

AN ABSTRACT OF THE THESIS OF

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Title: EFFECT OF N AND P FERTILIZERS ON GROWTH,
NITRATE REDUCTASE ACTIVITY, SEED PRODUCTION
AND SEED QUALITY OF SNAP BEAN (*Phaseolus vulgaris* L.)
Redacted for privacy

Abstract approved: _____
Don F. Grabe

The effects of N and P fertilization on growth, nitrate reductase activity, seed production and seed quality were investigated at six growth stages in 'Oregon 1604' snap beans (*Phaseolus vulgaris* L.) grown for seed. Plots were planted in a randomized block design with a factorial arrangement of treatments consisting of four nitrogen rates (0, 50, 150 and 300 kg/ha) as ammonium nitrate and three phosphorus levels (0, 50 and 150 kg/ha) as triple superphosphate with four replications.

ATP content/embryo axis and % seed water soluble carbohydrates were increased by both N and P fertilization. Seed yield, seed weight, seed size, % crude seed protein and seedling axis dry weight were increased by N but not by P, while ATP content/mg embryo axis and % germination were increased by P but not by N.

Dry weight of the 5-day old seedling axis was positively

correlated with ATP content/embryo axis and ATP content/mg embryo axis. The multiple correlation coefficient (R^2) indicated that ATP content/mg embryo axis was responsible for 12.5% of the variation in seedling axis dry weight.

Assays of nitrate reductase activity (NRA) and NO_3^- content were conducted on leaves at four maturity stages: (I) fully developed trifoliate, (II) floral bud, (III) flowering and (IV) seed development. NRA was low at stage I, high at II and III and low at IV. A marked decrease in NRA occurred when the levels of N were increased from 0 to 300 kg/ha. NRA was not affected by P applications.

Nitrate content in the leaf tissue and total protein yield increased with each increase in amount of N applied. Nitrate level generally decreased after stage I as a result of dilution by rapid plant growth.

N translocation efficiency was not affected by either N or P levels.

Yield was positively correlated with leaf NO_3^- content at all four maturity stages, but was correlated with NRA only at stage I.

No correlation was observed between NRA and NO_3^- content in any of the four stages assayed, however, indicating that once NR is induced other factors control NRA in vivo.

N increased dry matter (DM), leaf area (LA) and leaf area index (LAI) at all growth stages except the first fully developed

trifoliate leaf stage. Crop growth rate ($\overline{\text{CGR}}$) was increased by N at the floral bud and seed development stages. Chlorophyll a, b and a+b content at the seed development stage, WSC at maturity, and yield were increased by N.

Responses to P were not as large or as consistent as for N. P increased DM at the fully developed trifoliate, floral bud and flowering stages, LA at the fully developed trifoliate and floral bud stage, LAI at the unifoliate, fully developed trifoliate, and floral bud stage and $\overline{\text{CGR}}$ at the fully developed trifoliate and floral bud stages of growth. Chlorophyll a, b, and a+b, water soluble carbohydrate (WSC) and yield were not influenced by P fertilization.

The multiple correlation coefficient (R^2) indicated that LAI at maturity was responsible for 41.6% of the variation in yield. WSC, $\overline{\text{CGR}}$ at the fully developed trifoliate stage, and LA at the flowering stage contributed an additional 13.5, 4.6 and 6.1%, respectively.

Effect of N and P Fertilizers on Growth, Nitrate
Reductase Activity, Seed Production and
Seed Quality of Snap Bean
(Phaseolus vulgaris L.)

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DEDICATION

To GOD, above all, that gave me strength, faith and courage in the moments that I most needed them.

To BRAZIL, my country, to retribute it for the high price paid for my intellectual and scientific development.

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To MY WIFE ARLETE, for her love, help and understanding manifested, which allowed me the accomplishment of my goal.

I wish to dedicate this thesis.

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EFFECT OF N AND P FERTILIZERS ON GROWTH,
NITRATE REDUCTASE ACTIVITY, SEED
PRODUCTION AND SEED QUALITY OF
SNAP BEAN (Phaseolus vulgaris L.)

INTRODUCTION

Large acreages of snap beans are grown each year in the Willamette Valley for processing purposes. Over 3,000,000 pounds of seed are required each year to plant this acreage. To achieve maximum yields of processing beans, the seed planted should possess the highest possible performance potential.

Several seed quality attributes have been associated with optimum seed vigor and performance in snap beans. Many of these attributes, such as size and protein content, are associated with soil nutrient levels. While fertilizer practices of achieving optimum yields are well developed, information on the effects of fertilizer levels on bean seed quality is limited.

If maximum performance is to be obtained from snap bean seed, the effects of fertilizer application on seed quality need to be identified and seed production practices geared toward maximizing those effects. Additional information is needed on how fertilizer applications affect certain aspects of plant growth and development leading to seed production.

The general objectives of these studies were to determine the

effects of N and P fertilization on plant development and on the yield and quality of snap bean seed. Specific objectives were to determine the effects of N and P fertilization on (1) yield and quality of snap bean seed, (2) nitrate reductase activity and nitrate content of snap bean leaves, and (3) growth attributes of snap bean plants.

This thesis is divided into five sections: a general introduction and literature review; a manuscript on the effects of N and P fertilization on snap bean seed yield and quality; a manuscript on the effect of N and P fertilization on nitrate reductase activity and nitrate content of snap bean leaves; a manuscript on the effects of N and P fertilizers on growth attributes of snap bean plants; and an appendix containing a general bibliography, meteorological data, statistical analyses and other information not reported in the manuscripts.

LITERATURE REVIEW

Seed Quality

The quality of seed is a product of their history.

Environmental conditions during the plant life cycle will directly or indirectly affect the quality of seed produced. As a result seed differ in size, weight, color, composition and biological properties. All these conditions will affect seedling metabolism and performance in the next generation.

In the literature the idea of seed quality components has varied with time and authors (Filgueiras, 1978). Delouche (1971) stated that quality of seed at any point in time reflects the significant factors that have acted upon them in the past. From fertilization through planting, seed are subjected to many conditions and operations which determine quality. These include: genetic characteristics of the seed, source of seed, land selection, cultural practices, growing conditions, pre-harvest environment, harvesting procedures, aeration and drying, handling and conveying, processing, storage, chronological age, and homogeneity.

The complete seed quality control program insures that the detrimental effects of the conditions and operations to which are subject are minimized.

Grabe (1979, personal communication) pointed out that seed

quality is constructed of many quality characteristics. In terms of individual seeds, these characteristics include viability, vigor, moisture content, maturity, mechanical damage, disease infections, size, appearances, length of life and performance. When extended to the seed lot, quality characteristics also include weed seed content and other foreign material, and uniformity of quality characteristics throughout the lot. He also considered that seed quality is at its highest when the seed reaches maturity on the plant and from maturity onward seed quality declines at a varying rate.

Delouche (1975) in a broad sense stated that seed quality involves all attributes of a seed lot including physical, physiological and genetic aspects, which implies that seed quality begins in the field, where the plant grows and matures its seeds.

Specifically considering beans Kerr (1962), emphasized that a good seed quality in beans should present a germination percentage not less than 90% and free from major seed-borne diseases.

Hanssen (1963) pointed out that quality characteristics for a bean seed lot should be: percent of pure seed, germination and hard seed, presence of noxious weeds, varietal purity, health conditions, moisture content, origin of production and bushel weight.

Galvez (1976) added that besides considering genetic, physical and physiological factors, we should include phytosanitary conditions in determining seed quality of beans. The presence of bacteria, fungi

and viruses in the seed also may affect germination seedling emergence, vigor, stand establishment and yield and represent a potential danger of spreading diseases to the next generation and to new areas.

More recently protein content of the seed has been related as a quality factor in beans. Ries (1971) studied the relationship of protein content and size of bean seed with growth and yield. In field studies he found that seedling size, yield and number of fruits were more highly correlated with seed protein than with seed size. Most importantly, there was also, with one exception, a significant increase in seedling size, yield and number of fruit from high protein seeds obtained by supplemental N applications the previous year.

Ries and Everson (1973) stated that environment and genetic factors could alter the protein content of wheat seed, but regardless of both parameters, seedling vigor measured by dry weight of shoots was related to seed protein.

Also Lowe and Ries (1972) pointed out in wheat that there is a significant positive correlation between seed protein content and dry matter after 3 weeks of growth. Seedlings grown from high protein seeds were more advanced in morphological development than seedlings grown from low protein seeds.

Adenosine Phosphates and Adenylate Energy Charge
and its Relation to Seed Vigor

Seed vigor is one of the factors determinant of seed quality. Although a concise definition satisfactory to most investigators has yet to be realized, the concept of vigor and its importance in crop development are well accepted. The concept of vigor and its measurement have been extensively investigated by many authors, among them Copeland (1976), MacDonald, Jr. (1975), Ching (1973), Woodstock (1973), Heydecker (1972), and Pollock and Roos (1972).

There are several vigor tests being used according to MacDonald, Jr. (1975) and they are divided primarily into physical, physiological and biochemical. Physical tests measure seed characteristics such as weight, size, density, etc. Physiological tests utilize some parameter of germination or growth and biochemical test for vigor monitor chemical reactions involved in cellular metabolism.

More emphasis will be placed on the discussion of a biochemical test for measuring seed vigor, so called "ATP test."

Atkinson (1968) first introduced the concept of Energy Charge (EC) as a regulatory parameter for cellular energy status. He suggested that EC of the adenylate system could measure the energy levels of the cell.

The energy levels of the cell are expressed in terms of

adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP). He defines EC as half of the number of anhydride-bound phosphate group per adenine moiety, therefore,

$$EC = \frac{ATP + 1/2 ADP}{ATP+ADP+AMP}$$

If all the adenine nucleotide in the cell is ATP, the system is fully charged and has an energy charge of one. ATP contains two anhydride-bound phosphates. ADP accordingly contains 0.5 energy charge. If all adenine nucleotide present in the cell is AMP, the system is empty of high-energy bonds and has an energy charge of 0.0.

With Escherichia coli, Chapman, Fall and Atkinson (1971) reported that growth occurred only at EC values greater than 0.8, and that at values between 0.8 and 0.5 viability was maintained; however, if the EC was less than 0.5 senescence and death of organism occurred. This energy charge is an index regulating the activity of various sequences related with energy utilization and regeneration of tissue.

Ching (1972a) pointed out that seed germination requires a tremendous amount of biological energy (ATP), not only for biogenesis of new cellular constituents in seedlings, but also for the formation of protein synthesizing machinery in producing enzymes for

degradation and conversion of storage compounds. The availability of ATP is a definite controlling factor in germination and early seedling growth. ATP supply does not appear to be limiting during germination in suitable temperature range and with normal oxygen supply. For survival reasons seeds are provided with apparatus (mitochondria) for ATP production and systems for ADP synthesis and production of inorganic phosphate to meet the energy requirement of growth activities. If the environmental conditions are changed to adverse ones, such as very low temperatures and anaerobic conditions, ATP would be limiting and germination arrested.

Also Ching (1972a) cited that ATP is formed during the light reaction of photosynthesis and during glycolysis, but the most important pathway during early stages of growth is through oxidative phosphorylation (mitochondria).

Ching and Danielson (1972c) cited that adenosine triphosphate (ATP) is needed for endergonic reactions, regulates biosynthesis and is required for protein synthesis during very early germination process. ATP content in the seeds could be a good biochemical index of growth potential or seedling vigor.

Working with 28 lots of nine cultivars of lettuce they have found highly significant correlations between ATP content in 4-hour - imbibed seeds and seed weight and seedling height.

Aging reduced ATP content as well as germination percent and

seedling size.

Ching and Kronstad (1972b), studying varietal differences in growth potential, adenylate energy level, and energy charge of wheat concluded that dynamic synthesis and utilization of adenosine phosphate in embryos and seedlings of a fast growing wheat variety "Yamhill," resulted in a higher overall energy level (ATP content) and energy charge (EC), than that of slower growing "Hyslop" seedlings. The varietal differences in growth potential appears to be related to energy metabolism and capacity of synthesis. During seed germination in both fast and slow growing seedlings, ATP content increases very rapidly providing the energy required for the early events of germination.

Significant correlations were found by Ching (1973a) between the ATP content in imbibed seeds and seed weight or seedling size in crimson clover, rape and ryegrass seeds. The correlation of ATP content and seed weight or seedling size was not affected by the seed chemical composition as crimson clover are proteinaceous, rape seeds fatty and ryegrass starchy. The need of energy supply for the initiation of germination and maintenance of growth is universal, so the chemical nature of seed reserves did not interfere with the energy synthesis at this early stage of germination. Also she stated that ATP content thus appears to be a useful biochemical

index of seed vigor.

Ching (1974) made a study of energy state and chemical composition of pod walls and seeds of maturing rape (Brassica napus L.) on two varieties, Victor and Gorczanski. Total adenosine phosphates, ATP and adenylate charge increased with increasing cell number and cellular synthesis, during the early stages, remained high at maximum dry weight accumulation, and maximum substrate influx time, and decreased with ripening.

Garay (1975) analyzed the effect of nitrogen fertilization of wheat on chemical and biochemical composition and performance of seeds. Applications of 150 and 300 Kg N/ha increased the grain yield and protein yield per hectare, seed size and percent and amount of protein per seed of Yamhill wheat. He stated that high protein wheat seeds frequently performed better than low protein seeds of the same variety. N also caused several biochemical changes in embryos resulting increases in ADP, ATP and energy charge (EC), and regardless of protein concentration the quantities of solubles and adenylate phosphate were higher in larger embryos. The larger seeds with high protein concentration produced seedlings with the highest ADP, ATP and total AP content, energy charge (EC) and growth potential.

Ching et al. (1975) reported for the first time the influence of

ammonium nitrate on adenylate energy state of wheat leaves.

The application of 150 kg/ha of N as an ammonium nitrate to young wheat plants increased the total leaf fresh weight, dry weight, water content and leaf area to 174, 150, 111, and 192% of control plants respectively. The contents of ATP, ADP and total adenosine phosphates (AP) per gram of leaf fresh weight averaged 25% higher in plants with fertilizer than without. AMP content, however, was 40% lower in fertilized plants. When the contents were compared on per leaf basis, the fertilizer-stimulated total increases reached 32 to 281% of the control on all three nucleotides and the total. These increases in pool size of nucleotides indicate that not only the phosphorylation of ADP to ATP via photosynthesis is more efficient in fertilized plants but also more synthesis of nucleotides.

Structural components and amino acid pool are larger but sucrose, total sugars and starch content are smaller, indicating efficient biosynthesis and facilitated transport of photosynthetic products in leaves of fertilized plants.

The concentration of glucose including glucose-6-phosphate, free amino acids and chlorophylls were elevated in fertilized plants to 240, 196 and 192%, respectively, of those of control.

Investigations were conducted by Vahabian (1977) at three environmentally diverse experimental sites and in the laboratory to determine the effects of seed source, seed size, adenosine

triphosphate (ATP) and adenylate energy charge (EC) on stand establishment, yield and yield components of wheat cultivars. Specifically he concluded that ATP and EC of cultivars varied among and within cultivars depending on the seed source and the seed size. Effect of seed source was found to be cultivar dependent. Larger seeds produced higher ATP than smaller seed in all cultivars. When the field data were combined over locations ATP showed fairly high total effect on yield but the effect of this trait on the yield of cultivars grown at the Moro site was not so pronounced.

Ching et al. (1977) compared thirteen seed vigor parameters in two 2-row and four 6-row barley (Hordeum vulgare L.) cultivars to determine selection criteria for predicting rate of field emergence. Based on the results of stepwise multiple regression analysis, it appeared that seed weight, 3-day seedling ATP, TAP content of hydrated embryo and 7-day seedling dry weight would be good seed vigor indices for predicting field emergence rate.

Nitrate Reductase, Nitrate Content, and its
Relationship to Grain Yield and Protein

The process of nitrate assimilation is of considerable importance to plant growth because nitrate is the main inorganic nitrogen compound which enters in plant cell and is incorporated to organic nitrogen into the form of amino acids. Nitrate is the most common source of nitrogen for crop plants and is the primary form of nitrogen absorbed by the plant's roots and reduced before it combines with the keto acids to form amino acids, and finally protein (Hageman et al., 1976; Magalhães, 1975; Tisdale and Nelson, 1975; Liu and Hadley, 1971; and Beevers and Hageman, 1969).

Lovato (1978) cited that other forms of nitrogenous compounds are converted to nitrate after rapid nitrification by microorganisms when the temperature is above 5°C.

Nitrate reductase is the enzyme responsible for the reduction of nitrate to nitrite, the first step in the nitrate metabolism pathway; then nitrite will be reduced to ammonia, which will be incorporated into amino acids and finally into proteins.

The general characteristics of nitrate reductase have been the object of extensive review by several authors (Hageman et al., 1976; Hewitt, 1975; Hageman and Hucklesby, 1971; and Beevers and Hageman, 1969).

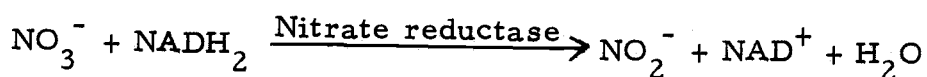
Nitrate reductase is considered by Hageman et al. (1976) and

Beevers and Hageman (1969), the rate limiting step between nitrate and amino acids, and is a factor limiting protein synthesis.

Lovato (1978) emphasized that this statement is substantiated by the observation that no intermediates are found free in plant tissue between nitrate and amino acids, although theoretically at least six intermediate compounds exist.

According to Magalhães (1975), nitrate reductase is found in the cell cytoplasm and has been extracted from diverse tissues such as leaves, stems, petioles, roots, cotyledons, etc. Hageman and Hucklesby (1971) and Magalhães (1975), indicate that there are several lines of evidence that the reduction of nitrate in green tissues occur in the cell cytoplasm and is associated with the oxidation of metabolites derived from photosynthesis.

The reaction catalyzed by nitrate reductase involves the transfer of two electrons from reduced nicotinamide adenine dinucleotide (NADH) generated via a NAD glycerinaldehyde and/or a NAD-malate dehydrogenase.



Klepper et al. (1971) found that sugars formed in photosynthesis which migrated from the chloroplast to the cytoplasm were the prime source of energy for the production of the reducing power (NADH), and the oxidation of glycerinaldehyde-3-phosphate was ultimately the

in vitro source of NADH for nitrate reduction.

Sanderson and Cocking (1965), working with tomatoes, pointed out that in general all tissues show nitrate reductase activity but stems and roots are much lower in activity than that observed in leaves.

Nitrate reductase is low in the initial leaf expanding phase and reaches a peak when the leaf is fully expanded and in soybean, nitrate reductase is maximum at full blossom stage, thus enzyme activity level appears to be closely associated with physiological growth stage (Harper et al., 1972).

However, Liu and Hadley (1971) observed in a soybean experiment that maximum activity of nitrate reductase was observed near the beginning of flowering and this is probably related to the high nitrogen rate during flowering.

The level of nitrate reductase decreases as the plant approaches physiological maturity in soybeans (Liu and Hadley, 1971) and wheat (Harper and Paulsen, 1967).

The amount of extractable nitrate reductase varies drastically with plant species, varieties within a species, plant age, plant tissue and cultural techniques (Hageman and Hucklesby, 1971).

Lavoy and Hageman (1971), working with wheat, also observed that the level of the enzyme varies from season to season and year to year. Nitrate nitrogen is the inducer of both nitrate and nitrite

reductase, according to Ingle et al. (1966).

Different levels of nitrate are required for optimum induction in different plant species.

Nitrate reductase being a substrate inducible enzyme is subjected to end product repression by ammonia and various amino acids according to Smith and Thompson (1971) and Shen (1969).

Shen (1969) suggested that the ammonium ion inhibits the first stage of nitrate reduction from nitrate to nitrite but does not inhibit the assimilation of nitrate. However, there are evidences that ammonium may act directly in the nitrate uptake process (Minotti et al., 1969).

Induction of nitrate and nitrate reductase is prevented by inhibitors such as: actinomycin D, puromycin and cycloheximide, indicating a requirement for continuous RNA and protein synthesis (Ingle et al., 1966).

Nitrate reductase is very sensitive to the environment conditions, and shows diurnal changes (Beevers and Hageman, 1969). Also this enzyme can be inactivated by environmental stresses such as moisture and temperature (Younis et al., 1965). Mattas and Pauli (1965) pointed out that moisture stress in corn greatly affects its activity but after the stress it returns to normal.

Huffaker et al. (1970), working with barley, concluded that almost 60% of nitrate reductase activity (NRA) was lost in four days

of water stress but 24 hours after watering NRA had recovered completely.

Temperature around 40°C according to Onwueme et al. (1971), and Mattas and Pauli (1965), can inactivate the nitrate reductase activity. Increase of temperature beyond an optimum reduces its activity and causes subsequent nitrate accumulation (Younis et al., 1965; and Mattas and Pauli, 1965).

Nitrate reductase activity is also affected by plant hormones. Lips and Roth-Bejerano (1969), and Roth-Bejerano and Lips (1970), found that kinetin and gibberellic acid (GA) permitted the induction of nitrate reductase in leaves of tobacco in the dark. Hormone sprays eliminated the need for light induction of the enzyme. Kinetin and gibberellin indirectly affected the activity of the enzyme. The larger the amount of kinetin added, the smaller the concentration of gibberellic acid required to obtain a maximum amount of nitrate reduction. However the effect of both growth substances on this enzyme was not due to general protein synthesis stimulation.

Light is required for the continuous activity of nitrate reductase and the enzyme activity is proportional to light intensity (Travis et al., 1970). The enzyme could be induced at the beginning of the dark period until the effect of the previous light treatment was exhausted.

Upon turning the leaves to light, enzymatic activity increased

again.

Hageman and Hucklesby (1971), stated that because the enzyme is substrate inducible and exhibits a diurnal variation, highest activities are obtained with cotyledons and leaf tissue from vigorous young seedlings grown on a high (at least 15 mM) nitrate medium, and with adequate illumination (at least 2500-fc, 12-hour photoperiod).

Since minimal activity would be encountered at the termination of the dark period, a 3- or 4-hour period of illumination prior to harvest is suggested.

Nitrate reductase activity is clearly under genetic control and its level according to Shrader et al. (1966) is highly heritable and can be a useful tool in breeding programs as a criterion for selecting and developing high yielding and high protein varieties. Nitrate reductase activity varies considerably among genotypes of a same species.

Harper et al. (1972) in soybeans, found a genetic variation in nitrate reductase activity up to 1.8 between cultivars and variations up to fivefold were observed in corn inbreds (Zeiserl and Hageman, 1962).

Hageman (1976) stated that the correlation between enzyme activity and nitrate content of the tissue is not close throughout most of the life cycle of the plant. The flux of nitrate to the enzyme

induction site is probably the major factor regulating the level of nitrate reductase activity.

It can be argued that the mechanism of protein synthesis, rather than aminoacid supply, is the major factor regulating protein production.

However, the pronounced effect of increased and supplemental nitrogen supply on vegetative growth, grain yield, and grain protein suggests that nitrogen supply is the primary limiting factor under commercial field conditions.

