THE RELATION BETWEEN MODE OF LEGUME NITROGEN NUTRITION, YIELD DETERMINANTS AND N ASSIMILATION EFFICIENCY

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To my parents

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ABSTRACT

Results of the effect of nitrogen (N) on legume dry matter (DM) and N accumulation are mixed. This study measured whole plant DM and N accumulation by legumes grown at different levels of soil N. Two field experiments tested whether reducing N_2 fixation by increasing soil mineral N results in: greater DM accumulation due to increased allocation of DM to leaves; increased N assimilation due to greater N uptake efficiency; and similarity between three species of plant response to N. CROPGRO V3.0 predictions of legume growth under different levels of soil N were also tested with observed data.

In 1991 soybeans [*Glycine max* Merr. (L.) cv. Clark] were grown with 0 (N0), 256 (N1) and 430 (N2) kg applied N ha^{-1}. Leaf area and weight and N concentration of leaves, stems, and pods were determined at 10, 22, 30, 39 and 51 days after emergence (DAE). At 22 and 51 DAE root and nodule weight and root length were also measured. In 1993 soybean, peanut (*Arachis hypogaea* cv. Pronto) and common bean (*Phaseolus vulgaris* cv. Porillo Sintetico) were grown with either 0 (N0) or 275 (375 for peanut) (N1) kg applied N ha^{-1}. Leaf area and weight and N concentration of above and below ground components were measured at 10, 23 and 44 DAE. Shoot and seed weight were measured at harvest maturity.

On average nitrogen reduced N_Z fixation to, approximately 20% of N0 plants. Nitrogen application resulted in greater grain yield and DM accumulation but harvest index was unaffected. With N application there was an average increase in above ground DM and leaf area index of 23 and 20%, respectively. This was due primarily to greater allocation of DM to leaves in N1 than N0 during early vegetative growth. Nitrogen assimilation was

17% more in N1 than N0 plants due to increased root weight (17%) and root surface area (27%) and greater N assimilated per unit below ground in N1 plants compared to N0. CROPGRO simulations of total N also increased with application of N. Simulated above ground DM, however, was either unresponsive or lower with N Application. This indicates a need for further development of CROPGRO's simulation of DM accumulation with an increase in available soil N.

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DISSERTATION INRODUCTION

BACKGROUND

Legumes are a major source of dietary protein, especially in developing countries, where direct consumption of vegetable protein accounts for 75% of total protein consumption (FAO, 1990). These countries produce 92, 47 and 85% of the world's production of peanut (*Arachis hypogaea*), soybean (*Glycine max*) and common bean (*Phaseolus vulgaris*), respectively (FAO, 1990). Legume productivity in developing countries lags behind that of developed countries. Yield of dry beans, soybean and peanut are 577, 1636 and 1119 kg ha^{-1} in developing countries compared to 1020, 2248 and 1885 kg ha^{-1} in developed countries, respectively (FAO, 1990).

For years, researchers and agriculture development specialists alike have tried to improve tropical agriculture productivity with minimal success (Uehara, 1989). Poor results indicate deficiencies in two areas: technology transfer (Uehara, 1989) and knowledge of tropical crop production. This thesis will address both areas by generating additional data on tropical legume growth and using data collected to improve the accuracy of legume simulation models, a new tool in technology transfer (Uehara, 1989).

Crop models can be used to predict the performance of crops in new environments. Computer simulation, as compared to field trials, may save time, money and labor by allowing the user (farmer, management consultant, development planner or agriculture researcher) to assess the impact of changes in management and weather patterns on crop productivity (Dent and Blackie, 1979; Uehara, 1989; and Singh, 1991).

Crop simulation models are a series of linked algorithms, based on current theory, arranged in a defined hierarchical order. Models do not include all the components of a defined system. Only the variables considered critical to meet the aims of the model developer are included (Dent and Blackie, 1979).

Crop models range from single or multiple equations to complex hierarchical structures of several algorithms. Models could be described as being made up of two basic levels. The first level is theoretical. The second level is made up of algorithms linking theoretical concepts. Theory is compiled from basic and applied sciences and observations in the study of plant growth and development such as, the influence of temperature and photoperiod on soybean development (Seddigh et al., 1989).

Disagreement between observed and predicted values of crop growth and development could be a result of an excluded system variable, inaccurate coefficients of physiological processes (Dent and Blackie, 1979; Singh, 1991), incorrect equations or inaccurate observations. Additional theory could improve the models ability to mimic various biological processes in a system resulting in better agreement between observed and predicted values. One of the objectives of this thesis was to generate theory that could be used to improve CROPGRO V3.0 (Jones et al., unpublished), a legume simulation model. CROPGRO simulates growth of three legumes: peanut, soybean and common bean, which account for the majority of legume production.

CROPGRO MODEL

Many decision support crop models are under continuous development and evolution. Testing models (comparing model predictions to an independent data set) determine the level of confidence one can have in model predictions for the crop and site tested. Models for which testing have shown reasonable predictions could be used as a base for development of other models. SOYGRO (a soybean simulation model), for example, was used as a base for the development of BEANGRO (common bean model) and PNUTGRO (peanut model) models (Hoogenboom et al., 1992). These three crop models were incorporated into a single legume model CROPGRO. A major difference between the three species models and CROPGRO is the incorporation of a nitrogen (N) sub-model (Hoogenboom et al., 1993) to simulate N uptake, mobilization and N_2 fixation in CROPGRO.

To simulate growth and yield of particular crop using CROPGRO, one first selects a species, then a cultivar parameter file. Each file contains genetic coefficients for several physiological aspects of crop growth and development (Hoogenboom et al. 1991). Thus, the model does not simulate a generic legume but maintains species and cultivar identities that were used for BEANGRO, PNUTGRO and SOYGRO models. In addition, the user has to input site information on weather, soil type, management (such as irrigation and fertilizer schedule) and initial, soil N availability.

To predict plant growth and development, CROPGRO simulates numerous physiological processes such as gross photosynthesis and N accumulation. Estimated respiration costs of growth and maintenance are subtracted from gross photosynthesis to estimate net photosynthate. The daily net photosynthate is allocated to plant parts according to developmental stage

(Hoogenboom et al, 1992). Nitrogen is partitioned to plant parts based on the growth rate and N concentration of the organ (Jones at al., unpublished). Partitioning of dry matter (DM) and N are dependent on stage of development (Thibodeau and Jaworski, 1975) as well as environmental factors, such as N availability (Tolley-Henry and Raper, 1986a).

Earlier versions of the GRO models excluded N uptake and fixation. Thies (1990) documented that mode of legume nutrition (N uptake versus N_2 fixation) had a differential effect on above ground DM accumulation, seed yield and phenology of Clark soybean at four elevations on the island of Maui, Hawaii. Prior to comparing SOYGRO V5.42 simulations with observed data, Thies adjusted crop and cultivar genetic coefficients so that predicted and observed phenology for emergence and R1 (first flowering) were the same. Under native conditions she estimated soybean N_2 fixation provided 15 to 82.0% of total N. She found that SOYGRO V5.42 biomass predictions had a good fit to observed data but over-predicted seed yield of soybeans at different elevations.

Thies (1990) showed that a decline in N_2 fixation (associated with N application) increases DM and N accumulation and agrees with responses measured by other researchers (Allos and Bartholomew, 1959; George and Singleton, 1992). SOYGRO V5.42 assumes that mineral N availability or N from N_2 fixation does not limit plant growth and yield. Thus, Thies (1990) indicated the need to incorporate a N response sub-model in SOYGRO to account for variations in soil N and its influence on growth and development of legumes. This model should account for both mineral N uptake and N_2 fixation.

In addition to CROPGRO there are two other simulation models which account for the effect of available soil N on N_2 fixation and growth response: a soybean model by T.R. Sinclair (1986) and EPIC (Williams et al., 1984). In Sinclair's soybean model N_2 fixation is a linear function of total vegetative biomass. The level of N_2 fixation is reduced when the soils are dehydrated or flooded. EPIC estimates growth parameters, N_2 fixation and N accumulation based on phenology, soil water and soil N for soybean (Bouniols et al., 1991). The parameters used in the EPIC N sub-model where derived from experiments conducted in France. Bouniols et al. (1991) recommended further development of the N sub-model should be done for different soils, climate and legumes. The N sub-model incorporated into CROPGRO takes all the above factors into account.

Nitrogen demand in the CROPGRO model is based on an estimation of potential growth, N concentration and N required to re-fill N mined from old tissue. Nitrogen for plant growth, in CROPGRO, is derived from three sources: N uptake from the soil, N mobilization from plant organs and N_2 fixation. Nitrogen uptake is dependent on soil-solution nitrate and ammonium, soil water content and root length density in each soil layer in which roots are present. Nitrogen mobilization is based on a fraction of mobilizable protein in each organ, except seeds. The N demand that is not met by uptake and mobilization is obtained from N_Z fixation. Dinitrogen fixation is dependent on available carbon and growth of nodules, both are influenced by soil water and soil temperature (Hoogenboom et al., 1990a).

The response of CROPGRO to changes in soil N was tested with data from NifTAL by Hoogenboom et al. (1991). Soybean was grown at two elevations and three N levels on the island of Maui, Hawaii. Improvements in the CROPGRO model, such as, incorporation of a N sub-model, resulted in a better fit of model simulation to field data. However, some discrepancies remained in the following areas of above ground biomass and nodule weight (Hoogenboom et al., 1991), leaf area index (LAI) and leaf weight and response to changes in soil N. This thesis is part of the development of crop models to handle nutrient stress with a focus on N. The following is a summary of the problems identified in preliminary versions of CROPGRO:

1. Predication of legume growth and development under different levels of soil N.
2. Partitioning of DN and N within legume species (peanut, soybean and common bean) in response to soil N.
3. Biomass and N accumulation over different levels of applied N.
4. Estimation of leaf area index.

LEGUME RESPONSE TO NITROGEN SOURCE

The effect of applied N on legume yield is a controversial area. A review of the literature indicates mixed results: no effect on soybean (Schweitzer and Harper, 1985; Buttery, 1986) and peanut (Selamat and Gardner, 1985); negative response by soybean (Harper, 1974; Imsande, 1988), peanut (Thies et al, 1991a); and grain yield increase by soybeans (Lathwell and Evans, 1951; Bhangoo and Albritton, 1976; George, 1988; Wallace et al., 1990; Thies et al, 1991a), peanut (Ball et al. 1983), and common bean (George, 1988; Thies et al, 1991a). A decrease or no change in yield in response to fertilizer N could be explained by low P availability (Cassman et al., 1980), high soil N availability (Stone et al., 1985) and species differences in mineral N assimilation (George and Singleton, 1992). When N is supplied in sufficient quantity to decrease N_2 fixation, growth is promoted in soybean and common bean (George and Singleton, 1992), and in lima bean, cowpea, leucaena, alfalfa and clover (Thies et al., 1991a).

Grain yield of legumes is proportional to DM and N accumulation (Thies et al., 1991a; Muchow et al., 1993). During pod fill 70% of N required for seed development in legumes is derived from remobilization (Pate, 1985). The pool of N within the plant available for remobilization and seed development is dependent on the rate of N assimilation during vegetative and early pod development.

Most research on legume growth and development has concentrated on the reproductive phase. Yet, yield and biomass accumulation in the reproductive phases are influenced by early growth and development. Assimilation of N is dependent on the growth of root and nodule tissues during both vegetative and reproductive stages. Their growth and function is proportional to the quantity of assimilates partitioned to them (Brown, 1984). Under equal conditions greater DM allocation above ground results in higher yield potential. When N is the slain factor limiting growth, the relative amount of photoassimilate partitioned below ground increases (Tolley-Henry and Raper, 1986a).

Available soil N and plant N demand will determine the quantity of total plant N derived from N_2 fixation. The level of N_2 fixation influences the allocation of photosynthate within a legume. In pot and solution culture studies it has been found that suppression of N_2 fixation by N application results in greater allocation of N above ground (Cassamn et al., 1980; George and Singleton, 1992). Thus, in order for a legume crop model to accurately simulate yield in different soils it must correctly predict the following: the level of N_2 fixation in response to plant demand and available soil N, and photosynthate allocation in response to the level of N_2 fixation determined.

GENETICS, GROWTH STAGE AND ENVIRONMENTAL INFLUENCES ON CROP NITROGEN AND DRY MATTER PARTITIONING

Dry matter and N partitioning are interrelated. When N limits growth, more N is partitioned to the roots (Vessey and Layzell, 1987) and less N is available for protein development in the shoots. Consequently there is less ribulose biphosphate carboxylase in the canopy. Photosynthesis rate and leaf expansion decline, and there is less photoassimilation (Tolley-Henry and Raper, 1986b; Greenwood et al., 1991). Dry matter and N partitioning among plant tissues are affected by plant genotype, growth stage and environmental factors.

Genetics

Muchow et al. (1993) found that the fraction of above ground vegetative biomass and N partitioned to leaves varied little across three legume species (soybean, mungbean and cowpea) and environments (water was the main environmental variable). However, calculations from a greenhouse study reported by Allos and Bartholomew (1959) indicate that partitioning of DM and N between shoot and roots in response to applied N differed between legume species. These two studies, Allos and Bartholomew (1959) and Muchow et al. (1993), indicate that partitioning of assimilate depends on the environment (Minchin et al, 1981), species being compared and whether roots have been included in the analysis.

