



Effects of N and P fertilizers on the growth, nodulation and N₂-fixation of fababean (*Vicia faba* L.), green pea (*Pisum sativum* L.) and dry bean (*Phaseolus vulgaris* L.)
by Saidou Koala

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in
Crop and Soil Science
Montana State University
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Abstract:

The most common grain legumes in temperate and sub tropical regions are *Pisum*, *Phaseolus* and *Vicia* beans. Their yields are often lower than the potential yield due to deficiencies in both P and N. The objectives of this research were to evaluate the relative effectiveness of different sources of phosphorus fertilizers, levels and methods of application on dry beans (*Phaseolus vulgaris* L.) and fababean (*Vicia faba* L.) and also to evaluate the effects of N and P fertilizers and their interaction on nodulation, N₂-fixation and growth of fababean, dry bean and green pea (*Pisum sativum* L.) grown in the field.

In 1980, a split plot, randomized complete block design with four replications was used. Main plots were 0 and 100 Kg ha⁻¹ N applied as ammonium nitrate (NH₄ NO₃). Sub plots were a no P control, two P sources, orthophosphoric acid (H₃ PO₄) as liquid P fertilizer and triple superphosphate. In 1981, the orthophosphoric acid was replaced by monoammonium phosphate. In 1982, 1983 and 1984, factorials in randomized complete block designs with four replications were used with varying levels of N and P fertilizers.

There were differential responses of fababean and dry bean grain yields to P sources and methods of application. Nodulation and N₂-fixation in fababean reached a maximum at pod filling and remained constant until pod filling was complete and then showed a decline. In dry bean, however, maximum nodulation and N₂fixation reached a maximum during pod set and declined rapidly during the final weeks of growth. Application of 100 Kg ha⁻¹ of fertilizer N reduced nitrogenase activity by 75, 72, 82 and 75 percent in dry bean at the four harvests but only 47,60,62 and 57 in fababean. Excellent positive linear correlations between acetylene reduction rates and nodule number and mass were found with both fababean and dry bean in 1980.

Increasing P supply increased nodule number and nodule dry weight but these increases paralleled increases in shoot and root dry weight and suggested that increasing P supply increases nodulation and N₂fixation in the three different species of host plants by stimulating the plant growth rather than by affecting nodule initiation and function. A model is proposed to explain the inhibitory effects of ammonia on nitrogenase activity. It suggests that ammonia acts as an uncoupler or ion ionophore and dissipates the electrochemical proton gradient created by the bacteriod respiratory chain. More importantly, the destruction of the membrane potential suppresses the low potential electrons that might be necessary in reduction reactions within the bacteroids.

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Saidou Koala

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Doctor of Philosophy

in

Crop and Soil Science

MONTANA STATE UNIVERSITY
Bozeman, Montana

June 1985

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APPROVAL

of a thesis submitted by

Saidou Koala

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citation, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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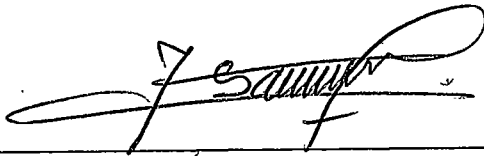
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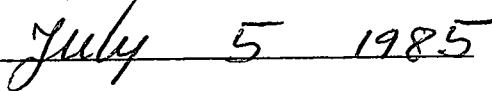
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Dedicated to my wife Bernadette and our children
Koutou, Kotima and Maimouna

VITA

Saidou Koala was born in 1951 to Rasmata and Issaka Koala, in Thyou, Burkina Faso. He attended elementary school at Thyou and secondary school at "Lycée Philippe Zinda Kaboré", OUA GADOUGOU, where he graduated in 1970. He received a B.Sc (Ag) degree from MacDonal'd College of McGill University, Montreal, Canada in 1976.

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ACKNOWLEDGMENTS

The author would like to thank the following people who directly or indirectly contributed in making my Ph.D. program successful: Dr. J. R. Sims, my major professor, for his guidance, inspiration and friendship during my graduate training; Drs. Ron Lockerman, Ray Ditterline, Hayden Ferguson, R. E. Lund and R. D. Dahl, members of my graduate committee, for sharing their time, efforts and enthusiasms; Dr. C. F. McGuire for his assistance in the lab; Drs. Hatim El-Attar and Mohammed El-Alfawi, postdoctoral fellows, for their invaluable help in the field as well as in the lab; the staff members of Plant and Soil Science Department for their teaching.

The Burkina Faso government and the SAFGRAD project for sponsoring my graduate training.

The USDA-SEA/USAID small BNF special grant No. 59-2301-0-5-001-0 and the Montana Wheat Research and Marketing Committee for partially funding the study.

My wife, Bernadette, and our children, Koutou, Kotima and Maimouna for their love, sacrifice and understanding during this entire program.

Mrs. Jean Julian for typing this manuscript.

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ABSTRACT

The most common grain legumes in temperate and sub-tropical regions are *Pisum*, *Phaseolus* and *Vicia* beans. Their yields are often lower than the potential yield due to deficiencies in both P and N. The objectives of this research were to evaluate the relative effectiveness of different sources of phosphorus fertilizers, levels and methods of application on dry beans (*Phaseolus vulgaris* L.) and fababean (*Vicia faba* L.) and also to evaluate the effects of N and P fertilizers and their interaction on nodulation, N₂-fixation and growth of fababean, dry bean and green pea (*Pisum sativum* L.) grown in the field.

In 1980, a split plot, randomized complete block design with four replications was used. Main plots were 0 and 100 Kg ha⁻¹ N applied as ammonium nitrate (NH₄NO₃). Sub plots were a no P control, two P sources, orthophosphoric acid (H₃PO₄) as liquid P fertilizer and triple superphosphate. In 1981, the orthophosphoric acid was replaced by monoammonium phosphate. In 1982, 1983 and 1984, factorials in randomized complete block designs with four replications were used with varying levels of N and P fertilizers.

There were differential responses of fababean and dry bean grain yields to P sources and methods of application. Nodulation and N₂-fixation in fababean reached a maximum at pod filling and remained constant until pod filling was complete and then showed a decline. In dry bean, however, maximum nodulation and N₂-fixation reached a maximum during pod set and declined rapidly during the final weeks of growth. Application of 100 Kg ha⁻¹ of fertilizer N reduced nitrogenase activity by 75, 72, 82 and 75 percent in dry bean at the four harvests but only 47, 60, 62 and 57 in fababean. Excellent positive linear correlations between acetylene reduction rates and nodule number and mass were found with both fababean and dry bean in 1980.

Increasing P supply increased nodule number and nodule dry weight but these increases paralleled increases in shoot and root dry weight and suggested that increasing P supply increases nodulation and N₂-fixation in the three different species of host plants by stimulating the plant growth rather than by affecting nodule initiation and function. A model is proposed to explain the inhibitory effects of ammonia on nitrogenase activity. It suggests that ammonia acts as an uncoupler or ion ionophore and dissipates the electrochemical proton gradient created by the bacteroid respiratory chain. More importantly, the destruction of the membrane potential suppresses the low potential electrons that might be necessary in reduction reactions within the bacteroids.

CHAPTER 1

INTRODUCTION

Grain legumes represent the most economic source of protein for human nutrition and many compete successfully with animal protein sources in relation to protein and essential amino-acid content (Macgillivry and Bosley, 1962). Protein production from 1 ha of land is 28.8 times greater from soybeans and 14.5 times greater from dry beans, than from beef (Harkness, 1967).

Pisum, *Phaseolus* and *Vicia* beans are the most common legume crops in the temperate and sub-tropical regions. Peas probably originated from the Middle East and are grown over the world on about 9 million hectares annually. The production is approximately 10 million metric tons per year (Allen and Allen, 1981).