Because nitrate reductase is induced by the substrate (NO_3^-) some investigators believe that NRA is not strongly related to nitrogen content of the grain and is only a reflection of availability of nitrate. There is a strong relationship between nitrate and NRA, but has been more success in using nitrate reductase activity as productivity index of grain and grain protein production than the tissue nitrate content. Deckard (1970) and Purvis (1972), cited by Hageman (1976), pointed out that genotypic differences in levels of nitrate reductase have been found to be independent of nitrate content of tissue.

Assay methods to measure nitrate reductase activity "in vivo" and "in vitro" were described by Hageman and Hucklesby (1971) and Hageman (1976). "In vitro" method means to isolate the enzyme from treated tissue and measure its activity for nitrate reductase

function. "In vivo" method uses excised plant tissue, providing the necessary substrate (NO_3^-), measures the disappearance of NO_3^- or appearance of NO_2^- in the suspending medium tissue.

Klepper et al. (1971) pointed out that an "in vivo" procedure for the measurement of nitrate reduction can offer several advantages over the "in vitro" analysis. These advantages would be (a) more rapid and simple analysis, (b) no aqueous extraction of enzymes is required, and (c) a better measure of the actual rates of nitrate reduction which are occurring within the plant.

In the procedure for the "in vivo" nitrate reductase assay, it was necessary to incubate the green leaf tissue in darkness. Photosynthetic electron flow is blocked by darkness which stops the energy generation necessary for nitrite reduction. When sections of green leaf tissue were incubated in the dark under the prescribed assay conditions, nitrite not only accumulated in the tissue but diffused into the incubation solution.

Normally, only traces of nitrite were detectable during incubation in the light. These small amounts of nitrite are thought to be due to leakage by peripheral, ruptured cells. Leaf discs incubated in the light did not accumulate nitrite until transferred to darkness. In contrast, leaf discs incubated in darkness, rapidly accumulated nitrite until they were transferred.

Upon exposure to light, nitrite is rapidly reduced to ammonia.

However, Hageman et al. (1976) stated that the "in vitro" assay shows 2 to 5 times more activity than the "in vivo" assay, correlations between the assay values obtained by the two methods are high ($r=0.85^{**}$ to 0.99^{**}) and neither the "in vivo" nor the "in vitro" assay is a true reflection of the "in situ" activity.

Many reports have shown that the correlation between nitrate reductase activity and production of grain and grain protein have been quite variable.

Zieserl et al. (1963) cited by Hageman et al. (1976), did the pioneer work associating NRA and grain yield and grain protein production. He tested four corn hybrids: Illinois 1996 and Hy2 x Oh 7, reported as high yielders and WF9 Oh 7 and WF9 x C 103, known as low yielders, especially at the high populations used.

There was no statistical difference in the nitrate reductase activity among the four hybrids. However, Shrader and Hageman (1965) using two of the above hybrids, Hy2 x Oh 7 and WF9 x C 103 found that nitrate reductase activity, grain yields, and grain protein production were significantly related.

Deckard et al. (1973), investigated whether a relationship between NRA, grain yields, and grain protein could be detected for six corn hybrids grown under field conditions with supplemental N and irrigation. The N-fertilizer treatments increased leaf blade nitrate concentration which resulted in a significant increase in NRA,

and increased in the amount of grain protein.

NRA showed a significant positive correlation with grain protein yield and total reduced N in the vegetative material (above ground) and grain and stover at maturity.

The highest correlations between NRA and yields of grain and protein were obtained during the stages of ear initiation and development. This suggests that a minimal number (1 to 3) of samplings would be as effective as the laborious full season sampling (12 or more dates) in selecting individual plants or varieties that have a high potential for grain yields or grain protein production.

Croy and Hageman (1970), working with two wheat varieties, Ponca and Monon, with and without supplemental nitrogen, tried to determine if nitrate reductase activity could be correlated with water soluble leaf protein, grain and grain protein production.

Their major conclusions were: (a) nitrate content of the tissue was a major factor in controlling the level of enzyme activity, (b) NRA was related though not numerically to leaf protein content, (c) Induction of nitrate reductase was achieved by supplemental N, (d) Increased enzyme activity from supplemental N-fertilizer treatments resulted in increases in grain protein (% and total), and (e) Significant correlation was found between the spring seasonal total of NRA and grain protein for both varieties.

Eilrich and Hageman (1973), in a wheat experiment, reported

a positive relationship between nitrate content and nitrate reductase activity, substantiating the dependency of enzyme activity upon availability of substrate. Since the translocation of vegetative nitrogen to the grain was uniform across treatments, a significant positive correlation was found between NRA and grain yield and grain nitrogen at maturity.

Harper et al. (1972), used an "in vivo" assay to determine if differences existed in NRA among 16 soybean (Glycine max L. Merr) varieties within four maturity groups. All varieties exhibited highest NRA per leaf x hour in upper leaves of the plant canopy during growth stages up to mid pod fill.

NRA of the total plants expressed in $\mu\text{moles NO}_2^-$ formed/plant/hour was maximal at approximately the full bloom stage, thus appearing that enzyme activity appeared to be closely associated with physiological growth stage.

NRA did not correlate with either seed yield ($r=0.51$) or seed protein content ($r=0.42$). They emphasized also that the ability of soybeans to incorporate N through utilization of nitrate via the nitrate reductase and through N fixation precludes an absolute dependence of the soybean plant on nitrogen derived from one source.

These two systems can be competitive because high soil NO_3^- inhibits nodulation, while high soil nitrate is required for maximum NRA. The possibility of NRA correlating with yield or percent grain

protein among a number of soybean varieties with diverse genetic backgrounds may be remote. Recent estimates indicate that 25 to 50% of the total nitrogen may be supplied via symbiotic N_2 fixation under field conditions, and thus a considerable portion of plant-N is dependent upon uptake of NO_3^- and subsequent reduction via nitrate reductase.

Liu and Hadley (1971) reported that nitrate reductase activity ("in vitro" assay) and nitrate content of nodulating soybean lines was higher than nonnodulating lines, especially in the late stage of the growing season. A significant positive correlation was observed between NR level and nitrate content ($r=0.59^{**}$) and between stem protein and seed protein ($r=0.65^{**}$).

Klepper (1975) pointed out that there are many factors involved in the accumulation of protein in wheat grain. Nitrate reductase activity is only a single factor, yet is one of the key factors. There are four factors that influence the incorporation and assimilation of nitrate into protein.

The first factor is nitrate uptake and translocation, if the plant roots are incapable of finding and taking up large amounts of soil nitrogen, protein cannot be synthesized regardless of how high its genetic potential for nitrate reduction.

The second factor is high genetic potential for nitrate reduction and protein synthesis. After nitrate uptake, nitrate must be reduced

for protein synthesis. The third factor under consideration is that the plant must have an efficient photosynthetic system, because a competition exists between carbohydrate and protein biosynthesis. The photosynthetic system must be able to provide the necessary reductive energy for both processes.

Finally, the last factor involved is an efficient vegetative protein breakdown and translocation to the grain. After protein has been synthesized, the plant must be able to transport this protein from the vegetative parts to the grain. In wheat the flag leaves are very important in mobilizing and translocating vegetative N to the developing grain.

Hageman et al. (1976) called the ability of plants to transport N from the vegetative parts of the plant to the grain "N transport efficiency" and is a function of the genotype. "Transport efficiency" according to them has two components involved: the total amount of straw and the percentage of nitrogen.

Transport efficiency is evaluated by the percentage of N retained by the straw and is a function of genotype as was stated before. Also they considered the term "enzyme efficiency" meaning the ratio between calculated input of reduced nitrogen from enzyme assay to actual input a very important factor that could affect the correlation between enzyme activity and yield of grain or total protein.

Streeter (1978), citing Sinclair and de Wit, stated that the

translocation of N from vegetative organs of soybean plants during reproductive development is a "self destructive" process.

Translocation of N is required because demand for N during seed growth exceeds the estimated maximum rate of N uptake.

Rapid translocation of N from leaves is presumed to result in degradation of cellular components required for photosynthesis, leading to a senescence. Also they calculated that about 130 kg N/ha can be supplied by vegetative organs.

During seed growth they estimated that 5 kg N/ha/day must be translocated from vegetative organs to seed and this will result in a depletion of supply and "self destruction" after a seed growth of only 26 days. Seed growth is stopped when N can no longer be translocated from vegetative organs, seed yield is largely a function of the length of seed growth period.

Egli et al. (1978) stated that the redistribution of N from vegetative to reproductive plant parts of soybean may be one of the factors causing leaf senescence and consequently it may have a direct influence on yield. Citing several other authors they reported that 50 to 64% of N in the seed of soybean comes from the redistribution of N from the vegetative portions of the plant during the filling period.

Egli et al. (1978) citing Hanway and Weber found that redistribution of nutrients, including N, from vegetative material to soybean

seed, occurred regardless of the rate of fertilizer application, and nutrients are translocated to the seed from vegetative material even though readily available in the soil.

As was pointed out before by Sanclair and Wit, cited by Streeter (1978), the redistribution of nutrients from vegetative parts of the plant plays an important role in the senescence process in plants such as soybeans, which produced high protein seeds.

Egli et al. (1978) performed an experiment with soybeans in a greenhouse by hydroponic system to investigate the influence of N removal from nutrient solution at various intervals during pod filling on the redistribution of N from vegetative plant parts to the seed and on leaf senescence. Removal of N early in the pod filling period reduced yield, primarily as a result of smaller seed. Also it hastened maturity and increased the proportion of N in the seed that came from redistribution. Early N removal resulted in earlier leaf and petiole abscission, but none of N removal treatments had a major effect on the concentration of N in the abscised material.

The data suggested that redistribution of N from vegetative to reproductive plant parts influences plant senescence; however, other factors are involved.

Influence of Fertilization on Plant Growth and
Development Measured by Growth Analysis

The living state of organisms is characterized by systematic increases in bulk and complexity over time.

The irreversible increase in size and weight over time characterizes the sigmoidal growth of an organism, while the development including growth and differentiation infers a high order of change that involves anatomical, physiological specialization and organization. Fertilizers among other factors (light, CO_2 , H_2O , temperature, hormones, enzymes, etc.) do influence plant growth and development. Growth in plants can be measured in many ways, the most straightforward is by the increase in dry weight that reflects the assimilation of photosynthate produced in the photosynthetic process.

Approximately 94% of all plant dry matter yield is the result of photosynthesis. Growth analysis, according to Watson (1968) provides a suitable method to measure growth, based on the principle that the increase in dry weight of plants in a given period is a measure of net photosynthesis.

The growth analysis procedure requires only measurement of plant dry weight and leaf area on samples of the crop at intervals throughout its growth, but counts and measurement of plant organs

on the same samples can give some information on the morphological constitution of the photosynthetic system, and the changes with time in its relation to plant parts other than the leaves.

Parameters of growth analysis such as crop growth rate (CGR), relative growth rate (RGR), leaf area (LA), leaf area index (LAI) and others with their respective formulae have been described by Wolf and Carson (1973), Wolf et al. (1972) and Radford (1967).

Magalhães et al. (1971) measured the effect of the soil incorporation of dry residues of perennial soybean alone or with fertilization upon the growth attributes and productivity of common beans.

The soil incorporation of dry organic matter positively affected growth during the early phases of plant development, causing a significant increase in LAI and CGR up to the time of flowering, causing leaf self shading and decreased growth in the later stages of development. In contrast, both the control plants and those that received NPK fertilization, showed lower values of LAI and CGR that continuously increased towards the end growing period.

The treatments incorporating organic matter induced higher values of NAR and RGR in the pre-blooming phase, and decreased rates in the period subsequent to flower formation. Only slight differences in grain yield were noticed despite remarkable increases of CGR, RGR, and NAR that result from the application of dry organic matter. The values of seed production in the control plots might be

attributed to the fairly good chemical properties of the soil in the experimental area, that appeared to be able to support a satisfactory grain yield.

Montojos and Magalhães (1971) pointed out that the dependence of photosynthesis on solar radiation reveals the possibility of increased crop yield by a more efficient utilization of sun energy by the leaves of chlorophyllous plants. If crops do not receive adequate fertilization, the efficiency of solar energy conversion into storage products may be limited by insufficient leaf area present during the periods of higher carbohydrate demand such as the time of grain filling.

They also cited that grain yield mainly in cereals is determined by the amount of leaf area actively photosynthesizing during the growth period after flowering the increase in leaf area duration after anthesis, between flowering and maturity, might be considered as an important factor for the improvement of grain yield. Alterations in leaf area duration can be obtained by fractionating the nitrogen applications throughout the growing season. They studied the effects of the intensity of solar radiation and the number of applications of nitrogen fertilization on the growth parameters and grain yield of dry beans.

Conditions of high intensity of solar radiation (400-500 cal. cm^2/day) promoted increasing growth rate, dry matter production

and consequently high seed production due to their effects on the increase of LAI which influenced the size of photosynthetic system.

The diminishing dry matter production noticed in the later growth stages in the treatment of high light-three nitrogen applications, might have been caused by the relatively high values of LAI reached during the early part of the growth cycle inducing mutual shading which caused the decrease of the photosynthetic capacity of the foliage.

The fractionation of N fertilization into two or three applications during the vegetative period induced modifications of the growth parameters which were most noticeable in the low radiation treatments.

Largest seed production was obtained when the N application was parcelled three times during the first 21 days of the growing period. This procedure delayed leaf senescence and improved photosynthetic capacity of bean crop because the increase in leaf area duration.

Buttery (1969a) evaluated the effects of plant population and fertilizer on the growth and yield of soybeans. High density (32 plants/m²) of soybeans resulted in small plants but high dry weight per unit area of ground, while low density (4 plants/m²) produced large plants with a small dry weight per unit of ground.

Intermediate densities (16 and 9 plants/m²) produced

intermediate plant sizes and yields. Shoot/root, bean/shoot and leaf area ratio were all decreased by increasing density. The effects of density on plant growth were detectable 30 to 40 days from planting, and increased rapidly thereafter.

Fertilizer in moderate quantity depressed the growth of the whole plant in the early stages but by maturity, fertilizer was associated with a small increase in weight of shoot and an increase in the proportion of beans to shoot.

High density reduced the proportion of flowers forming mature pods while fertilization application increased it. There was no interaction between fertilizer level and density of planting on any plant characteristic.

Effects of fertilizer on later stages of growth could be attributed mostly to N; effects on early stages of growth are more difficult to interpret but suggest an extreme sensitivity to fertilizer, which may be involved in the generally observed poor response of soybeans to fertilizer.

Also Buttery (1969b) conducted an experiment to observe how growth parameters such as NAR, LAI, RGR, CGR etc., and yield are affected by plant population and fertilizer treatments.

Net assimilation rate (NAR), relative growth rate (RGR) and relative leaf growth rate (RLGR) of soybeans declined throughout the season; much of this decline and most effects of plant population

on growth could be attributed to a rise in leaf area index (LAI). Increase in LAI from 0.3 to 1.0 was associated with a marked decrease in NAR and maximum LAI was attained between 70 and 80 days from planting in all densities used.

Crop growth rate (CGR) increased for the first 50 to 60 days falling sharply thereafter. There was no optimum value of LAI.

LAI was increased slightly by fertilization application, presumably because a higher RLGR between 50 and 70 days.

Hanway and Weber (1971), studied the dry matter accumulation in different parts of eight soybean varieties determined at developmental stages of growth during two growing seasons.

They pointed out that within varieties, rates of dry matter accumulation in different plant parts were similar in both years.

The rate of leaf fall was similar for all varieties, but time of leaf fall varied among varieties.

Koller et al. (1970), used growth analysis techniques to study the components of dry matter accumulation in field soybean.

They made an analysis of dry matter accumulation in leaf, supporting pod wall, and seed fractions, as well as the total above-ground portion of the crop.

Also were calculated during the growing season values of relative growth rate (RGR), crop growth rate (CGR), net assimilation rate (NAR) and leaf area ratio (LAR). They concluded that RGR of

each individual plant fraction steadily decreased at a decreasing rate as the season progressed.

At any given time the most recently initiated plant fraction had the greatest RGR. The CGR of each fraction rose to a peak and then declined. The increases in RGR and CGR during August are attributable to a concurrent increase in NAR. The increase in NAR is interpreted as a response of the photosynthetic apparatus to an increase for assimilates was due to rapid growth of the seed fraction.

Weber et al. (1966) reported an experiment relating the effect of plant population and row spacing on soybean development and production. They measured leaf area and dry weight of soybean plants at various stages of development and found that dry weight production (DW) was positively and highly correlated with leaf area index (LAI). Those plant populations arrangement combinations favoring a rapid attainment of high LAI (i. e., high plant population and narrow spacings) were those also having the greatest DW accumulation.

Maximum seed yield occurred at less than maximum LAI, and at generally lower populations and narrower lower row spacings.

Plants produced at highest densities were taller, more sparsely branched, lodge more and set fewer pods and seed than

those plants at lower densities. Thus, the seed yield reduction resulting more severe plant competition at higher plant densities. Plant spacing and population had a small effect on protein and oil content.

Wallace and Munger (1965) compared the leaf area (LA), leaf area ratio (LAR) and relative growth rate (RGR) for two varieties of each of the dry bean types, pea, marrow and yelloweye. Within each type a variety considered to have the higher mean seed yield was compared with a standard variety. Dry weight and leaf area samples were obtained from the aerial shoots at intervals of approximately two weeks. Within the marrow and yelloweye types the higher yielding variety was found to have both the large leaf and the larger leaf area and the larger leaf area ratio. The higher overall RGR and leaf growth rate (RLGR) of the pea bean varieties was accompanied by a much higher leaf area ratio (LAR) suggesting that LAR be the factor chiefly responsible for these differences in growth rates.

Watson (1952), considering variations in leaf area pointed out that leaf area and LAI changes with time, climatic factors, intraspecific differences, supply of mineral nutrients and water. As far as supply of mineral nutrients and water it is a familiar fact that the leaf area of plants, in common with other growth attributes is greatly dependent on nutrition.

Variation in nitrogen supply affects all the phases of leaf growth. Increase in leaf area produced by application of a nitrogenous fertilizer results from increase in both leaf number and leaf size and potassium appear to be more transitory.

Phosphate application was found to increase the leaf area of cereal crops mainly by increasing tillering, and its effect was greatest near to time of maximum shoot number.

Potassium had little effect on leaf area of wheat or barley during tillering phase, but increased leaf area during the subsequent phase of shoot extension, presumably by increasing leaf size or by delaying senescence.

Hammond and Kirkham (1949) measured dry matter accumulation of soybean plants grown under both field and greenhouse conditions with and without nitrogen added and calculated relative growth rate (RGR) for different stages of development. They conclude that at the growth curve of soybeans, relating dry weight versus time is made up of three linear segments which coincide with distinct growth stages of the plant (preflowering, flowering to cessation of vegetative growth and seed development).

Seed Yield

A profitable high yielding bean crop is the product of the interactions between genetic factors, soil, plants, diseases, insects, weeds, weather and the producer itself.

Also an adequate supply of plant nutrients combined with other management practices is essential to insure bean plant growth, top seed quality, and yield.

Reports in the literature have presented no consistency as far as bean yield response to applied N fertilizer.

Robertson and Frazier (1978) cited that even though a good soil test for N is not available there is much experimental evidence to support nitrogen fertilization of the dry edible bean. There was a bean yield response to added N. Dry edible beans fix their own nitrogen but not in sufficient quantities for maximum production and for this reason, they respond well to N fertilizer.

Mack (1977a) evaluated the performance of snap bean seed produced in 1976 under two row spacing (12 and 36 inches) and four nitrogen rates (0, 50, 100, 150 and 200 lb N/A) and concluded that there was a slight but not significant increase in seed yield when N-rates were increased to 200 lb N/A. There were no marked differences in the mineral content of seeds due to treatments but

there was a higher N content of seeds at the highest N fertilizer rate but a slightly lower phosphorus content. Plant heights were decreased at the higher N-rates.

Mack (1977b) reported that "OSU-1604" bush snap beans grown at four N fertilizer rates (50, 100, 150 and 200 lb N/A) have shown that average pod yield was highest at 100 lb N/A but there was no significant difference between N rates.

Jansen (1977) reported a research conducted with snap beans for a three year period and using three rates of N (0, 40, 80 lb N/A) concluded that the yield was highest at 40 lb N/A and excess nitrogen will tend to keep the plants in a vegetative state, will reduce pod set and may even, on occasion, lower yield. When the 80 lb N/A was used the bean plants remained dark green until after harvest and where not enough nitrogen was applied such as no N treatment, the mature foliage became excessive pale.

Westerman et al. (1977) found that N fertilization significantly increased bean seed yield but N_2 fixation estimated by relative seasonal acetylene reduction was reduced 2-10 fold by N fertilization.

However, Westerman et al. (1978) trying to evaluate the effects of N fertilization (0, 50, 150 lb N/A) observed no significant effects on bean seed yields.

Studies were conducted by Mack (1975) for five years on effect of fertilizers on yield and chemical composition of bush snap beans.

Results have shown that in 1966, yield of "Tendercrop" bush beans was not affected by fertilizer rates, but the elemental composition of leaves was increased by an increase in fertilizer rates.

Data of 1967 have shown highest yield for the 100-500-500 fertilizer rate and there was a general trend for an increase in N, P, K and Zn content of leaves as fertilizer rates were increased whereas levels of Ca, Mg, and Mn were reduced slightly.

In 1970 an increase in N fertilizer reduced yield slightly.

Results in 1973 have shown that the yield of "OSU 1604" bush beans were increased as N rates were increased and finally, higher rates of N fertilizer in 1974 caused some reduction in stand and early growth of plants which was reflected in lower yields. Increase of N rates generally increase N, Mg and Mg content of plants and there was an increase in yield.

Peck and Van Buren (1975) conducted a PK factorial field experiment in snap beans. They concluded that concentrated superphosphate (CSP) increased the concentration of P and Mg in parts of snap beans plants and generally decreased the Fe and Zn contents.

Concentrated superphosphate increased the fresh and dry weights per snap bean plants.

There is no fertilizer recommendation for snap bean seed production in Oregon; however, Mack and Jackson (1973) in their fertilizer guide for bush beans in Western Oregon recommended

rates of 40 to 70 lb N/A if beans are grown on fields having a history of heavy N fertilization and intensive culture. Rates of 70 to 100 lb/A are recommended where forage legumes or heavily fertilized vegetable crops were not grown the preceding year. Phosphorus and potassium fertilizer rates are recommended according to soil analysis.

Bulisani et al. (1973) studied the effect of leaf spray application of a commercial NPK fertilizer compared with a usual rate of NPK soil applied fertilizer and control (no fertilizer) on dry bean seed production and nutrient content of leaves in a field trial carried out at Campinas, S. Paulo State (Brazil).

They concluded that NPK soil applied fertilizer plots produced 41% more than the leaf spray application and 78% more than control. The foliar application treatments produced 26% more in average as compared with those of untreated.

Hegwood (1972) carried out an experiment growing eleven cultivars of snap beans on acidic Tifton loamy sand soil to compare yielding ability and leaf and fruit mineral element composition and to determine if leaf composition was associated with yield. He found that there is a strong cultivar effect on leaf mineral composition at full bloom. The Ca and Sr level of leaf tissue at harvest was positively associated with yield, and yield varied significantly with cultivars.