Differences in partitioning of DM by legume species could be related to the efficiency of carbon use for N_2 fixation (Layzell et al, 1979; Pate, 1985), and plant morphology characteristics such as root mass (Minchin et al, 1981). The variation in N partitioning among species could be due to genetically determined differences in N concentration at a point in time and over time. Nitrogen concentration in seeds of common bean and soybean are approximately 0.04 and 0.05 g N g seed^{-1}, respectively. During podfill, N concentration of common bean varies (Dubois and Burris, 1986) while Ohyama (1983) found that it is stable for soybean. Inherent differences in partitioning of assimilates and the efficiency of their use have been used by plant breeders to create higher economic yielding crop varieties (Gifford et al., 1984).

Legume cultivars display different N partitioning patterns. Selamat and Gardner (1985) demonstrated this in peanut cultivars. Reports in the literature on DM partitioning to different plant parts as a result of genetic differences between legume cultivars vary.

Partitioning of soybean above ground DM in field experiments was the same for cultivars at the same growth stage (Egli et al., 1985; Hintz and Albrecht, 1994) and the same under different environment conditions (Egli et al., 1985). Kerr et al. (1984), in a solution culture study, found that soybean DM partitioning was influenced by cultivar. Westerman et al. (1985), however, under green house conditions, found partitioning of DM and N to be similar for two common bean cultivars.

Growth stage

The distribution of DM and N among plant tissue is dependent on the stage of development. Early in the plant life cycle vegetative tissues (primarily leaves) are the principal sinks; as plants progress from early to mid to late reproductive stages the pods develop into stronger sinks for carbon (Thibodeau and Jaworski, 1975; Scott et al., 1983) and N as seed protein content increases (Pate, 1985).

The rate of N and DM accumulation changes with growth stage. Initially DM accumulation is slow, increases exponentially and declines as the plants start to senesce (Brown, 1984). The period of greatest accumulation of both DM and N in soybean and common bean is between full bloom (R2) and late pod fill (R5) (George and Singleton, 1992).

Environment

The major environmental factors that influence allocation of DM and N within a plant are temperature and N availability (Tolley-Henry and Raper, 1986a). The level of N availability in a soil determines the relative contributions of mineral versus symbiotic sources of N for a legume (Streeter et al., 1988).

Studies have shown that N metabolism varies with different N sources (Yoneyama and Ishizuka, 1982; Ohyama, 1983), but the authors did not state what effects N source would have on seed yield or crop DM production. As stated earlier application of fertilizer N in amounts sufficient to suppress N_2 fixation does increase the yield and biomass of soybeans (Bhangoo and Albritton, 1976; George, 1988; Thies et al., 1991a) and common bean (George, 1988).

Increased partitioning of DM and N to leaves potentially increases plant productivity (Sinciair and Horie, 1989; Muchow et al., 1993). Under conditions of low N availability and inadequate leaf N concentration, roots are a relatively stronger sink for N than above ground plant parts in peanut (Selamat and Gardner, 1985) and soybean (Vessey and Layzell, 1987).

In soybean, Tolley-Henry and Raper (1986a) reported N stress reduces leaf expansion more than photosynthetic rate, making relatively more photoassimilate available to the roots. In addition, Vessey and Layzell (1987) found sink strength for soybean in vegetative stages followed the order (from highest to lowest): roots, leaves, and stems and petioles. This indicates that under N stress roots continue to develop at the expense of shoots. Thus, when N limits growth, the shoot to root ratio is lower than with adequate N availability (Allos and Bartholomew, 1959; Kerr et al., 1984; Tolley-Henry and Raper, 1986a; Vessey and Layzell, 1987). In legumes, sink strength below ground also increases with a decline in soil N as nodules grow and develop in response to the degree mineral N limits growth. The quantity of photoassimilate partitioned to the root is dependent on the level of N_2 fixation (Singleton and van Kessel, 1987). This competition for carbon between shoots and N assimilating tissues can reduce above ground development and photosynthetic potential, ultimately limiting yield potential as a function of legume reliance on N_2 fixation.

Agreement between solution culture and pot studies, summarized above, implies that a similar response should occur in the field. Yet no field studies have been designed to test this hypothesis. Hence, one of the aims of this thesis was to test that under N deficient conditions roots are a greater sink for DN than under N sufficient conditions.

ROOT GROWTH

Roots anchor plants and absorb water and nutrients from surrounding soil. Legume roots obtain N via uptake of N from soil solution or via N_2 fixation in root nodules. The quantity of N potentially accumulated through N_2 fixation is a balance between plant demand for N and the amount of N available from the soil (Altos and Bartholomew, 1959; George and Singleton, 1992). When uptake of N from the soil is insufficient to meet plant requirements, N_2 fixation will occur to meet the deficit, providing there are no factors limiting N_2 fixation. Thus, N_2 fixation only occurs in plants when growth is limited first by N deficiency. The proportion of total crop N from N_2 fixation is directly related to the level of N deficit. Despite the ability of legumes to fix large quantities of N_2 from the air, N_2 fixation is unable to supply all the N required by legumes. This is supported by findings that symbiotic legumes frequently respond to applied N (Lathwell and Evans, 1951; Thies, 1990; George and Singleton, 1992).

Plants deriving most of their N from N_2 fixation tend to have lower biomass and yield (Allos and Bartholomew, 1959; George and Singleton, 1992). The response to applied N may be indicative of the higher total energy costs of N_2 fixation compared to that of mineral N and metabolism. Although at cellular and enzymatic levels the cost of nitrate assimilation is not greatly different than that of N_2 fixation, on a whole plant level the cost of N_2 fixation is greater (Pate et al., 1979). In solution culture nodulated roots respire more carbon per gram N assimilated than non-nodulated roots (Pate et al., 1979; Ryle at al., 1979; Finke et al., 1982). Increased below ground respiratory cost for N_2 assimilation may be due to the fact that legumes must support highly specialized structures, root nodules, that unlike roots, have no other purpose than N_2 assimilation. This carbon lost via respiration represents a loss in potential biomass. There are, however, no field experiments that test whether or not the suppression of N_2 fixation would result in greater root and shoot growth.

Legumes have a feedback mechanism that controls the level of N_2 fixation in response to available soil N. Soil nitrate concentrations are negatively correlated with N_2 fixation. Nitrogenase activity declines with applied N (Streeter, 1988) decreasing the sink strength of, and hence, the quantity of photoassimilate partitioned to nodules. A decline in nodule weight occurs as a result (Buttery, 1986; Abaidoo et al., 1989).

With an increase in available N, root (nodules not included) growth increases (Allos and Bartholomew, 1959; George and Singleton, 1992) and root length increases (Cassman et al., 1980). Thus legumes in higher N environments have more roots and a greater area over which to absorb N. This study measures the effect of mineral N on growth and development of roots and nodules within and between three legume species: peanut, soybean and common bean.

OBJECTIVES

Some of the problems identified in CROPGRO are a result of insufficient research from which simulation coefficients could be developed such as, early growth of legumes (Jim Jones, Personal Communication). Models, like plants, build on earlier performance. Improved simulation of early growth could improve model precision of final yield and biomass estimation.

Further, a review of the literature indicated that there was little field data to explain legume growth above and below ground in response to applied N. Coefficients for models simulating growth of legumes in farmers fields should be based on field data. Experiments were designed to generate data to meet two objectives. The first objective was to better understand the mechanisms for enhanced DM and N accumulation by legumes obtaining most of their N from the soil as opposed to legumes that fix most of their N. The second objective was to compare data collected to values predicted by CROPGRO, and to make recommendations for further development based on a comparison of predicted and observed values and legume growth as conditioned by N levels.

Chapter 2

THE RELATION BETWEEN MODE OF N ASSIMILATION IN PEANUT, SOYBEAN AND COMMON BEAN TO:

I. YIELD AND YIELD DETERMINANTS.

ABSTRACT

Results for legume yield response to suppression of N_2 fixation, due to an increase in available nitrogen (N), are variable. It has been shown, however, in solution culture studies that on a whole plant level N_2 fixation consumes more energy than assimilation of soil N. A decline in N_2 fixation, in response to elevated soil N, should increase the level of photoassimilates available for shoots or root growth. There are no explanations based on field data as to why legumes in which N_2 fixation is suppressed have greater shoot weight compared to legumes more reliant of N_2 fixation. Two experiments, 1991 and 1993, tested two hypotheses: response of legume growth to applied N (causing lower N_2 fixation) would have the same trend across species and; 2) increased dry matter (DM) partitioning to legume leaves is the primary factor determining increased shoot weight in plants less reliant on N_2 fixation. In 1991, soybean (*Glycine max* Merr. (L.) cv. Clark) was grown at three levels of applied N: 0, 256 and 430 kg ha^{-1} defined as N0, N1 and N2, respectively. Weight and N concentration of leaves, stems, pods, cotyledons, nodules and leaf area were measured at 10, 22, 30, 39 and 51 days after emergence (DAE). At 22 and 51 DAE root weight was measured. In 1993 peanut (*Arachis hypogasa* cv. Pronto), soybean and common beam (*Phaseolus vulgaris* cv. Porillo Sintotico) were grown at either

0 (N0) or 275 (375 for peanut) (N1) kg N ha^{-1}. Weight and N concentration of leaves, stems, pods, cotyledons, roots and nodules were measured at 10, 23 and 44 DAE. Application of N decreased the fraction of plant N derived from fixation (Ndfa) by all species. In 1993, at 44 DAE, application of N decreased Ndfa to 60% of the value measured for plants grown with native soil N. Compared to N0, average shoot weight and deaf area index (across years and species) of N1 plants were greater by 23 and 20%, respectively. This response was measured as early as 10 DAE and remained consistent throughout the growth of the legumes. Increased allocation of DM to leaves at 10 DAE resulted in greater leaf area that was maintained throughout crop growth. For all observations plants that were more reliant on N_2 fixation partitioned a greater fraction of total DM below ground. There were three slain conclusions. One, soybean, common bean and peanut, despite active fixation, can be N limited. Two, allocation of plant DM in early vegetative stages was an important determinant in shoot DM accumulation when N_2 fixation was suppressed by N application. Three, the response of actively fixing soybean, common bean and peanut to applied N was similar.

INTRODUCTION

Greater legume growth has been reported when available soil nitrogen (N) increases, even when the legume is well nodulated (Thies et al., 1991a; George and Singleton, 1992). Legumes assimilate N from both root uptake of NH_4^+ and NO_3^- from the soil and reduction of N_2 in root nodules through symbiotic N_2 fixation. Energy use by N_2 fixation (growth and maintenance of nodules and the process of N_2 fixation) is less efficient than assimilation of soil N (Pate et al., 1979; Ryle et al., 1979; Pinks et al., 1982). The amount of N derived from N_2 fixation is determined by the difference in total crop N requirements and N available in the soil. As the level of soil N increases, the quantity of crop N derived from N_2 fixation declines (Streeter, 1988). As N_2 fixation declines, with an increase in soil N, photoassimilate that would have been used for N_2 fixation is more available for shoot and root growth.

The literature, however, reports mixed results of the effect of applied N on legume growth and grain yield. The response of legumes to applied N is dependent on two factors. Firstly, total N requirement for plant growth is determined by the interaction between genetic yield potential and a host of environmental factors. In field (George and Singleton, 1992) and pot (Allos and Bartholomew, 1959) studies, the degree of suppression of N_2 fixation by application of N was not constant across species. This could be due to varying N requirements or genetic differences in the quantity of carbon required to reduce a mole of N_2 (Layzell et al., 1979) between the species. In some locations crop N demand can be met by available soil N, where environmental effects reduce crop N demand, such as low temperature and solar radiation (Thies et al., 1991a) or at sites where soil N availability is high (Stone et al., 1985). Nutritional status of the soil, other than N, is also influential in determining crop response to applied N. Cassman et al. (1980) studied growth of soybeans at different levels of phosphorus (P) and N. When P was no longer limiting to plant growth, an increase in soybean biomass to applied N was measured. Thus, if an essential element, other than N, is the main limiting factor crop growth and yield response to applied N will be attenuated.

The second factor affecting crop response to fertilizer N is the method of N application. Experiments in which N was applied in regular intervals, such as Ball et al. (1983) George and Singleton (1992) and Thies et al. (1991a), throughout the growing period measured a positive effect of N on growth. A reduction or no effect of N on growth was measured in experiments that applied fertilizer N at planting such as Buttery (1986). Lack of greater growth to applied N is probably due to loss of N over time, as applied N may have been leached from the soil profile.

Most research on the role of N assimilation on legume growth and development has concentrated on the reproductive phase. Yet, yield and biomass accumulation in the reproductive phases are influenced by early growth and development. During pod fill 70% of the N and 2-5% of the carbon (C) previously assimilated are remobilized for legume seed development (Pate, 1985). For high yield, therefore, legumes must accumulate a large N reserve and leaf area to supply N and C to the seed. Leaves are the sole C source and pods, roots and nodules are net sinks. Increasing leaf area would increase the photoassimilate available for whole plant development. The experiments presented in this thesis compare the effect of N assimilation from mineral or atmospheric sources on partitioning of DM and N and subsequently leaf area and crop growth, from early vegetative stages to mid-podfill.

Dry matter (DM) and N partitioning among plant tissues is affected by plant genotype, growth stage and the environmental factors. Muchow et al. (1993) concluded under similar soil field conditions the fraction of above ground DM and N partitioned to leaves varied little across species (soybean, mungbean and cowpea) and soil moisture regimes. Calculations from a greenhouse pot study reported by Allos and Bartholomew (1959) indicate that partitioning of DM and N to shoots or roots in response to applied N differed between legume species. Comparing these two studies indicates that differences in assimilate partitioning may be dependent on the environment and species being analyzed, as well as the inclusion of roots in the analysis.

The distribution of DM and N among plant tissue is also dependent on the stage of development. Early in the plant life cycle vegetative tissues (primarily leaves) are the principal C and N sinks. As plants progress from early to mid to late reproductive stages the pods develop into stronger sinks for C (Thibodeau and Jaworski, 1975; Scott et al., 1983) and N (Pate, 1985) as seeds increase their protein reserves.

