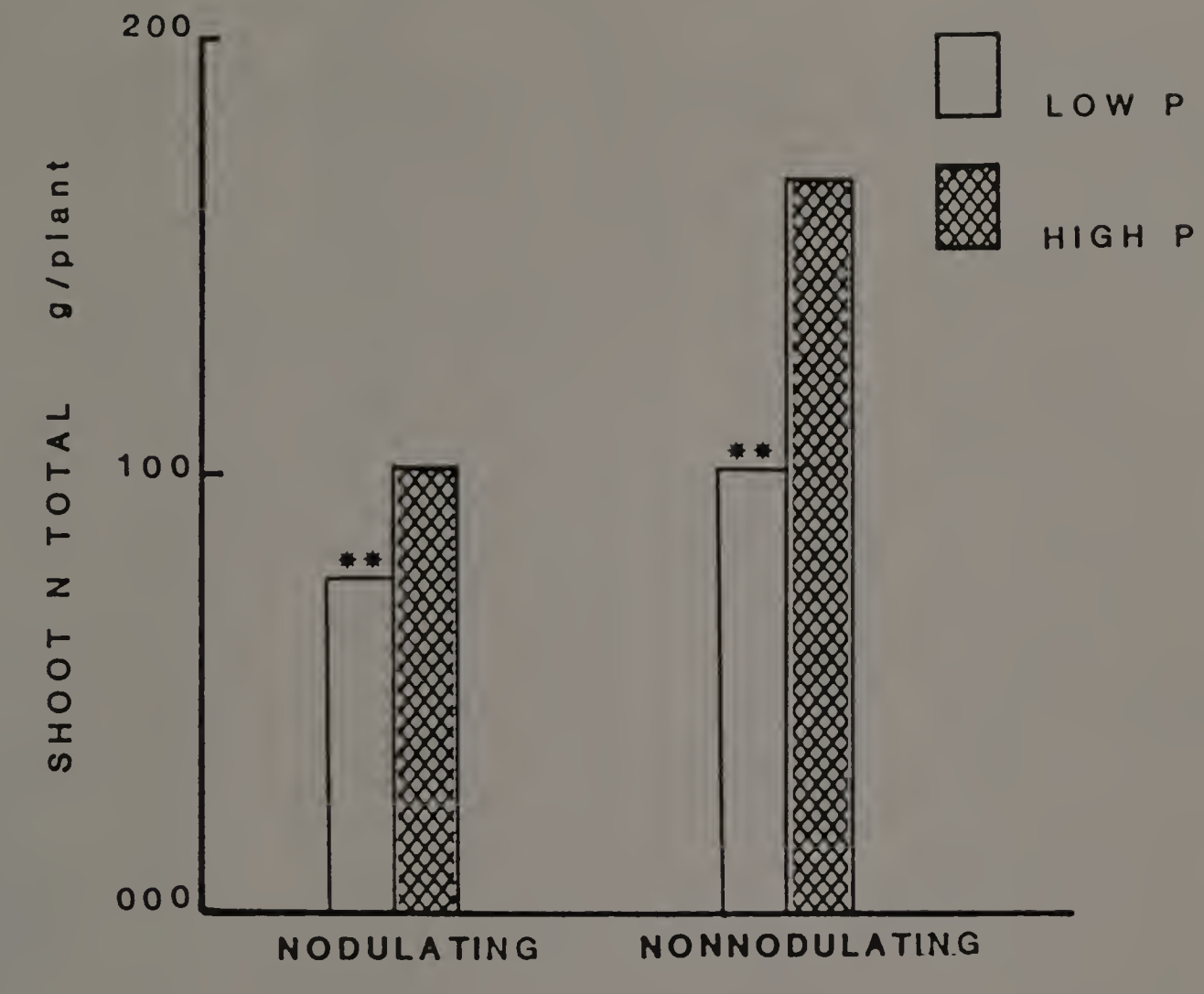


Figure 4.13 Shoot N accumulation as affected by P regime within soybean isoline. *Significant at 5% level. Low and high levels of N refer to 5mM and 10mM N for nodulating plants and 10mM and 20mM N for nonnodulating plants.

showed a dramatic reduction in shoot P totals , paralleling a reduction in shoot N totals (Fig. 4.5 and 4.8).

The depressions in tissue P percentage that accompanied the higher level of P supply (Table 4.2) could not be accounted for by a tissue dilution effect, since shoot dry weights also decreased (Table 4.1). These decreases in shoot dry weights were coupled with sharply increased N percentages of the root and shoot that clearly exceeded the sufficient N (4.26-5.5%) range (Small and Ohlrogge, 1977). Presumably, the elevated N levels, lead to nutritional imbalances (Hiatt and leggget, 1976), that were detrimental to the plant. Lowered P accumulation and sharply depressed growth were evidence of these nutritional imbalances (Fig. 4.3 and 4.8).

Comparison of Growth of Nodulating and Nonnodulating Plants

The nonnodulating isoline had higher shoot dry weights than the nodulating isoline (Fig. 4.14). The higher level of $NO_3^-$ supply did not lead to increased shoot dry weights, shoot N totals, or shoot P totals of either isoline (Fig. 4.3, 4.5, and 4.8). The higher level of urea supply led to increased shoot dry weights, shoot N totals, and shoot P totals of the nodulating plants (Fig. 4,5 and 4.8). Presumably, any advantage gained by increased nitrate supply to the nodulating plants was offset by the inhibitory effect of $NO_3^-$ on $N_2$ fixation (Streeter, 1985). Supply of a higher level of urea must have been deleterious to the nonnodulating isoline, since it led to

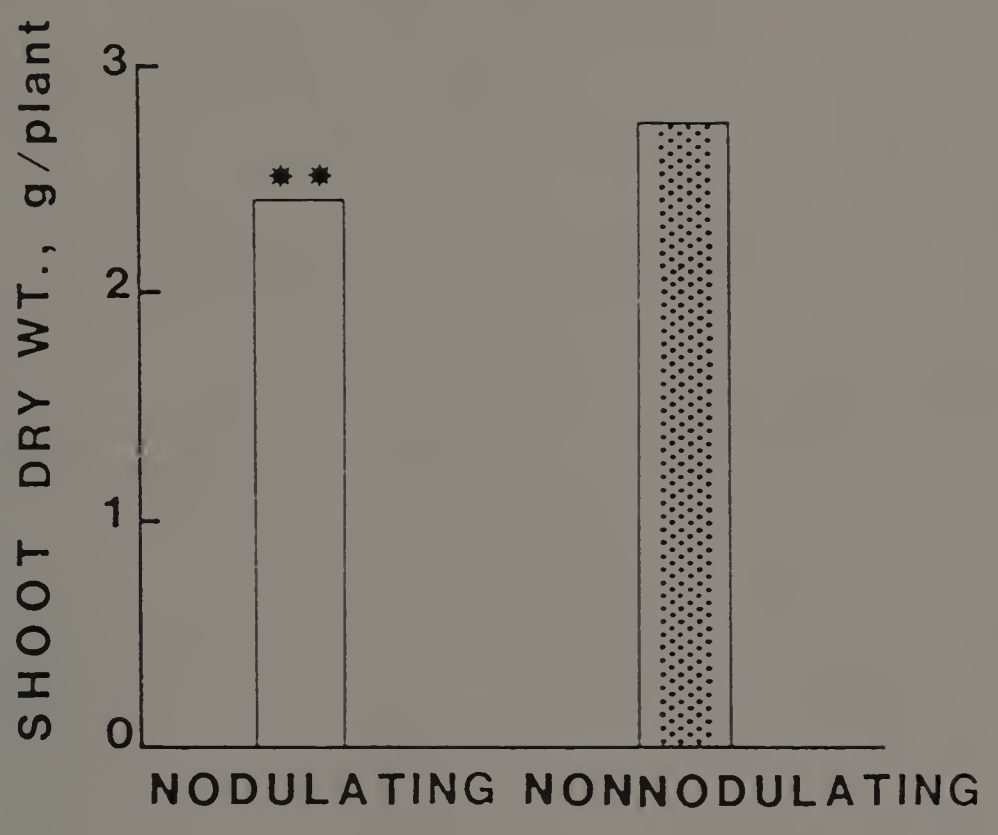


Figure 4.14 Effect of soybean isoline on shoot dry weights. **Significant at 1% level.

decreased shoot dry weights and shoot and root N totals (Fig. 4.5, and 4.8). The improved growth of the nodulating plants, associated with the higher level of urea nutrition, supports the theory of synergism between urea nutrition and nitrogen fixation. Nodulating soybean showed enhancements of shoot dry weights and shoot N and P totals under both P regimes, when urea was provided at the higher level (Fig. 4.5 and 4.8 and Table 4.1). Failure of shoot dry weights of the nodulating soybean to increase when the nitrate supply was increased, in spite of an enhanced P level may be because the tissue N levels did not increase parallel to the increae in tissue P levels (Fig. 4.5 and 4.8). Adverse effects of nitrate ion on $N_2$ fixation and other metabolic events could account for the absence of a positive response to increased nitrate supply by the nodulating plants treated with the higher level of nitrate (Haynes and Goh, 1977; Streeter, 1985).