Almeida et al. (1971) in field trials conducted at research stations at Monte Alegre do Sul, Ribeirão Preto and Pindorama S. Paulo State (Brazil) studied the effects of irrigation, mineral fertilization and incorporation in the soil of partially decomposed vegetative matter of Crotalaria juncea L. on the yield of dry beans. The results showed that among the fertilizers there was a yield response only to phosphorus at three locations.

Le Baron et al. (1971), referring to the fertilization for bean production in Idaho, stated that phosphorus and zinc fertilizers in the proper amount and combination are essential for good yield of bean seeds and should be included in any bean production program in Idaho.

Nitrogen is not generally needed for beans unless a large amount of crop residue returned to the soil has not decomposed sufficiently to release nutrition for the bean plant. The inoculation of bean seed with N fixing bacteria has not proven to be of value in the bean producing areas of Southern Idaho.

Roberts and Weaver (1970) reported an experiment of five different N rates respectively 0, 40, 80, 120 on 160 lb/A as ammonium nitrate and its effect on growth and yield of three field dry beans varieties.

They concluded that application rates of N produced no significant increase in bean yield at 88 day harvesting, but average over

varieties, there was a significant response to applied N when the crop was harvested 108 days after seeding. The lower yields at 88 days harvest were largely explained by plant immaturity. N fertilizer had more effect on dry matter production than on yield of beans. Fertilization with N increased dry matter at all samplings.

Hiroce et al. (1970) conducted an NP factorial experiment in dry beans, in a Red Yellow Latosol in the presence and absence of lime at Paiquera Acu, S. Paulo (Brazil) and concluded that phosphorus fertilization increased linear and significantly both dry bean yield and phosphorus content of the leaflets, independently of liming.

Brown et al. (1969) studied the effect of two levels of lime, phosphorus, potassium and three levels of nitrogen and observed no green bean yield response from the applied treatments. The 200 lb N/A treatment caused the greatest NO_3^- accumulated in the green bean pods.

Hiroce et al. (1969) evaluated the effects of additions of fresh organic matter and NK fertilizers on yield and nitrogen and potassium content of the leaves of dry beans. Results of four experiments showed that there was a significant increase in the N- NO_3 or total N content of the leaves associated with the addition of N fertilizer to the soil.

Dry bean yield was correlated with the N contents of the petiole and with the K content of leaflets.

Mascarenhas et al. (1968) studied the influence of NP fertilizer on the bean seed production as well as the mineral composition of leaves and seeds of bean plants. There was no response to nitrogen while phosphorus and lime induced yield increases mainly when applied in the presence of one another. The lime applied increased the content of Ca, Mg, and N, while phosphate fertilizer increased the content of P, Ca and Mg and depressed the K content of the bean leaves.

Paterson et al. (1966) evaluated the effect of N fertilizer applications on yield, quality and mineral uptake of "Harvester" snap beans at several locations in the United States. Maximum yields in Michigan, New York, Oklahoma and Texas occurred with 50 lb N/A, but in Florida the highest yields resulted from the application of 75 lb N/A. Nitrogen fertilizer application not only resulted in a significant increase in the N, Mg, P and Zn content of the bean leaves but also gave a significant decrease in the boron content of the bean plant.

Cackett (1965), working with fertilization on field beans at Salbi Experimental Station in Rhodesia (Africa), stated that in spite of the fact the crop is a legume, it has shown consistent yield responses to high levels of nitrogen up to 100 lb N/A.

A feature of bean growth in the station is that nodulation does not occur until flowering, about five to seven weeks after planting. An experiment conducted in 1964 to investigate the effect of seed inoculation showed that inoculation did not produce significantly higher yields than N control, and that it certainly did not preclude the necessity for nitrogen fertilization.

Results indicated that all (or most) of the nitrogen requirements should be applied at planting and that no real benefit could be expected from top dressing.

Rhizobia were presented in the soil but appeared to remain inactive until flowering, and this probably accounted for the marked responses to applied nitrogen which were required during the early growth stages up until rhizobial activity commenced.

MANUSCRIPT I

EFFECT OF N AND P FERTILIZER RATES ON YIELD AND QUALITY
OF SNAP BEAN (Phaseolus vulgaris L.) SEEDS.

ABSTRACT

Studies were conducted to determine the effects of N and P fertilization rates on snap bean (Phaseolus vulgaris L.), seed production, seed quality attributes, and the effect of these attributes on early stages of seedling growth.

Field plots were laid out in a randomized block design with a factorial arrangement of treatments consisting of four nitrogen rates (0, 50, 150 and 300 kg/ha) and three phosphorus levels (0, 50, and 150 kg/ha) with four replications.

ATP content/embryo axis and % seed water soluble carbohydrates were increased by both N and P fertilization. Seed yield, seed weight, seed size, % seed crude protein and seedling axis dry weight were increased by N but not by P, while ATP content/mg embryo axis and % germination were increased by P but not by N.

Dry weight of the 5-day old seedling axis was positively correlated with ATP content/embryo axis and ATP content/mg embryo axis.

A stepwise multiple regression analysis was conducted to assess the contribution of the seed quality factors to seedling axis dry weight. The multiple correlation coefficient (R^2) indicated that ATP content/mg embryo axis was responsible for 12.5% of the variation in seedling axis dry weight. The other seed quality factors did

not contribute enough to be of predictive value and are not included in the regression model.

Additional index words: seed vigor, seed weight, seed size, ATP, seed protein, seed soluble carbohydrate, seed germination, seedling dry weight.

INTRODUCTION

The amount of nitrogen supplied to the snap bean plant by nitrogen-fixing bacteria is not sufficient for maximum bean seed production (16); therefore, application of fertilizer N for seed production is a common practice. However, positive (2, 20) as well as negative responses (12, 15, 19) have been reported for N effects on bean yield.

Several seed quality attributes are associated with rates of nitrogen application. These attributes in turn are related to seed vigor and performance in snap beans. Ries (14) reported that seedling vigor was related to seed protein content. Vigor of snap bean seed was found to be influenced by initial seed moisture, maturity and mechanical damage (13) and can be reduced by unfavorable storage conditions (17). The effect of bean seed size on crop yield is not conclusive (10).

ATP content has been related to seed performance in lettuce (5), wheat (6), crimson clover, annual ryegrass and rape (7), corn (3), and barley (9). Such findings suggest the possibility of using ATP content as a seed vigor index in snap beans.

While fertilizer practices for achieving optimum yields are well developed, information on the effects of fertilizer levels on bean seed quality is limited.

The objectives of this study were to determine the effects of N

and P fertilization rates on bean seed quality attributes and the effects of these attributes on early stages of seedling growth.

MATERIALS AND METHODS

Plots of "Oregon 1604" snap beans were established at the Vegetable Research Farm near Corvallis, Oregon on a Chehalis silty clay loam soil. Each plot consisted of six rows 10.4 long spaced 0.91 m apart. The plots were planted in a randomized block design with a factorial arrangement of treatments consisting of four nitrogen rates (0, 50, 150 and 300 kg/ha) and three phosphorus rates (0, 50 and 150 kg/ha), with four replications.

Seeds were planted on May 12, 1977 at the rate of 82 kg/ha with a belt-type hand-pushed planter and seedlings were thinned to a population of 26 plants/m. The seeds were treated with Lorsban insecticide and Captan fungicide but were not inoculated. Difonate insecticide at the rate of 22.4 kg/ha was incorporated in the soil before planting. For weed control, 0.84 kg/ha trifluralin was incorporated preplant and 3.3 kg/ha dinitramine was applied after planting. Irrigation was provided every 8-12 days as needed.

Soil analysis before fertilizer application indicated a pH of 6.3, P=49 ppm, K=250 ppm, Ca=18.7 meq/100 g, and Mg=6.2 meq/100 g.

Potassium sulfate (50% K_2O and 17% S) was applied before planting to provide a uniform rate of 60 kg K/ha on all plots.

Triple superphosphate (45% P_2O_5) was hand broadcast before planting to provide 50 and 150 kg P/ha on appropriate plots.

Split applications of ammonium nitrate (34% N) were made according to the developmental stages of the plants. The 50 kg/ha rate was applied before planting. For the 150 kg/ha rate, an initial application of 100 kg/ha was made before planting and 50 kg/ha was applied on June 17 when plants were in the first trifoliate leaf stage. For the 300 kg/ha rate, 100 kg/ha was applied before planting, 50 kg/ha at the first trifoliate leaf stage, 50 kg/ha on June 27 at the floral bud stage, 50 kg/ha on July 11 at flowering, and 50 kg/ha on July 19 at the small pod stage.

Several agronomic traits were measured. Seedling emergence counts were made in 1 m of two middle rows of each plot. Seed moisture was determined prior to harvest. Seed yield was measured by harvesting 6.1 m of the two central rows of each plot.

Seed quality components were evaluated as follows: Average seed weight was obtained from a plot of 1 m². The percentage of seeds larger than screen size 12/64 x 3/4 in (4.76 mm x 19.05 mm) was determined by shaking 200 g seed over a 4.76 mm x 19.05 mm slotted hand screen. Seed germination percentage was determined after 7 days in rolled towels at 20-30 C (1). Seedling axis dry weight was determined after 5 days growth in rolled towels at 20-30 C. Cotyledons were removed before drying the seedling axis at 100 C for 1 hr and 75 C for 23 hrs.

Analysis of seed nitrogen content was made by a modified

Kjeldahl procedure, using a Technicon Auto Analyzer. Percent crude protein was calculated by multiplying % N by 6.25.

Seed water soluble carbohydrate of the aqueous fraction of alcohol solubles from dry seeds was determined by the Anthrone method (21). ATP content was determined by the luciferin-luciferase method (18) following extraction by the method described by Ching (6).

RESULTS AND DISCUSSION

Seed Yield

The highest seed yield of 2,729 kg/ha was obtained with an application of 150 kg N and 150 kg P/ha (Table 1).

The three N rates (50, 150 and 300 kg/ha) increased yields by 10.2, 14.1 and 15.7% over the control but the difference in yield between N rates was not significant. Others (2, 20) have also reported increased seed yield from N fertilization, while N has not increased seed yield in some studies (12, 15, 19).

There was no yield response to added P, probably because of the high initial soil P content.

Seed Quality Components

Seed Weight and Size

Seed weight was increased 5.5, 7.4 and 7.1% by application of 50, 150 and 300 kg N/ha (Figure 3), while there was no response to P.

Applications of N increased the proportion of the seed lots held over a 12/64 x 3/4 in. (4.76 x 19.05 mm) screen by 26.8, 38.3 and 59.3% over the control. P had no effect on seed size (Table 1).

ATP Content

Although N and P are both constituents of the ATP molecule, P fertilizer made the major contribution to changes in ATP content of the embryo.

Table 1. Effect of N and P fertilizers on yield and quality components of snap bean (*Phaseolus vulgaris* L.) seed.

NP treatments		Seed yield	Seed weight	Larger than screen size 12 ‡	ATP content		Crude seed protein	Water sol. carb. (WSC)	Germination	Seedling axis dry weight
kg/ha		kg/ha	mg/seed	%	n moles/embryo	n moles/mg embryo	%	%	%	mg/seedling
N ₀	PO	2,219	200	23.90	28.8	2.12	24.60	4.42	95	52.2
	P ₅₀	2,421	228	26.91	50.1	3.43	25.66	4.20	96	59.8
	P ₁₅₀	2,324	215	18.20	101.0	6.72	25.89	4.92	98	57.8
N ₅₀	PO	2,612	225	29.44	44.5	3.56	27.30	5.35	94	58.9
	P ₅₀	2,612	235	32.18	33.9	2.46	26.18	4.33	96	56.1
	P ₁₅₀	2,453	218	25.90	49.4	4.40	26.47	4.96	95	58.5
N ₁₅₀	PO	2,641	235	32.71	36.7	2.90	26.08	5.28	94	54.4
	P ₅₀	2,577	228	32.18	74.5	5.60	26.36	5.38	94	59.8
	P ₁₅₀	2,729	228	32.18	74.5	5.60	26.36	5.38	94	59.8
N ₃₀₀	PO	2,649	225	34.77	63.4	4.19	27.03	5.03	95	61.3
	P ₅₀	2,728	230	36.24	56.3	4.01	27.82	5.26	96	58.8
	P ₁₅₀	2,678	232	28.92	50.2	3.59	27.30	5.56	96	59.0
Overall mean		2,554	225	30.15	53.8	3.92	26.48	4.96	95	57.8
LSD _{0.05}		233	22	5.42	15.1	1.10	1.47	0.61		4.13
LSD _{0.01}		313	30	7.28	20.3	1.48	1.97	0.82		5.55
Grand mean										
N ₀		2,322	214	23.00	60.0	4.09	25.38	4.51	96	56.6
N ₅₀		2,559	226	29.17	42.6	3.47	26.64	4.88	95	57.9
N ₁₅₀		2,649	230	31.81	56.2	4.18	26.50	5.17	95	57.1
N ₃₀₀		2,685	229	36.64	56.6	3.93	27.38	5.28	95	59.7
LSD _{0.05}		134	13	4.15	8.7		0.86	0.35		2.38
LSD _{0.01}		181	17	6.57	11.7		1.15	0.47		3.20
PO		2,530	221	30.20	43.3	3.19	26.25	5.02	94	56.7
P ₅₀		2,584	230	31.47	49.4	3.49	26.67	4.66	96	58.0
P ₁₅₀		2,546	223	28.79	68.8	5.08	26.50	5.20	96	58.8
LSD _{0.05}					7.53	0.55		0.31	0.01	
LSD _{0.01}					10.12	0.74		0.41	0.02	

‡ Percentage of seed held over a 4.76 mm x 19.05 (12/64 x 3/4 in) screen.

Increasing the rate of P to 50 and 150 kg/ha increased the ATP content of the embryo axis by 14.0 and 58.7%, respectively, over the control (Figure 1). When ATP content was calculated on the basis of embryo weight, the increases were 9.4 and 59.3% (Figure 1).

N had no effect on ATP content, although Garay (11) reported increase in ATP content of wheat embryos when N was applied.

ATP is required during seed germination for biosynthesis of new cellular constituents in seedlings and for the formation of enzymes for degradation and conversion of storage materials. Thus, ATP content in the embryo may be considered as a good biochemical index of growth potential in snap bean seeds as reported for lettuce (5), wheat (6), crimson clover, annual ryegrass and rape (7), and corn (3).

Protein Content

Increasing N rates to 50, 150 and 300 kg/ha caused increases in the protein content of the bean seed of 4.96, 4.41 and 7.88% respectively, over the control (Figure 2). Ries (14) also reported an increase in protein content of bean seed with supplemental N fertilization.

Water Soluble Carbohydrates (WSC)

Increasing N rates to 50, 150, and 300 kg/ha resulted in an increase in seed WSC% of 8.20, 14.63 and 17.07% respectively

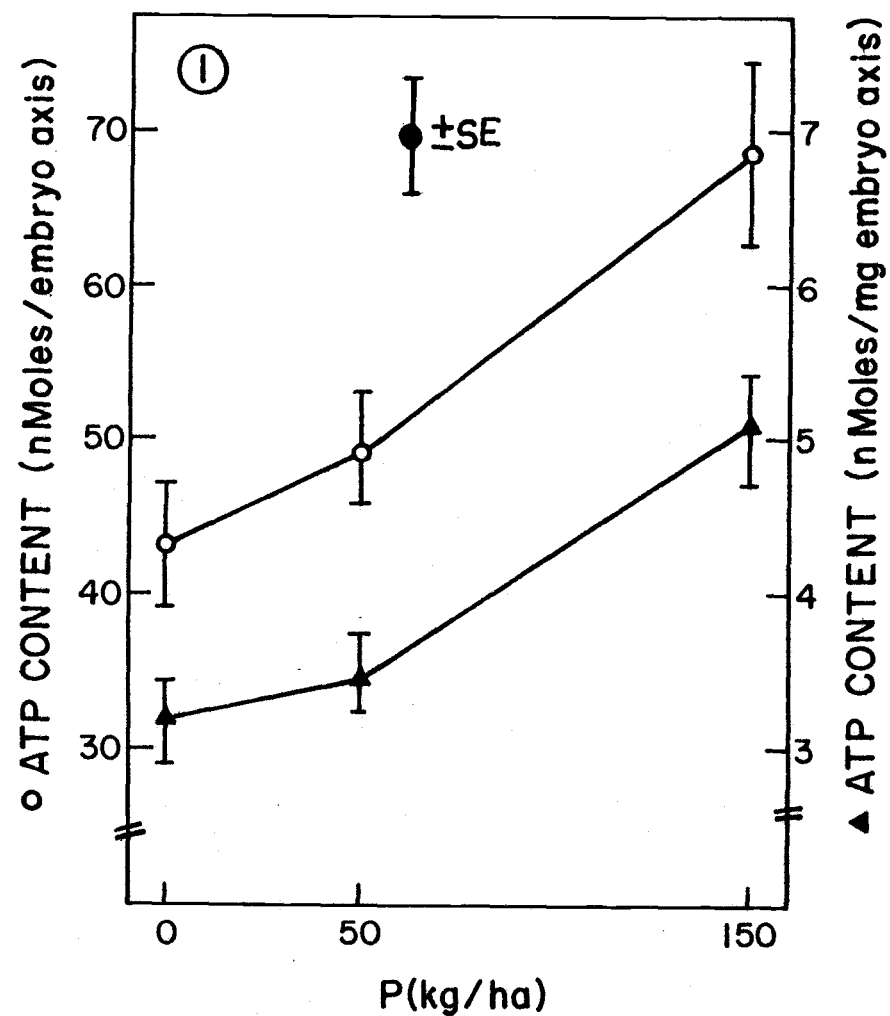


Figure 1 - Effect of N and P fertilizer rates on the ATP content/embryo axis and ATP content/mg embryo axis of snap bean seeds

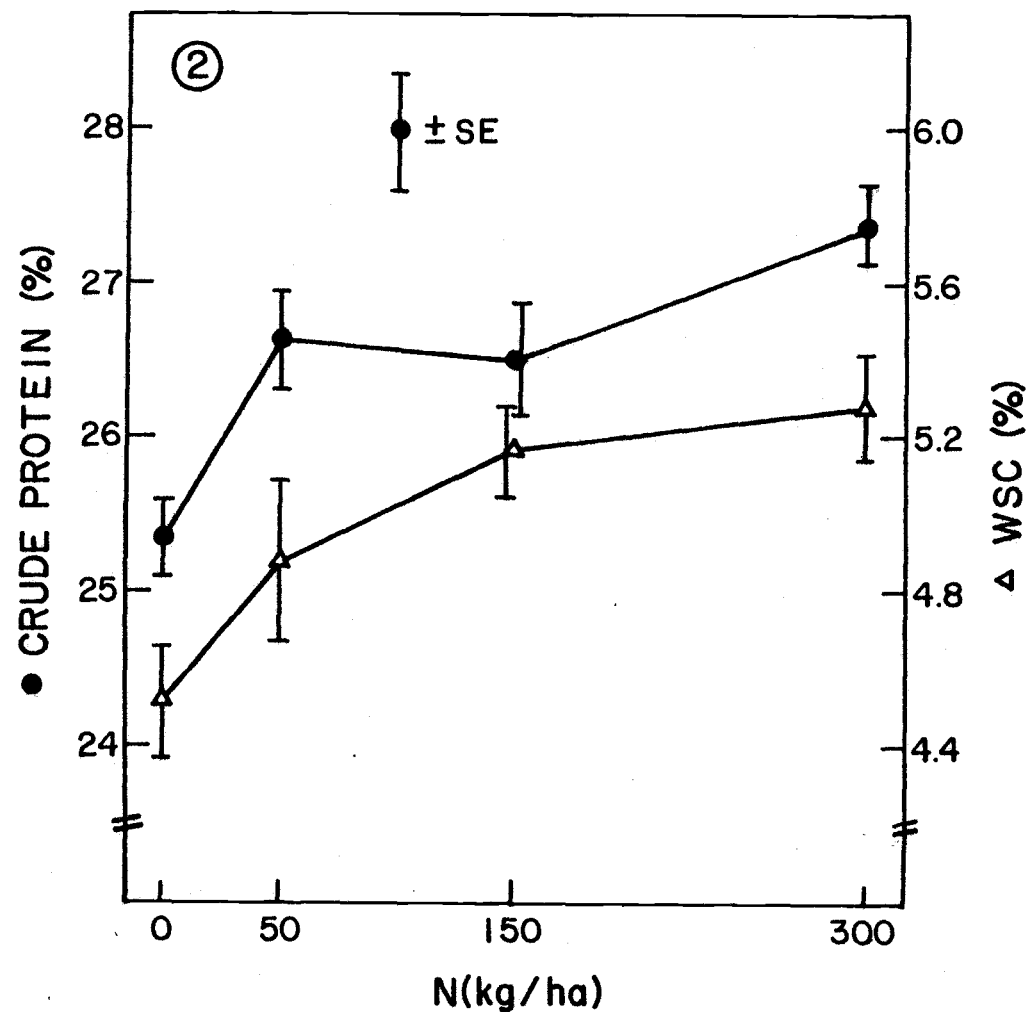


Figure 2 - Effect of N and P fertilizer rates on the crude protein % and WSC% of snap bean seeds

over the control (Figure 2).

Cantliffe (3) pointed out that the most important factors limiting synthesis of ATP may be the total and type of carbohydrates available to the embryo. Starch is broken down to glucose or glucose 1-phosphate which is then converted to sucrose. Sucrose is split into glucose and fructose which can then enter glycolysis and the TCA cycle of respiration. Energy in the form of ATP is produced from these metabolic pathways. If the total available carbohydrates are reduced, less total ATP would be synthesized. During the early phases of germination, ATP is needed for synthesis of protein, enzymes and nucleic acids, which are used to produce a vigorously growing seedling. Low amount of ATP produced by the seed would reduce seedling growth.

N fertilizer rates increased the seed WSC and this could result in more readily available substrate for ATP synthesis and consequently lead to an increase in ATP content in the embryo of snap bean seed. Garay (11) reported that the amount of soluble sugars in wheat increased slightly and starch remained constant with high rates of N.

Germination Percentage

Increasing P rates to 50 and 150 kg P/ha resulted in a slight but significant increase in the germination percentage of snap bean seed of 1.7 and 1.5%, respectively (Figure 4).