It has also been found in solution culture (Tolley-Henry and Raper, 1986b; Vessey and Layzell, 1987) and greenhouse (Cassman et al., 1980; George and Singleton, 1992) studies that N availability is a major factor in allocation of DM and N. Tolley-Henry and Raper (1986a) concluded N stress in soybean reduces leaf expansion more than photosynthetic rate and increases photoassimilate available to the roots. Vessey and Layzell (1987) measured a hierarchy of sink strength for soybean tissues (in descending order): roots, leaves, stems and petioles. In legumes, sink strength below ground also increases with a decline in soil N as nodules grow and develop in direct proportion to the degree to which mineral N availability limits growth. When N limits growth, root development continues at the expense of shoot development, resulting in a lower ratio of shoot weight to root weight than with adequate N availability (Kerr et al., 1984; Tolley-Henry and Raper, 1986a and 1986b; Vessey and Layzell, 1987). Thus under N-luxuriant conditions there is an increase in shoot to root ratios, shoot growth rate (Kerr et al., 1986; Tolley-Henry and Raper, 1986a) and yield (Thies et al., 1991a; George and Singleton, 1992).

Despite the evidence under controlled conditions, there are no reports of field studies on the effect of variable soil N on DM and N partitioning in legumes. Thus, two field experiments were designed to test whether greater allocation of DM to leaves would result in greater shoot weight in legumes less reliant on N_2 fixation, and the response i.e., more or less growth, of each legume to applied N is the same across species.

MATERIALS AND METHODS

The first experiment measured growth of soybean under a range of different soil N regimes. The aim of the second experiment was to determine if trends measured in the first experiment could be repeated in soybean and two other legume species. In both experiments soil N levels were altered (by application of N) so that legumes were differentially dependent on soil N and N_2 fixation as a source of N.

Site description

Two experiments in adjacent sites were conducted on the island of Maui, the first in 1991 and the second in 1993. The soil is classified as Haiku Clay (clayey, oxidic, isohyperthermic typic palehumult). The elevation of the sites was 320m. Soil pH was 5.67 and 6.5 for experiments one and two, respectively. In 1991 and 1993 the average maximum temperatures were 26.5 and 26.6^{0}C, and the average minimum temperatures were 18.8 and 20.3^{0}C, respectively.

Experimental and treatment design

Experiment 1, 1991.

Treatments were arranged in a split-split block design with four replications. Two soybean isolines (nodulating and non-nodulating) cv. Clark (USDA, Beltsville, MD) were randomly assigned to main plots, N treatments were assigned to sub-plots at random and harvest time (10, 22, 30, 39 and 51 days after emergence [DAE]) to sub-sub plots selected at random (see Appendix 1).

Applications of N were made over time, based on the pattern and quantity of soybean N uptake as reported by George and Singleton (1992) and soil N availability by Cassman (1979) for 1991. Nitrogen (as sulphate of ammonia) was applied at the rates of 0, 267 and 430 Kg N ha^{-1} (coded N091, N191 and N291, respectively). Nitrogen was split into four applications. At planting 60% was applied, 15% after the first two harvests and the remaining ten percent between the second and third harvest. In 1991, 22 Mg ha^{-1} of sugar cane bagasse was applied to the soil a week prior to planting to reduce soil N availability. The bagasse was disked into the soil and rotavated to a depth of 20 cm.

Experiment 2, 1993

The experimental design was a split-split-split-plot, with four replicates. Treatments at each level were randomly assigned; main plots were three legume species: peanut (*Arachis hypogaea* cv. Pronto, University of Oklahoma), soybean (two isolines as in experiment 1) and common bean (*Phaseolus vulgaris* cv. Porillo Sintetico, CIAT, Colombia), sub-plots were inoculated and uninoculated treatments, two N application rates were sub-sub plots and sub-sub-sub plots were harvests (see Appendix 2). There were four harvests, the first three harvests were at 10, 23 and 44 DAE corresponding to V1, V5 and R5, respectively, in soybean and common bean (Fehr and Caviness, 1977), and to V3, V6 and R3 for peanuts (Boote, 1982).

There were two N treatments, native soil N (N093) and applied N, from urea, (N193). A total of 275 kg N ha^{-1} was applied to soybean and common bean and 375 kg N ha^{-1} to peanut over the period of crop growth. Seven N applications were made (kg N ha^{-1}); 75, 50, 30, 35, 35, 25 and 25 at planting then 12, 24, 31, 38, 46 and 62 DAE (peanuts emerged two days later than soybean and common bean). Two additional applications of 50 kg N ha^{-1} each were made to peanut at 82 and 104 DAE.

Soil amendments

Other fertilizers were applied to all plots (kg ha^{-1}): 350 P as treble superphosphate, 285 K as K_2SO_4, 60 Mg as $MgSO_4 \cdot 7H_20$, 10 Zn as $ZnSO_4 \cdot 7H_20$, 5 B as H_3BO_3 and 2 Mo as Na_2MoO_4. In 1993 5.5 Mg ha^{-1} of $Ca(OH)_2$ was applied to the soil a month prior to planting.

Planting, inoculation and plant culture

In 1991 soybean seeds were planted on the 10th of July. Prior to planting, the seeds of the nodulating isoline were coated with a three strain mix of Bradyrhizobium japonicum; USDA 110 (TAL102), USDA 136 (TAL379) and YCK213 (TAL1906) using gum arabic as a sticker. The seeds were inoculated at the rate of 10^7 cells per seed.

In 1993 liquid inoculum was applied to furrows at the rate of 10^4 to 10^5 cells per cm of row, after planting and prior to covering. A separate three strain mix of rhizobia was used for each species. For peanut TAL 1000, Nitragin 176A22 (TAL 169) and Nitragin 8A11 (TAL 1371) were used. For common bean CIAT 632 (TAL 1383), TAL 182 and CIAT 899 (TAL 1797) were used. Soybean strains were the same as in experiment 1.

Each plot consisted of 6 rows 60 cm apart and plants in the rows were spaced to obtain a final population of 400,000 plants ha^{-1} for soybean and 300,000 for peanut and common bean. Moisture tension was monitored with tensiometers placed at 25 cm depth and maintained at - 0.2 bars with drip irrigation supplementing rainfall. Air and soil temperature, total solar radiation, and rainfall were recorded by a Campbell Scientific CR-21 micrologger (Campbell Scientific, Inc., Logan UT).

Shoot and root sampling procedures

Shoot sampling

Photosynthesis of the most recent fully expanded trifoliate was measured In experiment 1 at R3 using a LICOR portable photosynthesis system model LI-6000. Three leaves were measured in each block. Two blocks were measured per day between 12 and 2 pm.

In both experiments harvest plots were 2.4 m^2. Plants were cut at ground level and the fresh weight of the entire plot was measured. A sub-sample of 10-20 plants (for early harvests) and 4-10 (for later harvests) was removed to determine dry weight fraction of the whole plot fresh weight and leaf area index (LAI). Sub-sample plants were separated into: cotyledon, leaf, stem and pod. Leaf (plus cotyledon) area was measured with a Licor LI-3100. For all the harvests ten random root systems of the nodulating plants were collected to measure nodule weight.

The final (fourth) harvest was done at harvest maturity, seed and stover were collected. Harvest maturity was 120, 84, 80 DAE for peanut, soybean and common bean, respectively.

Root sampling

In 1991 at 22 and 51 DAE root weight was estimated by collection of soil cores at two depths: 0-25 cm and 25-50 cm. Half the cores were taken between and half within the rows for both depths. For the harvest at 22 DAE 6 cores were collected. At 51 DAE ten root cores were taken at each depth. Thirty per cent of the soil collected for the 0-25 cm layer was sub-sampled for root extraction. Roots and other plant debris were separated from the soil using the hydropneumatic elutriation system (Smucker et al., 1982), and stored in 20% propanol at 4^0C. The roots were manually separated from other organic matter by suspending the mixture in a plastic container with water. The container was placed over a light box and translucent roots collected. Tap roots (coarse lateral root were clipped off) collected for nodule weight measurement were included in the estimation of root weight. In 1993 (experiment 2) root mass was estimated as described for the second harvest in experiment one. At 10 DAE root mass was estimated to be zero for 25-50 cm depth.

Plant analysis

Nitrogen concentration was measured for all plant parts with a Leco CHN analyzer after drying at 70^{0}C to constant weight and grinding to fine powder.

Growth analysis

Dinitrogen fixation was estimated by the difference method as described by Peoples et al. (1989). The uninoculated plots were used as reference plants. Leaf area index (LAI, m^2 m^{-2}) was expressed as the leaf area per unit of land area. Specific leaf area (SLA, cm^2 g^{-1}) was calculated by dividing leaf area of a sample by its weight. Unit leaf rate (ULR, g m^{-2} d^{-1}) was calculated by dividing the incremental increase in Ddb (DM2-DM1) over the average leaf area for the same time period ([LAI1+LAI2]*(T2-T1)/2) (Hunt, 1978).

All statistical analysis was done using SAS (SAS Institute, 1985). Treatment differences were considered significant at a probability level of 5%. The following analysis was done for presentation of F-tests in the tables. The tables only include the statistical analysis of the inoculated N treatments for both years (data for the uninoculated treatment can be found in the appendices). In 1991, N treatments were tested with the error term *replicate (R) X N*. In 1993, the analysis of variance was organized as follow: *R species(S) Ea=R*S N N*S Eb=N*S*R.* Species were tested with the error term *EA*. Nitrogen and *N*S* interaction were tested with error term *Eb*.

RESULTS

Effect of N nutrition on yield and yield determinants

In both years and across species there was a significant reduction in N_2 fixation when N was applied (Fig. 2.1 and Table 2.1). In 1991, N_2 fixation by soybean N1 treatment at 51 DAE was 27% of the level of N_2 fixation in N0 (Fig. 2.1). Dinitrogen fixation, at 44 DAE, estimated in 93N1 treatments were lower than measured in the 93N0 treatments by 0.38, 0.79 and 0.92% for peanut, soybean and common bean, respectively (Table 2.1). At 44 DAE, the fraction of N derived from N_2 fixation in peanuts was probably under estimated as uninoculated peanut, in later growth, formed some effective nodules with native rhizobia.

Of the three species grown in 1993 peanut had the highest seed yield at harvest maturity (Table 2.2). In addition, a significant increase in yield to applied N was measured in soybean and common bean but not peanut. Total DM and N accumulation, and seed yield were proportional to length of time to harvest maturity.

Of all the species, soybean had the highest proportion of seed harvest index (HI) and seed N harvest index [NHI] (Table 2.2). There were no significant effects of N fertilization within species for HI or NHI. There was a strong correlation between seed yield and total DN above ground (r=0.96).

Above ground DM (Table 2.3), leaf area and total leaf N (kg N ha^{-1}) (Table 2.4) increased in response to applied soil N (or decline in N_2 fixation) for all species, and both years for soybean. There was no significant treatment effect on SLA within a species (data not shown).

In 1991, photosynthesis per unit area measured at R3 was not significantly different between N treatments, except non-nodulating N0 (Appendix A-3). In 1991, there were significant differences in specific leaf N at 10 (V1) and 22 (V5) DAE (SLN; leaf N per unit leaf area) but not for the other harvests, which may explain why no difference in photosynthesis was measured at R3. It cannot be concluded that N fertilization had no effect on photosynthesis. Perhaps if measurements were taken when SLN was significantly different, an affect of N on photosynthesis may have been measured. In 1993 there were significant differences in SLN across species.

For each percentage decline in N_2 fixation at harvest maturity there was an increase of above ground DM of 20.5 and 7.6 kg ha^{-1} for soybean and common bean, respectively. This suggests that N_2 fixation in soybean uses more energy than common bean. This indirect measure, however, is not an absolute estimate of N_2 fixation as leaf area development between the two treatments (N0 and N1) was not the same.

Influence of N source on partitioning of N and DM

The degree to which applied N increased DN partitioning to leaves was species dependent (Table 2.5). Common bean partitioned the greatest amount of DM to leaves and soybean the least at 10 and 23 DAE. The N1 soybean treatments consistently allocated more DM to leaves across harvests and both years. For peanut and common bean the trend was not that consistent. There were no differences in partitioning of DM to leaves at 23 DAE for peanut, and 44 DAE for peanut and common bean across N treatments.

The fraction of DM allocated to leaves changed over time across species. From 10 to 23 DAE more DM was partitioned to leaves of soybean and peanut, in 1993, and declined thereafter. The same occurred in partitioning of DM to leaves in soybean in 1991. In common bean the fraction of DM partitioned to leaves was greatest at 10 DAE.

In the N0 treatments relatively more DM and N was partitioned below ground to roots and nodules for all the harvests (Table 2.6). In 1993, the effect of N application on shoot to root plus nodule ratio was not significant at 22 and 44 DAE but across harvests the effect was significant. The relative differences in shoot to root ratios of DM and N were greatest for common lean. There were greater differences in partitioning of N than DM.

DISCUSSION

The experiments described in this chapter measured growth response and N_2 fixation of three legumes under two soil N regimes. The quantity of N derived from N_2 fixation or mineral sources differed in the two-regimes. In the first regime (N0) N was obtained from N_2 fixation and native soil N. In the second regime (N1) the addition of fertilizer N decreased the proportion of N assimilated through N_2 fixation. Thus for N1 treatments more plant N was derived from the soil compared to N0. This resulted in plants with low and high levels of N_2 fixation (N1 and N0, respectively). The proportion of plant N derived from the atmosphere (Ndfa) was also dependent on phenoiogical stage and species.