When nodulating and nonnodulating plants were fertilized with identical concentrations of N in the form of urea, nodulating plants showed the poorer shoot dry weights (Fig. 4.15). This observation cannot be attributed to P limitation since P percentage of these plants fell within the adequate range unlike the previous experiment. Possibly less energy is available for increases in dry matter in the nodulating plants since some energy is used for $N_2$ fixation. The recently confirmed reports of denitrification by rhizobial bacteria may account for some lack of fertilizer efficiency of this isoline (O'Hara and Daniel, 1985). Nonnodulating isolines have been reported previously to give greater increases in attributes such as seed and

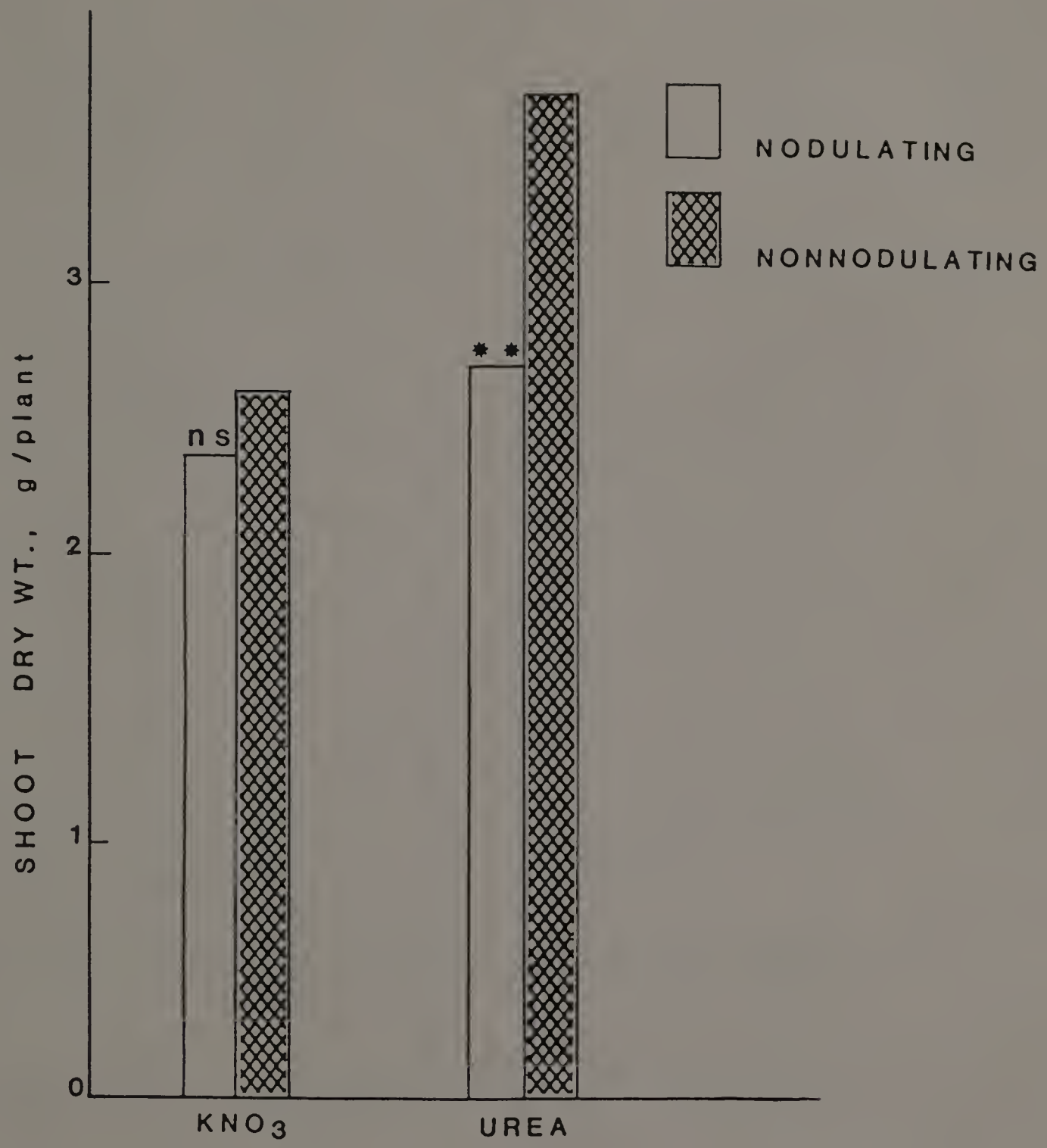


Figure 4.15 Effect of N source and soybean isoline on shoot dry weights of the nodulating and nonnodulating plants supplied by identical concentrations of nitrogen. ns, **Nonsignficant or significant at 1% level.

dry matter yields, seed size and plant height relative to nodulating isolines, when increased amounts of N were supplied, under the same conditions (Weber, 1966). However, these attributes of the two isolines are reported to become equalized with stresses for moisture, N or both.

Shoot P accumulation of both isolines depended upon a significant 3-way interaction of soybean isoline, N source, and level of N. When P supply was low, all plants treated with the higher level of N had significantly enhanced shoot and root P totals (Fig. 4.15 and 4.16) relative to the plants treated with the lower level of N. When P supply was high, level of N did not have a significant influence on the shoot P totals (Fig. 4.16). Root P totals, however, were decreased in parallel with increased N supply under the high P regime (Fig. 4.17). Thus under conditions of restricted P supply, enhancing the N supply led to a greater accumulation of P. Nitrogen and phosphorous uptake appear to be synergistic, when P is limiting.

A delicate balance among the N source, its availability, and P regime appeared to govern the capacity of the soybean plant to accumulate P and N. The results reveal the following fertilizer combinations to be effective for nodulating soybean:(1). A lower $NO_3^-$ level combined with a higher P regime, or (2). A higher urea level combined with a lower P regime. The lower level of N combined with the higher P regime was more effective for the nonnodulating soybean. An explanation for the preference of a lower $NO_3^-$ level by the nodulating plants is available since higher $NO_3^-$ levels are inhibitory