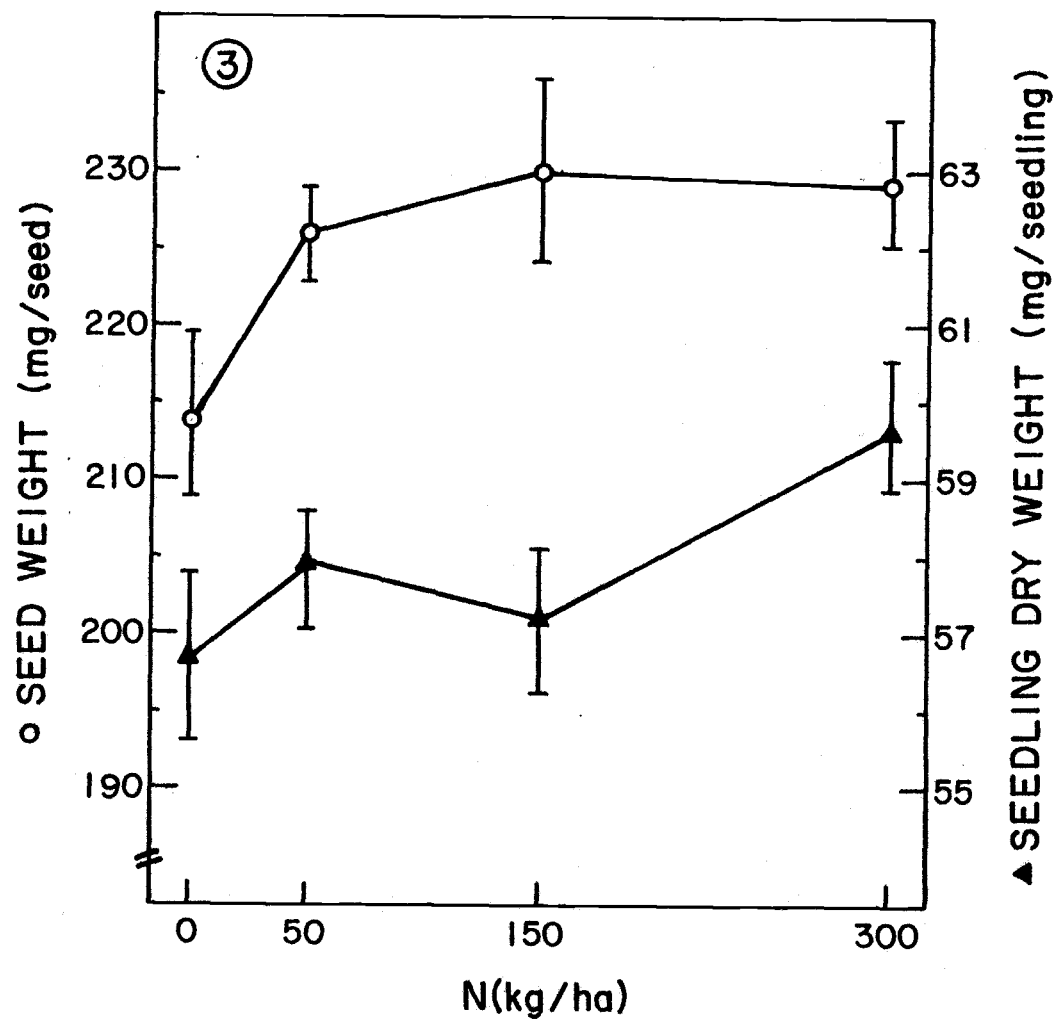


Figure 3 - Effect of N and P fertilizer rates on seed weight and dry weight of 5-day old seedling axis of snap bean

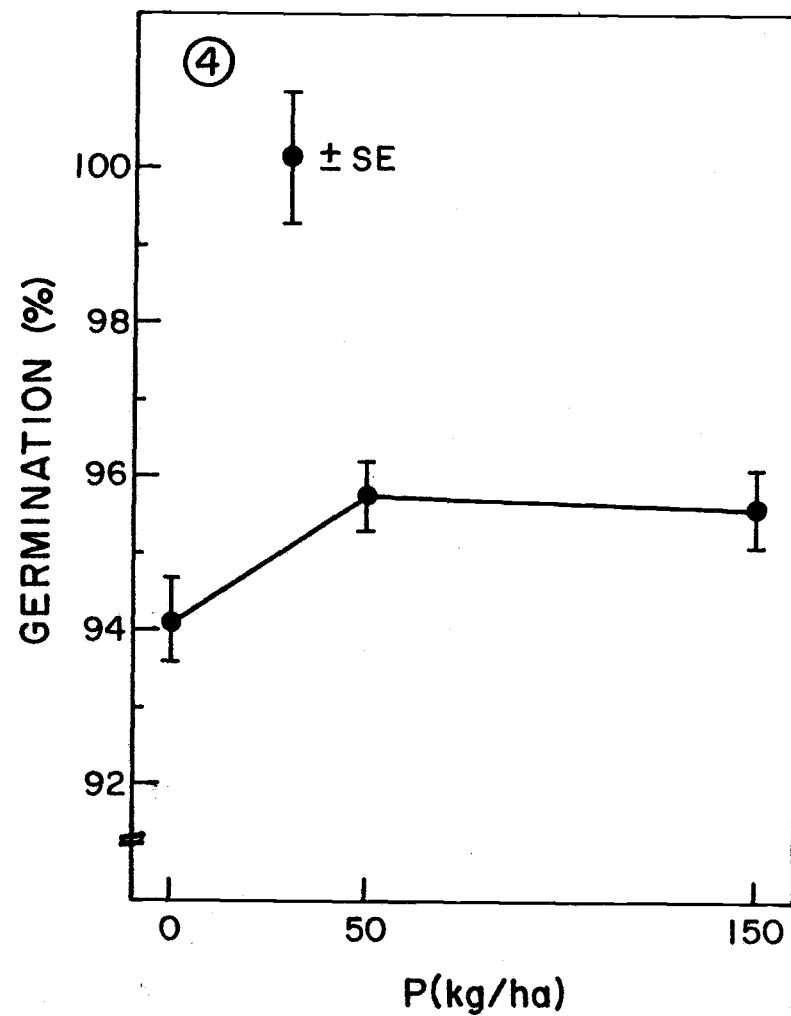


Figure 4 - Effect of N and P fertilizer rates on germination percentage of snap bean

N rates had no effect on germination percentage.

Dry Weight of 5-Day Old Seedling Axis

Seedling axis dry weight was increased by N but not by P (Table 1). N rates of 50, 150 and 300 kg/ha increased seedling dry weight by 2.2, 0.9 and 5.5%, respectively (Figure 3).

Seedling axis dry weight was positively correlated with ATP content per embryo axis ($r=0.33^*$) and ATP content per mg of embryo axis ($r=0.35^{**}$), confirming previous findings with lettuce (5), crimson clover, annual ryegrass, rape (7), and barley (9). This positive correlation could be explained by the need for ATP in the very early stages of germination and the requirement of high energy for maintenance and seedling growth. However, no correlation was found between seedling axis dry weight and seed weight, germination percentage, WSC and % crude seed protein.

Seedling dry weight or seedling vigor is frequently related to several of the seed quality factors studied such as size, weight, ATP content and % crude protein, and can be used to predict the growth potential or performance of seeds.

A stepwise multiple regression analysis was conducted to assess the contribution of seed weight, seed size, % crude seed protein, % seed WSC and ATP content/mg embryo axis to seedling axis dry weight.

The multiple correlation coefficient (R^2) indicated that ATP

content/mg embryo axis (X) was responsible for 12.5% of the variation in seedling axis dry weight. The other seed quality factors did not contribute enough to be of value for prediction and are not included in the regression model. The regression equation of seedling axis dry weight (Y) is:

$$Y = 54.40 + 0.87 (X)$$

Whether this model can be used for predicting seedling axis dry weight as a function of ATP content/mg embryo axis needs further investigation.

It is surprising that seedling axis weight was not related to additional seed quality factors since other studies have shown seed size (10) and seed protein content (14) to be related to seedling vigor in snap beans. It is probable that significant relationships were not demonstrated because of the extremely small differences in quality factors of seeds from fertilized and non-fertilized plots.

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MANUSCRIPT II

EFFECT OF N AND P FERTILIZERS ON NITRATE REDUCTASE
ACTIVITY AND NITRATE CONTENT OF SNAP BEAN
(Phaseolus vulgaris L.) LEAVES

ABSTRACT

The effect of N and P fertilization rates on nitrate reductase activity (NRA), nitrate content (NO_3^-), crude seed protein percentage, N-translocation efficiency and yield was studied in field grown snap beans (Phaseolus vulgaris L.).

Plots were planted in a randomized block design with a factorial arrangement of treatments consisting of four nitrogen rates (0, 50, 150 and 300 kg/ha) and three phosphorus levels (0, 50, and 150 kg/ha) with four replications.

Assays of NRA and NO_3^- content were conducted on leaves at four maturity stages: (I) fully developed trifoliate, (II) floral bud, (III) flowering and (IV) seed development.

NRA was low at stage I, high at II and III and low at IV.

A marked decrease in NRA occurred when the levels of N were increased from 0 to 300 kg/ha. NRA was not affected by P applications.

Nitrate content in the leaf tissue increased with each increase in amount of N applied. Nitrate level generally decreased after stage I as a result of dilution by rapid plant growth.

Increases in N-fertilizer levels increased seed yield, crude seed protein percentage and total protein yield, but there was no effect of P on these characteristics.

N translocation efficiency was not affected by either N or P

levels.

Yield was positively correlated with NO_3^- content at all four maturity stages, but was correlated with NRA only at stage I.

No correlation was observed between NRA and NO_3^- content in any of the four stages assayed, however, indicating that once NR is induced other factors control NRA in vivo.

Additional index words: Nitrate metabolism, harvest N index, mobilization of plant nitrogen, snap bean seed yield.

INTRODUCTION

Plants absorb nitrate mainly from the soil by the root system, from where it is translocated throughout the plant and reduced to ammonia before it can combine with ketoacids to form aminoacids and finally protein, nucleic acids, and other nitrogenous compounds (1, 9, 19).

Nitrate reductase is considered to be the rate limiting step in the transformation of nitrate to aminoacids and is a factor limiting protein synthesis (1, 9). The enzyme is located in cytosol of leaves, stems, petioles, cotyledons and roots. In general stems and roots are much lower in activity than are leaves (22).

Nitrate reductase activity (NRA) is low in the initial leaf expansion phase and reaches a peak when the leaf is fully expanded and maximum activity occurs at flowering and decreases as the plants approach physiological maturity (12, 16).

NRA has been studied in several crops including corn (24, 29), wheat (3) and soybeans (12). Little information is available on the influence of N and P fertilizer on: (a) leaf nitrate reductase activity, (b) nitrogen translocation efficiency, (c) seed protein content or (d) total protein yield in snap beans. The objective of this study was to investigate the effect of N and P fertilization rates on NRA and NO_3^- content of snap bean leaves.

MATERIALS AND METHODS

Plots of "Oregon 1604" snap beans were established at the Vegetable Research Farm near Corvallis, Oregon on a Chehalis silty clay loam soil. Each plot consisted of six rows 10.4 m long spaced 0.91 m apart. The plots were planted in a randomized block design with a factorial arrangement of treatments consisting of four nitrogen rates (0, 50, 150 and 300 kg/ha) and three phosphorus rates (0, 50 and 150 kg/ha), with four replications.

Seeds were planted on May 12, 1977 at the rate of 82 kg/ha with a belt-type hand-pushed planter and seedlings were thinned to a population of 26 plants/m. The seeds were treated with Lorsban insecticide and Captan fungicide but were not inoculated. Difonate insecticide at the rate of 22.4 kg/ha was incorporated in the soil before planting. For weed control, 0.84 kg/ha trifluralin was incorporated preplant and 3.3 kg/ha dinitramine was applied after planting. Irrigation was provided every 8-12 days as needed.

Soil analysis before fertilizer application indicated a pH of 6.3, P = 49 ppm, K = 250 ppm, Ca = 18.7 meq/100 g, and Mg = 6.2 meq/100 g.

Potassium sulfate (50% K_2O and 17% S) was applied before planting to provide a uniform rate of 60 kg K/ha on all plots.

Triple superphosphate (45% P_2O_5) was hand broadcast before

planting to provide 50 and 150 kg P/ha on appropriate plots.

Split applications of ammonium nitrate (34% N) were made according to the developmental stages of the plants. The 50 kg/ha rate was applied before planting. For the 150 kg/ha rate, an initial application of 100 kg/ha was made before planting and 50 kg/ha was applied on June 17 when the plants were in the first trifoliate leaf stage. For the 300 kg/ha rate, 100 kg/ha was applied before planting, 50 kg/ha at the first trifoliate leaf stage, 50 kg/ha on June 27 at the floral bud stage 50 kg/ha on July 11 at flowering, and 50 kg/ha July 19 at the small pod stage.

Seed yield was determined by hand harvesting 6.1 m of two central rows of each plot when seed moisture was 11-13%. Threshing was done with a rubber-belt thresher.

Nitrate Reductase Assay

Leaf samples to assay NRA were taken at four growth stages: (I) fully developed trifoliate leaves (34 days after emergence), (II) floral bud stage (45 days after emergence), (III) flowering stage (57 days after emergence) and (IV) seed development (71 days after emergence). The most recent fully developed leaves were collected at each stage from the four external rows of a plot and the two central rows were reserved for seed yield.

Leaf samples were collected between 1:00-2:00 pm on clear and sunny days to eliminate diurnal variation in NRA. Usually 16

fully developed trifoliate leaves were collected at random from eight plants, placed in a plastic bag, stored in ice in a styrofoam chest and immediately brought to the laboratory for analysis. To avoid enzyme degradation over time, only one replication of twelve treatments was assayed per day. The three remaining replications were assayed on the three subsequent days.

After material was taken for the NRA assay, the remaining leaves were dried at 70 C for approximately 48 hours, ground in a Wiley mill to pass a 40 mesh-screen, and used for nitrate determination.

A modified "in vivo" method of Street and Bossler (26) and Jaworski (14) was used for the NR assay. Four replications of 200 mg of leaf-discs were used per sample. NRA was calculated by the following formula:

$$\text{NRA} = \mu\text{moles NO}_3^- \text{ red./g FW/10 min.} = \frac{(\text{A sample} - \text{A blank}) \times 0.1}{(\text{A stand.} - \text{A blank}) \times 0.2}$$

The standard consisted of 0.1 μmole of KNO_2 .

Nitrate Determination

The nitrate content in the leaves was determined by the steam distillation method described by Bremner (2).

Nitrogen Analysis

Nitrogen analysis of the dried seed and above ground plant material was made with a modified Kjeldahl-nitrogen procedure and

the Technicon Auto Analyzer. The crude protein percentage in the seed was obtained by multiplying the % N by 6.25.

Protein Yield

Protein yield was obtained by multiplying the seed yield (kg/ha) by % seed protein.

Nitrogen Translocation Efficiency Percent

or Harvest N Index

The N. T. E. % was determined in the above ground plant material harvested at plant maturity (83 days after emergence) according to the following formula:

$$\text{N. T. E. \%} = \frac{\text{total N seed/ha}}{\text{total N plant + seeds/ha}}$$

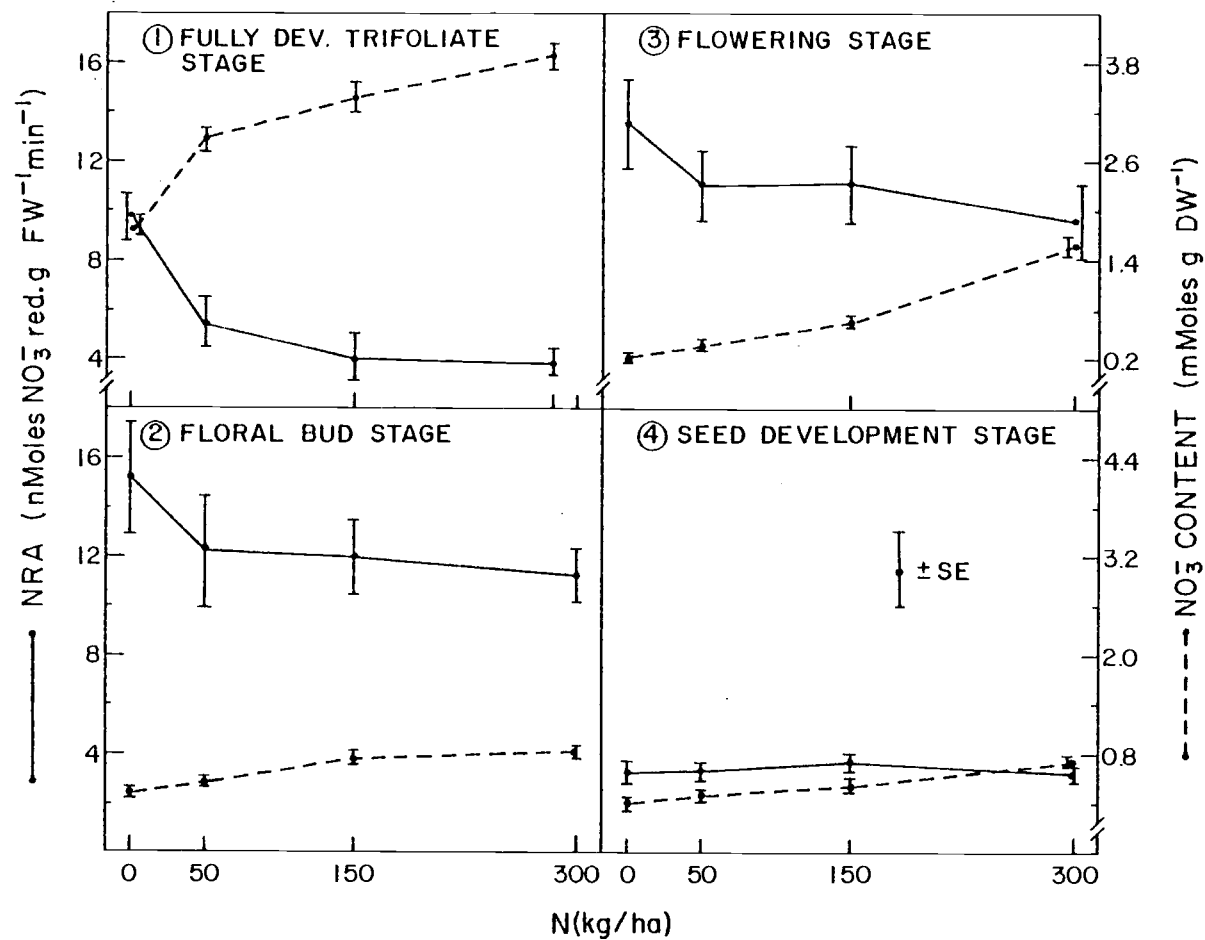
RESULTS AND DISCUSSION

NRA AND NO_3^- Content

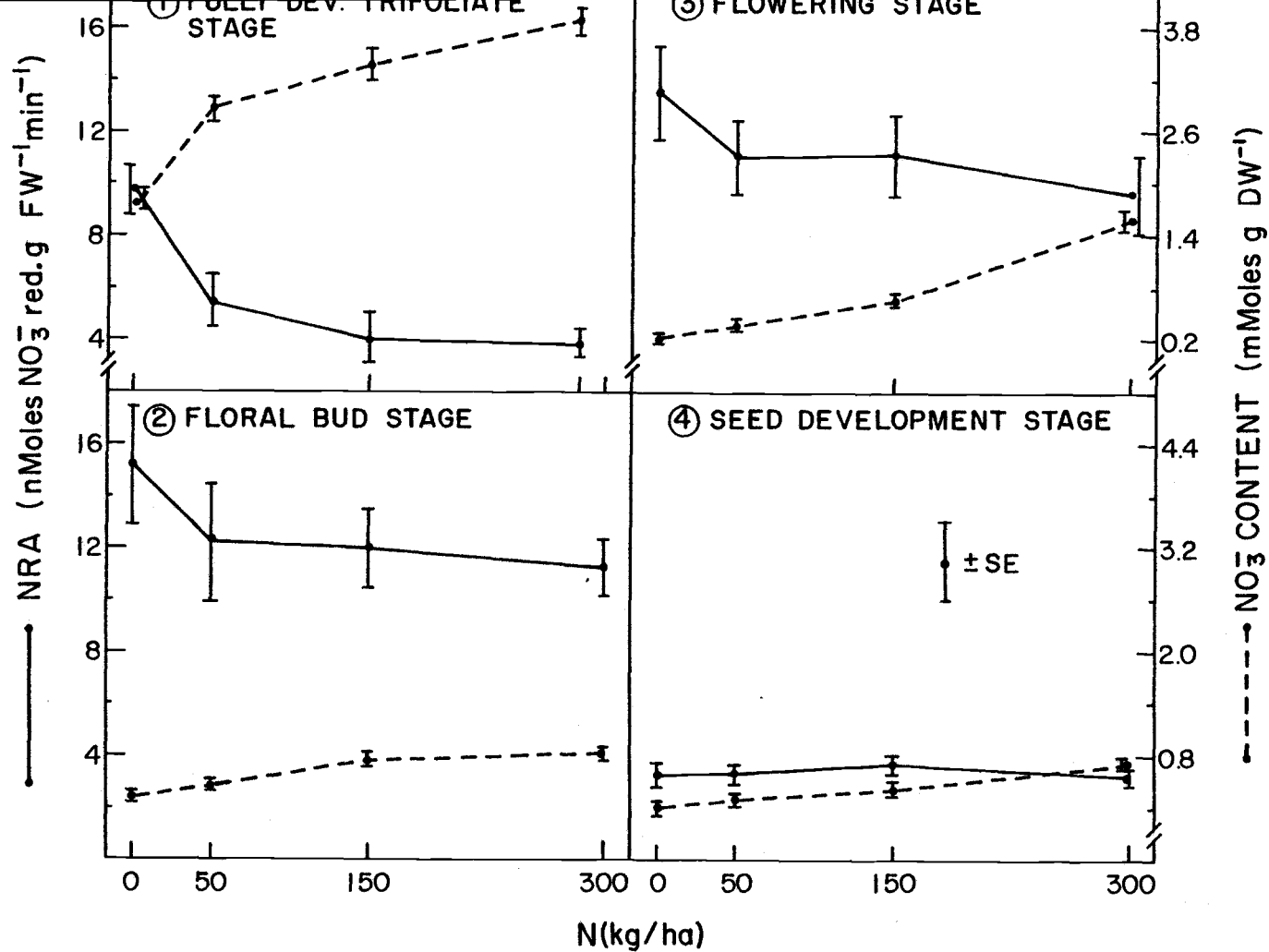
Stage I - Fully developed trifoliate leaves. A marked decrease in NRA was observed when the levels of N (as ammonium nitrate) were increased from 0 to 300 kg/ha (Figure 1). P levels had no effect on NRA so the N effects shown are averaged over P levels. The control material had 44.1%, 58.5% and 60.1% higher NRA than that of 50, 150 and 300 kg N/ha respectively. Differently research done in dry beans (8) did not show a reduction in NRA and probably was due to smaller levels of ammonium nitrate fertilizer used.

The decrease in NRA probably is explained by the feedback inhibition by NH_4^+ ion contained in the N fertilizer source used for the excess of NH_4^+ in leaf tissue may act as a repressor of NRA. Shen (25) suggested that NH_4^+ ion inhibits the first stage of nitrate reduction from NO_3^- to NO_2^- but does not inhibit the uptake of nitrate. However, Minotti *et al.* (20) stated that there is evidence that NH_4^+ may act directly on NO_3^- uptake.