The common bean (*Phaseolus vulgaris* L.) is frequently cultivated in most agricultural areas of the world and is the primary food staple in many developing countries, especially Latin America (Bazan, 1975; Pinchinat, 1977).

Fababean (*Vicia faba*) is a major food legume in the Middle East and is used as a forage in cereal-legume rotations throughout much of the Canadian, Northern Great Plains. Fababean is capable of achieving high seed yields (7 metric tons/ha) and protein contents (23 to 32%) (Koala, 1982; Evans et al., 1972). They require large quantities of nitrogen to attain full yield and protein potential and may be influenced by phosphorus availability (McEween, 1970; Richards, 1977).

Bean yields are often lower than the potential yield due to deficiencies in both P and N in the tropics (FAO, 1979; Hernandez-Bravo, 1973; Graham, 1978). An alternative to

extensive fertilizer application which is too costly for many small farmers is to utilize N_2 -fixation through the legume-*Rhizobium* symbiosis. It is also assumed that P deficiency is the most important single limiting factor for N_2 -fixation and legume production.

About 85 percent of the cultivated soils in Montana tested medium, low or very low in available P (Sims, 1971). Consequently, most cultivated soils in Montana will give an economic response to P fertilizer additions unless there is a more limiting factor. The most common phosphorus fertilizer used in Egypt is a low quality ordinary superphosphate which is broadcast applied (El-Attar, 1981; Sims, 1981, personal communication). Many current methods in both developed and developing countries do not include banding P fertilizer near the seed or placement with the seed. Therefore, source, rate and method of application of P may be negatively influencing symbiotic N_2 -fixation in those conditions.

There are many questions regarding the role of phosphorus in nodulation and N_2 -fixation. Studies with some of these species do not separate out the effects of phosphorus from other factors such as host plant effects. It is well documented that high rates of combined N inhibit nodulation and N_2 -fixation (Oghoghorie and Pate, 1971). The inhibition mechanism is still unclear even though a few studies have considered the P×N interaction as a means of overcoming some of the ammonia inhibition of nodulation and N_2 -fixation.

The literature is limited on the effect and mechanism of P and P×N interactions on food legumes. The objectives of this research were to: (1) evaluate the relative effectiveness of different sources and levels of phosphorus fertilizers; (2) evaluate methods of application on dry bean and fababean in soils of low P availability; and (3) to evaluate the effects of N and P fertilizers and their interaction on nodulation N_2 -fixation and growth of fababean, dry bean and green pea.

CHAPTER 2

LITERATURE REVIEW

Effect of P Placement on Nodulation, N₂-Fixation and Growth

Cummings (1943) stated that the "most efficient and most effective placement of fertilizer is that which provides for an adequate supply of soluble nutrients in a well aerated zone of moist soil occupied by actively absorbing plant roots at periods of growth when the demands of the plant for nutrients are most acute." These factors have been recognized, but they have not been quantitatively characterized for many legumes, such as fababean and dry bean. A major problem has been the difficulty in achieving the ideal conditions which Cummings described.

Band application of fertilizer places the fertilizer in a smaller soil volume than broadcast application when the fertilizer is added at the same rate. Consequently, roots in contact with banded fertilizer will be in zones of higher fertilizer concentration than roots with broadcast application. However, broadcast application usually results in more zones of root-fertilizer contact.

Diwit (1953) developed an equation that expresses the relation between plant uptake of both banded and broadcast fertilizer, whereby the smaller volume of fertilized soil with banded placement is compensated by the higher concentration that results in greater fertilizer uptake per unit volume of fertilized soil. Singh and Black (1964) confirmed that Diwit's compensation function represents the results to a first approximation.

Currently, there is little information available to compare the efficiency of broadcast versus band placement of P on nodulation, N₂-fixation and growth of fababean and dry

bean. However, there is considerable information available involving the effect of fertilizer placement on the corn growth (*Zea mays* L.). Bates et al. (1965) reported that the growth and nutrient content of corn was substantially increased by fertilizers placed with the seed in comparison to the same rate located either 5 cm below or beside the seed. Nelson (1956) concluded that 224 kg/ha of fertilizer in the row is frequently as effective as 448 kg/ha broadcast for increasing corn yields. Werkhoven et al. (1967) reported that banding 28 was as effective as broadcasting 56 kg P/ha. Nelson and Randall (1968) found a significant response in early growth and yield when the fertilizer was placed in a band near or in direct contact with the seed without significant difference between the two treatments.

Radioactive P has permitted determination of the quantity of fertilizer P absorbed by plants with different placement methods. Nelson et al. (1949) reported that fertilizer P absorbed by plants was less for broadcast than for placement with the seed or for mixing in the row. However, broadcast was equal to the other placement methods with respect to corn yield and total P content.

The effects on plant uptake of banded versus broadcast P are dependent on such factors as soil structure, temperature, moisture, and chemical form of the P. Olsen et al. (1967) reported that absorption of P by corn seedlings was inversely related to the soil moisture tension. The decreased P uptake with increased soil moisture tension may play an important role when comparing banded and broadcast application. Banded P is near the plant and the soil moisture develops high tension sooner than an area further removed from the plant due to water uptake by the plant. Consequently, broadcast and incorporated P might be more readily absorbed than banded P during dry periods.

Robinson et al. (1959) studied the effect of temperature on response of red clover to banded P in a P-deficient soil. They noted a 22% yield increase to band application at 10°C and only 34% at 27°C. This was not due to the banded application being less effective at higher temperatures, but due to the broadcast being more effective at higher temperatures.

It was concluded that band placement was apparently the more effective because of an increased concentration of phosphorus in a small portion of the root zone. Furthermore a band application would be particularly important on soils low in available phosphate, especially if they were high in P fixing capacity. Ketcheson (1957) found that fertilizers distributed in bands compared to seed placement resulted in a greater increase in yield of dry matter at 13°C than at 20°C for greenhouse grown corn.

Some investigators have not found band application to be superior to broadcast. Ham et al. (1973) reported that soybean seed yields increased with increasing rates of applied P and the yield from seed placed fertilizer was greater per unit P than the yield from band and broadcast P. Ham et al. (1978) also studied the effects of fertilizer placement on soybean seed yield, N₂-fixation, and ³³P uptake in soybean. Seed yield and total plant P increased significantly from adding P fertilizer, although no differences were found among the various placements. It was speculated that the lack of differences among fertilizer placements may have been due to the warm soil temperatures on a well-drained soil with a pH of approximately 7.0.

Yost et al. (1979) reported that broadcast treatments gave greater yields than band treatments at the same rates for the first corn crop grown. However, total yields in the field and P uptake at the end of four seasons were very similar for broadcast and band treatments in which the same total amount of P had been applied to a high P-fixation capacity soil.

Duell (1974) reviewed the literature on P fertilization for forage establishment and found that legume seedlings are usually less capable than grasses to obtain P from the low soil-P concentrations associated with broadcast fertilizer applications. Seedling growth of both grasses and legumes is often enhanced by placing P in concentrated bands directly underneath the seed row (band seeding). Moving the fertilizer band as little as 2 or 3 cm to the side of the seed row is often sufficient to significantly reduce early growth of legume

seedlings. Brown (1959) seeded alfalfa with triple superphosphate banded and broadcast at rates ranging from 100 to 800 kg/ha. All banded rates except the lowest resulted in a doubling of alfalfa seedling size. However, only the highest broadcast rate increased the alfalfa seedling growth rate.

Sleight et al. (1984) reported that the amount of P uptake by oats (*Avena sativa* L.) was nearly proportional to the volume of the soil containing the applied P fertilizer. Apparently, the early beneficial effects of banding are obtained primarily from placing all of the fertilizer where contact by active roots is more likely, rather than from any increase in availability that may be obtained from decreased soil-fertilizer contact associated with banding. This suggests that the most efficient use of P fertilizer in which P is relatively immobile in soils will be made by the young plants if the fertilizer is mixed thoroughly with the soil near the seed.