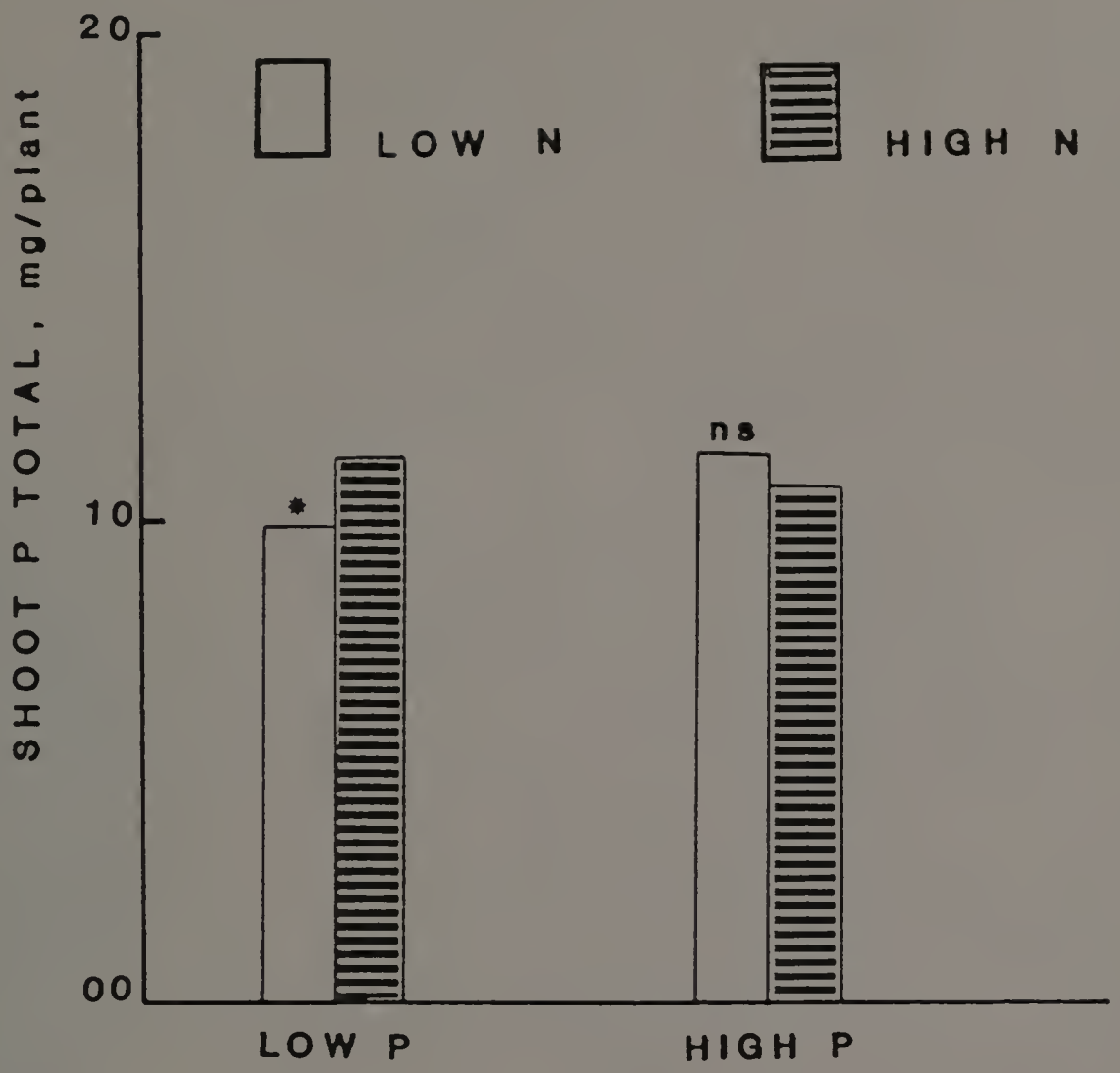


Figure 4.16 Shoot P accumulation as affected by N rate within P regime. ns,*nonsignificant or significant at 5% (*) level. Low and high levels of N refer to 5mM and 10mM N, for nodulating plants and 10mM and 20mM N for nonnodulating plants.

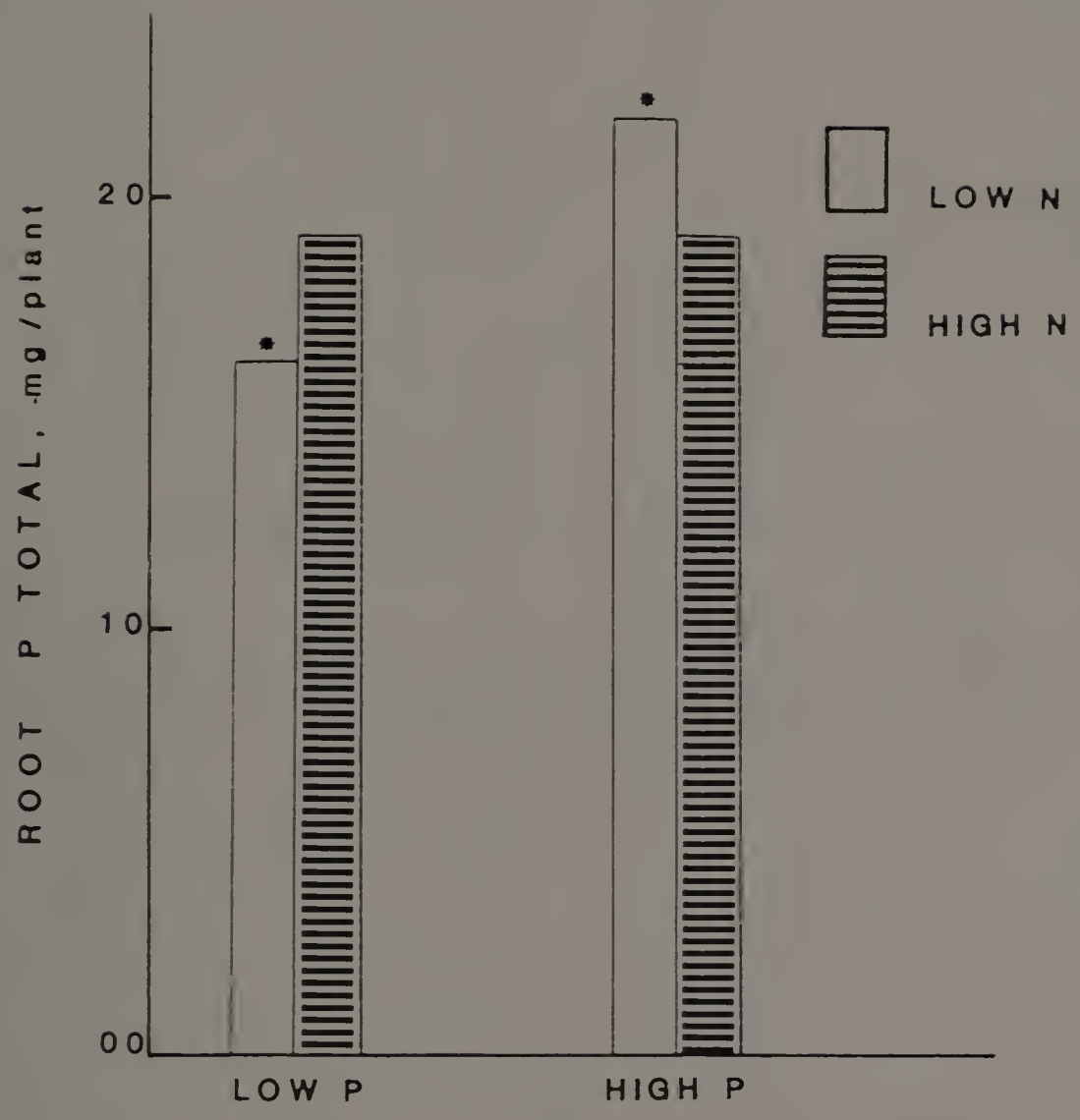


Figure 4.17 Effect of N rate within P regime on root P accumulation.

to $N_2$ fixation. Concomitantly, a higher P regime would supply more energy for $N_2$ fixation. Alternately, urea would be preferred at enhanced levels by the nodulating plants, since urea supplements the plant with N, without inhibiting $N_2$ fixation. For the nonnodulating plants the lower level of N obviously was preferable, since the higher level of N led to less growth (Fig. 4.3).

Root N/P Ratios

Root N/P ratio of urea-treated plants were higher within both isolines, than the root N/P ratios of nitrate-treated plants (Table 4.3). Within the nodulating isoline, the lower levels of both N sources favored the higher root N/P ratios (Table 4.4). Although a drastic increase in root N/P ratio occurred in the nonnodulating, urea-treated plants, when they were subjected to the higher p regime, positive conclusions cannot be drawn from this result, since the plants suffered ammonium toxicity at this stage. Generally, the higher P supply did not cause a significant difference to the root N/P ratios (Table 4.5).

The mechanism by which the root N/P balance was adjusted in response to N source differed for the 2 isolines. In the nonnodulating isoline, the increase in root N/P ratios in response to urea ntrition was brought about by an increase in the root N totals (Fig. 4.12), while the root P totals remained the same as with nitrate nutrition (Fig. 4.9). In the nodulating isoline, this increase in

Table 4.3 Effects of soybean isoline, and N source on root N/P ratios.

| | $KNO_3$ | UREA |
|---|---|---|
| Nodulating | 8.51 | 12.31** |
| Nonnodulating | 11.31 | 17.89** |

**Signficant at 1% level, rows.

Table 4.4 Effects of soybean isoline, nitrogen source, and nitrogen rate on root N/P ratio.

| N source | N rate | Nodulating | Nonnodulating |
|---|---|---|---|
| $KNO_3$ | 1 | 9.65 | 10.97 |
| | 2 | 7.37 | 11.65 |
| urea | 1 | 13.00 | 13.70 |
| | 2 | 11.57 | 22.07 |

$Lsd_{(.01)}$ = 1.43, for soybean isoline, and nitrogen rate. 1 and 2 refer to low and high levels of N, corresponding to 5 and 10mM for nodulating plants, and 10 and 20mM for nonnodulating plants.