N fertilizer increased NO_3^- content over the control by 67.5%, 99.4% and 129.0%, respectively, at 50, 150 and 300 kg/ha. P levels had no effect on NO_3^- content. The NO_3^- content in snap bean leaves was higher at the trifoliate stage than at other stages of plant



Figures 1-4. Effect of N fertilizer on nitrate reductase activity (NRA) and NO₃⁻ content of snap beans at: 1. fully developed trifoliolate stage (34 days after emergence) 2. floral bud stage (45 days after emergence) 3. flowering stage (57 days after emergence) and 4. seed development stage (71 days after emergence).



Figures 1-4. Effect of N fertilizer on nitrate reductase activity (NRA) and NO₃⁻ content of snap beans at: 1. fully developed trifoliolate stage (34 days after emergence) 2. floral bud stage (45 days after emergence) 3. flowering stage (57 days after emergence) and 4. seed development stage (71 days after emergence).

development (Fig. 1-4). Apparently there was enough NO_3^- available even in the control plot for NR induction.

Yield was correlated with NRA ($r=0.51^{**}$) and NO_3^- content ($r=0.64^{**}$) at the trifoliate stage. A negative correlation between NRA and NO_3^- content ($r=-0.59^{**}$) also was noted (Table 1). A positive relationship between NRA and grain yield was also found in corn (23) and wheat (7) but not in soybean (12).

Stage II - Floral bud stage. A maximum NRA was observed at the floral bud stage (Fig. 2) in snap beans.

A similar situation occurred in soybeans where maximum NRA was observed at the beginning of flowering (16) or at full blossom (11) and dry beans at the postflowering stage (8). Differences due to N level in leaf NRA at this stage were not significant, probably due to a feedback inhibition of NRA by NH_4^+ as discussed previously. This is in contrast to dry beans (4) in which no reduction of NRA was observed.

Nitrate content in leaf tissue reflected N fertilizer treatment in that the higher the level of nitrogen applied, the higher the nitrate content (Fig. 2). In general there was a decrease in nitrate concentration after leaves were fully developed, probably due to a dilution effect from accumulation of insoluble materials and the continuous utilization of nitrate by NR.

Yield was correlated with NO_3^- content at the floral bud stage

($r=0.58^{**}$) but not with NRA.

No correlation existed between NRA and NO_3^- content at this stage (Table 1).

Stage III - Flowering stage. Differences observed in NRA at the flowering stage due to N levels were not significant (Fig. 3), but the nitrate content in leaves was enhanced by increased N rates.

Yield was correlated with NO_3^- content at this stage ($r=0.43^{**}$) indicating the importance of total N supply to yield.

Stage IV - Seed development. N and P fertilization had no positive effect on NRA at seed development stage (Fig. 4). Nitrate content in leaf tissue reflected N fertilizer treatments. The higher the level of nitrogen applied, the higher the concentration of nitrate (Fig. 4).

Yield was correlated with NO_3^- content ($r=0.57^{**}$) whereas no significant correlation was observed between NRA and NO_3^- at seed development or between NRA and yield.

Effect of N and P fertilizer levels on seed yield. N fertilizer increased seed yield over the control by 10.2%, 14.1% and 15.7% at 50, 150 and 300 kg N/ha (Fig. 5).

The N fertilizer effect may be somewhat masked since legume plants have symbiotic root nodules which fix atmospheric nitrogen. Consequently positive (4, 8, 28) as well as negative (18, 27) responses have been reported for N effects on snap beans by other

Table 1. Simple correlation coefficients (r) between seed yield, % crude seed protein, total protein yield, % N translocation efficiency, nitrate reductase activity (NRA) and NO_3^- content of snap beans "Oregon 1604" (*Phaseolus vulgaris* L.).

	Seed yield	Crude seed protein	Total protein yield	N-translocation efficiency	NRA				NO_3^- content			
					Fully dev. trif.	Floral bud	Flowering	Seed dev.	Fully dev. trif.	Floral bud	Flowering	Seed dev.
Crude seed prot.	0.21											
Total prot. yield	0.90**	0.62**										
N. translocation efficiency	-0.43**	-0.14	-0.40**									
NRA	Fully dev. trif.	0.52**	-0.15	-0.48**	0.34*							
	Floral bud	0.10	-0.24	-0.03	-0.21	-0.07						
	Flowering	-0.11	-0.26	-0.21	0.10	0.12	-0.13					
	Seed dev.	0.04	-0.08	-0.01	0.19	-0.17	-0.32*	-0.13				
NO_3^- content	Fully dev. trif.	0.64**	0.54**	0.76**	-0.22	-0.59**	-0.11	-0.002	-0.07			
	Floral bud	0.58**	0.46**	0.67**	-0.27	-0.55**	-0.04	-0.14	-0.09	0.84**		
	Flowering	0.43**	0.48**	0.57**	-0.18	-0.32*	-0.17	-0.17	0.05	0.58**	0.63**	
	Seed dev.	0.57**	0.54**	0.70**	-0.30	-0.50**	-0.12	-0.27	0.001	0.77**	0.78**	0.79**

*, **Significant at 5 and 1% level of probability, respectively

authors.

Crude Seed Protein

Increasing N rates from 0 to 50, 150 and 300 kg N/ha resulted in increases in seed protein content of 4.96%, 4.41% and 7.88% over the control (Fig. 5). Ries (21) also obtained an increase in protein content of snap bean seed by supplemental N fertilization. He reported that seedling size, yield, and number of fruits were more correlated with protein per seed than with seed size, and that within a genotype, seedling vigor was related to the seed protein content.

No correlation was found between yield and crude seed protein. However, crude seed protein was correlated with NO_3^- content at the fully developed ($r=0.54^{**}$), floral bud ($r=0.46^{**}$), flowering ($r=0.48^{**}$) and seed development ($r=0.54^{**}$) stages (Table 1).

Total Protein Yield

Increasing N rates from 0 to 50, 150 and 300 kg N/ha increased total protein yield by 15.6%, 18.9% and 24.7% over the control (Fig. 5). P had no effect on total protein yield.

N Translocation Efficiency

N translocation efficiency percentage or harvest N index, was not affected by N or P fertilization levels. Jeppson et al. (15), working with soybeans, and Lovato (17) with barley, also reported that translocation of plant nitrogen to the seed was not influenced by N fertilization but was controlled by the genetic capability of cultivars.

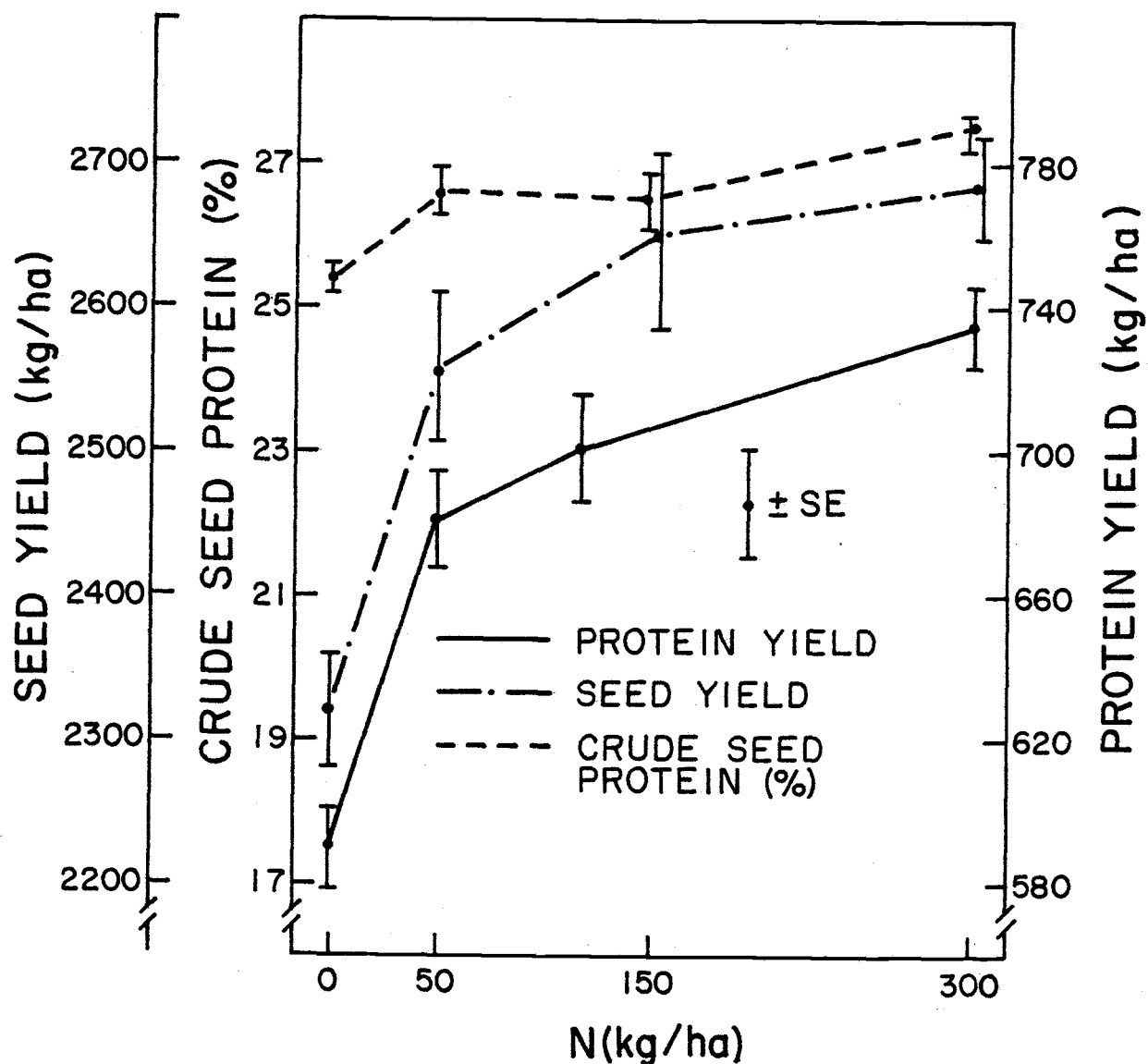


Figure 5. Effect of N fertilizer on seed yield, % crude seed protein and protein yield of "Oregon 1604" snap beans (*Phaseolus vulgaris* L.).

N translocation efficiency was negatively correlated with seed yield ($r=-0.43^{**}$, Table 1). This result is confirmed by similar finding in soybean (15). The percentage of total above ground plant nitrogen that is retained in the snap bean plant, or conversely, the proportion that is transferred to the seed, is an indication of N transport efficiency. A higher transport efficiency from the plant to the seed is a desirable characteristic for high seed protein production.

Egli et al. (6) stated that the redistribution of N from vegetative to reproductive plant parts of soybeans may be one of the factors causing leaf senescence, and, consequently, it has a direct influence on yield. Results with snap beans showed that redistribution of N from the vegetative plant material to the snap bean seed occurred regardless of the rate of fertilizer application. This is in agreement with the results obtained by Hanway and Weber (10) with soybean.

Ammonium nitrate fertilizer probably caused a repression and/or feedback inhibition of NR and a decrease in NRA in snap bean leaves. Further research is suggested to determine the effect of various sources of nitrate fertilizer on NRA and NO_3^- content in snap bean leaves.

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MANUSCRIPT III

Effect of N and P fertilization rates on growth attributes of the snap bean (Phaseolus vulgaris L.) seed crop.

ABSTRACT

The effects of N and P fertilization rates on growth attributes were investigated at six growth stages in "Oregon 1604" snap beans (Phaseolus vulgaris L.) grown for seed. Plots were planted in a randomized block design with a factorial arrangement of treatments consisting of four nitrogen rates (0, 50, 150 and 300 kg/ha) and three phosphorus levels (0, 50 and 150 kg/ha) with four replications.

N increased dry matter (DM), leaf area (LA) and leaf area index (LAI) at all growth stages except the first fully developed trifoliolate leaf stage. Crop growth rate ($\overline{\text{CGR}}$) was increased by N at the floral bud and seed development stages. Chlorophyll a, b and a+b content at the seed development stage, water soluble carbohydrate (WSC) at maturity, and yield were increased by N.

Responses to P were not as large or as consistent as for N. P increased DM at the fully developed trifoliolate, floral bud and flowering stages, LA at the fully developed trifoliolate, and floral bud stages, and $\overline{\text{CGR}}$ at the fully developed trifoliolate and floral bud stages of growth. Chlorophyll a, b and a+b, WSC and yield were not influenced by P fertilization.

The multiple correlation coefficient (R^2) indicated that LAI at maturity was responsible for 41.6% of the variation in yield. WSC, $\overline{\text{CGR}}$ at the fully developed trifoliolate stage, and LA at the flowering

stage contributed an additional 13.5, 4.6 and 6.1%, respectively.

Additional index words: Dry matter, leaf area, leaf area index,
mean crop growth rate.

INTRODUCTION

The problem of accounting for variation in yield in terms of growth and development of the crop plant is very complex. For ultimately it involves the effect of external factors on all the physiological processes of the plant, the interrelation between different processes, and their dependence on internal factors which are determined by the genetic constitution of the plant.

Growth in plants can be measured in many ways. The most direct method is by measuring the increase in plant dry weight over time, which in turn reflects the assimilation of photosynthate produced by the plant. Growth analysis is based on the principle that the increase in dry weight of plants is a measure of net photosynthesis (15), and the procedure requires the measurement of dry weight and leaf area of the plant throughout its growth period. Techniques and formulae have been described by several investigators (12, 14, 18).

Although growth analysis techniques have been used for a long time and have made a substantial contribution to current concepts of the physiological basis of crop yield, there have been few attempts to apply these techniques to snap beans (Phaseolus vulgaris L.) grown for seed.

Magalhães et al. (9) concluded that dry organic matter and NPK fertilization affected growth of dry beans during the early

phases of plant development, causing a significant increase in leaf area index (LAI) and mean crop growth rate (CGR) up to the time of flowering, but decreased growth in the later stages. In contrast, both the control plants and those that received NPK fertilization showed continuous increases in LAI and $\overline{\text{CGR}}$ toward the end of the growing period. Only slight differences in seed yield were noticed among treatments despite remarkable increases in $\overline{\text{CGR}}$, RGR and NAR. Montojos and Magalhães (11) found that high intensity solar radiation ($400\text{-}500 \text{ cal/cm}^2/\text{day}$) promoted increased growth rate, dry matter production and seed production of dry beans because of increased LAI which influenced the size of the photosynthetic system. Largest seed production was obtained when N was applied three times during the first 21 days of the growing season. This procedure delayed leaf senescence and improved the photosynthetic capacity of the bean crop because of the increase in leaf area duration.

The objective of this study was to investigate the effects of N and P fertilization rates on growth analysis parameters of the snap bean seed crop at various stages of crop development. Growth attributes measured were leaf area (LA), leaf area index (LAI), dry matter (DM), mean crop growth rate ($\overline{\text{CGR}}$), chlorophyll content, water soluble carbohydrate content (WSC), and yield.

MATERIALS AND METHODS

Plots of "Oregon 1604" snap beans were established at the Vegetable Research Farm near Corvallis, Oregon on a Chehalis silty clay loam soil. Each plot consisted of six rows 10.4 m long spaced 0.91 m apart. The plots were planted in a randomized block design with a factorial arrangement of treatments consisting of four nitrogen rates (0, 50, 150 and 300 kg/ha) and three phosphorus rates (0, 50 and 150 kg/ha) with four replications.

Seeds were planted on May 12, 1977 at the rate of 82 kg/ha with a belt-type hand-pushed planter and seedlings were thinned to a population of 26 plants/m. The seeds were treated with Lorsban insecticide and Captan fungicide but were not inoculated. Difonate insecticide at the rate of 22.4 kg/ha was incorporated in the soil before planting. For weed control, 0.84 kg/ha trifluralin was incorporated preplant and 3.3 kg/ha dinitramine was applied after planting. Irrigation was provided every 8-12 days as needed.

Soil analysis before fertilizer application indicated a pH of 6.3, P = 49 ppm K = 250 ppm, Ca = 18.7 meg/100 g, and Mg = 6.2 meg/100 g.

Potassium sulfate (50% K_2O and 17% S) was applied before planting to provide a uniform rate of 60 kg K/ha on all plots.

Triple superphosphate (45% P_2O_5) was hand broadcast before planting to provide 50 and 150 kg P/ha on appropriate plots.

Split applications of ammonium nitrate (34% N) were made according to the developmental stages of the plants. The 50 kg/ha rate was applied before planting. For the 150 kg/ha rate, an initial application of 100 kg/ha was made before planting and 50 kg/ha was applied on June 17 when the plants were in the first trifoliate leaf stage. For the 300 kg/ha rate, 100 kg/ha was applied before planting, 50 kg/ha at the first trifoliate leaf stage, 50 kg/ha on June 27 at the floral bud stage, 50 kg/ha on July 11 at flowering, and 50 kg/ha July 19 at the small pod stage.

Seed yield was determined by hand harvesting 6.1 m of two central rows of each plot when seed moisture was 11-13%. Threshing was done with a rubber-belt thresher.

Growth Analysis

Growth analysis was conducted at six different stages of plant development: (I) unifoliate (15 days after emergence), (II) fully developed trifoliate (27 days after emergence), (III) floral bud (43 days after emergence), (IV) flowering (50 days after emergence), (V) seed development (63 days after emergence), and (VI) maturity (82 days after emergence). For stages I, II, and III, eight bean plants, equivalent to a sampling area of 0.28 m^2 were used for analysis. For stages IV, V and VI, four bean plants from an area of 0.14 m^2 were used. Similar to the procedure used by Koller et al. (7) no attempt

was made to recover leaves that had abscised.

The plants were collected, placed in a plastic bag, stored in ice in a styrofoam chest and immediately brought to the laboratory for analysis.

Leaf area (LA) was measured with a portable area meter (Model LI-3000 LAMBDA Inst. Co.).

Dry matter (DM) of components parts was determined after placing plants in a forced air oven at 70 C for 48 hours.

Leaf area index (LAI) and mean crop growth ($\overline{\text{CGR}}$) were estimated by the methods proposed by Watson (14) and the formulae listed by Radford (12).

Chlorophyll (a, b and a+b) content of fully developed trifoliate leaves was determined during the seed development stage (68 days after emergence) by the method of Bruisma (1).

Content of water soluble carbohydrate (WSC) in the plant was determined by the Anthrone method described by Yemm and Willis (19). Above ground plant parts (except seeds) were collected at maturity, dried at 70 C for 48 hours, ground in a Wiley mill to pass a 40 mesh screen, and analyzed for WSC.

RESULTS AND DISCUSSION

Leaf Area and Leaf Area Index

LA (Figure 1) and LAI (Figure 2) increased rapidly, attaining maximum values during the seed development stage 63 days after emergence. Thereafter, LA and LAI declined because of abscission of the lower leaves. Similar responses have been reported for dry beans (9, 11, 13) and soybeans (2, 3).

N fertilizer increased LA and LAI at nearly all growth stages while P increased LA and LAI only through the floral bud stage.

Leaf senescence was delayed in fertilized plots, resulting in an increase in leaf area duration.

Dry Matter (DM) and Mean Crop Growth Rate (CGR)

DM production increased continuously until the end of the growing season, reaching its maximum value at maturation 82 days after emergence (Figure 3). N increased DM production at all stages except the first fully developed trifoliate leaf stage, with the greatest amount of DM produced at the 300 kg/ha N rate.

P increased DM production through the flowering stage but not during the later stages.

Increases in DM production as a consequence of N and P fertilization can be explained by increases in Net Assimilation Rate (NAR), although Heath and Gregory, cited by Watson (14), pointed out that

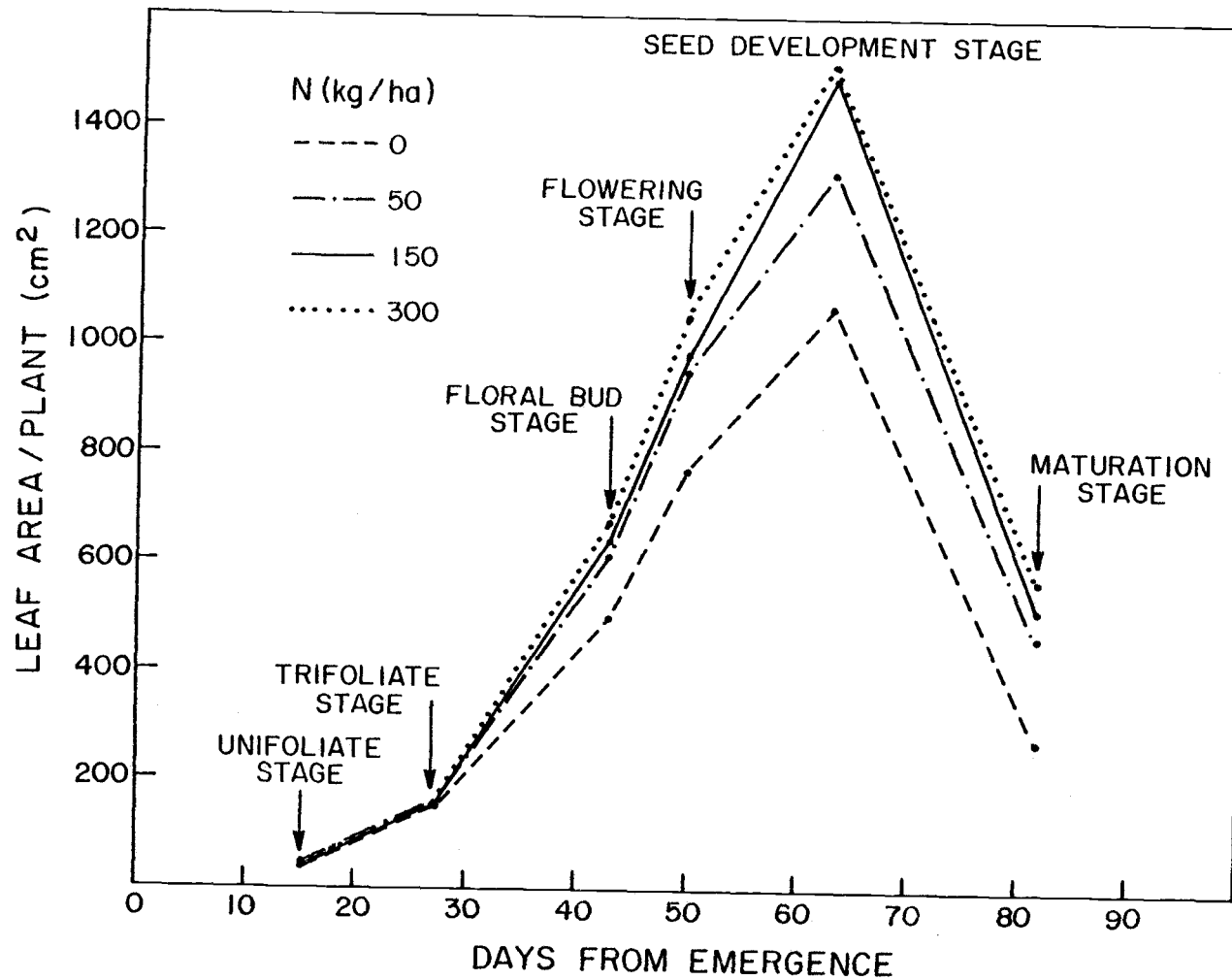


Figure 1 - Effect of N fertilizer rates on the leaf area (LA) of snap bean plants at various stages of plant development.