Effects of band and broadcast placements might be affected by N availability. Miller et al. (1958) reported that placement of N fertilizer caused a relative increase in the feeding power of the root system on band-placed phosphorus. N had a greater influence when mixed with P than when placed in a band 3 to 4 inches from the band. The influence was nearly independent of the soil phosphate level when the N was mixed in the band phosphorus. However, it was not independent of the soil phosphate level when the nitrogen was separated from the phosphorus band.

In a split-root experiment with corn, Engelstad and Allen (1971) showed that P applied to one side of the root system was translocated throughout the entire root system and was effective in promoting root and top growth. They found that the presence of ammonium N enhanced the uptake of P from a band, but had no effect on uptake of P mixed throughout the soil.

The management of P placement might be different on the tropics with soils of high P fixing capacity. The traditional way to cope with the high P fixation is to apply the fertilizer in bands to minimize the volume of soil with which it will react. The high cost of superphosphate and other energy-dependent inputs has led to exploring additional ways of managing high P-fixing soils with limited capital resources, particularly in small farming systems in the tropics. The results are completely different in soils with extremely high fixation capacity and very low levels of available P. Studies by Yost et al. (1979) on a Brazilian oxisol which requires 750 ppm P at the standard solution concentration indicate that banded applications are inferior to broadcast application for the first corn crop. The available P in the soil was so low that root development was limited to the regions where P was applied. However, the very limited root development around the banded treatments caused the plants to be less resistant to periods of moisture stress. Similar results were reported by Hansen (1979). The best method for applying P to these high adsorbing soils appears to be an initial broadcast application followed by maintenance band applications.

Effect of P Sources on Nodulation, N₂-Fixation and Growth

Any soil condition may cause some degree of reversion from soluble to insoluble forms. Divalent and polyvalent cations in the soil cause reversion of water soluble phosphates to less soluble forms when P fertilizer is applied to soil. The term fixation therefore refers to the degree of reversion which adversely affects the recovery of applied P by plants or chemical extractants (McLean and Logan, 1970). Soils differ greatly in P fixing capacity, plants respond differentially to a given source of P depending on the P fixing capacity of the soil. This implies that there is a best source of P for a given soil condition. This has not been evaluated for fababeans and dry beans with respect to nodulation, N₂-fixation and yield in Montana.

McLean and Logan (1970) evaluated the sources of P for plants grown in soils with differing phosphorus fixation tendencies. They found that P content of corn seedlings increased in direct proportion to water solubility of "available" P in relatively low fixation soils. However, P content decreased with increased water solubility of P in high fixation soils.

Many other workers have reported similar results. Increased yields or P availability to crops has been obtained with increased water solubility of P fertilizer in low P fixation soils (Lawton et al., 1956; Webb and Pesek, 1958; Webb et al., 1961). However, several reports (McLean and Wheeler, 1964; Webb and Pesek, 1959) indicated that increased water solubility is of little or no benefit on acid soils. Rock phosphate under acid conditions has produced crop yields equal to or better than those from superphosphate (McLean et al., 1952).

The development of superphosphoric acid, containing approximately equal amounts of ortho and condensed phosphates has also created much interest in the agronomic effectiveness of condensed phosphates (Gordon and Kamprath, 1971). However, their effectiveness as fertilizers is considered to be almost entirely dependent upon their hydrolysis to orthophosphate (OP) (Sutton and Larsen, 1964). Although several factors affect the hydrolysis rate. The above authors found that the level of biological activity was the most important factor in soils. In soils with low levels of biological activity, P uptake by rye grass (*Lolium multiflorum* Lam.) was significantly lower with pyrophosphate than with OP. Pyrophosphate was a relatively ineffective source of P prior to hydrolysis to the orthophosphate form. Differences between P sources in soils with higher levels of biological activity were detectable only in the first cutting. Uptake of P by barley (*Hordeum vulgare* L.) from solutions containing pyrophosphate was lower by a factor of 2.4 than that from OP solutions.

Soil phosphorus levels, formulations, and plant growth stage might also explain differences obtained with different P sources. Bureau et al. (1953) found that superphosphate and double superphosphate were equally available as a source of phosphorus on the high phosphorus soil throughout the growing season. However, superphosphate was slightly superior on the medium and low phosphorus soils. Calcium metaphosphate was less available than superphosphate or double superphosphate in the early portion of the season, but equaled the availability of the superphosphate carriers during the latter part of the season. However, calcium metaphosphate furnished less phosphorus to plants throughout the season than either of the above sources on the high phosphorus soil.

Robertson and Hutton (1972) evaluated ten phosphorus sources on the growth of corn, peanut (*Arachis hypogae* L.), oat and soybean (*Glycine max.* L.) and found that these crops responded differentially. However, the phosphorus sources could be arranged in the following descending order of response: superphosphate, dinitra phosphate (17-22-0), fused tricalcium phosphate (< 40 mesh), dinitraphosphate (17-33-0), fused tri-calcium phosphate (< 40 mesh), concentrated superphosphate, calcium metaphosphate, potassium metaphosphate and rock phosphate.

Terman et al. (1964) compared liquids and solid P fertilizers and reported that crop yields with liquids and suspensions produced from superphosphoric acid were equal to those obtained with other water-soluble solid phosphates when they contacted the same amount of soil and supplied the same quantities of N and P. Lathwell et al. (1960) compared liquid and granular formulations of several water-soluble P sources for supplying P to corn and small grains in New York, Iowa, and several southeastern states. They found that responses to the liquid forms on P-deficient soils were similar to those obtained with CSP (concentrated superphosphate solid). Additionally, response to P was similar when ammonium polyphosphate (APP) was applied in either liquid or granular form. However, a

difference existed between the solid and liquid forms when the P was applied to high fixing soils due to the greater initial soil contact by the liquid form.

Effect of Combined N on Nodulation, N₂-Fixation and Legume Growth

Establishment of the N₂-fixing symbiosis between legumes and rhizobia involves four main phases, during each of which the host and the microsymbiont must be in close association. These phases (1) establishment of the microsymbiont on the root surface, (2) infection, (3) nodule initiation and development, and (4) N₂-fixation are all subject to a number of factors, intrinsic in the symbionts or in the environment including nutrition that can promote or inhibit the successful development of the symbiotic association. The factor most widely studied in relation to this is inorganic nitrogen. X

Early research reviewed by Fred, Baldwin, and McCoy (1932) suggested that nodulation and subsequent fixation can be inhibited by concentration of available inorganic nitrogen. Burk and Lineweaver (1930), and Wilson, Hull, and Burri (1943), showed that fixation by *Azotobacter* could be prevented by the presence of sufficient inorganic nitrogen. X

Recently, studies have been performed to evaluate the influence of varying quantities of available nitrogen on the fixation process in legumes with the advent of the use of the nitrogen isotope of mass 15 as a tracer. The presence of inorganic nitrogen diminished symbiotic N₂-fixation in soybean, Norman and Krampitz (1946); soybean and lespedeza (*Lepedeza* sp.), Thorton (1946); peanut, Thorton and Broadbent (1948); soybean, peanut, alfalfa (*Medicago sativa* L.), lespedeza, ladino clover (*Trifolium repens* L.), and birdsfoot trefoil (*Lotus corniculatus* L.), Allos and Bartholomew (1955). Much more work has been done on soybean, but findings are similar: inhibition of nodulation and N₂-fixation by the addition of mineral N (Williamson and Diatloff, 1975; Johnson et al., 1975; Bhangoo and Albritton, 1976; Criswell et al., 1976). X

Soybean apparently cannot fix sufficient nitrogen for maximum growth response although combined nitrogen normally reduced the amount of N fixed symbiotically. This indicates the essentiality of combined nitrogen. Allos and Bartholomew (1959) found that soybean, peanut, alfalfa, lespedeza, ladino clover and birdsfoot trefoil exhibited an apparent capacity to supply by fixation, only about one-half to three-fourths of the total nitrogen which could be used by the plant. However, combined nitrogen cannot be used as a supplement to the fixation system, because as soil N or fertilizer N increases, fixation decreases.