Table 4.5 Effects of soybean isoline, nitrogen rate and phosphorous rate on root N/P ratio.

| N source | N rate | LOW P | HIGH P |
|---|---|---|---|
| Nodulating isoline | | | |
| $KNO_3$ | 5 | 9.63 | 9.66 |
| | 10 | 6.14 | 8.60 |
| UREA | 5 | 13.39 | 12.62 |
| | 10 | 12.32 | 10.83 |
| Nonnodulating isoline | | | |
| $KNO_3$ | 10 | 10.86 | 11.09 |
| | 20 | 10.95 | 12.37 |
| UREA | 10 | 14.43 | 12.98 |
| | 20 | 19.70 | 24.44 |

$Lsd_{(.01)}$ = 2.0234, rows. Low P and high P refer to 100 and 200 ppm, phosphorous supplied as dicalcium phosphate.

root N/P ratios was accomplished by a reduction in root P totals while root N totals remained the same, as with nitrate nutrition (Fig. 4.9 and 4.12).

The final root N:P balance derived under a urea/low P combination may be a unique physiological factor that give urea-treated plants a specific vulnerability to VAM infection. A low root P status and a high root N content are the favored prerequisites for successful VAM infection (Hepper, 1983). Soybean plants supplied with urea appear to achieve this favorable root N:P composition, as indicated by the previous experiment and confirmed by the results of this experiment where plants were grown under P sufficiency.

# C H A P T E R V

## ROLE OF FOLIAR OR SOIL APPLIED PHOSPHOROUS ON ESTABLISHMENT OF MYCORRHIZAL INFECTION

### Materials and Methods

Greenhouse experiments were conducted to compare the effect of foliar P and soil P on establishment of mycorrhizal infection in nodulating (experiment 1), and nonnodulating (experiment 2), soybean plants. Foliar P applications were used to raise tissue P levels without affecting the level of P in the soil.

Surface-sterilized seeds were germinated in sand and inoculated with mycorrhizae according to the procedures given in Chapters II and III. Nutrients other than P were supplied to all plants as recommended by Johnson et al. (1957). Nutrient solutions were supplied to the plants 5 days of the week, and the medium was flushed with water on 2 days of the week. Foliar sprays contained 0.01% Tween 80, a surfactant to facilitate uniform spreading and penetration. Stomatal penetration with associated water logging of leaf tissue was not observed. The spray was maintained at pH 5. Foliar spraying was started at the 7-leaf stage. Application of foliar fertilizer were made 3 times a day with a hand sprayer, early morning, late afternoon,

and night. Upper and under surfaces of leaves were sprayed to runoff. A portion of the solution added was retained by the leaves, and the rest dripped off. To prevent drops of solution falling off the leaves reaching the soil, the pots were covered with plastic sheets at the time of application. All plants were given a basic soil dressing of 150 mg of hydroxyapatite per pot, to ensure that the foliar P supplied plants were not P deficient at the juvenile stage, when P demand was highest and leaf surface area a minimum. Also foliar P applications were not begun until the 7 leaf stage. Solutions for the low and high foliar applications of P contained 0.05 percent and 0.1 percent P. Low and high soil P applictions contained 1mM and 2 mM solutions of $KH_2PO_4$. Plants were harvested biweekly beginning the fourth week. Final harvest was at the mid-podset stage. Roots and shoots were separated and analyzed for P as described in Chapter III. Tissue zinc levels were measured by atomic absorption spectrophotometry after dry ashing the tissue and dissolving the ash in HCl. Data were analysed using Analysis of Variance. If the F test was significant at $P< 0.01$ or $P< 0.05$, pairwise comparisons among treatment means were undertaken using the method of Least Significant Difference.

## Results and Discussion

Trends in the establishment of VAM infection were similar in

nodulating and nonnodulating plants in response to the different treatments applied.

Tissue P levels in plants treated with high soil P (Table 5.1 and Table 5.2 ) exceeded levels of P favourable for normal growth (Small and Ohlrogge, 1977). These plants also exhibited symptoms of a mineral disorder (Fig. 5.1) similar to Zn deficiency. Reportedly soybean plants with excessively high tissue P contents suffer Zn deficiency (Paulsen and Rotimi, 1968). Analysis for zinc however did not indicate deficient shoot or root zinc levels (Table 5.3 and 5.4). Sharpe et al. (1984) observed a linear reduction in soybean leaf zinc with increasing levels of applied P, but the tissue Zn levels did not fall below the sufficiency range. Takkar et al. (1975) found the P-Zn disorder to be related to the P/Zn ratio in different parts of the plant than either with the P or Zn content of tissues. An incipient chlorosis appeared in the tips of older leaves treated with foliar P at the high rate (Fig. 5.2). Symptoms of mineral disorder however were absent in the plants treated with foliar P at the low rate (Fig. 5.2).

The consistently low percentage of VAM infection found in plants treated with soil P at the high rate (Table 5.5 and 5.6) can be attributed to a combination of a high tissue P level and a high soil P level.

The intramatrical mycelium of the roots with high root P contents appeared less clear (Fig. 5.3) and stained more lightly than the mycelium of the roots treated with lower levels of P (Fig. 5.4).

Table 5.1 Shoot phosphorous concentration in nodulating isoline as affected by harvest, foliar or soil feeding and, concentration of phosphorous applied.

| Treatment | Harvest | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Foliar low P | 0.27 | 0.31 | 0.28 | 0.30 |
| Soil low P | 0.28 | 0.32 | 0.28 | 0.32 |
| Foliar high P | 0.44 | 0.52 | 0.40 | 0.51 |
| Soil high P | 0.48 | 0.57 | 0.62 | 0.64 |

$LSD_{(.05)}$ = .04, columns.

Table 5.2 Shoot phosphorous concentration in nonnodulating isoline, as affected by harvest, foliar or soil phosphorous feeding, and concentration of phosphorous applied.

| Treatment | Harvest | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Foliar low P | 0.25 | 0.30 | 0.33 | 0.31 |
| Soil low P | 0.28 | 0.32 | 0.37 | 0.36 |
| Foliar high P | 0.39 | 0.37 | 0.37 | 0.38 |
| Soil high P | 0.39 | 0.40 | 0.56 | 0.67 |

$LSD_{(.01)}$ = .04, columns.


Figure 5.1 Plant treated with soil P at the high rate exhibits symptoms of mineral disorder. Plant treated with foliar P at the high rate lack deficiency symptoms, to a large extent.

Table 5.3 Shoot and root zinc concentrations in the nodulating isoline.

| Treatment | Harvest | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| | ppm | | | |
| Shoot | | | | |
| Foliar low P | 32 | 37 | 49 | 37 |
| Soil low P | 31 | 53 | 46 | 30 |
| Foliar high P | 30 | 46 | 53 | 37 |
| Soil high P | 28 | 28 | 37 | 20 |
| Root | | | | |
| Foliar low P | 41 | 43 | 45 | 40 |
| Soil low P | 48 | 43 | 34 | 34 |
| Foliar high P | 41 | 33 | 45 | 43 |
| Soil high P | 30 | 42 | 28 | 32 |

Table 5.4 Shoot and root zinc concentrations in the nonnodulating isoline.

| Treatment | Harvest | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| | ppm | | | |
| Shoot | | | | |
| Foliar low P | 41 | 33 | 35 | 60 |
| Soil low P | 33 | 30 | 67 | 49 |
| Foiar high P | 59 | 33 | 59 | 46 |
| Soil high P | 27 | 18 | 59 | 40 |
| Root | | | | |
| Foliar low P | 52 | 38 | 43 | 47 |
| Soil low P | 41 | 37 | 39 | 46 |
| Foliar high P | 53 | 36 | 61 | 59 |
| Soil high P | 40 | 33 | 39 | 35 |


Figure 5.2 Symptoms of mineral disorder are absent from the plant treated with foliar P at the low rate. Plant treated with foliar P at the high rate exhibit incipient necrosis at the tips of older leaves.

Table 5.5 Percentage of VAM infection in the nodulating isoline as affected by harvest, foliar or soil phosphorous feeding and concentration of phosphorous applied.

| | H1 | H2 | H3 | H4 |
|---|---|---|---|---|
| Foliar low P | 39 | 57 | 70 | 78 |
| Soil low P | 22 | 44 | 60 | 67 |
| Foliar high P | 18 | 41 | 45 | 22 |
| Soil high P | 11 | 13 | 11 | 11 |

$LSD_{(.05)}$ =7, columns.