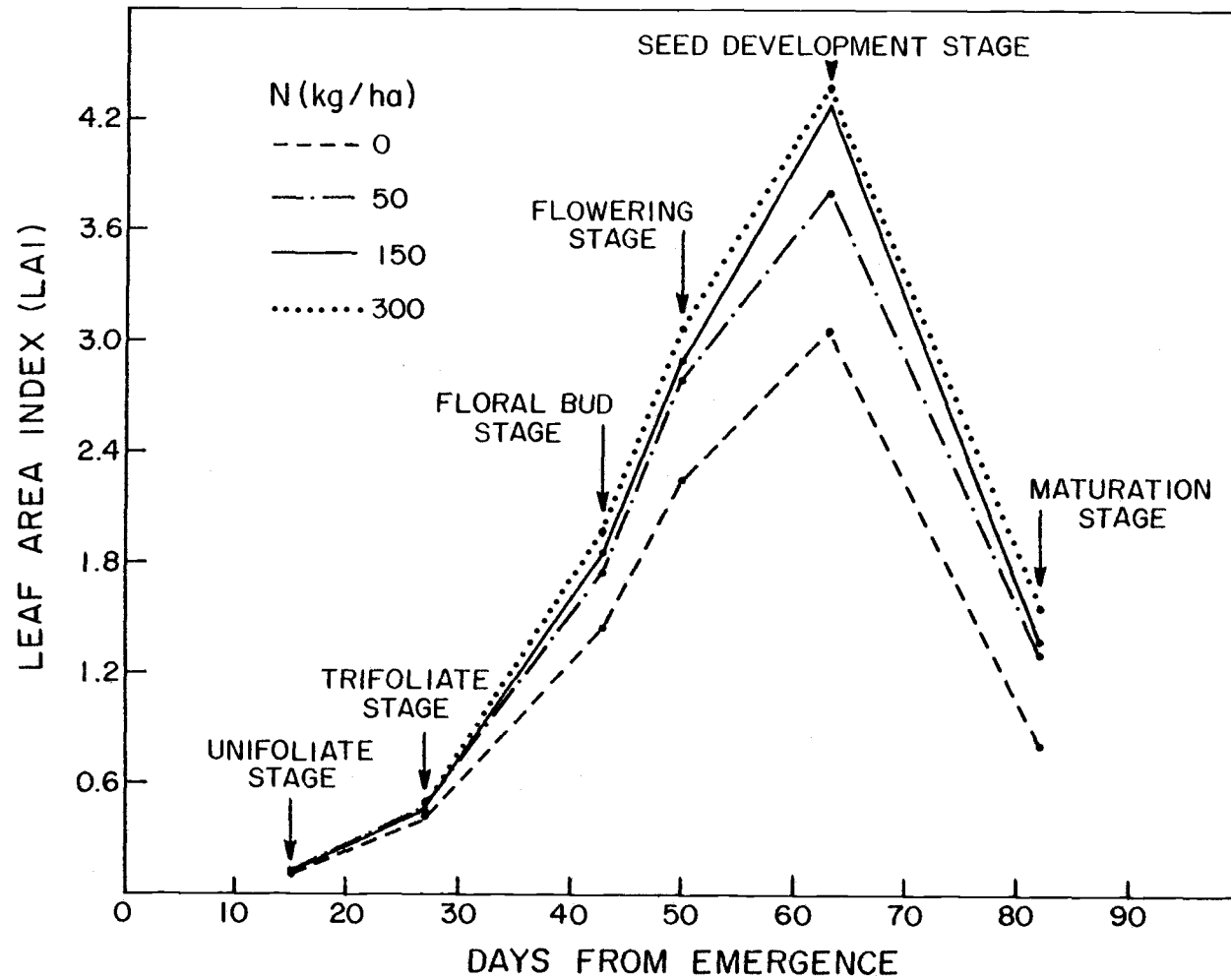


Figure 2 - Effect of N fertilizer rates on the leaf area index (LAI) of snap bean plants at various stages of plant development.

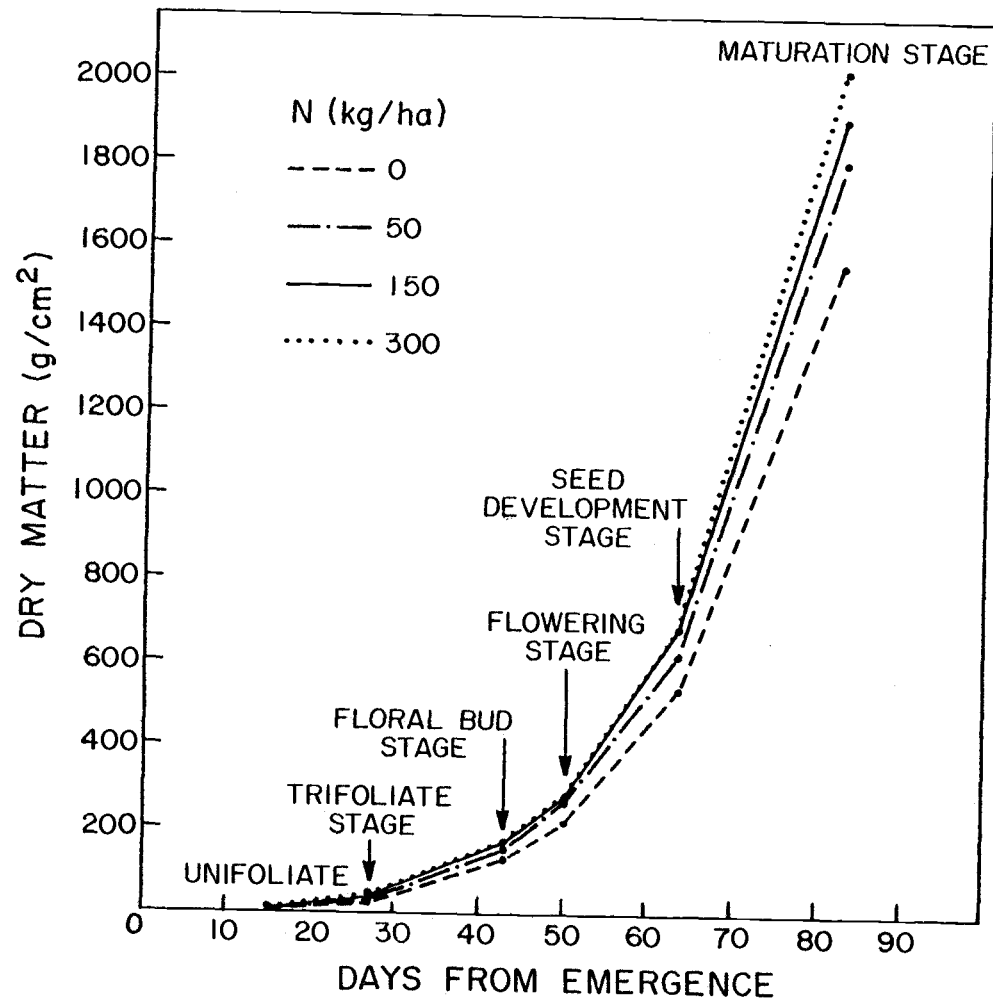


Figure 3 - Effect of N fertilizer rates on dry matter (DM) accumulation of the above ground portions of snap bean plants at various stages of plant development.

the major contribution is due to an increase in LA rather than NAR. Enhancement of DM production by fertilizer application has been reported in dry beans (11) and soybeans (3) and has been associated with increases in LAI, $\overline{\text{CGR}}$ and NAR.

The $\overline{\text{CGR}}$ increased continuously as the season progressed (Figure 4). This continuous increase in $\overline{\text{CGR}}$ over time can be explained by the increases in LA, LAI and NAR. $\overline{\text{CGR}}$ is related to LAI and NAR by the formula $\overline{\text{CGR}} = \text{NAR} \times \text{LAI}$, and any increase in NAR or LAI consequently resulted in increases in $\overline{\text{CGR}}$ and DM accumulation in the plant.

The $\overline{\text{CGR}}$ of fertilized plots was consistently higher than non-fertilized plots, although increases due to N were significant only at the floral bud and seed development stages.

P increased $\overline{\text{CGR}}$ up to the floral bud stage, but not at later stages.

These results are similar to those obtained with dry beans by Magalhães et al. (9). They reported continuous increases in $\overline{\text{CGR}}$ toward the end of the growing period due to NPK fertilization. Buttery (2), however, pointed out that $\overline{\text{CGR}}$ in soybeans increased for the first 50 to 60 days and fell sharply thereafter.

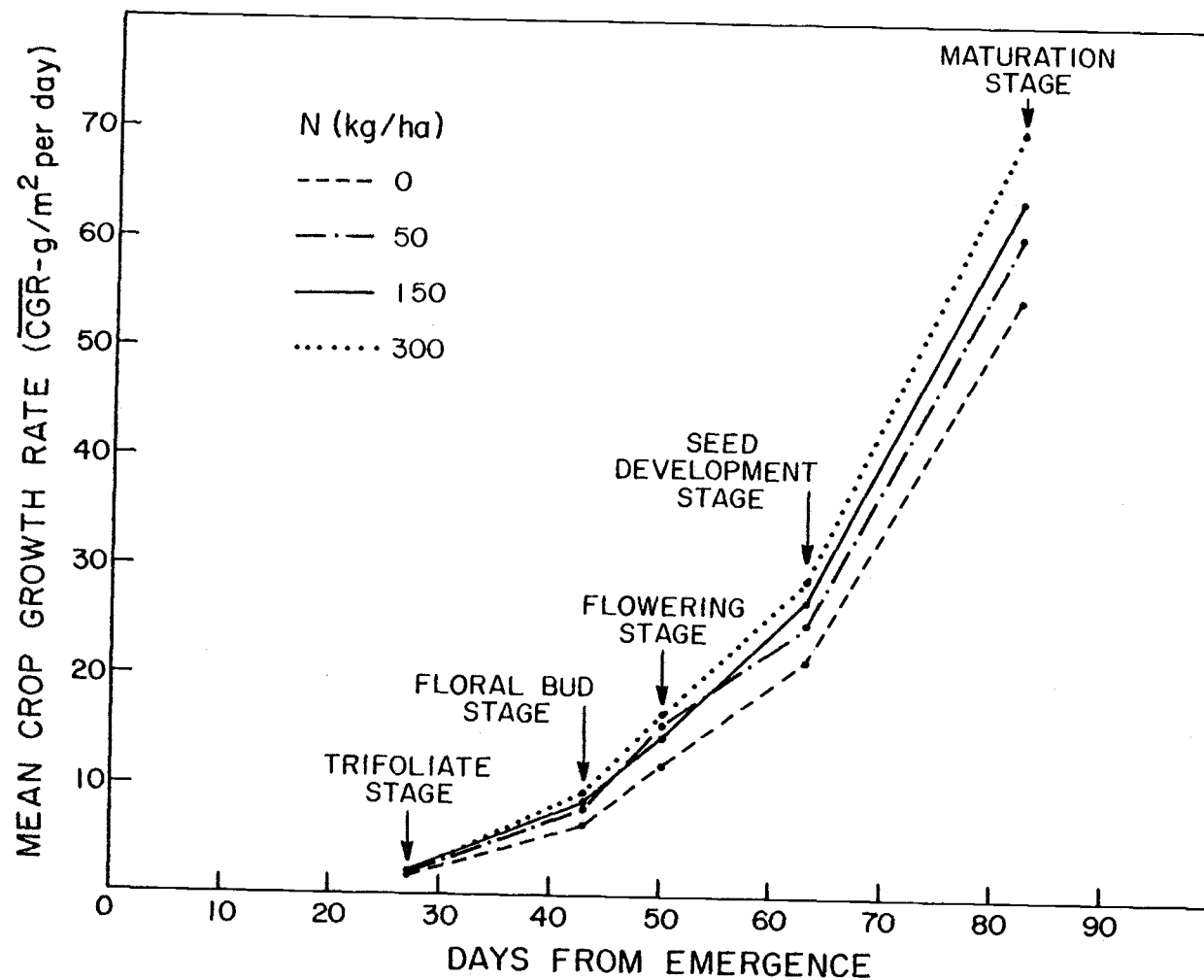


Figure 4 - Effect of N fertilizer rates on the mean crop growth rate ($\overline{\text{CGR}}$) of snap bean plants at various stages of plant development.

Chlorophyll Content

N increased the content of chlorophyll a, b and a+b at the seed development stage (Figure 5). Application of 50, 150 and 300 kg N/ha increased chlorophyll a by 7.7, 23.1 and 57.7%, chlorophyll b by 17.7, 29.4 and 52.9%, and chlorophyll a+b by 16.7, 40.0 and 59.5%, respectively. Similar results have been reported for wheat (5) in which chlorophyll increases of 192% were observed in fertilized plants.

P had no effect on chlorophyll content.

Water Soluble Carbohydrates (WSC)

WSC of the above ground plant parts (except seeds) at maturity was increased by N but not by P (Figure 6). Maximum content of WSC was obtained with 150 kg N/ha and 150 kg P/ha (3.35%), and with 300 kg N/ha and 150 kg P/ha (3.37%).

Increasing N rates to 50, 150 and 300 kg/ha resulted in increases in WSC of 45.4, 72.1 and 66.1%, respectively.

WSC analysis conducted at various stages of soybean development confirm that substantial quantities of carbohydrates accumulate in leaves, petioles and stems prior to seed development and later are utilized in seed growth (6). Application of ammonium nitrate to wheat resulted in increases of up to 24.0% in the concentration of glucose (5); however, sucrose, total soluble sugars and non-structural polysaccharides were decreased to 58, 49 and 64%,

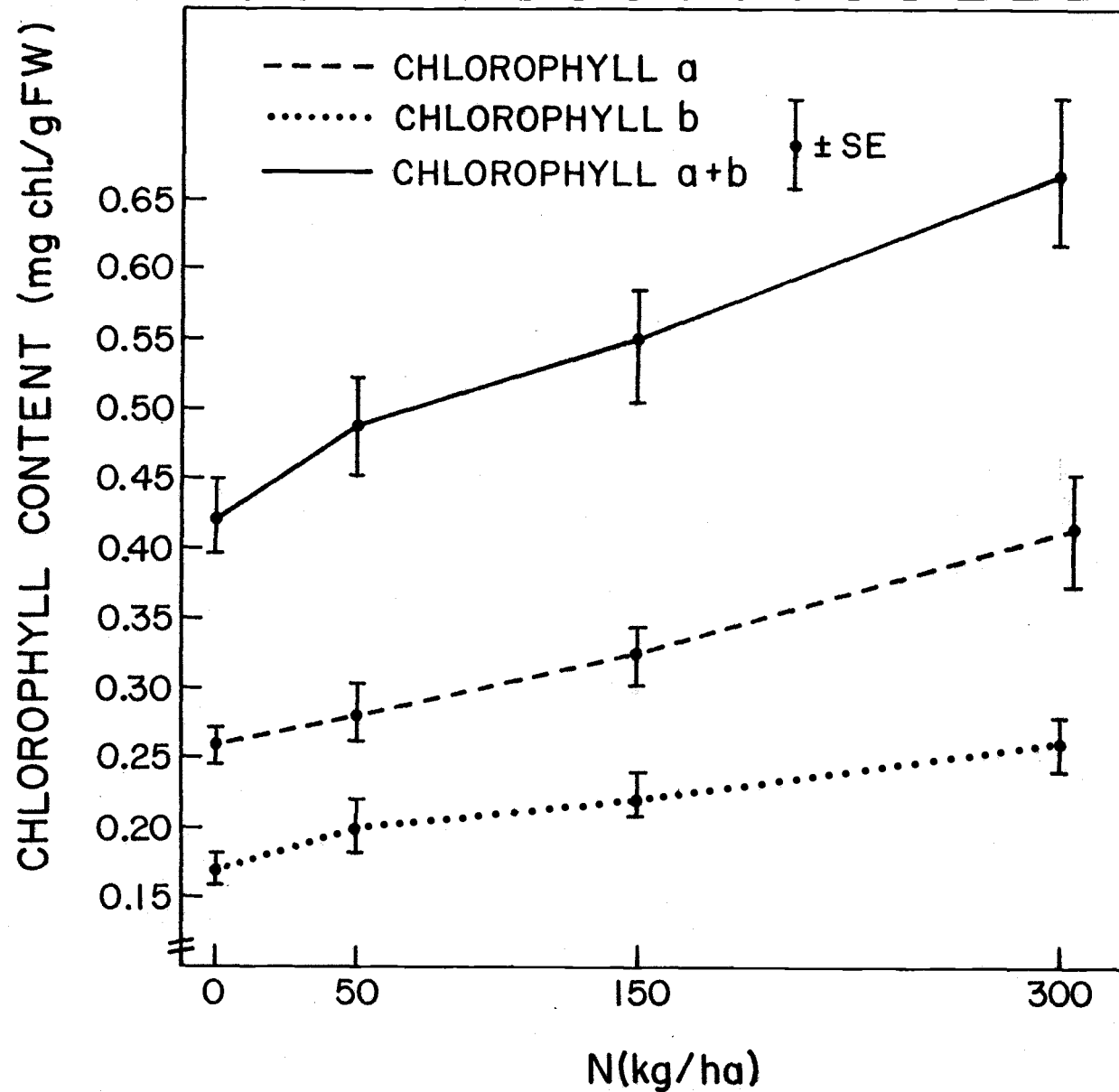


Figure 5 - Effect of N fertilizer rates on the chlorophyll (a, b, and a+b) content of the leaves of snap bean plants at the seed development stage.

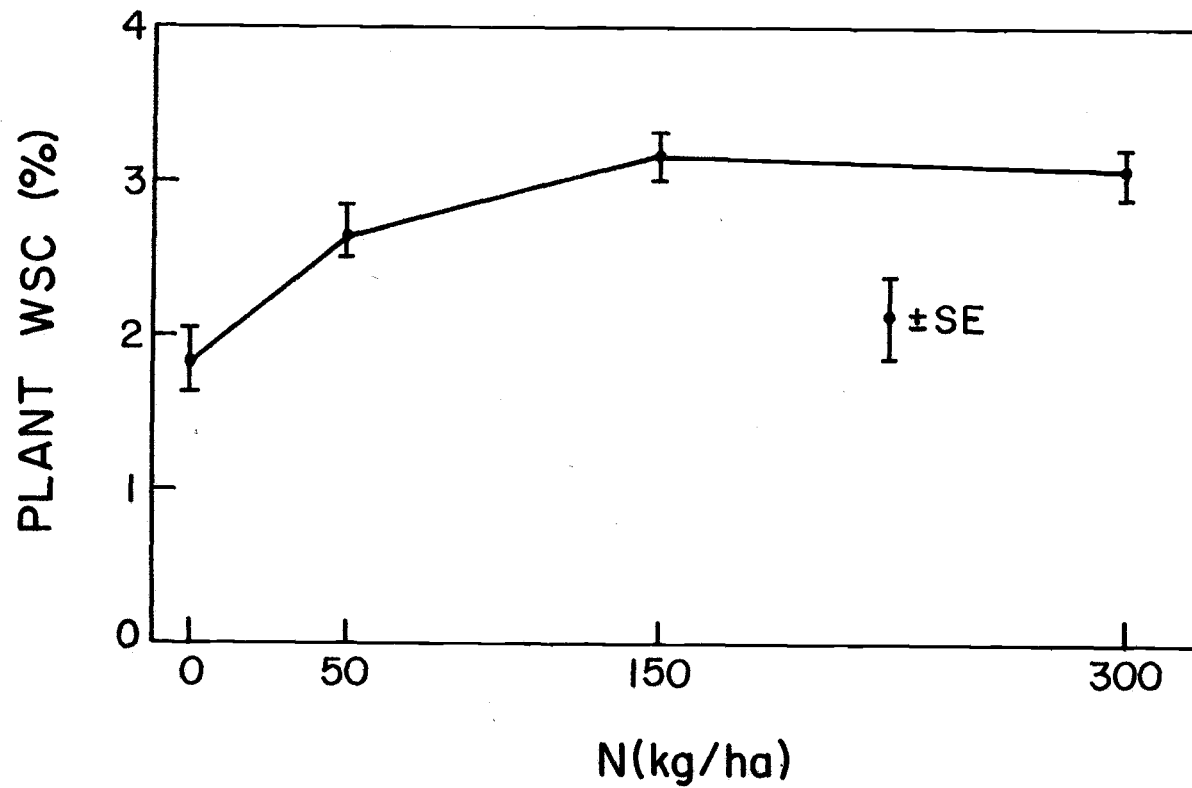


Figure 6 - Effect of N fertilizer rates on water soluble carbohydrate (WSC) content of the above ground portions (except seeds) of snap bean plants at maturity.

respectively, of the control.

Seed Yield

Seed yield increased 10.2, 14.1 and 15.7% with N applications of 50, 150 and 300 kg/ha, respectively (Figure 7). Despite large N effects on LA, LAI, DM and $\overline{\text{CGR}}$, seed yield increases were not proportionally as great. Some previous studies have also shown increases in snap bean seed yield from application of N (4, 17) while other studies (8, 16) have not.

Yield was positively correlated with LA and LAI at most of the growth stages. Montojos and Magalhães (11) pointed out that LAI values of approximately 3 when measured after flowering were associated with high seed yields of dry beans. Nichiporovich, cited by Mitchell (10), suggested that an optimum LAI is definable for every economic crop and he considered the optimum range to be between 2.5 and 5.0.

In this study, the highest snap bean seed yield of 2,729 kg/ha was associated with a LAI of 4.64 at the seed development stage.

Seed yield was also positively correlated with DM at the unifoliate ($r=0.48^{**}$), floral bud ($r=0.41^{**}$), flowering ($r=0.48^{**}$), seed development ($r=0.42^{**}$) and maturation ($r=0.36^{**}$) stages. Yield was not correlated with chlorophyll a+b content at the seed development stage.