During the phase from emergence to R1 the rate of N assimilation was 20 to 22% of the rate between R1 and R5 for all species (data not shown). These findings were similar to those of George and Singleton (1992). Low demand for N in early growth stages and/or greater soil N availability in the N1 treatments kept %Ndfa below 20% for most time and N treatment combinations. Nitrogen application suppressed N_2 fixation more in common bean than soybean and peanut. Dinitrogen fixation increased in soybean (91N2) and peanut (93N1) at 10 DAE possibly as a result of starter N effect.

In all the treatments in which N application suppressed N_2 fixation there was greater yield. In peanut, however, increased yield in the N1 treatments was not statistically significant. Perhaps insufficient N was applied to measure treatment differences. Other field studies have measured an increase (Ball et al., 1983) and decrease (Thies et al., 1991a) on peanut yield in response to applied N. Further work is needed to better understand the interaction between peanut cultivars, climate and available soil N to yield and yield determinants.

Increased legume yield could not be explained by HI or NHI which were the same within species at both levels of applied N and is consistent with data collected by Jeppson et al. (1978), and Egli (1988). This supports the hypothesis that variation in soil N does not influence the expression of genes determining DM and N partitioning to seeds at harvest maturity.

Egli (1988) suggested that yield may be more closely related to crop growth rate than partitioning of DM and N at harvest maturity. The plants to which N was applied did have greater growth rates. Greater growth rates for legumes less dependent on N_2 fixation were not a result of increased efficiency of DM accumulation per unit leaf area (see Appendix Table A-4). Growth rate differences were due to measured differences in partitioning. In 1991, there was a significant increase in the fraction of DM allocated to soybean leaves at 22 DAE. The results of the 1991 experiment supports the hypothesis that increased DN partitioning to leaves in legumes less dependent on N_2 fixation was the main determinant of increased crop growth measured in this and other experiments. The experiment in 1993 tested whether this hypothesis would be consistent across years for soybean and across three legume species. Data collected in 1993 supported the above hypothesis. Increased partitioning of DN to leaves in 1993 at 10 DAE resulted in a greater biomass that was maintained throughout crop growth.

A review article by Gifford et al. (1984) recommended two ways to improve yield; increasing photosynthesis or partitioning more carbon to yield. All the legume species used the former strategy. Increased LAI from early growth would have increased the quantity of solar radiation intercepted and subsequently photosynthetic capacity of the legume. Sinclair and Horie (1989) found that the quantity of N per unit leaf area was indicative of the carbon dioxide assimilation rate. The combination of greater N per unit leaf area and more leaf area measured for N1 plants likely created plants with a greater capacity to fix C.

Treatment differences in C-fixation were measured through biomass accumulation. As early as 10 DAE and continuing through at least early pod-fill there were significant differences in LAI between the treatments. Treatments in which N_2 fixation was suppressed by N application had higher LAI than N0 treatments. At 10 DAE, plants in the N0 treatment were experiencing some degree of N stress, as seed N was depleted and the plants became more reliant on N from the soil or N_2 fixation.

Growth, maintenance and N assimilation of nodules are a major sink of photoassimilate (Finke et al., 1982). These observations of the sink strength of nodulated roots being higher than roots with fewer nodules agree with data collected in this experiment. The greater the level of N_2 fixation the more photoassimilate was allocated below ground, probably for growth of nodules. Allocation of assimilate below ground appears to limit leaf area development, which in turn limits photoassimilate supply for overall plant growth.

The positive response of legume growth to N measured in the above experiments and by other researchers within a species (Ball et al., 1983), in multiple legume species and on more than one site (Thies et al., 1991a; George and Singleton, 1992) presents convincing evidence to support the theory that well nodulated soybean, peanut and common bean can be N limited. The data presented in this paper indicate that N limitation of legumes relying on N_2 fixation was manifested in lower grain yield. Lower yield was due to lower shoot weight as a result of lower leaf area. This was due to a lower proportion of DN allocated to leaves during the early vegetative stages as compared to plants less reliant on N_2 fixation. Shoot and leaf area growth appear to be limited by competition with nodulated roots for photoassimilate. The greater the N stress the more DN was partitioned below ground to roots and nodules, the organs obtaining the factor most limiting to plant growth.

Increasing N_2 fixation to meet N demand results in lower yield than can be achieved by non-symbiotic N assimilation. This is due to more preferential partitioning of photoassimilate to root and nodules for growth and maintenance at the expense of shoot development. This study, however, is not to recommend the use of N as a means of increasing legume yield. Rather, legume yield could be increased by improving the efficiency of the rhizobium symbiosis so that less C is used per gram N fixed, decreasing sink strength of the nodules. In turn this would elevate the quantity of photoassimilate for growth and maintenance of other plant organs.

Table 2.1 The effect of nitrogen application on proportion of nitrogen derived from the atmosphere (%Ndfa) by three legumes grown in Maui, Hawaii in 1993.

	N levels	Days after emergence		
		10	23	44
			%Ndfa	
Peanut	N093¶	0.9	16.4	17.0
	N193	2.7	0.0	11.3
Soybean	N093	7.2	19.7	57.1
	N193	0.0	19.8	11.9
Common bean	N093	4.2	32.7	34.1
	N193	0.0	3.5	2.6
F-test	Species (S)	NS	NS	NS
	Nitrogen (N)	NS	*	*
	S X N	NS	NS	NS

¶ 93 refers to the year 1993.
NO, N1 refer to an application of 0 or 225 kg N ha^{-1}.

Table 2.2 The effect of nitrogen application on the proportion of nitrogen derived from the atmosphere (%Ndfa), seed yield, seed harvest index (HI) and seed nitrogen harvest index (NHI) of three legumes grown in Maui, Hawaii in 1993.

	N levels	Ndfa	Seed yield	HI	NHI
		%	Mg ha^{-1}	g^{seed} g^{-1}	g^{seed} g^{-1}
Peanut	N093¶	-#	6.5	0.48	0.72
	N193	-	6.7	0.48	0.70
Soybean	N093	41	3.8	0.63	0.94
	N193	25	4.3	0.63	0.94
Common bean	N093	24	3.0	0.47	0.75
	N193	5	3.4	0.48	0.74
F-test	Species (S)	NS	***	***	***
	Nitrogen (N)	*	**	NS	NS
	S X N	NS	NS	NS	NS

¶ 93 refers to the year 1993.
N0, N1 refer to the N treatment native and applied N: 275 kg N ha^{-1} was applied to soybean and common bean and 375 kg N ha^{-1} was applied to peanut.

Not determined at harvest, uninoculated peanut was effectively nodulated.

Table 2.3 The effect of nitrogen application and time on above ground dry matter accumulation of three legumes grown in Maui, Hawaii.

	N levels	Days after emergence		
		10	23 22 (1991)	44 51 (1991)
			kg ha^{-1}	
Peanut	N093	157	569	3425
	N193	169	625	4104
Soybean	N093	109	570	3276
	N193	114	637	3652
	N091	89	457	4035
	N191	122	753	5681
Common bean	N093	74	443	2808
	N193	83	570	3622
F-test 93§	Species (S)	***	NS	**
	Nitrogen (N)	***	*	**
	S X N	NS	NS	NS
91	N	**	**	*

¶ 91, 93 refer to the years 1991 and 1993, respectively. N0, N1 refer to an N application of 0 or 225 (256 in 1991) kg N ha^{-1}.

§ Statistical analysis was done within years.

Table 2.4 The effect of nitrogen application and time on leaf nitrogen and leaf area index (LAI, m^2 m^{-2}) of three legumes grown in Maui, Hawaii.

	N levels	Days after emergence					
		10		23 22 (1991)		44 51 (1991)	
		Leaf N	LAI	Leaf N	LAI	Leaf N	LAI
		Kg N ha^{-1}	m^2 m^{-2}	Kg N ha^{-1}	m^2 m^{-2}	Kg N ha^{-1}	m^2 m^{-2}
Peanut	N093¶	4.3	0.24	13.2	0.88	72.4	4.41
	N193	5.1	0.26	14.8	0.96	90.9	5.56
Soybean	N093	3.5	0.25	17.6	1.23	73.8	4.26
	N193	4.0	0.26	19.7	1.32	84.6	4.72
	N091	2.1	0.16	14.7	0.88	68.4	3.99
	N191	4.5	0.20	29.4	1.33	100.8	5.36
Common bean	N093	2.9	0.22	12.3	0.96	58.4	5.16
	N193	4.0	0.24	15.1	1.22	74.3	6.85
F-test§ 93	Species (S)	**	*	*	*	**	*
	Nitrogen (N)	***	**	*	*	**	**
	S X N	NS	NS	NS	NS	NS	NS
91	N	**	*	**	*	NS	NS

¶ 91, 93 refer to the years 1991 and 1993, respectively.
NO, N1 refer to an application of 0 or 225 (256 in 1991) kg N ha^{-1}.
§ Statistical analysis was done within years.

Table 2.5 The effect of nitrogen application and time on the fraction of total dry matter (shoots+roots+nodules) allocated to leaves for three legumes grown in Maui, Hawaii.

	N levels	Days after emergence		
		10	23 22 (1991)	44 51 (1991)
			g g^{-1}	
Peanut	N093¶	0.41	0.46	0.41
	N193	0.42	0.46	0.40
Soybean	N093	0.37	0.38	0.30
	N193	0.40	0.39	0.32
	N091	-≠	0.42	0.26
	N191	-	0.48	0.27
Common bean	N093	0.52	0.49	0.37
	N193	0.60	0.48	0.37
F-test§ 93	Species (S)	**	***	***
	Nitrogen (N)	***	NS	NS
	S X N	**	NS	NS
91	N		*	NS

¶ 91/93 refers to the years 1991/1993 respectively.
NO, N1 refer to an application of 0 or 225 (256 in 1991) kg N ha^{-1}

≠ Not determined.

§ Statistical analysis was done within years.

Table 2.6 The effect of nitrogen application and time on the ratio of shoot weight to root plus nodule weight (RD) and ratio of total shoot N¶ to root plus nodule N (RN) of three legumes grown in Maui, Hawaii.

	N levels	Days after emergence					
		10		23 22 (1991)		44 51 (1991)	
		g g^{-1}					
		RN	RD	RN	RD	RN	RD
Peanut	93N0	4.3	3.7	5.1	4.8	10.4	11.9
	93N1	5.5	4.5	5.8	5.3	12.5	12.5
Soybean	93N0	3.4	2.2	3.3	2.4	6.6	5.2
	93N1	4.4	2.8	3.8	2.5	8.0	5.8
	91N0	-#	-	3.9	2.4	8.5	5.3
	91N1	-	-	9.7	4.0	20.1##	6.7
Common bean	93N0	2.7	2.3	3.7	3.2	6.4	5.8
	93N1	5.3	3.8	4.1	3.4	8.4	6.6
F-test§ 93	Species (S)	NS	**	*	**	***	***
	Nitrogen (N)	***	***	NS	NS	NS	NS
	S X N	*	*	NS	NS	NS	NS
91	N			**	**	*	NS

¶ 91, 93 refer to the years 1991 and 1993, respectively. § Statistical analysis was within years. N0, N1 refer to an application of 0 or 225 (256 in 1991)kg N ha^{-1}.

Not determined. ## In 1991 root N was based only on N% root crowns N which is lower than N% of fine roots collected by coring.

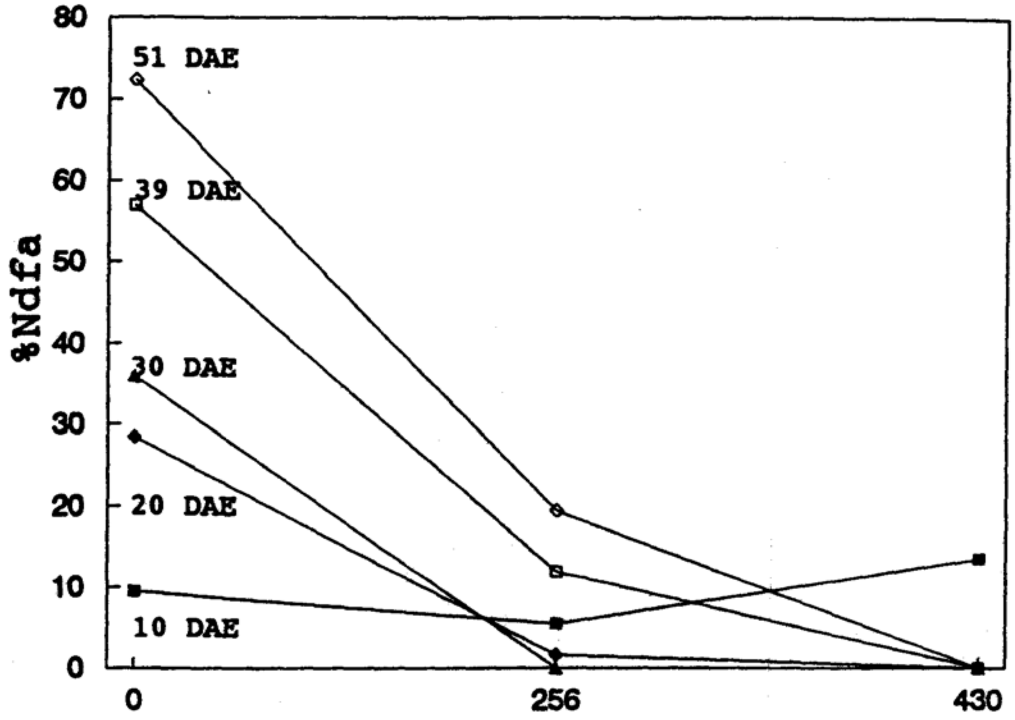


Figure 2.1 Proportion of crop N derived from atmospheric N (%Ndfa) calculated from total shoot N of soybeans grown in Maui, Hawaii 1991. The standard error of the mean is 7.92. DAE is days after emergence.