Table 5.6 Percentage of VAM infection in the nonnodulating isoline, as affected by harvest, foliar or soil phosphorous feeding and concentration of phosphorous applied.

| | H1 | H2 | H3 | H4 |
|---|---|---|---|---|
| Foliar low P | 31 | 50 | 62 | 74 |
| Soil low P | 18 | 30 | 53 | 65 |
| Foliar high P | 14 | 26 | 25 | 25 |
| Soil high P | 05 | 09 | 09 | 08 |

$Lsd_{(.01)}$ = 8, columns.

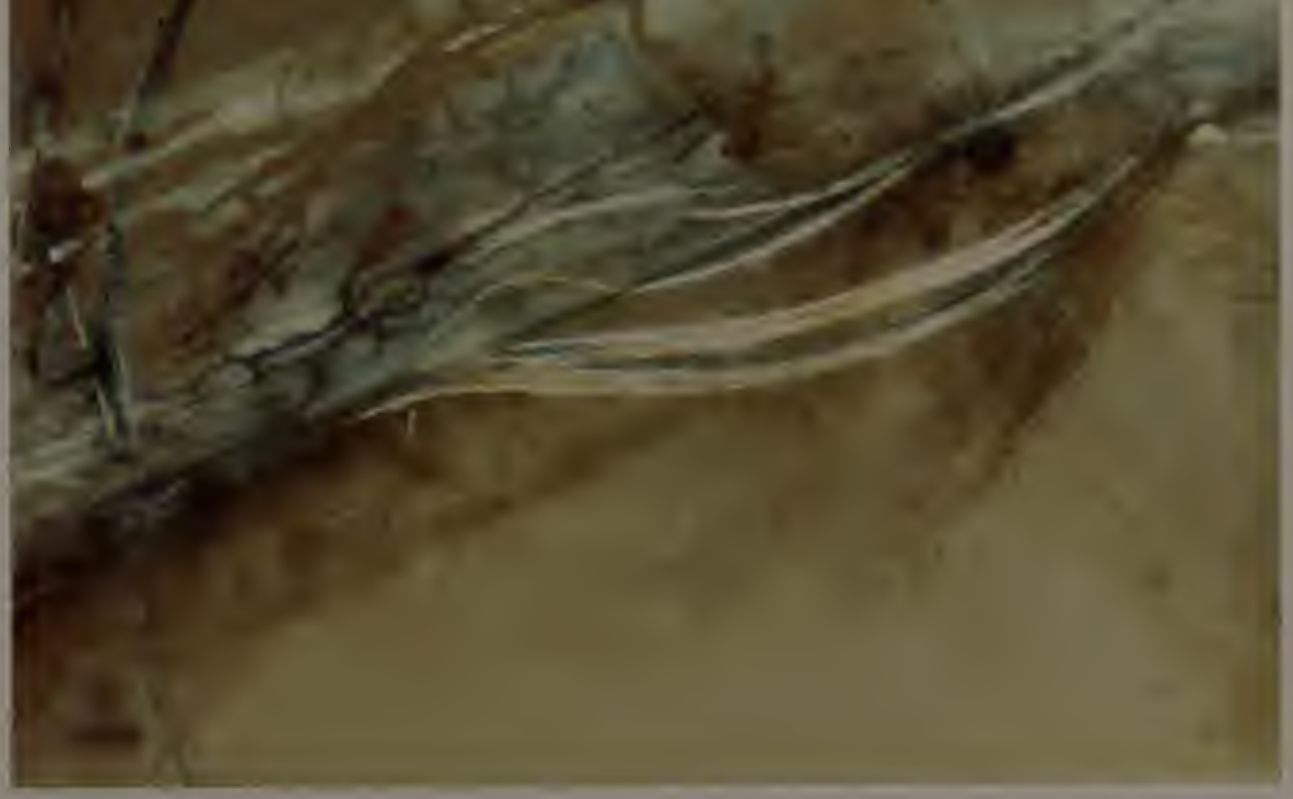

Figure 5.3 Roots of plant having high root P levels, show lightly stained mycelium.

Figure 5.4 Roots of plant having low P levels, show darkly stained mycelium.

Mosse (1973) reported similar results and attributed the lighter stain to thinner walls of the internal mycelium of roots treated with a high level of P.

At the first harvest infection percentage was highest for plants, that received the low foliar application of P, and were lowest for plants that received the high soil application of P (Table 5.5 and 5.6). Root P concentrations were similar in plants that received P foliarly and as a soil dressing at the low rate (Table 5.7 and 5.8). In spite of the similar root P concentration, VAM infection was lower in the plants that received P as a soil dressing (Table 5.5 and 5.6). Infection percentage should have been similar in the low foliar P and low soil P treated roots, if tissue P was the only factor that determined VAM infection.

The infection pattern for the second harvest was similar to that of the first harvest. The plants supplied with P foliarly at the low rate had the highest pecentage of VAM infection (Table 5.5 and 5.6). Plants treated with P foliarly at the high rate and plants treated with P as a soil dressing at the low rate had similar levels of infection (Table 5.5 and 5.6).

At the third harvest, plants having lower root P percentages (Table 5.7 and 5.8) had higher infection levels (Table 5.5 and 5.6) than the plants that had higher root P percentages. Unlike the first two harvests the plants treated with low soil P (having relatively low tissue P) now had higher infection percentage than plants that received high P foliarly (having relatively high tissue P) (Table 5.5

Table 5.7 Root phosphorous concentration in nodulating plants as affected by harvest, foliar or soil phosphorous feeding, and concentration of phosphorous applied.

| | H1 | H2 | H3 | H4 |
|---|---|---|---|---|
| Foliar low P | 0.24 | 0.29 | 0.23 | 0.28 |
| Soil low P | 0.26 | 0.32 | 0.26 | 0.32 |
| Foliar high P | 0.39 | 0.42 | 0.32 | 0.47 |
| Soil high P | 0.48 | 0.49 | 0.43 | 0.58 |

$Lsd_{(.05)}$ = .05, columns.

Table 5.8 Root phosphorous concentration in nonnodulating plants as affected by harvest, foliar or soil phosphorous feeding, and concentration of phosphorous applied.

| | | | | |
|---|---|---|---|---|
| Foliar low P | 0.22 | 0.27 | 0.30 | 0.29 |
| Soil low P | 0.23 | 0.28 | 0.31 | 0.31 |
| Foliar high P | 0.35 | 0.37 | 0.42 | 0.43 |
| Soil high P | 0.51 | 0.49 | 0.55 | 0.49 |

$LSD_{(.01)}$ = .08, columns.

and 5.6). Apparently, at this stage soil P was less important in inhibiting VAM infection perhaps because increase of infection mainly was due to spread of already established infection cushions. The trends in infection pattern obtained at the third harvest were unaltered at the final harvest indicating that tissue P was the primary factor in the determination of infection spread (Table 5.5 and 5.6), when infection was advanced.

Results of this experiment indicate that soil P or foliar P can exert inhibitory effects on infection. The inhibitory effects due to high soil P may operate indirectly via an inhibition on infection establishment. Mechanisms of inhibition due to soil P or tissue P may exist in a state of dynamic equilibrium. The stage of development of the mycorrhizal root presumably determines the dominance of one mechanism over the other, e.g., at the 3rd and 4th harvests, tissues with lower P concentrations had consistently higher VAM infection percentages. At the 1st and 2nd harvests, however, soil P apparently exerted a greater inhibitory effect than tissue P because the infection remained low in the plants that recived P as a soil dressing at the low rate, in spite of low root P levels (Table 5.7 and 5.8). Infection percentages of the roots of these plants were similar to the infection percentages of the roots of plants that received P foliarly at a high rate and which also contained higher tissue P levels (Table 5.7 and 5.8).

Sanders (1975) supplied P as a foliar dressing to onion plants and observed a substantial reduction in VAM infection. Based on his

data, Sanders concluded that the effects of soil P in reducing infection were root mediated. Menge et al. (1978) applied P to one half of a split root system, and found a reduction in VAM infection in the half of the root to which P was not applied. These experiments supported the hypothesis that high tissue P inhibited VAM infection. Increasing the P content of the soils into which plants were transferred reduced the percentage of VAM infection (Azcon et al., 1982) implying an inhibition of VAM infection by soil P.