The reasons for the greater growth from combined nitrogen are not clear. Some have attributed it to a greater energy cost for fixation (Allam, 1931; Ryle et al., 1978), though others have suggested that the major effect of nitrogen addition is to overcome nitrogen stress which occurs as the seed nitrogen reserves are depleted and before fixation is of sufficient magnitude to meet the demands of the growing plant (Pate and Dar, 1961; Hoglund, 1973; Gibson, 1966, 1976).

Gas exchange studies have identified a large respiratory CO_2 loss associated with symbiotic fixation (Mahon, 1977b, 1979; Ryle et al., 1978). This increased respiration occurs throughout the fixation period, and in soybean was sufficient to account for a 10 to 15% loss of the daily assimilates supply (Ryle et al., 1978). A carbon loss of this magnitude would be expected to have a significant effect on growth. However, other studies have shown similar respiration rates (Minchin and Pate, 1973) and growth (Gibson, 1966, 1976) on atmospheric and combined nitrogen.

Legume response to N is confounded by the ability of the plants to utilize both nitrate and N_2 . Nitrate is considered the primary source of nitrogen available from the soil. Nitrate uptake and subsequent reduction by nitrate reductase is the primary pathway of soil nitrogen utilization. Harper and Hageman (1972) reported that the utilization of N_2

through the symbiotic relationship with *rhizobia* affords a second major pathway of nitrogen input to legumes.

Effect of N Fertilizer on Fababean Nodulation, N₂-Fixation and Growth

Nitrogen fertilization of non-leguminous crops generally leads to an increase in dry matter produced. However, N₂-fixation by root nodules complicates the effect in legumes.

With fababean (*Vicia faba* L.), McEwen (1970) reported decreased nodulation and increased yield from the addition of N. Candlish and Clark (1975) demonstrated increased suppression of N₂-fixation in fababean with increased increments of nitrate on greenhouse-grown plants. Dean and Clark (1977, 1979) reported reduced N fixation in fields high in nitrate in Manitoba, Canada. They also studied the effect of low level nitrogen fertilization on nodulation, acetylene reduction and dry matter in fababeans and three other legumes (green pea, soybean and dry bean). The addition of 30 kg N/ha as ammonium nitrate depressed nodulation in all species, especially dry bean. Acetylene reduction was also depressed in all species. However, acetylene reduction in fababean was significantly greater than in pea and dry bean. More than 90% of fababean and pea produced nodules, but only 77% of dry bean. However, in spite of low nodulation and fixation rates, dry bean yielded significantly more dry matter and N than fababean and green pea. Nitrogen fertilizer increased dry matter in all species, except fababean. Richards and Soper (1979) also found that fababean aerial yield was not affected by N fertilizer up to 600 mg N/pot (200 mg N/kg soil) applied at seeding. Only the highest rate of N employed (900 mg N/pot at seeding), significantly increased fababean yield (13.2%). However, protein content and total N uptake into fababean shoots were unaffected by all N applications used. Symbiotic fixation on low nitrate soils accounted for up to a maximum of 146.0 kg N/ha, but with high

nitrate soils, acetylene reduction was 37% less than the maximum rate found at the low nitrate location (Dean and Clark, 1977).

Fababean, grown in the Canadian prairies, have responded to N applications. Rogalsky (1972) in Manitoba, and Sadler (1975) in Saskatchewan reported that N broadcast on the surface at seeding significantly increased fababean seed yields, suggesting symbiotic N fixation was not fully able to satisfy the fababean requirements.

Kralova and Mouchova (1974) in Czechoslovakia reported the maximum aerial yield of fababean harvested at flowering occurred when 105 to 210 mg N/kg soil had been applied at seeding. Fababean receiving the higher rates of N fertilizer had fewer nodules than fababeans receiving no supplemental N. However, researchers in Great Britain noted that fababean were capable of fixing all of their N requirements. Rates of N fertilizer broadcast on the surface at seeding in excess of that which could be symbiotically fixed by fababean, resulted in seed yield increases of less than 10% with protein content being unaffected (McEwen, 1970b). Split N applications and single large mid-season N applications also did not affect seed yield and protein content (McEwen, 1970b). In identical growth chamber experiments with *Pisum sativum* L. and *Vicia faba* L., Rinno et al. (1973) reported that single large N applications at the onset of flowering significantly increased aerial yields of green pea but had no effect upon fababean. They concluded that fababean derived sufficient N from symbiotic fixation.

Effect of N Fertilization on Green Pea Nodulation, N₂-Fixation and Growth

In work reported by Chen and Phillips (1977), N fixation in green pea was reduced by NH₄ and NO₃ ions which also caused earlier nodule senescence. Sosulski and Buchan X (1978) found that N fertilizer depressed N fixation in pea, while increasing seed yield and N content.

Combined nitrogen inhibited nodulation (Pate and Dart, 1961), translocation of photosynthate to the nodules (Small and Leonard, 1969), and symbiotic nitrogen fixation in green pea (Bethlenfalvay and Phillips, 1978; Ogoghorie and Pate, 1971; Mahon, 1977a, 1977b) but generally stimulates growth above inoculated control levels. Sosulski and Buchan (1978) found in Canada that nitrogen fertilization of 106 kg N/ha at seeding severely depressed nitrogenase activity but markedly increased forage and seed yields as well as protein contents.

It is unlikely that the only effect of supplemental nitrogen is the alleviation of the early nitrogen stress since nitrate application after 4 weeks of symbiotic growth rapidly increased dry-matter production in pea (Mahon, 1977b) and nitrogen source altered plant morphology (Minchin and Pate, 1973).

Effect of N Fertilization on Dry Bean Nodulation, N₂-Fixation and Growth

The ability of dry bean to support *Rhizobium phaseoli* and to subsequently benefit from symbiotically fixed N₂ has been defined by Rennie (1981) as *nis*, the nitrogen fixation supportive trait.

Dry bean is inferior in *nis* (nitrogen fixation supportive trait as defined by Rennie (1981) and Rennie et al. (1982)), i.e., in their ability to support and benefit from their symbiotic association with N₂-fixing *Rhizobium phaseoli*.

Poor and variable N fixation is not well understood, but has been attributed to the inhibition of nodulation by antibiotics leaching from the testa (Kreaman, Abel-Ahaffar, and Elgabaly, 1972), delay in rhizobial activity until flowering (Cackett, 1965), short growing season of bean (Gallagher, 1968), and seasonal variation (Masefield, 1971). Thompson also attributed poor nodulation to seed coat antibiotics in subterranean clover (*Trifolium subterranean* L.). However, Rennie and Kemp (1983) reported that the use of acetylene reduction assays underestimate N₂-fixation in dry bean. To support their view,

they cited published data, using direct (Rushel et al., 1982; Rennie and Kemp, 1983) or indirect (Westermann et al., 1981) ^{15}N techniques to quantify N_2 fixation in the field, which showed that some bean cultivars may obtain approximately 50% of their plant N requirements from N_2 fixation and may fix up to 100 kg N fixed ha^{-1} per annum.

The response of dry bean to N fertilization under some field conditions is also dependent upon the cultivar (Burke and Nelson, 1967, 1969) or/and the Rhizobium (Rennie and Kemp, 1983) indicating that there may be a range of effectiveness for the rhizobia-cultivar relationship (or N_2 fixation limitation due to the characteristics of the cultivar itself).