Chapter 3

THE RELATION BETWEEN MODE OF N ASSIMILATION IN PEANUT, SOYBEAN AND COMMON BEAN TO: II. ROOT GROWTH, MORPHOLOGY AND N UPTAKE

ABSTRACT

Suppression of legume N_2 fixation, by elevated levels of available nitrogen (N), can result in increased dry matter (DN) and nitrogen (N) accumulation compared to plants more dependent on N_2 fixation. Work done in solution and pot studies has demonstrated that changes in root mass and structure and uptake of N per gram of root account for increased N accumulation in plants less dependent on N_2 fixation. There are no comprehensive field studies testing this theory within and across different legume species. Field experiments, in 1991 and 1993, were, therefore, designed to test whether: 1) suppression of N_2 fixation, by N application, increases efficiency of N accumulation per gram DM below ground and root surface area (RSA) due to greater root growth, accounting for more N accumulation in legumes less dependent on N_2 fixation; 2) different legume species would have similar responses to applied N. In 1991, soybean (*Glycine max* Merr. (L.) cv. Clark) was grown at two levels of applied N: 0 and 256 kg N ha^{-1}. Weight and N concentration of shoot and roots (subdivided into components) and root length were measured at 22 and 51 days after emergence (DAE). For the second field experiment, in 1993, soybean, peanut (*Arachis hypogaea* cv. Pronto) and common bean (*Phaseolus vulgaris* cv. Porillo Sintetico) were grown at two levels of applied soil N: 0 and 225 kg N ha^{-1}. Plant samples were collected at 10, 23, 44 DAE as in 1991 and at harvest maturity. The response to applied N was the same in all legumes. Common bean had the greatest relative increase in N accumulation. The legumes to which N was applied accumulated 17% more N compared to plants grown at native soil N. Average nodule weight across years and species of fertilized plants was half of the weight in the unfertilized plots. At 10 DAE in 1993, root mass was less in the fertilized plots compared to unfertilized plots. In 1993 and 1991, an average increase of 17, 27 and 19% was measured for root weight, RSA and N uptake per gram DM below ground at 23 nd 44 DAE. The main conclusions of this study are: one, over time root weight in response to N application changes, lower at 10 DAE and higher thereafter compared to unfertilized plots; Two, increased efficiency of N accumulation per unit DM below ground and RSA due to greater root weight were the main factors accounting for an increase in N accumulation; Three, crop response to applied N was similar across species.

INTRODUCTION

Chapter two demonstrated that well nodulated soybean, common bean and peanut can be nitrogen (N) limited. This was demonstrated by a consistent increase in growth across species with applications of fertilizer N. Application of N reduced the quantity of legume N that was derived from fixation (Ndfa). Legumes with lower Ndfa partitioned a greater proportion of their dry matter (DM) to leaves early in the growing season (prior to 10 DAE). This preferential allocation of DM to leaves resulted in greater DM accumulation in legumes less dependent on N_2 fixation. Previous research has shown that an increase in DM corresponds to greater N accumulation (Allos and Bartholomew, 1959; George and Singleton, 1992). The aim of this chapter was to demonstrate on a whole plant level the mechanisms for greater N accumulation in legumes less dependent on N_2 fixation.

Legumes obtain N via either uptake of N from soil solution or through N_2 fixation. The quantity of N accumulated from either source is a result of the difference between quantity of legume N demand and the amount of available soil N (George and Singleton, 1992). When available soil N is inadequate to meet plant demands, N_2 fixation will occur. Thus, N_2 fixation occurs when there is insufficient soil N to meet growth potential. Accumulation of N, however, by legumes more dependent on N_2 fixation is not equivalent to legumes in N-sufficient environments (George and Singleton, 1992).

Legumes more dependent on N_2 fixation, as an N source, tend to have lower DM and yield compared to plants deriving the majority of their N from the soil (Allos and Bartholomew, 1989; Thies et al., 1991b; George and Singleton, 1992). This indicates that N_2 fixation requires more energy than uptake of soil N. At cellular levels the cost of N assimilation is not very different from that of N_2 fixation. On a whole plant level, however, the cost of N_2 fixation is greater (Pate et al., 1979). Solution culture experiments demonstrated that nodulated roots respire more carbon per gram N assimilated than non-modulated roots (Pate et al., 1979; Ryle at al., 1979; Finks et al., 1982). Increased respiratory cost of the N_2 fixation system represents a potential loss of DM.

Dinitrogen fixation declines with increasing soil N availability. This is a result of a decline in nitrogenase activity with elevated levels of soil N (Sweeter, 1988). This decline in enzyme activity may decrease sink strength of nodules, accounting for lower nodule weight at elevated levels of soil N

(Buttery, 1986; Abaidoo et al., 1989; Thies et al., 1991b). The sink strength of roots and nodules has been demonstrated by decreasing shoot to root ratios as available N decreases (Tolley-Henry and Raper, 1986a; George and Singleton 1992; Chapter 2). In some cases this effect of mineral N on DM partitioning does not translate into less root mass due to an increase in absolute DM accumulation.

An increase in root weight and N accumulation with applied N has been measured in many studies (Allos and Bartholomew, 1959; Cassman et al., 1980; George and Singleton, 1992). George and Singleton (1992) using data collected in pot studies estimated greater accumulation of N per gram DM in roots with application of N. Increased N accumulation under N-sufficient conditions could be a result of greater root absorptive area (Cassman et al., 1980; George and Singleton, 1992) and higher soil N concentrations. Greater absorptive area of legume roots could result from greater root mass and changes in root length (Cassman et al., 1980).

There are, however, no comprehensive field studies on legume root and nodule growth as conditioned by available N levels. This may be due to the difficulty of collecting roots in field studies. Pot and solution culture studies provide valuable data on the patterns of plant response, but coefficients for use in legume simulation models should be derived from field experiments.

Two field experiments were designed, therefore, to test the following two hypotheses:

1. When N_2 fixation is suppressed by mineral N application increased efficiency of N accumulation per gram root and greater root surface area due

to greater root growth account for elevated levels of accumulated plant N as compared to plants obtaining most of their N from N_2 fixation.

2. The pattern of legume response is the same across all three species.

MATERIALS AND METHODS

Treatment design, plant culture and sampling techniques are described in chapter 2. Additional data collected is described below.

Available soil nitrogen

Soil suspensions, of 7.5 g (oven dry weight equivalent) soil in 75 ml of 2M KCl, were shaken for 2 hours on a mechanical shaker. The suspensions were filtered and extracts taken. A sample of the extract was sent to the soils lab at Stanford for determination of NH_4^+-N and NO_3^--N. The methods used were A303-S071-00 and A303-S170-21 as described by Alpkem (now Perstorp Analytical Company) for NH_4^+-N and NO_3^--N respectively.

Root length

Roots collected were evenly spread on a sheet of clear glass and photocopied. A clear plastic sheet with a 1 or 2 cm grid pattern was placed over the photocopy. The number of intersections between the grid and roots were counted and root length estimated using the protocol outlined by Tennant (1975).

Growth analysis

Efficiency of DM and N assimilation per fraction of DM allocated to roots and nodules was calculated by modifying the equation for unit leaf rate (Hunt, 1982). The DM or N increment during a given growth period was divided by the average root plus nodule weight over the same time period; $d(DM)/[(R1+R2)*(T2-T1)/2]$, where T is time and R1 is the weight of roots plus nodules at time 1.

The above formula was used to estimate soil mineral N influx into legume roots (Nflux). Incremental increase in Nflux (total N accumulated minus N from fixation) was divided by average root length during the period to determine N influx per root length. R1 in this equation is the average of root length over the two depths (0-25cm and 25-50 cm).

Root surface area (RSA) was calculated using equations derived from Hallmark and Barber (1984):
Fresh weight of roots was assumed to have e density of 1.0 Mg m^{-3} to calculate volume.
r is radius = $(V/\pi L)^{1/2}$
where L is root length
RSA= $2\pi rL$ cm^{2} root cm^{-3} soil

Roots (excluding root crown) were placed between two paper towels to which a 525 g weight (plastic flask filled with water) was applied for 30 seconds. Then fresh weight was measured.

RESULTS AND DISCUSSION

Root weight, RSA and root length density (RLD, cm root cm^{-3} soil) are interrelated. Thus, it follows that an increase in one will result in an increase in the others.

An increase in RSA, similar to mycorrhizae associations, results in a greater area over which essential nutrients are absorbed. Thus, changes in RSA, as affected by N availability are important in the understanding of mineral nutrition of all plants.

Effect of fertilizer N on N_2 fixation and N assimilation

This study was to identify plant characteristics associated with increased N assimilation by legumes less dependent on N_2 fixation compared to legumes relying primarily on N_2 fixation as an N source. Two experiments were conducted in which mineral N was applied to the soil to create different levels of N_2 fixation. Plants in the N0 treatment (0 kg applied N ha^{-1}) were more reliant on N_2 fixation than plants in treatment N1 (225-256 kg applied N ha^{-1}).

The effect of N on N_2 fixation was described in chapter 2 and is summarized in the following paragraph. In 1991 and 1993, N_2 fixation by peanut, soybean and common bean increased as legume plant phonology went from early to late vegetative to reproductive stages and with a decline in available N. The magnitude of suppression of N_2 fixation by N fertilizer application was greatest in common bean and the least in peanut. The effect of N fertilizer application was consistent for soybean across years and across three legume species.

Imsande (1989) reported soybeans supplied with mineral N in solution culture accumulated less N than plants relying on N_2 fixation. Imsande's findings were not supported by the data collected in our field experiments. When N_2 fixation was suppressed by the addition of mineral N there was a significant increase in the quantity of N assimilated across all species in 1993 and in both years for soybean (Table 3.1). Similar responses have been reported for peanut (Selemat and Gardner, 1985), soybean (Alloy and Bartholomew, 1959; George and Singleton, 1992) and common bean (George and Singleton, 1992).

The effect of N application on N assimilation was statistically significant as early as 10 days after emergence (DAE) and continued throughout the growth cycle of all the legumes. The increase in N accumulation at 10 DAE indicated that soil N was not sufficient to meet plant requirements even in very early crop growth. The data collected in this experiment indicate N deficiency in nodulated legumes can occur prior to 10 days after emergence (DAE) for the three legumes grown.

The majority of N accumulation, by all three species, occurred during the reproductive phase and is consistent with data collected by George and Singleton (1992). The percentage of final shoot N accumulated by 23 DAE (1993) was 5, 11 and 12% for peanut, soybean and common bean respectively. The magnitude of the increased N accumulation in response to applied N was significantly different across species. The average increase in total plant N, in response to applied N, was 8, 17 and 27% for soybean, peanut and common bean, respectively, averaged for the first three harvests (10, 23 and 44 DAE). The stage of growth with the greatest relative increase in N to applied N was V5 for soybeans in both years, R3 for peanut and R5 for common bean (44 DAE). At 10 DAE peanut had the greatest accumulation of N, probably due to seed weight and N content in the seeds. In 1993 at 23 and 44 DAE, soybean had accumulated the most N and also had the highest level of N_2 fixation (reported in chapter 2). The differences in uptake indicate genetic differences across species in N demand and N assimilation.

Effect of fertilizer N on root growth and morphology

Nodule growth was adversely affected by application of mineral N across all species and both years for soybean (Table 3.2). This supports the conclusions of other researchers (Abaidoo et al., 1990; Thies at al., 1991a; Streeter, 1988). The average decline in nodule weight across the first three harvests in 1993 was 70, 56 and 30% for peanut, soybean and common bean.

In 1991, application of N significantly increased soybean root weight by an average of 21% at 22 and 51 DAE. In 1993 at 10 DAE both root and nodule mass of all the species were suppressed by N application. In the same year (1993) at 23 (V5) and 44 DAE (R5), N application promoted root growth by an average of 15% as compared to the N0 treatment: 12, 13 and 21% for soybean, peanut and common bean, respectively.

Plants that were less reliant on N_2 fixation (N1) were more efficient at N uptake per gram of DM allocated to roots and nodules. In 1991, soybeans to which N was applied accumulated 37 and 42% more total DM and N, respectively, per unit DM allocated below ground than soybeans to which no N was applied (Table 3.3). A similar trend was measured for DM and N in 1993 at 10, 23 and 44 DAE for all three species. The increased efficiency of DM accumulation averaged across harvests was 13% greater for peanut and soybean and 25% greater for common bean than their respective N0 controls. The relative increase in efficiency of N accumulation was 18, 16 and 49% for peanut, soybean and common bean, respectively, in the N1 compared to N0 treatment. These findings indicate that increased soil N availability resulted in increased efficiency of DM and N accumulation. The efficiency of DM and N assimilation were highly correlated (r=0.89) across all harvests.

Adding fertilizer N increased the quantity of mineral N (total N assimilated N derived from N_2 fixation) taken up per length of root (Nflux [Table 3.4]) at all harvests, across all species in 1993 and over two years for soybean (except at 23 DAE in 1993). In 1991, the rate of Nflux was twice as much in N1 than N0. In 1993, the relative increase in the Nflux for N1 versus N0 was 28, 58 and 82% for peanut, soybean and common bean respectively. George and Singleton (1992) hypothesized that N accumulation increases in legumes to which N has been applied are a result of higher soil N concentrations as well as increased root surface area. Data presented in the following paragraphs support this hypothesis.