Sanders (1975) described the process of development of mycorrhizal root system, in terms of three phases: an establishment phase, a phase when infection increases rapidly, and an equlibrium phase when percentage infection tends to a constant value. The sequence of infection seen in the plants treated with P foliarly at the high rate and those treated with soil P at the low rate may be explained in light of these observations. Since establishment of infection plays a major role in early infection stages, it is possible that soil P exerted stronger control than tissue P in inhibiting infection at this early stage. The lower infection of the plants treated with low soil P in spite of a low root P content is evidence of this. At the first two harvests, the plants treated with foliar P at the high rate had infection levels similar to the plants treated with soil P at the low rate even though the root tissue P was lower. This difference may have been because, VAM establishment was facilitated in the plants treated with foliar P at the high rate due to a lack of inhibition by externally applied phosphate. Importance

of tissue P as an inhibitory factor of VAM infection gained importance during the later stages, when infection failed to increase significantly in spite of high early establishment. In the plants treated with soil P at the low rate however infection continued to increase beyond the 3rd harvest, since tissue P was not an inhibitory factor here.

The lower level of infection of the plants treated with high soil P, than the plants treated with high foliar P cannot be attributed to soil P inhibition alone. The high soil P treated plants had higher levels of root P, in spite of the efforts made to maintain tissue P levels comparable. Uptake of P through the leaves is not as effective as uptake through the roots, for at least three reasons: 1, solution runs off when spraying, and consequently the short duration of contact between the fertilizer and leaves 2, phosphorous does not readily penetrate the cuticle, and 3, poor translocation of P from treated areas of soybean leaves (Neaumann, 1979). Sensitivity of soybean to foliar applied P does not allow application of high levels of foliar P (Barel and Black, 1979). Comparable root P levels however were obtained in the case of plants treated with soil P and foliar P at the low rate (Table 5.7 and 5.8).

# C H A P T E R VI

## PHOSPHATE UPTAKE BY MYCORRHIZAL AND NONMYCORRHIZAL ROOTS OF (GLYCINE MAX MERR. CV CLAY)

### Materials and Methods

Phosphorous influx was studied on mycorrhizal and nonmycorrhizal roots of soybean (Glycine max Merr. cv. Clay). Seeds were surface-sterilized as described in Chapter III. Plants were grown in 1.8 litre plastic pots, filled with a coarse quartz sand that had been washed repeatedly with a solution of 0.2mM $CaCl_2$ and 0.2mM KCl. Mycorrhizal inoculum was introduced as described in Chapter II. Several surface sterilized seeds were planted in each pot, irrigated with water until healthy cotyledons and growing points emerged and then thinned to 5 plants per pot. Plants were watered for 15 days and thereafter were provided with a nutrient solution as recommended by Johnson et al., (1957). Phosphorous was supplied at 1/4 the recommended level. At the end of 39 days (when substantial mycorrhizal infection was established in the roots), plants were uprooted from the sand, with minimum disturbance to the extramatrical mycelium. Roots were washed free of sand using deionized water. Five plants each were transplanted to pots containing 2.6 liter of aerated

nutrient solution (Table 6.1). The plants were grown according to the procedure given by Nielsen and Barber (1978). The electrical conductivity of the nutrient solution was maintained at 0.8mmhos at 25°C. This conductivity was reestablished daily by additions of solution 11 (Table 6.1), in which the ratios between nutrients were selected considering the ratio at which these nutrients are absorbed by plants. Solution pH was measured daily and adjusted to 5.5 with NaOH or HCL. The pH change did not deviate more than 0.5 pH units. The plants were grown in the greenhouse during March, April, and May for the first part of the experiment and transferred to a controlled climate chamber for the uptake study. The growth chamber temperature was 28°C during the 16 hour day and 21°C during the night. The greenhouse temperatures were similar, and supplemental light was used to give a 16-hour day.

When 54-days-old (15 days after transplanting to nutrient solution), the plants were transferred to a pretreatment solution (-P) in a controlled climate chamber. After 24 hours of pretreatment, plants were transferred to a solution containing 30uM of phosphorous. Uptake of P was measured by determining depletion of the ion from solution similar to that used by Olsen (1955). The solution was sampled every 15 min for the first 2 hours and then sampled every 20 min upto the eighth hour. The P concentration determined using the method of Olsen and Watanabe (1955) reached a minimum after about 6 hours. The sampling was continued for an additional 1 to 2 hours. The solution volume was maintained constant by replacing the amount of

Table 6.1 Composition of nutrient solutions 1 and 2.

| Solution | K | Ca | Mg | $NH_4$ | $NO_3$ | $SO_4$ | Fe | Mn | Zn | Cu | B | Mo |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mM | | | | | | uM | | | | | |
| 1 | 1 | 1 | 1 | 1 | 5 | 0.5 | 70 | 10 | 1 | 0.5 | 40 | 0.5 |
| 2 | 38 | 2 | 3 | 10 | 50 | 4 | 130 | 20 | 2 | 1 | 74 | 1 |

(Edwards and Barber, Ag. J.)

sample removed with deionized water. At the completion of the experiment, the plants were harvested and root fresh weights were determined. Root lengths were determined by the line intercept procedure, of Tennnant (1975). In this procedure, roots are spread over grid squares, and the number of interceptions of roots with horizontal and vertical grid lines are counted. When the grid dimension is 1 cm, the number of intercept times 11/14 gives the root length in cm. Mean root radius can be calculated by assuming that fresh roots have a density of $1Mg/m^3$.

The nutrient-depletion procedure of Claasen and Barber (1974) was used to measure the P absorption parameters $V_{max}$, maximal P influx rate; $K_m$, the concentration where influx was 0.5 $V_{max}$. Terms used to describe ion movement are: influx, movement of ions from the external solution into the root; I, the rate of influx per unit length of root; efflux, the movement of ions out of the root into the external solution; E, the rate of efflux per unit length of root and $I_n$, the net rate of influx per unit root length which is equal to I-E. When plants are grown in a solution of a constant volume, v, the decrease in ion content of the solution measures the net amount of the respective ions absorbed. The amount of ions in the nutrient solution, Q, is given by the equation 1 where c is the ion concentration in solution.

$$Q = cv \qquad (1)$$

A plot of Q versus time, gives a curve showing the depletion of the ion from solution resulting from plant absorption and is called

the depletion curve. The net influx per pot at any point on this curve is given by -dQ/dt. A rate equation can be developed that relates -dQ/dt to the flux parameters and integrated to obtain Q = f(t). The integrated rate equation can be fit to the experimental data of the depletion curve to estimate the parameters that characterize ion uptake kinetics. The degree to which the proposed rate equation describes ion uptake kinetics will be indicated by how well the experimental data follow the curve predicted from its integration.

When influx is expressed per unit root length, L,

$$I_n = -I\, dQ / L\, dt \qquad (2)$$

By definition:

$$I_n = I - E \qquad (3)$$

To use the rate equation procedure one needs to know the relation between I and c (which is Q/v) and E and c to substitute into equation 3. For many ions, influx over the low concentration range (for P < 1 mM) follows Michaelis-Menten kinetics (Epstein, 1972). Therefore the use of this model to calculate I is justified,

$$I = V_{max} c / K_m + c \qquad (4)$$

Relation between E and c has not been established. Michaelis-Menten kinetics assumes no efflux occurs by the carrier mechanism. Tentatively E has been assigned a constant value, since it is a passive process along the electrochemical gradient(Pitman and Saddler, 1967; Macklon and Higinbotham, 1970).

By using a constant value for E, combining equations 2, 3, and 4,

substituting Q/v for c as shown in equation 1, and rearranging, we obtain the expression describing the rate equation:

$$dQ/dt = -L(V_{max}\ Q/v)/(K_m + Q/v) + LE \qquad (5)$$

Equation 5 can also be expressed in terms of c where v remains constant.

$$dc/dt = -L/v\ (V_{max}c)/(K_m + c) + LE \qquad (6)$$

Integration of equation 5 gives a function that when fit to the experimental data by a least-squares procedure describes the depletion curve. The rate equation, equation 5, was integrated numerically and the experimental data were fit by coupling the numerical integration procedure to a nonlinear regression program. The output gave predicted values of Q and values of $V_{max}$, $K_m$, and E. The values of $V_{max}$, $K_m$, and E were substituted into the expanded form of equation 4:

$$I_n = (V_{max}c/K_m + c) - E \qquad (7)$$

to generate a curve of $I_n$ versus c. The maximum $I_n$ is $V_{max}$ -E and $K_m$ is equal to c where I equals one-half $V_{max}$ and not where $I_n$ equals one-half $V_{max}$ -E.