Effect of Cultivar

Rennie and Kemp (1983) using 26 cultivars found that the addition of 40 kg fertilizer N ha^{-1} on a Typic Haploboroll soil caused a 10% reduction in percent N derived from atmosphere (% Ndfa) in most cultivars but had no effect on 'Redcloud'. In contrast, the cultivar 'Limelight' suffered a 60% reduction in % Ndfa. This indicated a host-specific reaction in *nis* to mineral N and potential for breeding this resistance into other bean lines. They also found that pole bean cultivars had higher % Ndfa and thus superior *nis* than bush cultivars. The actual amounts of N_2 fixed varied between 40 kg ha^{-1} and 125 kg ha^{-1} depending on the cultivar.

Effect of Strains of *Rhizobium*

Strains of *R. phaseoli* can significantly alter the amount of N_2 fixed (Rennie and Kemp, 1983) and therefore the yield of dry bean cultivars under conditions of N-free growth or in the field. Some strains fixed more than 100 kg N ha^{-1} in the variety Aurora resulting in dry matter and N yields in excess of uninoculated treatments receiving 40 or 100 kg fertilizer N ha^{-1} . Therefore, *R. phaseoli* are as efficient as other rhizobia in supplying fixed N_2 to their host plant and, in N_2 -fixing mode, certain dry bean cultivars can meet their genetic yield potential in the field without the addition of fertilizer N.

Mode of Action of Combined Nitrogen

Understanding how nitrate and ammonium decrease biological N reduction in legume root nodules is important for devising strategies for maximum N_2 fixation. One method for studying interactions between combined nitrogen and N_2 fixation is to supply excess nitrate or ammonium to legumes and examine the effect on root functioning. Morphological effects of supplying NH_4NO_3 to root nodules have been described (Dart and Mercer, 1966).

Large amounts of applied N reduce root-hair infection (Munns, 1968; Dazzo and Brill, 1978) and the root hair curling phenomenon which is thought to be an initial step in *Rhizobium* infection (Thornton, 1936). Root hairs were eliminated on *Medicago* roots at concentrations above 1 mM N as would be found in fertile soils and conventional nutrient solutions (Munns, 1968). In support of this theory, Tanner and Anderson (1963) showed that nitrate in the external medium catalyzed the destruction of indoleacetic acid (IAA), while NH_4^+ -N decreased the amount of tryptophan converted to IAA. Fahraeus and Ljunggren (1959) reported that formation of polygalacturonase (PA) by rhizobia in the legume root zone possibly plays a role in root hair infection and PA formation is suppressed by $NaNO_3$. Added IAA can partly offset nodule inhibition by nitrate (Munns, 1968; Valera and Alexander, 1965). Several strains of *Rhizobium* grown with nitrate cause nitrite accumulation and associated loss of IAA activity (Tanner and Anderson, 1964), at least under certain cultural conditions in bacteriological media. Vincent (1965) suggested that nitrite is the prime inhibitor of nodulation, operating in part by accelerating the destruction of IAA.

Combined N reduced nodule number (Dart and Mercer, 1965). Dart and Wildon (1970) observed that combined N restricted primary root nodulation in *Ungiculata sinensis* and *Vicia atropurpurea* but did not affect nodule number on the secondary roots. Recent

papers (Dart and Wildon, 1970; Raggio et al., 1965; Harper and Cooper, 1971; Munns, 1968a, 1968b) report inhibition or delay of nodulation by maintained concentrations of nitrate.

Combined N reduced nodule mass (Summerfield et al., 1977). Dart and Wildon (1970) reported that the presence of 100 ppm of inorganic N during the growing season significantly reduced nodule weight of soybeans at mid-pod fill stage, but no effect was noticed when N was applied at 200 ppm, 10 days before the harvest.

Nitrate has been reported to induce early nodule senescence. Pin-Ching et al. (1977) investigated the temporal sequence of nitrate-induced root nodule senescence in terms of nitrogenase activity and leghemoglobin content to determine whether CO₂ enrichment could alter that time course. Increasing CO₂ concentration from 0.00032 atm to 0.00120 atm did not significantly alter the time course of nitrate-induced root nodule senescence. These results suggest that in spite of the fact that cofactors derived from photosynthetic products presumably were more available to decrease hypothetical competition between nitrate reductase and nitrogenase, nitrate still induced a rapid senescence of root nodules under the higher CO₂ concentration.

Positive yield responses are associated with decreased N fixed during later growth stages due to nodule senescence (Pin-Ching et al., 1977) or competition between nodules and seed formation for plant assimilates. The N₂-fixing activity of nodulated roots (Gibson, 1974) and the total amount of N fixed (Allos and Bartholomew, 1959) is reduced by inorganic N as measured by ¹⁵N₂, C₂H₂, or nitrogen-balance studies.

Degree of inhibition varies with the form of the N-compound (Dart and Wildon, 1970). NO₃-N generally decreased acetylene reduction more than NH₄⁺-N.

Numerous factors affect the degree of inhibition. Inhibition varies with the species (Allos and Bartholomew, 1959), and cultivar (Gibson, 1974) and the rhizobium strain (Pate and Dart, 1961). The degree of inhibition varies also with the season (Pate and Dart,

1961), light intensity (Dart and Mercer, 1965), temperature (Gibson, 1974) and the nutritional conditions (Pankhurst, 1981).

The primary mechanism of inhibition of nodulation by nitrate nitrogen has yet to be identified, even if the morphological effects resulting from combined N are easily seen. Khan et al. (1981) concluded that the primary effect of high levels of $\text{NO}_3\text{-N}$ was a decreased translocation of photosynthates to the nodules. This resulted in depression of ^{14}C radioactivity in nodules, and nodule inhibition.

Small and Leonard (1969) reported data which suggested that ^{14}C -labelled photosynthate was retranslocated out of pea root nodules on *Pisum sativum* L. plants treated with NaNO_3 . Such movement of photosynthate could have a considerable effect on the energy available for N_2 -fixation (Rigaud, 1976).

Oghoghorie and Pate (1971) explored the concept that N_2 -fixing nodules and $\text{NO}_3\text{-}$ assimilating centers compete for supplies of reductant and carbon skeletons derived from photosynthate. This might be overcome by increasing the photosynthate available to root nodules if the competition results in root nodule senescence. When they induced leaf nitrate reductase by supplying nitrate to either the roots or the leaves, root nodule senescence, however, was observed only when nitrate was supplied to the roots, in spite of the fact that leaf nitrate reductase utilized significant amounts of photosynthate. This result suggests that nitrate does not induce root nodule senescence through competition between nitrate reductase and nitrogenase for products of photosynthesis.

Shanmugam and Morandi (1976) demonstrated a strong correlation between the suppression of nitrogenase biosynthesis by NH_4^+ and subsequent assimilation of NH_4^+ , presumably to the level of amino acids. This implies the overall mechanism of regulation of nitrogenase biosynthesis involves the conversion of NH_4^+ to the level of amino acids.

Virtanen et al. (1955), using a split-root technique, showed that nodulation was inhibited on the root system exposed to combined nitrogen, but was unaffected on the

part growing in nitrogen free media. Likewise, Raggio et al. (1965), working with excised roots of *Phaseolus*, showed that nitrate fed through the cut end with the organic nutrient may increase nodulation or reduce it only slightly, but nitrate in the external bathing solution reduces it drastically.

Effect of P on Nodulation, N₂-Fixation and Growth of Legumes

Heltz and Whiting (1928) found that 60 kg P/ha increased the average number of nodules per plant from 37.8 to 49.6 in field experiments. Fletcher (1961) also reported a significant effect of P on the nodule number on the P-tolerant soybean cultivar 'Chief' and P-sensitive cultivar 'Lincoln', grown in the greenhouse. In one experiment, a maximum number of nodules was found at 280 part per 2 million (pp2m) and in another case, at 870 pp2m. Maximum nodule number per plant varied between 12 to 25. However, Perkins (1924) reported that added P was not essential for nodulation. A sharp reduction in the number of nodules resulted when P was applied at 0 and 666 kg/ha in pot trials with two soils and quartz sand.