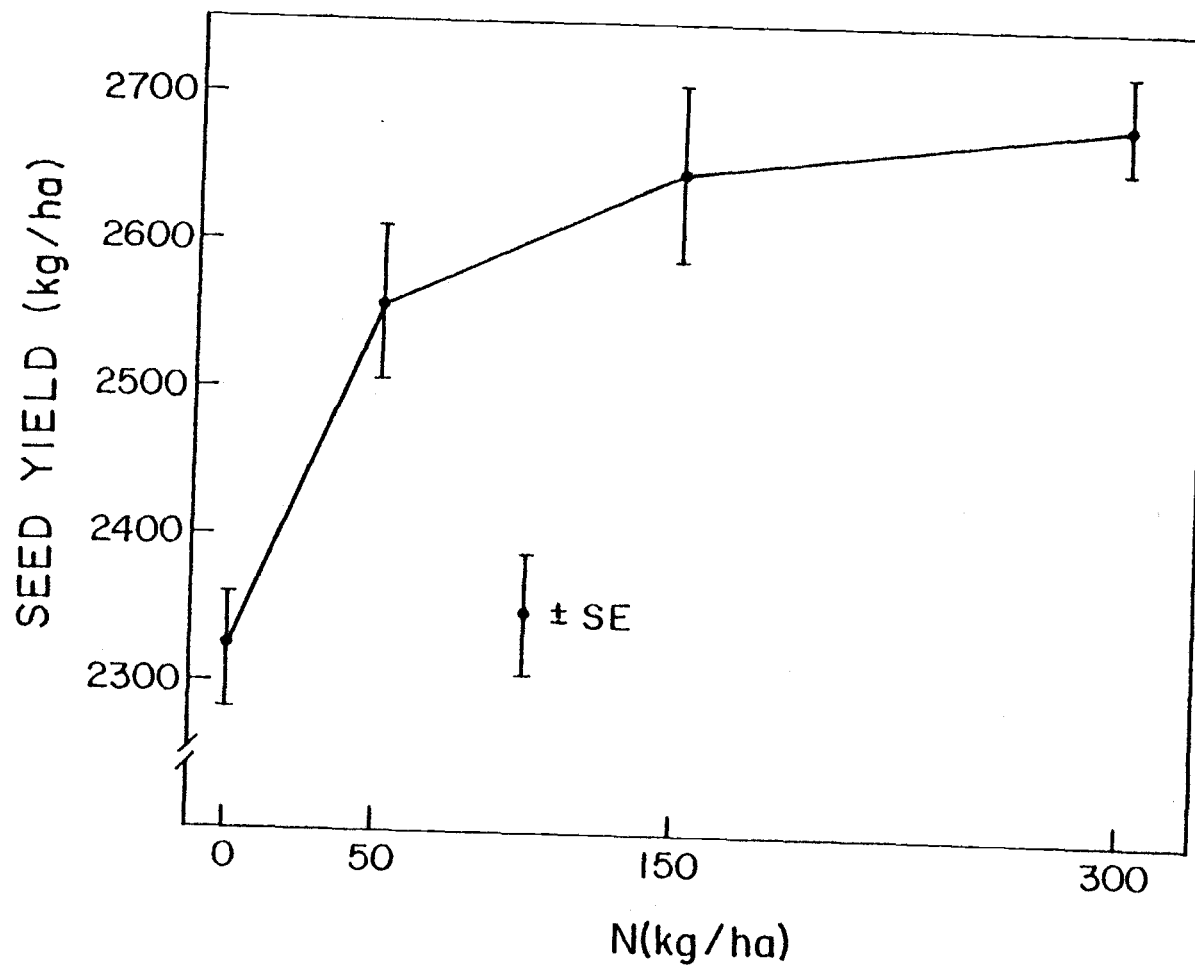


Figure 7 - Effect of N fertilizer rates on seed yields of "Oregon 1604" snap bean.

A stepwise multiple regression analysis was conducted to assess the contribution of LA, LAI, $\overline{\text{CGR}}$, DM, chlorophyll a+b content and WSC to yield. The multiple correlation coefficient (R^2) indicated that LAI at maturity was responsible for 41.6% of the variation in yield. Upon addition of WSC into the regression, an increase of 13.5% was achieved in predicting yield. Upon addition of WSC into the regression, an increase of 13.5% was achieved in predicting yield. Similarly, $\overline{\text{CGR}}$ and LA contribute an additional 4.6 and 6.1%, respectively. Altogether these four growth attributes were responsible for 65.8% of the variation in yield. Chlorophyll a+b content and WSC did not contribute enough to be of predictive value.

The regression equation for yield is:

$$Y = 1955.42 + 246.18 (X_1) + 74.81 (X_2) - 152.14 (X_3) + 0.38 (X_4),$$

where Y = Yield,

X_1 = LAI at maturity,

X_2 = WSC at maturity,

X_3 = $\overline{\text{CGR}}$ at the fully developed trifoliate stage,

and X_4 = LA at the flowering stage.

Whether this model can be used for predicting snap bean seed yield needs further research.

Further research on methods of increasing LAI and yield of snap bean is suggested. Photosynthetic activity might be improved

by adjusting row width and plant density for greater light interception. Changing the shape and orientation of the leaves through plant breeding is another potential area of research that could bring increases in LA, LAI, NAR and yield.

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APPENDIX

Appendix Table 1. Average monthly rainfall at Hyslop Farm and air and soil temperature at OSU Vegetable Research Farm during the 1977 growing season.

Month	Rainfall	Air temperature			Soil temperature at 10 cm depth		
		Min.	Max.	Mean	Min.	Max.	Mean
	(mm)	----- °C -----					
May	87.1	5.0	16.1	10.6	13.3	16.1	14.4
June	28.7	8.9	23.3	16.1	18.3	19.4	18.9
July	3.1	10.0	25.6	17.8	18.3	21.7	20.0
August	48.0	11.7	28.9	20.6	21.1	25.6	23.3
September	90.9	9.4	19.4	14.4	15.6	18.9	17.2

Appendix Table 2. Simple correlation coefficients (r) between quality components of snap bean (*Phaseolus vulgaris* L.) seeds.

Seed quality components	Seed wt.	Seedling dry wt.	Germination	ATP/embryo	ATP/mg embryo	Water sol. carbo-hydrate	Crude protein
Seed wt.	-						
Seedling dry wt.	0.06	-					
Germination	-0.21	0.03	-				
ATP/embryo	-0.10	0.33*	0.27	-			
ATP/mg embryo	-0.10	0.35*	0.19	0.96**	-		
Water soluble carbohydrate	0.17	0.07	-0.21	0.14	0.20	-	
Crude protein	0.27	0.18	0.09	0.09	0.12	0.45**	-

**, * Significant at the 1% and 5% levels of probability, respectively.

Appendix Table 3. Effect of N and P fertilizers on quality components of snap bean (*Phaseolus vulgaris* L.) seed.

NP treatments		Seed weight	Larger than screen size 12 ‡	ATP content		Crude protein	Water sol. carb. (WSC)	Germination	Seedling axis dry weight
kg/ha		mg/seed	%	n moles/embryo	n moles/mg embryo	%	%	%	mg/seedling
N ₀	PO	200	23.90	28.8	2.12	24.60	4.42	95	52.2
	P ₅₀	228	26.91	50.1	3.43	25.66	4.20	96	69.8
	P ₁₅₀	215	18.20	101.0	6.72	25.89	4.92	98	57.8
N ₁₅₀	PO	225	29.44	44.5	3.56	27.30	5.35	94	58.9
	P ₅₀	235	32.18	33.9	2.46	26.18	4.33	96	56.1
	P ₁₅₀	218	25.90	49.4	4.40	26.47	4.96	95	58.5
N ₁₅₀	PO	235	32.71	36.7	2.90	26.08	5.28	94	54.4
	P ₅₀	228	30.54	57.3	4.07	27.07	4.86	95	57.1
	P ₁₅₀	228	32.18	74.5	5.60	26.36	5.38	94	59.8
N ₃₀₀	PO	225	34.77	63.4	4.19	27.03	5.03	95	61.3
	P ₅₀	230	36.24	56.3	4.01	27.82	5.26	96	58.8
	P ₁₅₀	232	28.92	50.2	3.59	27.30	5.56	96	59.0
Overall Mean		225	30.15	53.8	3.92	26.48	4.96	95	57.8
F (N)		2.67+	27.30**	6.47**	2.04	7.89**	7.85**	1.57	2.71+
F (P)		1.41	2.01	25.74**	28.02**	0.71	6.75**	3.39*	2.05
F (N×P)		1.19	2.64	13.80**	10.01**	1.35	1.80	0.82	3.67**
CV %		7.51	21.35	38.95	35.66	4.58	12.09	9.00	5.98
S _x ²		7.76	1.88	5.24	0.40	0.51	0.21	0.010	1.44
S _d ²		10.98	2.66	7.40	0.54	0.72	0.30	0.015	2.03
LSD _{0.05}		22.36	5.42	15.07	1.10	1.47	0.61	0.03	4.13
LSD _{0.01}		30.04	7.28	20.25	1.48	1.97	0.82	0.04	5.55

**, *, + Significant at 1, 5 and 10% levels of probability, respectively.

‡ Percentage of seed lot held over a 4.76 mm x 12.05 mm (12/64 x 3/4 in) screen.

Appendix Table 4. Effect of N and P fertilizers on yield, yield components, and plant height of "Oregon 1604" snap bean (*Phaseolus vulgaris* L.).

NP treatments		Seed yield	Yield components				Plant height
			Plants/m ²	Pods/plant	Seeds/pod	Seed weight	
kg/ ha		kg/ ha				mg/ seed	cm
N ₀	PO	2, 219	28	7	5	200	45.6
	P ₅₀	2, 421	24	9	4	228	46.2
	P ₁₅₀	2, 324	25	9	4	215	49.8
N ₅₀	PO	2, 612	26	8	4	225	49.1
	P ₅₀	2, 612	27	8	4	235	52.2
	P ₁₅₀	2, 453	24	9	4	218	51.1
N ₁₅₀	PO	2, 641	29	9	4	235	50.6
	P ₅₀	2, 577	23	9	4	228	51.8
	P ₁₅₀	2, 729	26	9	4	228	53.2
N ₃₀₀	PO	2, 649	26	9	5	225	54.0
	P ₅₀	2, 728	23	10	4	230	54.4
	P ₁₅₀	2, 678	27	9	4	232	53.5
Overall mean		2, 554	26	9	4	225	51.0
F (N)		12.29**	0.14	1.40	0.53	2.66+	11.35**
F (P)		0.47	2.10	2.63	0.72	1.41	2.11
F (N×P)		1.17	0.75	0.59	0.80	1.19	0.78
CV %		8.46	16.90	14.88	8.16	7.51	7.52
S _x		80.81	2.19	0.61	0.19	7.76	1.46
S _d		114.29	3.09	0.87	0.27	10.98	2.06
LSD _{0.05}		232.69	6.29	1.77	0.55	22.36	4.19
LSD _{0.01}		312.70	7.49	2.38	0.74	30.04	5.64

**, *, + Significant at the 1%, 5% and 10% level of probability, respectively.

Appendix Table 5. Simple correlation coefficients (r) between seed yield and yield components of "Oregon 1604" snap bean (Phaseolus vulgaris L.).

Yield components	Seed yield	Yield components					
		Plants/m ²	No. pods	No. seeds	Pods/plant	Seeds/pod	Seed wt.
Plants/m ²	0.05						
No. pods	0.45**	0.42**					
No. seeds	0.42**	0.45**	0.77**				
Pods/plant	0.26	-0.78**	0.19	0.03			
Seeds/pod	0.06	0.08	-0.26	0.41**	-0.21		
Seed wt.	0.45**	-0.20	0.15	-0.17	0.37**	-0.41**	-

**Significant at the 1% level of probability.

Appendix Table 6. Effect of N and P fertilizers on nitrate reductase activity (NRA) and NO_3^- content at fully developed trifoliolate stage (34 days after emergence), floral bud stage (45 days after emergence), flowering stage (57 days after emergence), seed development stage (71 days after emergence), seed yield, protein yield and % N translocation efficiency (N. T. E.) of snap bean (*Phaseolus vulgaris* L.).

NP treatments		NRA				NO_3^- content				Seed yield	Crude seed protein	Protein yield	N. T. E.
		Fully dev. trifoliolate	Floral bud	Flowering	Seed dev.	Fully dev. trifoliolate	Floral bud	Flowering	Seed dev.				
kg/ha		-----n Moles NO_3^- red g FW ⁻¹ min ⁻¹ -----				-----m Moles g DW ⁻¹ -----				kg/ha	%	kg/ha	%
N ₀	PO	92.5	150.0	145.0	30.0	1.35	0.29	0.22	0.18	2,219	24.60	546	64.13
	P ₅₀	100.0	137.5	140.0	40.0	1.77	0.31	0.22	0.22	2,421	25.66	621	61.36
	P ₁₅₀	102.5	167.5	120.0	27.5	1.96	0.32	0.26	0.21	2,324	25.89	602	64.49
N ₅₀	PO	57.5	130.0	105.0	37.5	2.94	0.40	0.38	0.26	2,612	27.30	713	62.81
	P ₅₀	60.0	120.0	132.5	35.0	2.81	0.49	0.38	0.31	2,612	26.18	684	67.35
	P ₁₅₀	47.5	115.0	92.5	27.5	2.74	0.52	0.38	0.31	2,453	26.47	649	60.75
N ₁₅₀	PO	62.5	100.0	102.5	37.5	3.06	0.72	0.62	0.37	2,641	26.08	689	59.76
	P ₅₀	22.5	130.0	102.5	37.5	3.20	0.75	0.64	0.41	2,577	27.07	698	62.52
	P ₁₅₀	37.5	130.0	127.5	35.0	3.85	0.86	0.65	0.45	2,729	26.36	719	59.54
N ₃₀₀	PO	42.5	110.0	105.0	32.5	3.76	0.86	1.63	0.50	2,649	27.03	716	62.11
	P ₅₀	25.0	125.0	85.0	30.0	4.10	0.82	1.40	0.76	2,728	27.82	759	61.44
	P ₁₅₀	50.0	102.5	100.0	35.0	3.74	0.85	1.73	0.68	2,678	27.30	731	58.67
Overall Mean		58.3	126.5	113.1	33.7	2.94	0.60	0.71	0.39	2,554	26.48	676	62.08
F (N)		9.94**	2.59+	2.11	0.35	57.25**	101.39**	83.37**	294.51**	12.29**	7.89**	28.43**	1.86
F (P)		0.63	0.14	0.08	0.59	1.98	2.76	0.63	28.56**	0.47	0.71	1.53	1.25
F (Nx))		0.72	0.64	0.81	0.57	1.72	0.821	0.51	7.90**	1.17	1.35	1.35	1.18
CV %		63.78	50.02	46.89	36.39	32.39	41.11	55.32	47.13	8.46	4.58	10.31	7.21
S _x		15.16	18.60	19.01	5.84	0.21	0.04	0.12	0.04	80.81	0.51	20.25	2.07
S _T		21.44	26.31	26.89	8.26	30.30	0.06	0.16	0.03	114.29	0.70	28.63	2.92
LSD _{.005}		43.65	37.87	54.75	16.82	0.61	0.12	0.33	0.06	232.69	1.47	58.29	5.95
LSD _{.001}		58.66	71.98	73.57	22.60	0.82	0.12	0.44	0.08	312.70	1.97	78.33	7.99

**, *, + Significant at the 1, 5 and 10% level of probability, respectively.

Appendix Table 7. Effect of N and P fertilizers on the above ground dry matter content (DM), leaf area (LA), leaf area index (LAI), mean crop growth rate (CGR), chlorophyll content, plant-water soluble carbohydrate, and seed yield of snap beans (*Phaseolus vulgaris* L.).

NP treatments		Dry matter (DM)						Leaf area (LA)					
		Unifoliate stage	Trifoliate stage	Floral bud	Flowering	Seed dev.	Maturation	Unifoliate stage	Trifoliate stage	Floral bud	Flowering	Seed dev.	Maturation
kg/ha		g/m ²						cm ²					
N ₀	PO	6.54	23.97	121.39	200.68	505.00	1174.55	35.68	129.08	479.45	728.30	1000.23	174.83
	P ₅₀	6.98	25.99	119.33	228.68	548.55	1736.88	38.51	138.65	454.69	799.58	1121.50	382.98
	P ₁₅₀	7.23	30.13	155.70	227.79	558.97	1793.23	41.06	165.28	571.29	827.52	1010.16	285.21
N ₅₀	PO	7.59	25.10	142.52	266.77	597.02	1853.81	40.32	130.90	576.25	941.34	1329.14	397.14
	P ₅₀	7.63	29.56	155.55	248.20	593.59	1633.89	42.92	152.09	592.32	942.25	1351.41	485.79
	P ₁₅₀	7.82	34.28	171.29	284.61	649.16	1831.29	43.25	186.15	685.57	1003.96	1289.78	458.55
N ₁₅₀	PO	7.44	26.39	156.75	249.77	619.04	1816.47	40.39	139.33	601.54	932.71	1313.83	421.83
	P ₅₀	7.70	29.77	162.62	257.95	698.40	1918.14	41.05	150.93	619.99	934.87	1527.02	389.01
	P ₁₅₀	7.87	33.60	184.91	301.61	705.52	1885.82	43.44	174.46	698.03	1105.95	1615.70	548.86
N ₃₀₀	PO	7.76	25.64	164.97	255.75	656.70	1893.23	40.49	133.59	644.88	982.24	1440.49	575.65
	P ₅₀	7.77	28.02	177.91	291.52	686.84	2143.25	42.62	146.77	669.86	1059.73	1516.63	533.10
	P ₁₅₀	7.70	33.48	184.52	317.93	719.22	2024.00	42.24	174.72	698.51	1146.11	1599.48	529.17
Overall Mean		7.50	28.83	158.12	260.94	628.17	1808.71	41.00	151.83	607.70	950.38	1349.61	431.84
F (N)		5.63**	1.74	8.30**	7.48**	4.51**	5.68**	4.08*	1.31	9.12**	7.30**	7.05**	14.45**
F (P)		1.28	15.75**	6.26**	4.68*	1.31	2.47	4.95*	27.59**	5.22*	2.86+	1.06	1.88
F (NxP)		0.34	0.15	0.31	0.67	0.10	1.81	0.41	0.33	0.23	0.24	0.31	2.11
C. V. %		8.55	17.23	18.53	17.92	19.67	18.47	8.52	15.36	17.58	18.90	22.85	35.46
S _x		0.28	0.93	11.46	18.70	55.90	137.95	1.49	8.13	42.38	75.63	133.50	50.23
S _d		0.40	2.72	16.20	26.45	79.05	195.12	2.11	13.50	59.93	106.96	152.80	71.04
LSD _{0.05}		0.81	5.54	32.98	53.85	160.49	327.22	4.30	27.49	122.02	217.77	384.40	144.64
LSD _{0.01}		1.09	7.44	44.32	72.37	216.31	533.79	5.77	36.94	163.97	292.64	516.56	194.37

**, * Significant at 1% and 5% levels of probability, respectively.

Appendix Table 7. (Continued)

NP treatments		Leaf area index (LAI)						Mean crop growth rate (CGR)				
		Unifoliate stage	Trifoliate stage	Floral bud	Flowering	Seed dev.	Maturation	Trifoliate stage	Floral bud	Flowering	Seed dev.	Maturation
kg/ha		----- g/m ² /day -----										
N ₀	PO	0.10	0.37	1.38	2.09	2.87	0.50	1.45	6.09	11.33	19.08	37.20
	P ₅₀	0.11	0.40	1.31	2.30	3.22	1.10	1.59	5.83	15.62	22.85	62.54
	P ₁₅₀	0.12	0.48	1.64	2.38	3.13	0.82	1.91	7.85	10.30	23.66	64.96
N ₅₀	PO	0.12	0.38	1.66	2.70	3.82	1.14	1.46	7.34	17.75	23.59	66.15
	P ₅₀	0.12	0.44	1.70	2.70	3.89	1.40	1.83	7.88	13.24	24.84	50.64
	P ₁₅₀	0.13	0.53	1.97	2.88	3.70	1.32	2.21	8.57	16.19	26.04	63.53
N ₁₅₀	PO	0.12	0.40	1.73	2.69	3.77	1.21	1.58	8.06	13.29	26.38	63.02
	P ₅₀	0.12	0.44	1.78	2.68	4.38	1.12	1.84	8.31	13.62	27.12	67.40
	P ₁₅₀	0.13	0.50	2.00	3.18	4.64	1.66	2.15	9.46	16.67	28.85	62.12
N ₃₀₀	PO	0.12	0.38	1.85	2.82	4.14	1.65	1.49	8.71	12.97	28.64	64.67
	P ₅₀	0.12	0.42	1.92	3.04	4.35	1.53	1.69	9.37	16.23	29.45	76.66
	P ₁₅₀	0.12	0.50	2.01	3.29	4.59	1.52	2.15	9.44	19.06	28.67	68.67
Overall Mean		0.12	0.44	1.75	2.73	3.87	1.25	1.78	8.07	14.69	25.76	62.30
F (N)		2.86	1.39	9.10**	7.30**	7.07**	14.22**	0.92	7.49**	1.30	2.57*	2.06
F (P)		4.58*	28.40**	5.19**	2.83+	1.07	2.13	13.70**	3.62*	0.47	0.57	1.04
F (N×P)		0.48	0.31	0.23	0.25	0.31	2.37	0.19	0.34	1.22	0.10	1.62
C. V. %		9.04	15.25	17.56	18.93	22.83	35.91	22.99	21.39	34.18	26.69	27.22
S _x ²		0.005	0.002	0.12	0.22	0.38	0.15	0.17	0.70	0.79	3.36	7.71
S _d ²		0.007	0.03	0.17	0.31	0.54	0.21	0.23	0.99	1.22	4.75	10.90
LSD _{0.05}		0.01	0.06	0.35	0.63	1.10	0.43	0.47	2.02	2.28	9.67	22.19
LSD _{0.01}		0.02	0.08	0.47	0.85	1.48	0.57	0.63	2.71	3.06	13.00	29.82

**, *, + Significant at 1%, 5% and 10% level of probability, respectively.

Appendix Table 7. (Continued)

NP treatments		Chlorophyll content			Water sol. carbo- hydrate (WSC)	Seed yield
		a	b	a+b		
kg/ha		mg Chl. a/g FW	mg Chl. b/g FW	mg Chl. a+b/g FW	%	kg/ha
N ₀	PO	0.24	0.15	0.39	1.47	2,219
	P ₅₀	0.29	0.19	0.48	1.73	2,421
	P ₁₅₀	0.24	0.16	0.40	2.29	2,324
N ₅₀	PO	0.29	0.21	0.50	2.60	2,612
	P ₅₀	0.30	0.22	0.52	2.92	2,612
	P ₁₅₀	0.26	0.18	0.44	2.47	2,453
N ₁₅₀	PO	0.34	0.22	0.56	3.19	2,641
	P ₅₀	0.30	0.22	0.52	2.92	2,577
	P ₁₅₀	0.33	0.22	0.55	3.35	2,729
N ₃₀₀	PO	0.41	0.26	0.67	2.84	2,649
	P ₅₀	0.46	0.29	0.75	2.90	2,728
	P ₁₅₀	0.37	0.23	0.60	3.37	2,678
Overall Mean		0.32	0.21	0.53	2.67	2,554
F (N)		8.09**	8.93**	8.66**	12.06**	12.29**
F (P)		0.88	2.43	1.40	1.43	0.47
F (N×P)		0.52	0.51	0.51	0.86	1.17
C. V. %		34.69	28.62	31.62	29.49	8.46
S _{x̄}		0.04	0.02	0.06	0.30	80.81
S _d		0.06	0.03	0.09	0.42	114.29
LSD _{0.05}		0.12	0.06	0.18	0.86	232.69
LSD _{0.01}		0.16	0.08	0.25	1.15	312.70

**, *, + Significant at the 1%, 5% and 10% level of probability, respectively.

Appendix Table 8. Simple correlation coefficients (r) between yield, growth analysis attributes, chlorophyll a+b content and above ground plant-water soluble carbohydrate (except seeds) of snap bean (*Phaseolus vulgaris* L.).