In 1991, the length of root per mg of root (RL, cm mg^{-1}) was greater for the treatments to which N was applied as compared to soybean grown at native soil N at 22 DAE (Table 3.5). This was not the case at 51 DAE in 1991. In 1993, there was no consistent trend in the response of RL to applied N within species. The mean RL across treatments and harvests in 1993 was 18, 27 and 29 cm mg^{-1} for peanut, soybean and common bean respectively. The low RL of peanut combined with low root weight, resulted in peanut having the lowest RLD (Table 3.6) compared to common bean and soybean.

In 1991, RLD of soybean increased when N_2 fixation was suppressed, by N application, at bath 22 and 51 DAE. This response agrees with measurements made in pot studies done by other researchers (Weber, 1966; George and Singleton, 1992). In 1993, the trends were the same for all species at 23 and 44 DAE. There was, however, a decline in RLD at 10 DAE with N application reflecting lower root growth at that time. Thus, the response of legume roots to elevated levels of soil N changes over time. At 10 and 23 DAE soybean had the greatest RLD in the upper 25 cm soil layer. At 44 DAE the average RLD (pooled across N treatments) was 2.65, 1.87 and 1.00 cm cm^{-3} for common bean, soybean and peanut respectively.

Response of RSA was similar to RLD and root weight (Table 3.7). At 10 DAE, the average decline in RSA across species to applied N was 24%. Thereafter the average increase in RSA to applied N was 17, 29 and 34% for peanut soybean and common bean, respectively. Greater RSA and N uptake per unit DM allocated below ground (due to greater available soil N [Table 3.8]) accounts for measured increase in N assimilation in N fertilized plots. Increased N assimilation per length of root was most likely due to increased available soil N. Mugiwara et al. (1980) reported an increase in the rate of N uptake in triticale, wheat and rye as the level of solution nitrate increased.

Summary and conclusions

The decline in root mass at 10 DAE for legumes less reliant on N_2 fixation accounts for their increased allocation of DM to leaves described in Chapter 2. Despite the lower root weight there was an increase in assimilated N in the N1 treatment as a result of greater N uptake per unit DM below ground.

The response of N accumulation, root growth and root structure to N were consistent for soybean across two years and across the three legume species (peanut, soybean and common bean). Increased RSA due to greater root mass, and greater N uptake per DM below ground were the main reasons for the greater N accumulation in legumes less reliant on N_2 fixation to meet N demands. These effects occurred from the early vegetative stages and continued to mid-podfill.

Dinitrogen fixation was suppressed least in peanut by N application as compared to soybean and common bean. Of all the species common bean had the overall greatest relative increase (N1/N0) in N accumulation, DM and N uptake per unit DM below ground, mineral N uptake per length of root and RSA to N application. This may account for N_2 fixation in common bean being suppressed the most of all the species. The response of species to N application was the same (demonstrated by the non-significant interaction between N and legume species).

Table 3.1 The effect of nitrogen (N) application and time on N accumulation in shoots (SH) and roots (roots plus nodules, RN) of three legumes (S) grown in Maui, Hawaii.

	N levels	Days after emergence					
		10		23 22 (1991)		44 51 (1991)	
		kg ha^{-1}					
		SH	RN	SH	RN	SH	RN
Peanut	N093¶	5.5	1.6	18.6	3.7	114.5	11.3
	N193	7.8	1.4	20.8	3.8	147.2	12.1
Soybean	N093	5.9	1.7	23.7	7.4	148.2	22.7
	N193	6.8	1.5	26.8	7.3	159.5	20.2
	N091	-#	-	19.5	4.8	165.5	14.5
	N191	-	-	41.6	4.3	214.3	11.1
Common bean	N093	3.4	1.3	14.9	4.3	84.7	13.5
	N193	4.9	0.9	19.0	4.9	115.6	13.8
F-test§ 93	S	***	*	***	**	***	***
	N	***	**	**	NS	**	NS
	S X N	NS	NS	NS	NS	NS	NS
91	N			***	NS	**	NS

¶ 91, 93 refer to the years 1991 and 1993, respectively. N0, N1 refer to an application of 0 or 225 (256 in 1991) kg ha^{-1}.

Not determined.

§ Statistical analysis was done within years.

Table 3.2 The effect of nitrogen application and time on root (RT) and nodule (NOD) weight (kg ha^{-1}) of three legumes grown in Maui, Hawaii.

	N levels	Days after emergence					
		10		23 22 (1991)		44 51 (1991)	
		kg ha^{-1}					
		RT	NOD	RT	NOD	RT	NOD
Peanut	N093¶	43	0.9	112	9.3	257	33.9
	N193	38	0.6	118	7.1	312	22.9
Soybean	N093	46	3.0	209	36.5	484	162.4
	N193	40	1.3	237	24.0	530	97.2
	N091	-#	-	155	32.7	630	148.0
	N191	-	-	173	5.4	816	33.2
Common bean	N093	30	2.7	123	19.9	451	48.1
	N193	22	0.3	151	11.6	530	10.2
F-test§ 93	Species (S)	*	**	***	***	***	***
	Nitrogen (N)	*	***	*	***	NS	*
	S X N	NS	**	NS	*	NS	NS
91	N			**	*	*	***

¶ 91, 93 refer to the years 1991 and 1993, respectively. N0, N1 refer to an application of 0 or 225 (256 in 991) kg N ha^{-1}.

Not determined.

§ Statistical analysis was done within years.

Table 3.3 The effect of nitrogen application and time on the accumulation of total dry matter (DR) and nitrogen (NR) per unit dry matter below ground (ug N g^{-1} root d^{-1}) of three legumes grown in Maui, Hawaii.

	N levels	Days after emergence					
		0-10		10-23 0-22(91)		23-44 22-51(91)	
		mg g^{-1} root d^{-1}					
		DR	NR	DR	NR	DR	NR
Peanut	N093	942	38	466	14	706	24
	N193	1090	49	525	15	771	28
Soybean	N093	645	31	346	12	334	15
	N193	765	41	383	13	366	16
	N091	-#	-	312	12	290	12
	N191	-	-	455	22	370	12
Common bean	N093	659	30	429	13	418	12
	N193	965	54	515	15	452	14
F-test § 93	Species (S)	**	NS	NS	NS	***	***
	Nitrogen (N)	***	***	*	NS	NS	*
	S X N	*	**	NS	NS	NS	NS
91	N			**	**	NS	NS

¶ 91, 93 refer to the years 1991 and 1993, respectively
NO, N1 refer to an application of 0 or 225 (256 in 1991) kg N ha^{-1}.

Not determined.

§ Statistical analysis was done within years.

Estimates were calculated from the modified unit leaf rate equation. The DM and N increment during a given growth period was divided by the average root plus nodule weight over the same time period [(R1+R2)*(T2-T1]/2 where R1 is the weight of roots plus nodules at time 1 (T1).

Table 3.4 The effect of applied N on the uptake of mineral N (total N assimilated - N derived from N_2 fixation) per unit root length per day for three legumes grown in Maui, Hawaii.

	N levels	Days after emergence		
		10	23 22 (1991)	44 51 (1991)
		μg N cm^{-1} root d^{-1}		
Peanut	N093	2.05	0.97	1.92
	N193	2.45	1.44	2.21
Soybean	N093	1.37	0.92	0.66
	N193	2.29	0.92	1.36
	N091	-#	0.89	0.39
	N191	-	1.58	1.20
Common bean	N093	0.94	0.50	0.68
	N193	2.13	0.93	0.90
F-test§ 93	Species (S)	NS	**	***
	Nitrogen (N)	**	*	**
	S X N	NS	NS	NS
91	N		*	***

¶ 91, 93 refer to the years 1991 and 1993, respectively. N0, N1 refer to an application of 0 or 225 (256 in 1991) kg N ha^{-1}.

Not determined.

§ Statistical analysis was done within years.

Calculated from the modified unit leaf rate equation. The mineral N increment during a given growth period was divided by average root length density (RLD) over the same period of time [(R1+R2)*(T2-T1)]/2, where R1 is the RLD at time 1(T1).

Table 3.5 The effect of applied nitrogen and time on the length of root per mg root dry weight in the 0-25 cm soil layer for three legumes grown in Maui, Hawaii.

	N levels	Days after emergence		
		10	23 22 (1991)	44 51 (1991)
			cm mg^{-1}	
Peanut	N093¶	19	18	17
	N193	21	18	16
Soybean	N093	27	25	29
	N193	28	24	29
	N091	-#	23	28
	N191	-	34	27
Common bean	N093	33	29	25
	N193	30	26	29
F-test§ 93	Species (S)	*	**	***
	Nitrogen (N)	NS	NS	NS
	S X N	NS	NS	NS
91	N		**	NS

¶ 91, 93 refer to the years 1991 and 1993, respectively. N0, N1 refer to an application of 0 or 225 (256 in 1991) kg N ha^{-1}.

Not determined.

§ Statistical analysis was done within years.

Table 3.6 The effect of applied nitrogen and time on root length density in the 0-25 cm soil layer of three legumes grown in Maui, Hawaii.

	N levels	Days after emergence		
		10	23 22 (1991)	44 51 (1991)
		cm root cm^{-3} soil		
Peanut	N093¶	0.17	0.45	0.86
	N193	0.16	0.46	1.13
Soybean	N093	0.21	0.85	1.70
	N193	0.16	1.06	2.04
	N091	-#	0.50	1.90
	N191	-	0.90	2.40
Common bean	N093	0.21	0.80	2.15
	N193	0.12	1.00	3.15
F-test§ 93	Species (S)	NS	**	NS
	Nitrogen (N)	*	*	NS
	S X N	NS	NS	NS
91	N		*	NS

¶ 91, 93 refer to the years 1991 and 1993, respectively. N0, N1 refer to an application of 0 or 225 (256 in 1991) kg N ha^{-1}

Not determined.

§ Statistical analysis was done within years.

Table 3.7 The effect of N application and time on root surface area per volume of soil calculated for the 0-50cm soil layer (does not include root crown) for three legumes grown in Maui, Hawaii.

	N levels	Days after emergence		
		10	23 22 (1991)	44 51 (1991)
		cm^2 root cm^{-3} soil		
Peanut	N093[¶]	0.81	2.55	6.12
	N193	0.71	2.80	7.59
Soybean	N093	0.84	4.17	7.70
	N193	0.66	4.93	9.41
	N091	-[#]	2.61	10.28
	N191	-	3.78	13.60
Common bean	N093	0.76	3.14	9.61
	N193	0.47	4.05	13.30
F-test[§] 93	Species (S)	NS	*	**
	Nitrogen (N)	*	NS	**
	S X N	NS	NS	NS
91	N		NS	*

[¶] 91, 93 refer to the years 1991 and 1993, respectively. N0, N1 refer to an application of 0 or 225 (256 in 1991) kg N ha^{-1}

[#] Not determined.

[§] Statistical analysis was done within years.

Table 3.8 The quantity of KCl extractable nitrogen at three time points in the 0-25 cm soil layer for three legumes grown in Maui, Hawaii in 1993.

	N levels	Days after emergence		
		10	23	44
		g N kg soil^{-1}		
Peanut	N093¶	0.010	0.007	0.002
	N193	0.020	0.022	0.022
Soybean	N093	0.009	0.007	0.002
	N193	0.019	0.019	0.009
Common bean	N093	0.007	0.006	0.007
	N193	0.016	0.022	0.005
F-test	Species (S)	NS	NS	NS
	Nitrogen (N)	**	**	**
	S X N	NS	NS	NS

¶ 93 refer to the year 1993.
N0, N1 refer to an application of 0 or 225 kg N ha^{-1}.

CHAPTER 4

TESTING CROPGRO V3.0 SIMULATION OF LEGUME GROWTH WITH DIFFERENT LEVELS OF APPLIED NITROGEN

ABSTRACT

The mode of nitrogen (N) nutrition has a differential effect on legume growth and development. Thus, to accurately predict legume growth simulation models should be sensitive to available soil N. CROPGRO V3.0 is a mechanistic simulation model that has included a N balance sub-model to simulate N demand, soil N uptake and N_2 fixation. CROPGRO simulates growth of three species of legumes (soybean, peanut and common bean) and cultivars within the legumes by inputing genetic coefficients at the species and cultivar level. The aim was to test the ability of CROPGRO to model legume growth under different levels of soil N. In 1993, an experiment was designed to generate data and theory that can be used in the process of model development. In 1993, soybean (*Glycine max* Merr. (L.) cv. Clark), peanut (*Arachis hypogaea* cv. Pronto) and common bean (*Phaseolus vulgaris* cv. Porillo Sintetico), were grown with two levels of applied soil N: 0 and 275 (375) kg N ha^{-1}. Plant samples were collected at 10, 23, 44 days after emergence (DAE) and harvest maturity. Leaf area, and weights and N concentration of leaves, cotyledons, stems, roots (plus nodules) and pods were measured at the three first harvests. At harvest maturity weight and N concentrations were measured for shoots and seed. Between 10 and 44 DAE, total N accumulation had an average increase of 19 and 16% when N was applied for CROPGRO and field data, respectively. Between 10 and 44 DAE,

observed above ground dry matter (DM) accumulation increased by an average of 15% with N application, while simulated DM accumulation was, for the most part, unresponsive or produced lower values. The cause of this lack of simulated DM response to N application is not clear. Observed and predicted seed yield were in closer agreement. Simulated seed yield increased primarily as a result of greater harvest index (HI) with application of N. In the field N application increased grain yield as a result of greater DM accumulation and not HI. The consistency of legume growth response to N application across species and time justify changing CROPGRO’s response to N application. Increased grain yield with N application should be a result of greater DM accumulation. Greater DM should be due to greater leaf area development from the early vegetative stages. As long as available soil N is sufficient to decrease N_2 fixation differences in leaf area should be maintained throughout crop growth.