DeMooy and Pesek (1966, 1969, 1970) found that nodulation and growth of all plant parts including roots at all stages of development and production of seed at maturity increased considerably with very high rates of P and K, and required some 500 pp2m P and 700 pp2m K for maximum nodulation. Maximum yield production in the field required similarly high rates of fertilization: 280 to 450 kg P and 560 to 675 kg K/ha.

Gates (1974) studied the symbiotic response to phosphorus and sulfur of *Stylosanthes humilis* and found that phosphorus had a beneficial effect on the initiation of nodules, which were first detected at day 11 in high-phosphorus plants, but not until day 14 in low P-plant. Phosphorus increased nodule number, volume and dry weight. Nodule relative growth rates were stimulated from 0.3 g/g/day at low phosphorus levels to 0.7 g/g/day at high phosphorus levels over days 23-26. Nodules were pink earlier in response to

phosphorus. This suggested that from the earliest stages, phosphorus not only promoted the development of an increased mass of nodular tissue, but also favored an effective symbiosis.

In the tropics, several workers have found that P fertilization increased N concentrations in tropical legumes (Andrew and Robins, 1966; Dradu, 1974; Shaw et al., 1966; Graham and Rosas, 1979); while Falade (1973) reported no change.

The increase in N concentration in *Stylosanthes humilis* with P fertilization reported by Shaw et al. (1966) was greatly enhanced by the addition of S. Total N fixed also increased as P fertilization increased yield.

Dradu (1974) found that the application of 625 kg/ha of single superphosphate increased desmodium dry matter yield and N uptake by 75 and 99% on Buganda loam and by 198 and 372% on Kyebe red loam, respectively. He also reported that omission of P on these soils depressed nodulation, N yield in nodules, P concentrations in tops, seedling vigor, and seedling growth.

Graham and Rosas (1979) working in Latin America applied phosphorus from 0 to 315 kg/ha as triple superphosphate and measured plant and nodule development, P distribution and N_2 (C_2H_2) fixation 42 days after planting. Nodules were a strong sink for P, nodule weight increased ninefold and P concentration in nodules by almost 50% over the range of P fertilization used. Other plant tissues benefited less. Levels of N_2 (C_2H_2) fixation, specific nodule activity, and nonstructural carbohydrate in nodules were highly correlated with supply of P.

Whiteaker et al. (1976) demonstrated that the yield of dry bean to low P level was strain specific. They found that the most efficient line produced 74% more dry weight per unit of P than did the least efficient. Two types of response were evident when the bean lines were grown at high levels of P. Responders produced greater dry weight yield as P supply was increased; non-responders did not. Lines which were the most efficient at stress

P levels were not always responders. Selecting lines with the combined attributes of efficiency under element stress and response to high levels of the element should be of interest to plant breeders who must develop cultivars adapted to a wide range of environments. They concluded that the physiological basis for differences in efficiency of P utilization involved participation of P in metabolic processes and is based on factors other than absorption from the environment.

Sharma et al. (1973) reported that the beneficial effect of P fertilization was immediately clear and continued up to the 45th day after sowing in cowpea (*Vigna pinensis*). The maximum number of nodules occurred when P was applied at the rate of 74 to 111 kg/ha.

Wagner et al. (1978) reported that added phosphorus caused a significant increase in nodulation of annual medicago species. They showed that improved growth or photosynthetic output due to adding phosphorus was associated with better nodulation. Root growth was also increased by phosphorus addition. Potentially this would provide more sites for nodule development. The observed vegetative response associated with adding phosphate to the soil was partly due to additional nitrogen furnished through N fixation.

N and P Interaction on Nodulation, N₂-Fixation and Growth of Legumes

Few studies have been reported on the interaction of nitrogen and phosphorus on the nodulation and N₂ fixation of legumes. Gates and Wilson (1974) described an experiment in which a wide range of mineral nitrogen and phosphorus treatments were applied to *S. humilis* plants nodulated with the commercial strain of rhizobium. They deduced that mineral nitrogen is not necessarily detrimental to nodulation and that, when in balance with favorable levels of phosphorus, may have a beneficial effect on plant growth and nodulation. Phosphorus greatly stimulated growth and nodulation at all of 6 levels of P×5 levels of N. N depressed nodulation at P₀ to P₁₂₅ kg/ha, but was beneficial at P₂₅₀ to

P₁₀₀₀. Balanced combinations of N and P stimulated nodulation and allowed stylo to achieve higher yield and N content than could symbiotic N alone.

Methodology for Assessing Phosphorus Involvement in Nitrogen Fixation

Robson (1983) has outlined several approaches to assessing the relative requirements of host-legume growth and symbiotic nitrogen fixation: First, a negative interaction between a nutrient and nitrogen on the legume growth can indicate that symbiotic nitrogen fixation has a greater requirement than host-legume growth. Anderson (1956) proposed that whenever two treatments each produce a positive response and the interaction between them is negative, they correct the same deficiency. For example, negative interactions between nitrogen and the supply of molybdenum (Anderson and Spencer, 1950), cobalt (Ahmed and Evans, 1961), calcium (Loneragan, 1959; Andrew, 1976), and copper (Snowball et al., 1980) on legume growth have been observed. However, Gates and Wilson (1974), Zaroug and Munns (1979), Robson et al. (1981) working with small seed legumes, have observed a positive interaction between nitrogen and phosphorus on nodule number and dry weight. This positive interaction suggests that host-legume growth has a greater requirement for phosphorus than symbiotic nitrogen fixation.

A second approach is to examine the effect of nutrient supply on nitrogen concentrations in the plant. When a nutrient shows a negative interaction with combined nitrogen on the growth of a legume, correcting that deficiency should increase nitrogen concentrations in tops. This has been shown with cobalt by Ahmed and Evans (1960), Reddy and Raj (1975), Chatel et al. (1978), with copper by Greenwood and Hallsworth (1960), with molybdenum by Anderson and Spencer (1950).

A third approach is to assess the role of mineral nutrients in symbiotic nitrogen fixation is to study how nodule distribution, size, weight and number is affected by the alleviation of nutrient deficiencies. Nutrient deficiencies which are specifically involved in

nodule function may lead to increased nodule number and weight. Anderson and Spencer (1950) showed that molybdenum-deficient plants of *Trifolium subterraneum* had more than doubled total nodule weight in *Lupinus angustifolius* at flowering (Robson et al., 1979). Copper deficiency increased the number of nodules but decreased the size of individual nodules in *T. subterraneum*.

These effects of deficiencies of molybdenum, cobalt, and copper on nodule number and weight are probably associated with nitrogen deficiency in the plant leading to compensatory increases in nodule development. In contrast, deficiencies of other nutrients generally reduce nodule number and weight.

A fourth approach is to show that application of that nutrient increases rates of acetylene reduction prior to increasing growth. For example, cobalt application increased rates of acetylene reduction both per plant and per gram nodule before affecting growth of *L. angustifolius* (Dilworth et al., 1979). Similarly, copper application increased the rate of acetylene reduction per plant and per gram nodule prior to marked effects on growth of *T. subterraneum*.

Acetylene reduction rate studies have been measured usually only after a growth response (Robson, 1983). Acetylene reduction studies are generally expressed as moles of ethylene produced per plant per unit time which gives an absolute fixation rate. Robson (1983) stated that there is no indication as to whether differences in host-plant growth, nodulation, or nodule function are responsible for higher rates of fixation. Specific activity, however, which is the rate of acetylene reduction expressed on a nodule weight basis, may indicate whether the treatment affects nodule function rather than affecting the host-plant growth.