## Results and Discussion

Mycorrhizal soybean roots showed a substantial level of infection that ranged from 60 to 70%. Noninoculated roots were free of mycorrhizal infection. The mycorrhizal roots depleted the P of the

nutrient solution, at a faster rate, than the nonmycorrhizal plants (Fig. 6.1). Also, the lowest level to which roots can reduce P concentrations in solution was lower for the mycorrhizal plants than for the nonmycorrhizal plants indicating effective utilization of P by mycorrhizal plants when solution P concentrations are minimal. The enhanced rate of P depletion by mycorrhizal plants relative to the nonmycorrhizal plants was reflected partly in increased $V_{max}$ values that were 4 to 5 times higher in mycorrhizal plants than in nonmycorrhizal plants (Table 6.2). Because, increases of $V_{max}$ would accompany enhanced $K_m$ values, the higher $K_m$ values observed in this experiment partly is a consequence of the enhanced $V_{max}$ values of the mycorrhizal plants for $H_2PO_4^-$ ions.

The lower efflux exhibited by the mycorrhizal plants at the lower end of the concentration range, relative to the controls (Fig. 6.2) could confer on the mycorrhizal plants a greater capability of P retention in situations of very poor P availability, because the mycelium would act as an efficient mop for phosphate ions. Phosphorous forms sparingly soluble compounds with divalent and trivalent cations in soil. Because of these reactions, the amount of P in soil solution (even in sufficiently well-fertilized soils) is very small at any given time. The soil solution needs to be renewed several times a day, in order that crop requirements for P are met during the growing season, (Barber, 1962). A limiting soil factor in P uptake by plants is likely to be renewal of the soil solution near the plant roots by processes of dissolution and diffusion (Thomas and

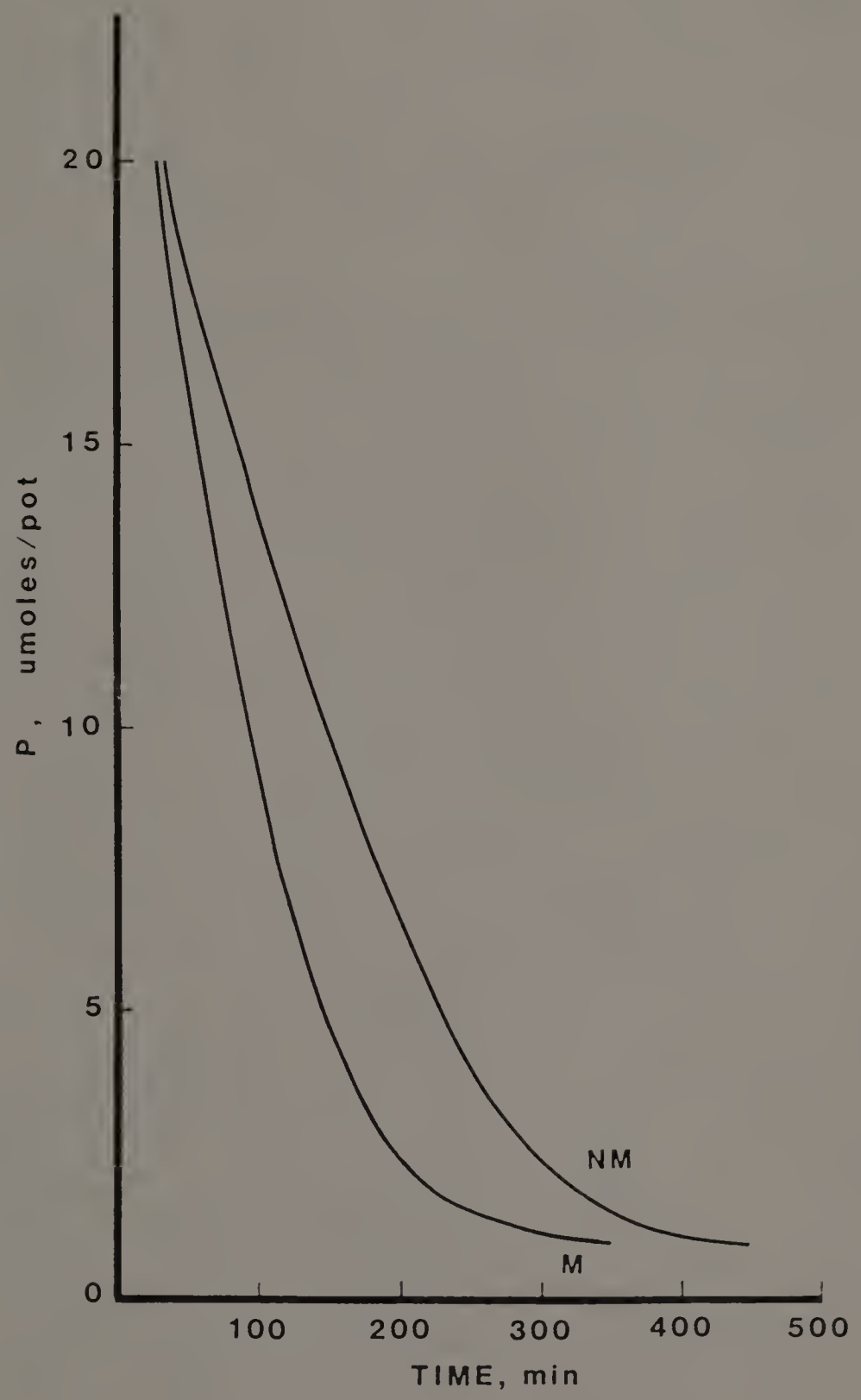


Figure 6.1 Depletion of Phosphorous from 2 litres of solution by mycorrhizal (M) and nonmycorrhizal (NM) soybean roots with time. The curve was calculated using an expression based on Michaelis -Menten kinetics.

Table 6.2 Kinetic constants of initial rates of P absorption by mycorrhizal and nonmycorrhizal soybean roots.

| | Concentration Range | | |
|---|---|---|---|
| | 1 to 30 uM $KH_2PO_4$ | | |
| | $K_m$ umol $L^{-1}$ | $V_{max}$ nmol $sec^{-1}$ $m^{-2}$ | E nmol $sec^{-1}$ $m^{-2}$ |
| Nonmycorrhizal plants | 3.5 | 19 | 3.8 |
| Mycorrhizal plants | 20.0 | 58 | 1.70 |