INTRODUCTION

Since mode of N nutrition affects legume growth (Chapter 2) legume simulation models should be sensitive to available soil nitrogen (N). Ritchie (1994) defined three types of simulation models: statistical which use recorded trends, functional which use simplified versions of plant processes, and mechanistic which incorporate major plant processes. CROPGRO V3.0 is a mechanistic model.

Mechanistic models attempt to consolidate existing data on physiological processes (Ritchie, 1994). Empirical data are synthesized into a hypothesis (Ritchie, 1994) which is subsequently used to develop an algorithm. Algorithm calculations use values derived from a single or multiple sources such as, genetic and environmental data and previous model computations. The result is an estimate of some physiological process in response to input variables. CROPGRO simulates many physiological attributes of crops and the soil environment such as water content, and available N.

CROPGRO is comprised of three legume models. These legume models are modified versions of previously released legume models: SOYGRO (soybean simulation model by Jones et al., 1989), BEANGRO (common bean simulation model by Hoogenboom at al., 1991a) and PNUTGRO (peanut simulation model by Boots et al., 1989) (Hoogenboom et al., 1993). CROPGRO simulations are not for a generic legume. To simulate one of the legumes (peanut, soybean or common bean) a user first selects the species to be simulated then the cultivar. Each selection inputs a file containing genetic coefficients for growth and development which are specific to the species and cultivar.

The three legumes simulated by CROPGRO are among the most important of the world's crops, especially soybean and peanut which have multiple uses. There are substantial physiological and field experimental data available for these three crops. Comparing model simulations to observed data can test model hypotheses and identify areas requiring further research and adjustment. Simulations of crop growth could improve decisions by predicting crop performance under changing farming practices and environments. For use in decision making it is imperative that legume growth and development are accurately simulated under a variety of conditions including different levels of available soil N.

Plant processes that most influence growth are included in simulation models. In CROPGRO growth is based on carbon (C), water and N balances (Hoogenboom et, al., 1992). Experimental evidence of legume response to available N is not consistent. It has been shown in solution culture studies, however, that on a whole plant level N_2 fixation uses more C than plants dependent on uptake of soil N in solution culture studies (Pate et al, 1979; Ryle et al., 1979; Finke et al., 1982). Their studies plus data in Chapters 2 and 3 show that peanut, soybean and common bean can be N limited, when dependent on N_2 fixation for a significant amount of total N. This is demonstrated by an increase in dry matter (DM) accumulation, like non-legumes, when available soil N is greater and N_2 fixation declines (Allos and Bartholomew, 1959; Thies et al., 1991b; George and Singleton, 1992; Chapter 2). This differential effect of mode of N nutrition on whole plant growth must be included in legume simulation models.

Soil type, climate and crop management are all influential on soil N content, and subsequently the level of N_2 fixation in a legume. Thies et al. (1991b) measured a range from 15 to 82% of soybean N derived from N_2 fixation under natural conditions at several field sites. Thus experiments were designed to induce different levels of N_2 fixation by applying fertilizer N. At higher levels of soil N the legumes fixed significantly less N_2.

One major difference between CROPGRO and the individual legume models (SOYGRO, BEANGRO and PNUTGRO), that form the basis of CROPGRO, is the incorporation of a N sub-model.

Two other simulation models also account for N_2 fixation by legumes: a soybean model developed by Sinclair (1986) and; Epic (Williams, 1983). In Sinclair's (1986) model, N_2 fixation is a linear function of vegetative biomass. In Epic, crop N assimilation requirements are estimated from the quantity of N required to raise plant N concentration to then optimum level for each growth stage (Bouniols et al., 1991). The fraction of daily N requirements, in EPIC, supplied by fixation is based on stage of development, water stress and soil mineral-N content.

In CROPGRO, N demand is based on growth mite, N concentration and N needed to re-fill N mined from old tissue. At present the model assumes that remobilized N can be replaced to the previous maximum N content defined in the species file (Jones et al., unpublished). To satisfy N demand the model simulates N assimilation from soil N, remobilization and N_2 fixation. If N from soil uptake and remobilization is inadequate to meet N demand for new growth, N_2 fixation will occur. The level of N_2 fixation is dependent on the crop demand for N, available plant C, soil temperature, aeration and nodule mass (Hoogenboom et al., 1993).

The previous chapters indicated that the directional response of soybeans to N application were similar in 1993 and 1991. The response of N and DM accumulation and partitioning were also similar in all three legumes (Chapter 2 and 3). Testing all three species would determine if the trends in the simulated legumes are as consistent across species as field observations.

This paper has two aims. The first is to test agreement between observed and CROPGRO predicted values for DM and N accumulation of the three component legumes. The second is to make recommendations for further development of CROPGRO. A single data set can indicate flaws in model concepts, however, many data sets are needed to develop coefficients. Thus recommendations will be at the conceptual level on a whole plant basis.

MATERIALS AND METHODS

Treatments, plant culture and data collected are described in Chapters 2 and 3.

Model description

To simulate growth of a legume, CROPGRO requires daily inputs of solar radiation, minimum and maximum temperature and rainfall. In a management file, used to simulate legume growth, values for initial soil N up to 25 cm, initial moisture content and planting time, depth and density were stipulated. The schedule for irrigation and fertilizer N application were also included in the management file.

The N sub-model included in CROPGRO is a modification of the CERES-Wheat N balance developed by Godwin et al., (1989). A N_2 fixation component was included in the N sub-model to simulate legume N assimilation (Hoogenboom et al., 1993). This sub-model simulates leaching of soil N, accumulation of N in the soil, and crop N demand and N accumulation.

Net photosynthesis, allocation of photosynthate to different plant parts and respiratory costs for growth and maintenance of different tissue influence legume growth in CROPGRO (Hoogenboom, 1992). Photosynthesis is determined by solar radiation, daily temperature, LAI, N content of the leaves, specific leaf area (SLA), plant water deficit, and row and plant spacing (Hoogenboom, 1992).

Phenology

The confounding effect of different phonological stages on comparison of values between observed and predicted were eliminated. Prior to testing CROPGRO, the simulated and measured phenological values of emergence and R1 (start of flowering) were compared. When there was a significant disagreement between the two, input parameters controlling legume phenology for the species in question were modified.

The genetic coefficients controlling phenology of common bean and peanut did not need adjustment. In both cases emergence was accurately predicted and R1 was two days earlier than observed in the field. For soybean, however, coefficients in the ecotype and cultivar files required modification. In the former file the time from planting to emergence was reduced from 3.6 to 2.0 and in the latter the time from emergence to flowering was increased from 18.0 to 20.0.

CROPGRO creates a number of simulation files. These files contain a wide range of information on aspects of crop growth and the environment such as, N accumulation, water and N stress, as well as soil conditions moisture and available N. Values from the ”growth.out” and “nitrogen.out” (Tsuji, Uehara and Balas, 1994) files were used for comparison to field data.

RESULTS AND DISCUSSION

To estimate yield CROPGRO simulates physiological processes using theory incorporated during model development. The following section compares

CROPGRO and field measurements to determine the validity of the theory included in the model.

N accumulation

The field data showed that there was a significant increase in N accumulation (N from N_2 fixation and uptake of soil N) in response to N application (Table 4.1). Nitrogen accumulation simulated by CROPGRO was far less responsive to N fertilization than field data during the vegetative stages (10 and 23 days after emergence [DAE]). At 44 DAE, however, simulated N accumulation values increased with N application by an average of 33% across species, compared to a 21% increase for observed values. The overall predicted trend, however, of an increase in N accumulation when N is applied agrees with field data and previous studies (George and Singleton, 1992; Thies et al., 1991b).

At 10 DAE, simulated values for N accumulation were lower than observed values for all three legume species tested. The simulation values for N accumulated, averaged over species, was twice than the observed values at 23 DAE. At 44 DAE simulated values for total N accumulation were 12 and 39% greater than observed for peanut and common bean, respectively. For soybean at 44 DAE, however, simulated N accumulation was 16% lower for the N0 treatment and 2% higher for N1 treatment compared to the observed values.

Application of N reduced N_2 fixation for all species at all three time points in the field. The quantity of N derived from N_2 fixation predicted by CROPGRO was 15% of observed values averaged for all three species at 44

DAE (Table 4.2). In each species application of N reduced predicted N_2 fixation at different times. The first reduction in predicted N_2 fixation with an application of N occurred at 10, 23 and 44 DAE for common bean, peanut and soybean, respectively.

There was an increase in simulated uptake of soil N when N was applied to the soil. In fact, CROPGRO simulated that the majority of crop N was derived from the soil, hence, the low values for N_2 fixation. At 23 and 44 DAE, the simulated values were on average 75% greater than the value measured in the field averaged over N treatments and species. At 44 DAE, uptake of soil N was 49% greater in the simulated values than observed (Tables 4.1 and 4.2).

Measured initial nitrate and ammonia values were high in the first 25 cm soil layer: 26.73 and 6.23 ug g^{-1}, respectively, due to wetting and drying and weed free environment prior to planting. Estimates of available soil N at 10, 23 and 44 DAE, from KCl extractions (for plots to which no N was applied), were much smaller than the values simulated by CROPGRO (data not shown). This may indicate that estimated losses of mineralized N were smaller than observed. There is insufficient data from this experiment to test leaching values in the model. Disagreement between observed and predicted values of soil N concentration indicates a need for further development of factors affecting available soil N before accurate predictions of N_2 fixation and mineral N assimilation can be made.

Root and nodule growth

CROPGRO values for root weight were higher than observed values for all species at 10 and 23 DAE and peanuts and soybean at 44 DAE (Table 4.3). At 44 DAE, simulated root weight for peanut and soybean was almost three times the value measured in the field, while simulated N uptake was 25% greater at the same time point. The large difference in the magnitude of simulated root growth to simulated N uptake indicates that the models predictions of N assimilation per gram of root was less in peanut and soybean than measured in the field. The opposite occurred for common bean. Simulated N uptake in common bean was 68% greater than field data, however, simulated root weight was 20% less than measured at 44 DAE. Thus the effect of N application on assimilation of N per gram of root is not the same across all legume species in CROPGRO.

Previous work has shown that in response to N application there is a decline in nodule weight and an increase in root weight (George and Singleton, 1992; Chapter 3). At 10 and 23 DAE, there was no effect of N application on simulated root weight. At 44 DAE, simulated root weight declined on average of 5% across species with application of N. In comparison, measured root weight at 44 DAE increased with N application by an average of 17% across species.

In the field it was a few days before nodules were visible or measurable CROPGRO, however, simulated nodule weight from the first day of emergence. Thus, despite the low CROPGRO values of N_2 fixation most simulated nodule weights (Table 4.4) were greater than the measured data at 10 DAE. There was a decline in simulated nodule weight between 10 and 23 DAE, and an increase between 23 and 44 DAE. Simulated nodule weights at 44 DAE, however, were 45 and 3% of the values measured in the field for treatments

N0 and N1, respectively. These low values corresponded to the low estimates of the quantity of N derived from N_2 fixation.

Simulated nodule weight in the N0 treatments declined during the start of the vegetative stage and increased at the end of that stage and over the reproductive period. For N1, simulated total nodule weight declined from emergence to harvest maturity, for all species. In contrast measured nodule weight showed that there was a steady increase in nodule weight over time, for both N treatments. Both observed and predicted nodule weight values declined with added N. This indicates that there is a feed back mechanism in the model, similar to the plant, that restricts nodule growth with N application.

Calculated values of N accumulation per gram dry matter (DM) below ground (NADM, Roots plus nodules) increased with applied N for both observed and predicted values. The similarity between observed and predicted NADM should be treated with caution. The resemblance of CROPGRO NADM to field measurements was due to an increase in simulated N accumulation and no increase or lower simulated root weight with application of N. Consequently, combining two simulated variables may mask simulation problems of each variable.

Above ground dry matter

Although predicted values of N accumulation increased with applied N (Table 4.1), simulations of above ground DM (Table 4.5) were either unresponsive at 10 and 23 DAE or lower with application of N at 44 DAE. Lower DM with N application was opposite to trends observed in the field and by other researchers (Thies et al., 1991b; George and Singleton, 1992). Nitrogen yields at 44 DAE were 20% greater than N0 while CROPGRO predicted an 8% decline in above ground DM when N was applied.

Predicted leaf weight was lower with application of N, similar to above ground DM (Table 4.6). CROPGRO had much higher values of leaf weight compared to observed values for all species at 23 DAE and for peanut and soybean at 44 DAE. In response to N application field data showed an increase in above ground DM, leaf weight and leaf area index (LAI) (Table 4.7) beginning at 10 DAE. The consistent and significant increase of these measured variables to N application indicated that all three legume species were N deficient (Chapter 2). In most cases the CROPGRO values of above ground DM, leaf weight and LAI were the same or lower for both N treatments. Despite the decline in simulated leaf weight, with applied N, for soybean and common bean there was a small increase in predicted LAI at 44 DAE for soybean and common bean (Table 4.7).

Of the parameters included in the photosynthesis routine solar radiation, temperature, plant water deficit and row and plant spacing were the same for both N treatments in addition, the field data showed no significant increase in SLA with N application (Chapter 2). Thus, in this study only two variables in the photosynthesis subroutine were affected by N application: leaf N content and LAI.

For both simulated and measured data total leaf N increased with application of N (Table 4.8). At 44 DAE, the increase in total leaf N with application of N was 23% in the field data and 39% for the simulated values. Nitrogen application increased observed leaf N as early as 10 DAE while CROPGRO simulated values did not respond until the reproductive stage at 44 DAE.