CHAPTER 3

MATERIALS AND METHODS

Field Experiment 1980

Field plots were established in the summer of 1980 at the Montana State University Arthur H. Post Field Research Laboratory. A split plot, randomized complete block design with four replications was used. Main plots were 0 and 100 kg ha⁻¹ N applied as ammonium nitrate (NH₄NO₃). Indigenous N in main plots not receiving fertilizer nitrogen was approximately 60 kg ha⁻¹ at planting. Subplots consisted of a no P control; two P sources, orthophosphoric acid (H₃PO₄) as liquid fertilizer (ortho), and triple superphosphate (TP); two P rates, 27 and 54 kg ha⁻¹; with two methods of application, involving broadcast on the surface and incorporation within the surface 15 cm of soil (B) or drilled near the seed in bands (S) (Table 1).

Table 1. Summary of Fertilizer Treatments used in Subplots at Bozeman, Montana, 1980.

| Treatments Number | Treatment Designation* | | |
|----------------------|------------------------|--------------------------|--------|
| | P Rate | Method of Application | Source |
| 1 | C ₁ | — | — |
| 2 | 15 | S | ortho |
| 3 | 15 | B | ortho |
| 4 | 50 | S | ortho |
| 5 | 50 | B | ortho |
| 6 | 27 | S | TP |
| 7 | 27 | B | TP |
| 8 | 54 | S | TP |
| 9 | 54 | B | TP |

*C₁ = inoculated control; S = banded with the seed; B = broadcast; ortho = orthophosphoric acid; TP = triple superphosphate.

The rates of orthophosphoric acid were reduced to 15 kg P/ha for the low rate and 50 kg P/ha for the high rate due to difficulties experienced with the application.

Two legume crops, fababean (*Vicia faba* L. cv. Ackerperle) and dry bean (*Phaseolus vulgaris* L. cv. UI 111) were used in two separate experiments. Seed was inoculated with appropriate commercial rhizobium inoculants in powdered peat carrier (*R. leguminosarum* for fababean and *R. phaseolii* for dry bean (Nitragin Co., Milwaukee, WI)).

Seed was planted May 1980, 2.54 cm deep, in four-row plots with a John Deere 71 Flexiplanter with a fertilizer attachment for the application of dry fertilizer. Liquid fertilizer (H_3PO_4) was applied with a gravity flow applicator attached to the planter. Broadcast applications were made by hand with the dry fertilizer and a plastic sprinkler can for the liquid. Row spacings were 60 cm apart with 15 seeds m^{-2} (250,000 seed ha^{-1}). Experimental design was a randomized block, split-plot arrangement with four replications. The nitrogen treatments formed the main plots and the phosphorus and control treatments the subplots.

Two plant samples per treatment were collected 24, 44, 64 and 85 days after seeding and represent first, second, third, and fourth harvest, respectively.

Acetylene reduction, nodule number, shoot and root dry weights, shoot nitrogen (% w/w), root nitrogen (% w/w) and percent P were determined at each harvest. Seed yield was measured at maturity and was based on the two external rows.

Total nitrogen percent was determined by the Kjeldahl procedure. Acetylene reduction assay was performed as described by Burris (1972) and Kisha (1983) with the following modifications. Root systems of two plants from each treatment and from each of two replicates were excavated carefully with a spade. Plants were taken from the middle four rows to exclude border effects. Root samples were washed, blotted dry, and incubated for one hour immediately after extraction in 500 ml, assay chambers (Mason jars). The jars were sealed with a stopper fitted with an air-tight tygon sleeve. Fifty milliliters

(50 ml) of air was withdrawn and replaced with 50 ml of high purity acetylene (C_2H_2) giving a final concentration of 10% of purified acetylene in each chamber.

Gas samples were withdrawn with a syringe and stored in 7 ml evacuated blood sampling tubes (Vacutainer[®]) after one hour and later injected into a Tracor Model 550 gas chromatograph with a dual H_2 flame ionization detector. Ethylene (C_2H_4) and C_2H_2 were separated in the 30 m \times .32 cm column of Porapak T[®]. Gas pressures entering the chromatograph were 30, 50 and 472 ml min^{-1} for He, H_2 , and compressed air, respectively.

Twenty-five microliter (25 μ l) gas samples were injected into the gas chromatograph with a Hamilton gas syringe with Chaney adaptor.

Ethylene peak heights were standardized with a dilution curve for C_2H_4 calibrating gas (1000 ppm C_2H_4 in He, Applied Sciences). Total nitrogenase activity was expressed as μ mole C_2H_4 $plant^{-1} hr^{-1}$. Shoot and root total percent nitrogen (% N) were determined by a semimicro Kjeldahl method (Bremner, 1965) and percent phosphorus (% P) by perchloric acid ($HClO_4$) digestion of plant samples followed by a colorimetric determination of the P in the digest (Olsen and Dean, 1965).

Soil samples were collected from twelve locations representing the site prior to planting. The soil samples were composited and analyzed for pH, electrical conductivity, and organic matter content as well as for nutrients (Table 2). The soil was homogeneous, non-saline, slightly acidic and was on an eroded field of Amsterdam Var. of silt loam (fine-silty, mixed family of Typic Haploborolls).

Meteorological Observations

Precipitation, evaporation and average temperatures were recorded daily approximately 300 m from the plots by the Weather Service Climatological Station and are summarized in Tables 105 and 106 (Appendix).

Table 2. Summary of Soil Properties at Experimental Site, 1980.

| Chemical Test | | Soil Depth 0-15 cm |
|-------------------|----------|-----------------------|
| Nitrate-N | ppm | 7.48 ± 1.09 |
| Phosphorus (Bray) | ppm | 65.00 ± 8.00 |
| K ⁺ | ppm | 409.20 ± 9.12 |
| Ca ⁺⁺ | ppm | 15.40 ± 1.52 |
| Mg ⁺⁺ | ppm | 4.98 ± 0.73 |
| Na ⁺ | ppm | 0.10 ± 0.00 |
| Fe | ppm | 24.34 ± 0.85 |
| Zn | ppm | 0.64 ± 0.13 |
| Cu ⁺⁺ | ppm | 3.24 ± 0.11 |
| Mn ⁺ | ppm | 74.00 ± 14.70 |
| pH | | 6.58 ± 0.11 |
| E-C | mmhos/cm | 0.80 ± 0.00 |
| O.M | % | 1.40 ± 0.10 |

Statistical Analysis

Statistics were based on contrast comparisons as shown in Table 3. The treatments were divided into 9 mutually orthogonal contrasts and 8 additional contrasts representing the interaction between C₁ and each of C₂, C₃, C₄, C₅, C₆, C₇, C₈ and C₉, respectively. The contrast comparison coefficients are reported in Table 4.

Means and error mean squares used in the comparisons were previously obtained by analyzing the experiment as a split plot. All programs used were from 'MSUSTAT' (Lund, 1979).