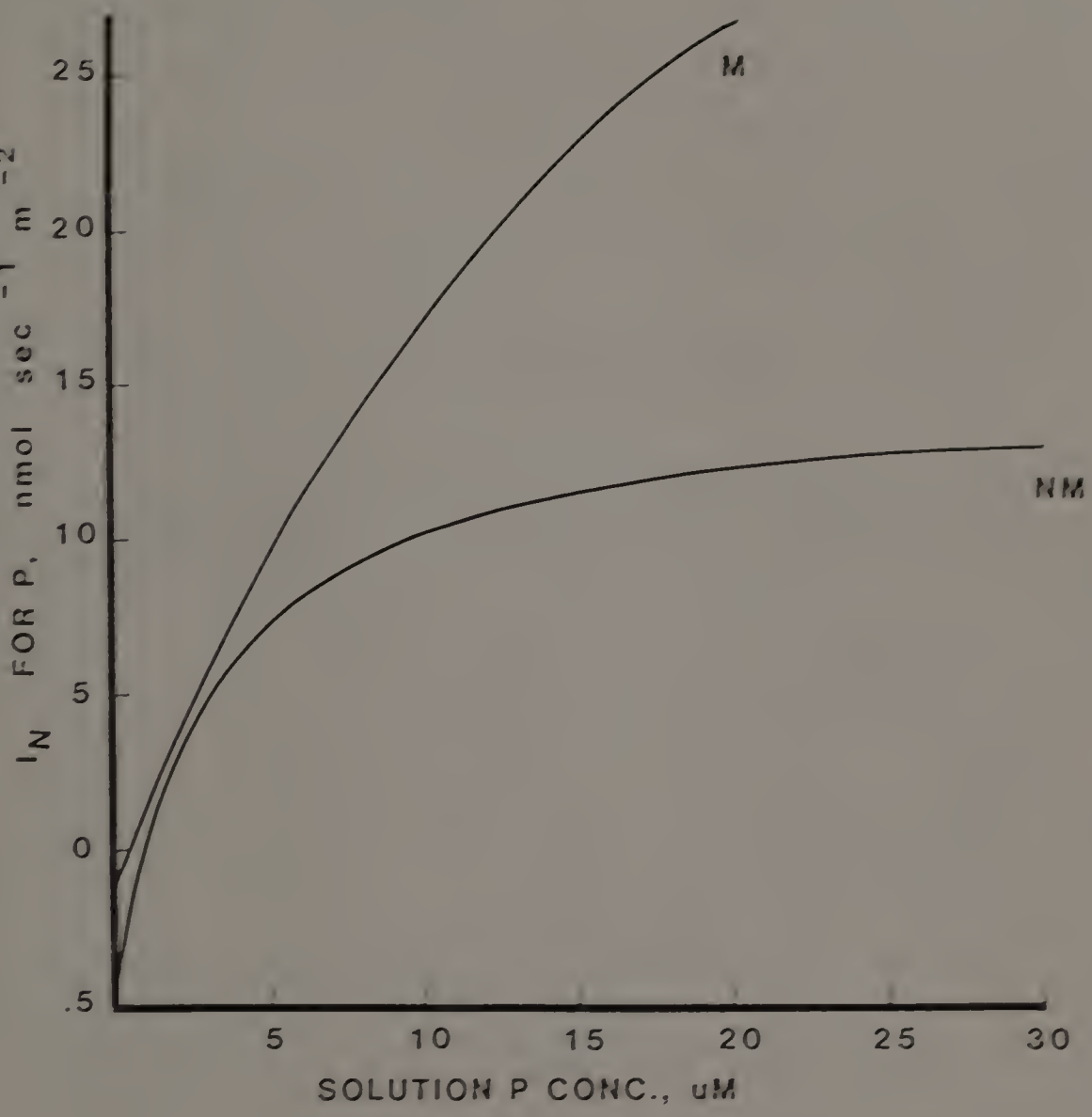


Figure 6.2 Mean $I_N$ of mycorrhizal (M) and nonmycorrhizal (NM) soybean roots as a function of solution P concentration. The curve was calculated using Michaelis-Menten kinetics. $I_N$ = Net P influx.

Peaslee, 1973). Under such cicumstances, ability at greater retention of P (through decreased efflux of phosphate ions by mycorrhizae) will be advantageous to the plant.

Ion uptake studies have been done on mycorrhizal plants, and the increased uptake observed was attributed to the greater surface area offered by the fungi (Gray and Gerdemann, 1967; Bowen et al., 1974). These studies however rarely have addressed the issue of kinetic parameters of mycorrhizal plants. Cress et al. (1979) applied the classical Epstein-Hagen Kinetics (Epstein, 1973) to examine the absorption of P by mycorrhizal plants. Their experiment was carried out at 2 different concentration ranges. The results show that $V_{max}$, the maximum rate of uptake was not changed by mycorrhizal infection, but that the $K_m$ of mycorrhizal plants was 2 to 3 times smaller than that of the nonmycorrhizal plants at the lower concentration range. Since apparent affinity ($K_m$) is independent of the surface area ($V_{max}$), they hypothesised that the mycorrhizal system did not depend exclusively on exploration of an increased soil volume. At the higher concentration range that they used, (>30uM $KH_2PO_4$), increased uptake sites (higher $V_{max}$) contributed to the efficiency of uptake by VAM while enhanced affinity contributed little. Because P concentrations of this higher range usually are absent in soil, their data imply that under normal conditions VAM contribution to ion uptake operates through increased affinity for P ion. Cress et al. (1979) justified their observations based on the report that a poor correlation existed between the P transport from hyphae of mycorrhizal clover or onion

plants and number and length of hyphae (Cooper and Tinker, 1981). A poor correlation between P transport and length of hyphae need not imply that hyphae are of little importance in P uptake. To be valid, uptake would have to be correlated with radially distributed hyphae, a measurement that is difficult to make. A large number of hyphae that run closely parallel to the root surface offers little advantage in gaining access to sites of undepleted P.

Cresse's data may have had some flaws in experimental technique. Prior to the uptake study, their plants were starved of P for 8 days. Usually in P uptake studies, plants are subjected to a period of P starvation that extend from 1 to 2 days (Edwards and Barber, 1976; Nielsen and Barber, 1978). During this period of P starvation, concentrations of carbohydrates increase in the roots. When the supply of the deficient nutrient is restored the stressed root takes up the deficient ion faster, facilitating the uptake study (Lee, 1982; Clarkson and Scattergood, 1982). Starving the plants for too long prior to the uptake study may have had an adverse affect on the root physiology. Plants starved of nitrogen for a week prior to an uptake study on nitrogen exhibited atypical kinetics (Lee, 1982). Plants employed for Cresse's uptake study were 124-days-old. The most active period of P uptake is during the juvenile stages of a plant (Neumann, 1979). Also, roots of older soybean plants showed lower $V_{max}$ and enhanced $K_m$ values than roots of younger plants. (Edwards and Barber, 1976).

My data differ from Cresse's data in that mycorrhizal plants in

my study did not exhibit an increased affinity for P ions. The five-fold increase in $V_{max}$ values found in my experiment contradicts their hypothesis that increased uptake of mycorrhizal plants was due solely to increased affinity. Therefore, I hypothesize that the enhanced uptake of $H_2PO_4^-$ ions by mycorrhizal plants is due to an increase in the number of uptake sites per unit root area in addition to an ability at greater soil exploration.

# C H A P T E R VII

## SUMMARY AND CONCLUSIONS

VAM benefits for nodulating plants were derived only when a number of factors were met satisfactorily: (1) greater than 70% infection with VAM; (2) a root N/P ratio approaching 15; (3) availability of a reduced N form and (4) a neutral root environment pH. These conditions were met best among the urea treated-nodulating plants. For nonnodulating soybeans a neutral root environment was not an essential factor for deriving VAM benefits, but other requirements were similar. Application of either $NH_4^+$ or urea were similarly effective in meeting these essential criteria for nonnodualting plants.

Establishment of mycorrhizal infection in soybean roots depended upon the interaction between P supply and N source. When $NH_4NO_3$ was the source of N, mycorrhizal infection was inhibited by all levels of P except the lowest applied. When urea was the source of N, mycorrhizal infection was obtained over the entire range of P applied. Plants subjected to the urea regime were characterized by an abundance of large vesicles in their root cortices. Roots of plants subjected to the $NH_4^+$ regime had high P and low N compositions whereas roots of urea-treated plants had low P and high N compositions. These results could explain why treatment with urea was conducive to VAM benefits

over a range of P concentrations, whereas benefits of VAM to $NH_4NO_3$-treated plants were limited to situations of low P supply.

$KNO_3$ was the least desirable N source where increases in growth or nutritional benefits from the symbiosis were desired either for nodulating or nonnodulating plants. Overall the $NO_3^-$ treated plants were least able to accumulate either P or N compared to the plants treated with the other 2 N sources. Plants subjected to $NH_4^+$ nutrition accumulated the greatest P content. Urea treated plants had the greatest ability to accumulate N.

Nodulating plants exhibited growth benefits due to VAM only when urea was the source of N. This could be explained by the lack of inhibition of $N_2$ fixation by urea and the consequent improvement in the N status of the host. The ineffectiveness of ammonium nutrition in gaining benefits from the tripartite symbiosis presumably is linked with the acid inhibition of $N_2$ fixation by $NH_4NO_3$ nutrition.

Nonnodulating plants exhibited a greater benefit from mycorrhizal infection than nodulating plants. The additional N supplied to the nonnodulating plants was primarily responsible in the greater mycorrhizal benefits to these plants. The absence of a response to mycorrhizal infection in nonnodulating plants in earliar experiments by other investigators presumably was due to N deficiency. The poorer growth of the nodulating plants relative to the nonnodulating plants may have been due to impaired $N_2$ fixation of the nodulating plants due to a low P supply. The P supply to the plants was maintained low to enhance mycorrhizal infection.

Application of $NH_4NO_3$ or urea led to increased availability of soil P, apparently due to 2 different mechanisms. Ammonium nitrate decreased soil pH, thereby increasing the solubility of the calcium phosphate salts. Increased availability of $HCO_3^-$ associated with urea nutrition could precipitate $CaCO_3$ and decrease Ca concentrations. The smaller Ca concentrations would permit greater P concentrations in equilibrium with the calcium phosphate salts, increasing P availability.

High levels of P either soil or foliarly applied inhibited VAM infection. The action of soil P in inhibiting VAM infection was limited to the stage of establishment of infection. Plants with a high tissue P content exhibited symptoms of a mineral disorder resembling Zn deficiency.

Mycorrhizally infected roots depleted P faster and were characterized by lower efflux values than nonmycorrhizal roots. Lower efflux by mycorrhizal roots could offer an advantage in P retention in situations of low P supply. Mycorrhizal roots had higher $V_{max}$ values than nonmycorrhizal roots but did not exhibit an enhanced affinity for $H_2PO_4^-$ ions in comparison to the nonmycorrhizal roots. The results contradict a previous report in which enhanced P uptake by mycorrhizal roots was attributed to an enhanced affinity for $H_2PO_4^-$ ions alone.

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