		Dry matter (DM)					Leaf area (LA)					Leaf area index (LAI)					Mean crop growth rate (CGR)								Chloro- phyll a+b	Plant water-sol carbo- hydrate (WSC %)
		Seed yield	Uni- foliate stage	Tri- foliate	Floral bud	Flower- ing	Seed dev.	Ma- tura- tion	Uni- foliate stage	Tri- foliate	Floral bud	Flower- ing	Seed dev.	Ma- tura- tion	Uni- foliate stage	Tri- foliate	Floral bud	Flower- ing	Seed dev.	Ma- tura- tion	Tri- foliate	Floral bud	Flower- ing	Seed dev.		
Dry matter (DM)	Unifoliate stage	0.48**																								
	Trifoliate	-0.01	0.16																							
	Floral bud	0.41**	0.49**	0.40**																						
	Flowering	0.48**	0.30*	0.43**	0.66**																					
	Seed dev.	0.42**	0.22	0.21	0.68**	0.58**																				
	maturation	0.36*	0.16	0.27	0.31*	0.46**	0.20																			
Leaf area (LA)	Unifoliate stage	0.42**	0.89**	0.32*	0.48**	0.35*	0.17	0.21																		
	Trifoliate	-0.03	0.17	0.94**	0.44**	0.45**	0.26	0.20	0.35*																	
	Floral bud	0.39**	0.44**	0.37**	0.95**	0.64**	0.66**	0.37**	0.40**	0.39**																
	Flowering	0.52**	0.32*	0.37**	0.71**	0.95**	0.66**	0.43**	0.33*	0.38**	0.72**															
	Seed dev.	0.58**	0.26	0.13	0.60**	0.51**	0.91**	0.24	0.18	0.15	0.62**	0.60**														
	maturation	0.63**	0.39**	0.16	0.28	0.29*	0.15	0.50**	0.38**	0.11ns	0.34*	0.30*	0.37**													
Leaf area index (LAI)	Unifoliate stage	0.35*	0.84**	0.31*	0.49**	0.32*	0.19	0.18	0.96**	0.34*	0.41**	0.29*	0.18	0.33*												
	Trifoliate	-0.02	0.18	0.93**	0.44**	0.46**	0.26	0.21	0.35*	0.99**	0.39**	0.38**	0.15	0.10	0.35*											
	Floral bud	0.39**	0.44**	0.38**	0.95**	0.64**	0.66**	0.37**	0.40**	0.40**	0.99**	0.72**	0.62**	0.33*	0.41**	0.39**										
	Flowering	0.52**	0.32*	0.38**	0.71**	0.95**	0.66**	0.43**	0.33**	0.38**	0.72**	0.99**	0.60**	0.30*	0.29*	0.38**	0.72**									
	Seed dev.	0.58**	0.26	0.13	0.61**	0.51**	0.91**	0.24	0.18	0.15	0.62**	0.60**	1.00**	0.31**	0.18	0.16	0.62**	0.60**								
	maturation	0.64**	0.41**	0.15	0.30*	0.28ns	0.16	0.47**	0.39**	0.09	0.34*	0.29*	0.38**	0.99**	0.34*	0.09	0.34*	0.29*	0.38**							
Mean crop growth rate	Trifoliate	0.07	0.03	0.99**	0.35*	0.40**	0.18	0.25	0.20	0.93**	0.32*	0.34*	0.10	0.11	0.21	0.93**	0.32*	0.33*	0.10	0.10						
	Floral bud	0.44**	0.49**	0.25	0.99**	0.63**	0.68**	0.28	0.45**	0.30*	0.94**	0.69**	0.62**	0.27	0.46**	0.30*	0.94**	0.69**	0.62**	0.29*	0.19					
	Flowering	0.29*	0.01	0.24	0.05	0.78**	0.21	0.35*	0.06	0.23	0.06	0.67**	0.18	0.10	0.02	0.24	0.06	0.67**	0.18	0.12	0.25	0.01				
	Seed dev.	0.38**	0.22	0.09	0.58**	0.35**	0.92**	0.14	0.16	0.15	0.56**	0.46**	0.85**	0.17	0.18	0.15	0.57**	0.46**	0.84**	0.18	0.06	0.59**	-0.02			
	maturation	0.16	0.06	0.20	0.08	0.24	-0.13	0.93**	0.12	0.11	0.16	0.19	-0.06	0.41**	0.08	0.13	0.16	0.19	0.06	0.37*	0.19	0.05	0.26	-0.18		
Chlorophyll a+b	0.27	0.04	0.09	0.16	0.11	0.04	0.39**	0.04	0.03	0.17	0.09	0.18	0.51**	0.01	0.02	0.17	0.09	0.18	0.49**	0.08	0.16	0.01	0.03	0.36*		
Plant-water soluble carbohydrate (WSC %)	0.52**	0.38**	0.27	0.50**	0.52**	0.59*	0.31*	0.36*	0.28	0.48**	0.58**	0.63**	0.25	0.33*	0.29*	0.48**	0.58**	0.63**	0.26	0.23	0.47**	0.28	0.51**	0.12	0.12	

**, * Significant at the 1% and 5% level of probability, respectively.

Appendix Table 9. Effect of N and P fertilizers on the mineral content of the snap bean (*Phaseolus vulgaris* L.) leaves at the unifoliate stage of growth (15 days after emergence).

NP treatments		N	K	P	Ca	Mg	Mn	Fe	Ca	B	Zn	Al
kg/ha		----- % -----						----- ppm -----				
N ₀	PO	5.05	1.78	0.47	1.53	0.62	46.00	693.50	10.50	20.75	45.00	658.00
	P ₅₀	5.08	1.71	0.46	1.62	0.58	38.50	581.75	10.75	17.25	41.25	556.25
	P ₁₅₀	5.04	1.92	0.52	1.56	0.56	45.75	568.00	9.50	17.75	43.00	794.25
N ₅₀	PO	5.52	1.68	0.38	1.44	0.62	39.75	709.25	8.25	16.50	38.75	687.75
	P ₅₀	6.14	1.85	0.45	1.90	0.70	40.25	567.25	8.75	17.50	47.00	546.50
	P ₁₅₀	5.77	2.00	0.43	1.86	0.66	40.75	645.00	8.00	15.25	42.75	661.25
N ₁₅₀	PO	6.01	1.91	0.32	1.72	0.57	37.25	482.00	5.75	13.50	39.25	521.00
	P ₅₀	5.98	2.12	0.40	1.72	0.63	44.75	704.00	7.25	15.50	43.75	719.25
	P ₁₅₀	6.05	2.02	0.48	1.81	0.67	48.00	744.50	7.00	16.00	47.25	752.25
N ₃₀₀	PO	5.86	1.86	0.36	1.67	0.60	46.75	710.00	7.25	15.00	45.25	744.00
	P ₅₀	6.07	1.94	0.38	1.84	0.60	41.25	634.00	8.00	14.00	43.75	661.00
	P ₁₅₀	5.85	1.97	0.45	1.73	0.66	48.00	685.00	7.75	17.00	43.75	714.25
Overall mean		5.70	1.90	0.42	1.70	0.62	43.08	643.69	8.23	16.33	43.40	667.98
F (N)		22.52**	1.22	7.49**	3.03*	1.26	2.07	0.29	6.39**	6.27**	0.23	0.40
F (P)		1.72	1.32	12.33**	5.02**	0.70	3.26	0.24	0.60	0.18	1.06	1.83
F (N×P)		0.90	0.34	1.75	1.72	0.69	2.12	1.50	0.23	2.32	2.10	1.18
C. V. %		8.58	15.07	18.54	12.09	18.34	17.82	31.88	40.98	19.66	12.77	31.82
S _{x̄}		0.16	0.15	0.03	0.09	0.05	0.25	81.16	1.04	1.12	0.71	0.70
S _d		0.23	0.21	0.04	0.12	0.06	0.36	114.77	1.46	1.58	1.01	1.18
LSD _{0.05}		0.47	0.43	0.08	0.24	0.12	0.73	233.67	2.97	3.22	2.06	2.40
LSD _{0.01}		0.63	0.57	0.11	0.33	0.16	0.98	314.01	3.99	4.32	2.76	3.23

**, * Significant at the 1% and the 5% level of probability, respectively.

Appendix Table 10. Effect of N and P fertilizers on the mineral content of snap bean (*Phaseolus vulgaris* L.) leaves at the first fully developed trifoliate stage of growth (27 days after emergence).

NP treatments		N	K	P	Ca	Mg	Mn	Fe	Cu	B	Zn	Al
kg/ha		----- % -----					----- ppm -----					
N ₀	PO	4.32	3.62	0.32	2.68	0.68	55.25	434.25	5.00	22.25	37.25	498.00
	P ₅₀	4.14	3.03	0.34	3.00	0.70	56.75	441.25	5.00	24.00	40.75	453.00
	P ₁₅₀	4.02	3.04	0.33	3.02	0.73	63.50	506.25	4.25	23.25	37.25	498.25
N ₅₀	PO	4.38	2.83	0.31	2.97	0.70	67.25	596.50	5.25	23.25	43.75	552.50
	P ₅₀	4.36	2.88	0.32	3.12	0.72	58.25	563.75	5.50	21.75	44.00	587.75
	P ₁₅₀	4.43	3.00	0.35	3.22	0.78	60.50	425.50	4.75	23.50	44.25	366.50
N ₁₅₀	PO	4.58	3.09	0.30	3.04	0.69	61.75	523.00	5.00	22.00	41.75	578.00
	P ₅₀	4.54	3.35	0.34	3.11	0.65	66.00	545.00	5.00	22.25	44.75	576.00
	P ₁₅₀	4.46	3.29	0.30	2.69	0.69	61.75	575.75	3.00	18.25	40.25	605.25
N ₃₀₀	PO	4.55	3.30	0.28	2.90	0.59	63.75	460.25	4.50	20.25	41.75	538.00
	P ₅₀	4.45	3.07	0.32	3.03	0.69	63.25	529.50	5.50	23.25	44.00	512.50
	P ₁₅₀	4.55	2.77	0.28	2.82	0.69	57.25	446.50	6.00	18.50	38.00	432.25
Overall mean		4.40	3.10	0.32	2.96	0.69	61.19	501.71	4.90	21.88	41.48	516.58
F (N)		14.6**	1.42	1.82	0.74	1.98	0.59	1.04	0.33	4.18**	2.24	1.24
F (P)		18.1	0.64	1.82	0.91	1.85	0.01	0.24	0.31	3.07	1.67	0.93
F (N×P)		1.20	1.04	0.67	0.83	0.61	0.95	0.73	0.31	2.36	0.29	0.80
C. V. %		4.89	15.06	12.99	18.79	12.52	15.83	30.35	50.43	12.35	14.02	32.44
S _e		0.08	0.24	0.02	0.18	0.04	0.45	66.88	1.35	1.10	2.70	73.79
S _d		0.11	0.33	0.03	0.26	0.06	0.63	94.59	1.91	1.57	3.82	104.35
LSD _{0.05}		0.22	0.67	0.06	0.53	0.12	1.28	192.58	3.89	3.20	7.78	212.46
LSD _{0.01}		0.30	0.90	0.08	0.71	0.16	1.72	258.80	5.23	4.30	10.45	285.50

**, * Significant at the 1% and 5% level of probability, respectively.

Appendix Table 11. Effect of N and P fertilizers on the mineral content of snap bean (*Phaseolus vulgaris* L.) leaves at the floral bud stage of growth (45 days after emergence).

NP treatments		N	K	P	Ca	Mg	Mn	Fe	Cu	B	Zn	Al
kg/ha		%						ppm				
N ₀	PO	3.76	2.14	0.37	2.37	0.74	74.00	598.75	8.50	26.00	39.25	521.75
	P ₅₀	4.03	2.48	0.31	2.40	0.65	63.75	407.00	6.25	23.00	37.25	368.00
	P ₁₅₀	3.96	2.39	0.33	2.54	0.66	77.50	745.00	6.25	22.75	36.50	742.75
N ₅₀	PO	4.08	2.83	0.30	2.57	0.58	52.00	315.00	5.75	19.00	33.00	366.50
	P ₅₀	4.19	2.57	0.29	2.55	0.60	59.25	479.25	7.75	18.75	36.75	516.50
	P ₁₅₀	3.95	2.40	0.30	2.68	0.65	59.50	346.75	5.00	19.00	35.50	308.25
N ₁₅₀	PO	4.27	2.51	0.33	2.80	0.57	61.75	428.75	7.25	21.25	42.50	409.50
	P ₅₀	4.42	2.43	0.34	2.76	0.68	77.00	534.25	7.00	21.50	40.75	508.25
	P ₁₅₀	4.37	2.65	0.33	2.98	0.69	83.75	590.75	6.50	21.75	42.00	627.50
N ₃₀₀	PO	4.33	2.38	0.33	2.76	0.68	74.50	472.50	7.75	21.00	43.75	437.50
	P ₅₀	4.34	2.48	0.33	2.88	0.69	65.00	349.75	7.50	21.00	43.00	316.00
	P ₁₅₀	4.44	2.46	0.33	2.78	0.73	80.25	429.75	6.00	21.75	48.25	395.75
Overall mean		4.18	2.48	0.32	2.67	0.67	69.02	474.79	6.79	21.40	39.88	459.85
FN		9.29**	1.04	1.48	4.07**	1.46	5.51**	1.57	0.66	5.32**	0.62	0.96
FP		1.17	0.02	0.23	0.61	0.23	3.25	0.52	0.96	0.25	0.54	0.50
F N×P		0.55	0.81	0.32	0.18	0.35	1.28	0.78	0.97	0.42	0.96	0.84
CV %		7.41	15.17	15.86	13.34	16.28	21.11	29.23	18.28	15.51	19.29	66.65
S _x		0.13	0.19	0.03	0.16	0.06	6.00	128.35	8.38	1.53	13.93	143.94
S _d		0.18	0.27	0.04	0.23	0.08	8.49	181.51	11.85	2.17	19.70	203.56
LSD _{0.05}		0.37	0.55	0.08	0.47	0.16	17.29	369.55	24.13	4.42	40.11	414.48
LSD _{0.01}		0.49	0.74	0.11	0.63	0.22	23.23	496.61	32.42	5.94	53.90	556.94

**, * Significant at the 1% and 5% level of probability, respectively.

Appendix Table 12. Effect of N and P fertilizers on the mineral content of snap bean (*Phaseolus vulgaris* L.) leaves at the flowering stage of growth (57 days after emergence).

NP treatments		N	K	P	Ca	Mg	Mn	Fe	Cu	B	Zn	Al
kg/ha		%					ppm					
N ₀	PO	4.13	1.62	0.28	2.00	0.66	53.75	197.00	8.00	27.00	31.75	121.25
	P ₅₀	4.02	1.37	0.30	2.47	0.82	78.00	243.25	9.25	27.75	33.50	108.00
	P ₁₅₀	3.93	1.59	0.29	2.23	0.70	81.50	304.50	7.75	25.75	37.00	129.50
N ₅₀	PO	4.38	1.81	0.30	2.39	0.71	82.25	220.00	8.50	25.50	39.00	142.50
	P ₅₀	4.26	1.64	0.28	2.52	0.72	57.50	244.50	7.25	26.00	53.25	153.25
	P ₁₅₀	4.15	1.67	0.28	2.56	0.71	65.00	213.25	7.50	26.25	35.25	115.25
N ₁₅₀	PO	4.37	1.91	0.27	2.36	0.71	62.25	197.25	7.75	24.50	51.25	119.75
	P ₅₀	4.45	1.91	0.31	2.36	0.68	67.25	190.50	8.00	25.75	37.75	105.75
	P ₁₅₀	4.45	1.84	0.29	2.29	0.73	77.00	203.50	8.25	25.50	35.50	122.25
N ₃₀₀	PO	4.38	1.73	0.30	2.41	0.72	68.75	233.00	10.25	26.25	40.00	142.00
	P ₅₀	4.44	1.66	0.29	2.54	0.84	65.25	228.50	9.75	26.00	38.25	136.75
	P ₁₅₀	4.47	2.02	0.31	2.34	0.78	63.75	206.50	8.50	25.75	37.00	132.00
Overall mean		4.28	1.73	0.29	2.37	0.73	66.85	223.48	8.40	26.00	39.13	127.35
FN		13.04**	4.00**	0.22	1.36	1.50	1.67	2.41	2.65	0.49	0.21	0.58
FP		0.57	1.17	0.09	1.27	1.70	3.35*	0.80	0.70	0.16	0.21	0.49
F N×P		0.95	0.80	0.44	0.48	0.97	2.25	1.82	0.73	0.16	1.32	1.23
CV %		5.63	17.88	13.70	17.90	15.39	18.78	24.89	22.62	11.16	34.61	23.89
S _{x̄}		0.09	0.13	0.02	0.17	0.05	5.50	23.39	0.80	1.60	14.04	31.72
S _t		0.13	0.19	0.03	0.24	0.07	7.78	33.09	1.16	2.26	19.86	44.86
LSD _{0.05}		0.26	0.39	0.06	0.49	0.14	15.84	67.37	2.36	4.60	40.43	91.33
LSD _{0.01}		0.36	0.52	0.08	0.66	0.19	21.29	90.53	3.17	6.18	54.34	122.74

**, * Significant at the 1% and 5% level of probability, respectively.

Appendix Table 13. Effect of N and P fertilizers on the mineral content of snap bean (*Phaseolus vulgaris* L.) leaves at the seed development stage of growth (71 days after emergence).

NP treatments		N	K	P	Ca	Mg	Mn	FE	Cu	B	Zn	Al
kg/ha		----- % -----						----- ppm -----				
N ₀	PO	3.41	1.04	0.27	3.12	0.88	58.75	194.75	8.50	29.50	36.75	101.25
	P ₅₀	3.33	1.14	0.25	3.10	0.82	55.75	193.75	5.75	26.75	34.25	116.00
	P ₁₅₀	3.10	1.02	0.24	3.23	0.87	69.50	250.75	7.00	26.50	35.50	158.50
N ₅₀	PO	3.57	1.32	0.25	3.84	0.90	60.00	178.00	6.25	26.00	38.00	111.25
	P ₅₀	3.50	1.25	0.26	3.88	0.90	54.75	208.25	7.00	27.75	34.00	149.75
	P ₁₅₀	3.58	1.28	0.23	3.98	0.74	55.25	147.00	3.75	24.25	34.25	93.75
N ₁₅₀	PO	3.76	1.53	0.20	4.16	0.76	52.00	146.25	4.50	21.00	34.25	125.50
	P ₅₀	3.77	1.46	0.23	3.83	0.82	59.25	149.00	4.50	23.00	33.75	96.25
	P ₁₅₀	3.80	1.45	0.25	3.31	0.88	69.00	138.50	4.00	23.25	33.25	78.25
N ₃₀₀	PO	3.80	1.51	0.24	3.50	0.81	76.75	170.00	6.00	23.00	40.50	118.75
	P ₅₀	3.98	1.54	0.22	3.22	0.79	60.25	170.50	5.00	23.75	34.25	129.75
	P ₁₅₀	3.86	1.24	0.26	3.39	0.94	71.25	241.50	8.75	25.50	40.25	152.75
FN		26.59**	9.13**	1.07	2.47	0.15	2.19	3.66**	3.89**	3.99**	0.88	1.52
FP		0.50	1.21	0.07	0.24	0.13	1.92	0.76	0.51	0.07	1.47	0.20
F N×P		1.39	0.63	1.12	0.40	1.03	1.06	1.56	2.4	0.77	0.81	2.04
C. V. %		9.36	20.75	16.57	25.23	19.68	21.96	22.07	41.96	16.27	32.19	38.27
S _x		0.09	0.11	0.02	0.38	0.08	6.32	8.27	1.06	1.95	9.57	20.16
S _d		0.13	0.15	0.03	0.54	0.11	8.94	11.69	1.50	2.76	13.54	28.52
LSD		0.26	0.31	0.06	1.10	0.22	18.20	23.80	3.05	5.62	27.57	58.07
LSD ^{0.05}		0.36	0.41	0.08	1.48	0.30	24.46	31.98	4.10	7.55	37.05	78.03
LSD ^{0.01}												

**, * Significant at the 1% and 5% level of probability, respectively.

Appendix Table 14. Effect of N and P fertilizers on the mineral content of snap bean (*Phaseolus vulgaris* L.) leaves in the maturation stage of growth (83 days after emergence).

NP treatments		N	K	P	Ca	Mg	Mn	Fe	Cu	B	Zn	Al
kg/ha		%					ppm					
N ₀	PO	2.60	0.71	0.21	4.20	0.85	47.50	134.75	5.00	33.50	27.75	103.75
	P ₅₀	2.88	1.01	0.19	4.24	0.78	53.75	142.25	6.25	28.75	25.50	129.25
	P ₁₅₀	2.88	1.06	0.17	4.22	0.75	49.00	146.25	4.75	28.50	23.25	135.00
N ₅₀	PO	3.01	0.94	0.22	4.59	1.02	71.75	149.75	6.75	31.25	35.00	116.75
	P ₅₀	2.85	0.90	0.19	5.06	0.95	53.75	123.75	4.75	26.75	29.00	105.00
	P ₁₅₀	2.84	0.87	0.19	5.19	0.92	61.00	148.50	5.25	29.25	30.25	122.50
N ₁₅₀	PO	3.16	0.97	0.18	5.56	0.95	66.75	150.75	6.75	22.00	34.50	120.00
	P ₅₀	2.85	0.94	0.17	5.73	0.88	68.00	135.25	4.75	21.50	30.25	121.25
	P ₅₀	3.23	1.15	0.19	5.69	0.99	71.00	133.50	5.00	23.00	37.50	85.50
N ₃₀₀	PO	3.08	1.18	0.18	5.68	0.94	85.75	122.50	4.50	20.50	33.75	105.00
	P ₅₀	2.99	1.01	0.19	6.39	1.07	76.25	150.50	5.50	23.50	38.50	114.75
	P ₁₅₀	3.20	1.15	0.19	5.72	1.03	88.75	154.50	4.25	23.50	33.50	118.25
Overall mean		2.96	0.99	0.19	5.19	0.93	65.10	141.04	5.29	26.00	31.56	114.67
FN		8.24**	3.30*	0.57	9.07**	5.29**	2.79**	0.01	0.49	15.73**	6.38**	0.47
FP		2.65	1.68	0.50	0.63	0.11	0.51	0.19	1.03	0.83	0.49	0.17
F NxP		2.74	1.74	0.62	0.25	0.17	0.61	0.54	0.96	1.32	1.20	1.18
CV %		7.98	21.26	19.11	21.33	16.30	29.13	23.99	34.42	19.95	21.66	27.32
S _{x̄}		0.09	0.09	0.02	0.44	0.07	7.72	18.70	0.93	1.86	2.38	15.02
S _d		0.13	0.13	0.03	0.63	0.09	10.92	26.45	1.31	2.63	4.22	21.24
LSD _{0.05}		0.26	0.26	0.06	1.28	0.18	22.23	53.85	2.67	5.35	8.59	43.24
LSD _{0.01}		0.36	0.36	0.08	1.72	0.25	29.88	72.37	3.58	7.20	11.55	58.11

**, * Significant at the 1% and 5% level of probability, respectively.