Field Experiment 1981

Field experiments were established on June 23, 1981 as described for 1980 with the following modifications. The two sources of phosphorus used were monoammonium phosphate, 11-48-0; (MP) and triple superphosphate, 0-45-0, (TP) at two levels of application, 60 kg P₂O₅/ha and 120 kg P₂O₅/ha applied either broadcast and incorporated prior to seeding (B) or banded with the seed (S). Treatments were split into main plots containing 0

Table 3. Summary of Contrast Comparisons used in Analysis, 1980.

| Contrast Designation | | Contrast Description |
|----------------------|---------------------------------|--|
| 1 | C ₁ | N vs no N |
| 2 | C ₂ | Control vs P treatments |
| 3 | C ₃ | Ortho vs TP |
| 4 | C ₄ | Banded vs Broadcast application |
| 5 | C ₅ | P ₁ low level vs P ₂ at high level |
| 6 | C ₆ | P ₁ vs P ₂ in Ortho |
| 7 | C ₇ | P ₁ vs P ₂ in TP |
| 8 | C ₈ | Banded vs Broadcast in Ortho |
| 9 | C ₉ | Banded vs Broadcast in TP |
| 10 | C ₁ × C ₂ | N × (control vs P treatments) |
| 11 | C ₁ × C ₃ | N × (Ortho vs TP) |
| 12 | C ₁ × C ₄ | N × (Banded vs Broadcast) |
| 13 | C ₁ × C ₅ | N × (P ₁ vs P ₂) |
| 14 | C ₁ × C ₆ | N × (P ₁ vs P ₂ in Ortho) |
| 15 | C ₁ × C ₇ | N × (P ₁ vs P ₂ in TP) |
| 16 | C ₁ × C ₈ | N × (Banded vs Broadcast in Ortho) |
| 17 | C ₁ × C ₉ | N × (Banded vs Broadcast in TP) |

and 100 kg ha⁻¹ nitrogen applied as ammonium nitrate. The experimental design was a 2X2X2 factorial in a split plot with the nitrogen levels as main plots and P sources, rates and methods of application as subplots. Three replications were used.

Two control treatments were included in each replication (uninoculated and no P, and inoculated no P). Two legume crops, fababean (cv. Ackerperle) and dry bean (cv. UI 111) were seeded with a John Deere 71 Flexiplanter. Except for the uninoculated control, seed was coated with peat cultures containing recommended strains of *Rhizobium* (Nitragin Co., Milwaukee, WI).

In addition to the variables measured during the 1980 field experiment, pods were counted and dry weight evaluated for dry bean in the third harvest, and dry bean and fababean for the fourth harvest.

All plots were irrigated to field capacity one day prior to the second, third and fourth harvests to allow easy excavation of roots and nodules.

Meteorological observations are reported in Tables 105 and 106 (Appendix).

Table 4. Summary of Contrast Comparison Coefficients, 1980.

| Contrasts | Treatments* | | | | | | | | | | | | | | | | | |
|------------------------------------|-------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| | +N | | | | | | | | | -N | | | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| 1 C ₁ | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 |
| 2 C ₂ | +8 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | +8 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 |
| 3 C ₃ | 0 | 1 | 1 | 1 | 1 | -1 | -1 | -1 | -1 | 0 | 1 | 1 | 1 | 1 | -1 | -1 | -1 | -1 |
| 4 C ₄ | 0 | -1 | 1 | -1 | 1 | -1 | 1 | -1 | 1 | 0 | -1 | +1 | -1 | +1 | -1 | +1 | -1 | +1 |
| 5 C ₅ | 0 | 1 | 1 | -1 | -1 | 1 | 1 | -1 | -1 | 0 | 1 | 1 | -1 | -1 | 1 | 1 | -1 | -1 |
| 6 C ₆ | 0 | 1 | 1 | -1 | -1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | -1 | -1 | 0 | 0 | 0 | 0 |
| 7 C ₇ | 0 | 0 | 0 | 0 | 0 | 1 | 1 | -1 | -1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | -1 | -1 |
| 8 C ₈ | 0 | -1 | 1 | -1 | 1 | 0 | 0 | 0 | 0 | 0 | -1 | 1 | -1 | 1 | 0 | 0 | 0 | 0 |
| 9 C ₉ | 0 | 0 | 0 | 0 | 0 | -1 | 1 | -1 | 1 | 0 | 0 | 0 | 0 | 0 | -1 | 1 | -1 | 1 |
| 10 C ₁ × C ₂ | +8 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -8 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 11 C ₁ × C ₃ | 0 | 1 | 1 | 1 | 1 | -1 | -1 | -1 | -1 | 0 | -1 | -1 | -1 | -1 | 1 | 1 | 1 | 1 |
| 12 C ₁ × C ₄ | 0 | -1 | 1 | -1 | 1 | -1 | 1 | -1 | 1 | 0 | +1 | -1 | +1 | -1 | +1 | -1 | +1 | -1 |
| 13 C ₁ × C ₅ | 0 | 1 | 1 | -1 | -1 | 1 | 1 | -1 | -1 | 0 | -1 | -1 | 1 | 1 | -1 | -1 | 1 | 1 |
| 14 C ₁ × C ₆ | 0 | 1 | 1 | -1 | -1 | 0 | 0 | 0 | 0 | 0 | -1 | -1 | 1 | 1 | 0 | 0 | 0 | 0 |
| 15 C ₁ × C ₇ | 0 | 0 | 0 | 0 | 0 | 1 | 1 | -1 | -1 | 0 | 0 | 0 | 0 | 0 | -1 | -1 | 1 | 1 |
| 16 C ₁ × C ₈ | 0 | -1 | 1 | -1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | -1 | 1 | -1 | 0 | 0 | 0 | 0 |
| 17 C ₁ × C ₉ | 0 | 0 | 0 | 0 | 0 | -1 | 1 | -1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | -1 | 1 | -1 |

*Treatment number refer to those of Table 1.

The field was similar to the one used in 1980. Soil samples were collected prior to planting as previously described and results of chemical analyses are reported in Table 5. The soil was a fine-silty, mixed family of Typic Haploborolls (Koala, 1982). The experimental area had been fallowed the previous year.

Statistical Analysis

Two separate analysis of variances were calculated. The experiment was first analyzed as a split plot design in a factorial arrangement omitting the two controls. This analysis allowed the determination of the N and P main effects and interactions. A second analysis was performed as a split plot in randomized complete block design with the two controls included. The nitrogen treatments served as main plots and all 10 subplots as independent

Table 5. Summary of Soil Properties at Experimental Site, 1981.

| Chemical Test | | Soil Depth | |
|-------------------|-----------|------------|----------|
| | | 0-15 cm | 15-30 cm |
| Nitrate-N | ppm | 6.6 | 6.6 |
| Phosphorus (Bray) | ppm | 40 | 33 |
| K ⁺ | ppm | 428 | 93 |
| Ca ⁺⁺ | meq/100 g | 20 | 46 |
| Mg | meq/100 g | 5.5 | 2.5 |
| Cu | ppm | 3.7 | 3.8 |
| Zn | ppm | 0.6 | 0.5 |
| Fe | ppm | 26 | 25 |
| Mn | ppm | 38 | 42 |
| B | ppm | 0.3 | 0.5 |
| SO ₄ | ppm | 72 | 80 |
| pH | | 6.4 | 6.7 |
| EC | mmhos/cm | 3.7 | 3.7 |
| O.M | % | 1.5 | 1.3 |

treatments. This analysis allowed the comparison between uninoculated treatment, inoculated and P treated plots. This second analysis also permitted the consideration of 3 rates of P, 0, 60 and 120 kg ha⁻¹ rather than two used previously.

Field Experiment 1982

The experiment was conducted at the Montana State University Arthur H. Post Field Research Laboratory as in previous years to evaluate the effect of N and P on nodulation, N₂ fixation, growth and yield of fababean cv. Diana, dry bean cv. UI 111 and green pea (*Pisum sativum* cv. Gardfield). The soil and its physical properties are as described previously in 1980.

The rates of phosphorus used in 1980 and 1981 were small and limited to two. This produced limited effects on nodulation and N₂ fixation. To test phosphorus effects on a wider range, eight rates were used in 1982, ranging from 0 to 210 kg ha⁻¹ in 30 kg ha⁻¹ increments. Nitrogen rates were either deficient 0 kg N/ha (plants primarily dependent on N₂ fixation) or sufficient, 200 kg N/ha (N fertilizer supplied at rates sufficient to satisfy