In CROPGRO the model uses C to convert assimilated N (from the soil or N_2 fixation) to protein. Dinitrogen fixation in CROPGRO also uses C for growth and maintenance of nodules, this is an additional carbon cost compared to soil N uptake. This greater allocation of C to N_2 fixation agrees with whole plant studies on the carbon cost of N_2 fixation (Ryle et al., 1979; Pate, 1979; Finks et al, 1982). Yet CROPGRO is simulating a decline in above ground DM with application of N.

It was not clear as to why simulated N accumulation increased and above ground DM declined with application of N. It follows that if there is a reduction in above ground DM weight there should be a corresponding reduction in total N. It appears that at higher levels of available soil N the model increased the quantity of N required for legume growth i.e. N concentration. Calculation of leaf N concentration using data from tables 4.7 and 4.9 agree with the above statement. A decline in above ground DM was also simulated when initial soil N values were input at half of what was measured. Thus lower DM accumulation in response to N application would also be simulated in areas with lower initial N values.

Simulated above ground DM of N0 was greater than N1 for common bean and soybean, from the start of the reproductive stage. At harvest maturity, however, simulated above ground DM was greater in the N1 treatment than the N0 (calculated from Table 4.9).

Yield

There was an increase in simulated and observed grain yield with an application of N (Table 4.9). Predicted grain yields of peanut (N0 and N1) and soybean (N0) was only 69% of observed values. CROPGRO simulated a higher value for yield of soybean N1 and both N treatments of common bean by an average of 23% compared to field data.

Greater yield in CROPGRO in response to N application was due to greater partitioning of accumulated DM to reproductive tissue. Previous research (Jeppson, et al., 1978; Egli, 1988) demonstrated that the fraction of DM allocated to reproductive soybean tissue is conservative i.e. despite changes in the environment the harvest index (HI, grain yield divided by total above ground DM) will not change. Thus, environmental conditions that result in increased above ground DM will result in increased yield (Egli, 1988). Data presented in chapter 2 agreed with the above studies. It also showed that the hypothesis of HI remaining the same across N treatments holds for both peanut and common bean. Thus, the change in simulated HI with N application is not accurate. CROPGRO should simulate greater yield with application of N as result of greater above ground DM and N accumulation.

Summary

This chapter compares CROPGRO's ability to simulate growth of three legumes at two levels of soil N. The lack of increased simulated above ground DM with N application plus low estimates of N_2 fixation for both N treatments suggests that there is little difference in the N conditions for the legumes simulated. Simulated grain yield, however, for all the legume species were substantially greater with N application. This was primarily due to greater HI with application of N. This effect of N on HI was not observed in the field data.

The three legume species and soybean over two years had similar directional responses to applied N (Chapters 2 and 3). The consistency in the response across species and time indicate that greater legume growth with N application are real and repeatable. Based on this evidence CROPGRO should make adjustments to simulation of legume growth in response to soil N to better correspond to field observations reported in this chapter and previously published literature.

CROPGRO should simulate an increase in grain yield with application of N as a result of more DM accumulation compared to unfertilized plots. This greater DM accumulation should be a result of greater partitioning of DM to leaves during the early vegetative stages. Maintenance of elevated soil N levels throughout crop growth should result in greater LAI and DM compared to unfertilized plots. Greater DM accumulation should corresponded to greater levels of total N with N application. Greater N accumulation should be a result of greater NADM and root weight at elevated levels of soil N.

Table 4.1 Comparison between observed and CROPGRO predicted values for total nitrogen accumulation of three legumes grown with two levels of applied nitrogen.

	N levels	Observed Days after emergence			CROPGRO Days after emergence		
		10	23	44	10	23	44
				kg N ha^{-1}			
Peanut	N0¶	8.1	22.3	125.9	4.5	36.9	138.0
	N1	7.8	24.6	159.3	5.8	36.4	181.8
Soybean	N0	7.6	31.0	170.8	4.2	39.9	142.9
	N1	8.4	34.1	179.7	4.2	41.1	183.8
Common bean	N0	4.7	19.2	98.2	4.3	43.9	132.8
	N1	6.0	23.9	129.4	4.4	50.8	185.1
LSD§		0.4	2.3	11.5			

¶ - N0, N1 refer to an application of 0 or 225 kg N ha^{-1}.

§ - LSD between N treatments.

Table 4.2 Comparison between observed and CROPGRO predicted values for nitrogen derived from fixation of three legumes grown with two levels of applied nitrogen.

	N levels	Observed Days after emergence			CROPGRO Days after emergence		
		10	23	44	10	23	44
				kg N ha^{-1}			
Peanut	N0¶	0.2	3.7	21.3	0.2	0.5	10.2
	N1	0.3	0.0	18.1	0.2	0.2	0.2
Soybean	N0	0.6	6.4	97.3	0.2	0.2	1.8
	N1	0.0	7.3	22.9	0.2	0.2	0.2
Common bean	N0	0.2	6.3	33.3	0.3	1.6	10.9
	N1	0.0	1.2	4.3	0.1	0.3	0.3
LSD§		1.0	3.1	36.5			

¶ - N0, N1 refer to an application of 0 or 225 kg N ha^{-1}.
§ - LSD between N treatments.

Table 4.3 Comparison between observed and CROPGRO predicted values for root weight of three legumes grown with two levels of applied nitrogen.

	N levels	Observed Days after emergence			CROPGRO Days after emergence		
		10	23	44	10	23	44
				kg ha^{-1}			
Peanut	N0¶	43	112	257	69	261	814
	N1	38	118	312	68	263	770
Soybean	N0	46	209	483	78	488	1468
	N1	39	237	530	78	489	1415
Common bean	N0	30	123	456	50	253	404
	N1	21	162	541	49	244	382
LSD§		4	32	12			

§ N0, N1 refer to an application of 0 or 225 kg N ha^{-1}.

¶ LSD between N treatments.

Table 4.4 Comparison between observed and CROPGRO predicted values for nodule weight of three legumes grown with two levels of applied nitrogen.

	N levels	Observed Days after emergence			CROPGRO Days after emergence		
		10	23	44	10	23	44
				kg ha^{-1}			
Peanut	N0¶	0.9	9.3	33.9	3.1	2.6	29.6
	N1	0.6	7.1	22.9	3.1	1.8	0.8
Soybean	N0	3.0	36.5	132.4	2.6	1.5	4.9
	N1	1.3	24.0	97.2	2.6	1.5	0.6
Common bean	N0	2.7	19.9	48.1	2.3	4.7	20.6
	N1	0.3	11.6	10.2	1.9	1.3	0.6
LSD§		0.4	3.6	27.6			

¶ N0, N1 refer to an application of 0 or 225 kg N ha^{-1}.

§ LSD between N treatments.

Table 4.5 Comparison between observed and CROPGRO predicted values for above ground dry matter of three legumes grown with two levels of applied nitrogen.

	N levels	Observed Days after emergence			CROPGRO Days after emergence		
		10	23	44	10	23	44
		kg ha^{-1}					
Peanut	N0¶	157	569	3425	116	782	4300
	N1	169	625	4103	115	783	3819
Soybean	N0	109	570	3276	81	735	3820
	N1	114	637	3652	81	738	3583
Common bean	N0	73	443	2808	125	1347	4915
	N1	83	570	3622	125	1267	4588
LSD§		4	52	372			

¶ N0, N1 refer to an application of 0 or 225 kg N ha^{-1}.
§ LSD between N treatments.

Table 4.6 Comparison between observed and CROPGRO predicted values for leaf (plus cotyledon) weight of three legumes grown with two levels of applied nitrogen.

	N levels	Observed Days after emergence			CROPGRO Days after emergence		
		10	23	44	10	23	44
				kg ha^{-1}			
Peanut	N0¶	107	339	1504	75	442	2031
	N1	113	366	1793	75	443	1823
Soybean	N0	80	325	1184	63	462	1545
	N1	85	361	1355	63	464	1541
Common bean	N0	55	288	1225	86	851	1618
	N1	63	356	1535	86	805	1602
LSD§		5	122	281			

¶ N0, N1 refer to an application of 0 or 225 kg N ha^{-1}.

§ LSD between N treatments.

Table 4.7 Comparison between observed and CROPGRO predicted values for leaf area index of three legumes grown with two levels of applied nitrogen.

	N levels	Observed Days after emergence			CROPGRO Days after emergence		
		10	23	44	10	23	44
				m^2 m^{-2}			
Peanut	N0¶	0.24	0.88	4.41	0.26	0.95	4.55
	N1	0.26	0.96	5.56	0.27	0.95	4.14
Soybean	N0	0.25	1.23	4.26	0.22	1.44	4.16
	N1	0.26	1.32	4.72	0.22	1.44	4.30
Common bean	N0	0.22	0.96	5.16	0.29	2.93	5.52
	N1	0.24	1.22	6.85	0.29	2.77	5.61
LSD§		0.03	0.38	1.17			

¶ N0, N1 refer to an application of 0 or 225 kg N ha^{-1}.

§ LSD between N treatments.

Table 4.8 Comparison between observed and CROPGRO predicted values for leaf nitrogen accumulation of three legumes grown with two levels of applied nitrogen.

	N levels	Observed Days after emergence			CROPGRO Days after emergence		
		10	23	44	10	23	44
				kg N ha^{-1}			
Peanut	N0¶	4.3	13.3	72.4	4.0	23.9	83.3
	N1	5.1	14.8	91.0	4.0	23.9	101.8
Soybean	N0	3.5	17.5	73.7	3.5	25.6	70.5
	N1	4.0	19.7	84.6	3.5	25.7	87.2
Common bean	N0	2.9	12.2	58.4	3.3	29.3	44.7
	N1	4.0	15.1	74.2	3.3	32.8	76.0
LSD§		0.3	1.6	6.8			

¶ N0, N1 refer to an application of 0 or 225 kg N ha^{-1}.
§ LSD between N treatments.

Table 4.9 Comparison between observed and CROPGRO predicted values for grain yield and harvest index (HI) of three legumes grown with two levels of applied nitrogen.

	N levels	Observed		CROPGRO	
		HI	Grain	HI	Grain
		g g^{-1}	kg ha^{-1}	g g^{-1}	kg ha^{-1}
Peanut	N0¶	0.48	6.5	0.30	3.8
	N1	0.48	6.7	0.39	5.2
Soybean	N0	0.63	3.8	0.39	2.7
	N1	0.63	4.3	0.65	4.5
Common bean	N0	0.47	3.0	0.53	3.7
	N1	0.48	3.4	0.64	4.8
LSD§		0.01	0.3		

¶ N0, N1 refer to an application of 0 or 225 kg N ha^{-1}.
§ LSD between N treatments.

Chapter 5

SUMMARY AND CONCLUSIONS

The fraction of total legume nitrogen (N) derived from the soil or the atmosphere (dinitrogen fixation) is determined by the level of crop N demand and the level of available soil N. With an increase in soil N, the quantity of crop N derived from N_2 fixation will decline. Solution culture studies of legume roots obtaining N via nodules or solution found mineral N assimilation had less respiratory cost than N_2 fixation. Thus, a reduction in N_2 fixation should result in more photosynthate available for shoot growth and N accumulation. The literature, however, reports mixed results of the effect of applied N on legume growth and grain yield.

Nutrient solution and potted soil studies have demonstrated that changes in root mass and root morphology result in greater uptake of N in plants less dependent on N_2 fixation. There are, however, no comprehensive field studies testing the whole plant mechanism as to why there is greater DM and N accumulation in legumes less dependent on N_2 fixation.

Two experiments, the first in 1991 and the second in 1993, were designed to test whether:

1. increased dry matter (DM) partitioning to legume leaves is the primary factor determining increased shoot weight in plants less reliant on N_2 fixation.
2. suppression of N_2 fixation by application of N would result in increased root mass, root surface area and efficiency of N assimilation per gram of root.

3. the nature of legume response is similar across species.

Detailed field data of legume response to N application could be used to improve the precision of crop simulation models such as CROPGRO V3.0. Simulation models predict crop growth on a daily basis. Predictions are derived from a series of linked algorithms describing plant growth processes and the environment. These algorithms are based on theory generated by both field and greenhouse experiments. The data collected and reported in this thesis were used to test the ability of CROPGRO to simulate legume growth under different soil N conditions.

In 1991, soybeans were grown at three levels of applied N: 0 (N0), 256 (N2) and 430 (N2) kg N ha^{-1} and sampled at 22 and 51 days after emergence (DAE). In 1993, three legumes soybean: (*Glycine max* Merr. (L) cv. Clark), peanut (*Arachis hypogaea* cv. Pronto) and common bean (*Phaseolus vulgaris* cv. Porillo Sintetico), were grown at two levels of applied soil N: 0 (N0) and 225 (N1) kg N ha^{-1}. Plant samples were collected at 10, 23 and 44 DAE. Weights of shoots, roots and nodules were estimated at each sampling time. Samples collected were analyzed for N concentration and root length was determined. Site characteristics and management regimes such as: irrigation, weather, soil characteristics and cultivars were entered into the CROPGRO model to generate predicted values. Model parameters determining phonology were adjusted so that observed and predicted days to seed emergence and first flower where the same.

Nitrogen was added to the soil to alter the relative dependence on N_2 fixation for N accumulation. Estimation of N_2 fixation using the difference method showed that application of fertilizer nitrogen at regular intervals resulted in a suppression of N_2 fixation, compared to unfertilized plots, throughout the duration of the experiments. Although magnitudes varied, DM and N accumulation of all species increased with fertilizer N. Plants less dependent on N_2 fixation had greater grain yield and above ground DM at harvest maturity. A significant increase in DM in response to applied N was measured from 10 days after emergence (DAE) and throughout crop growth. This indicates legumes were undergoing N stress prior to 10 DAE. Response of legumes to available soil N as early as 10 DAE is influential in determining differences in crop growth. At 10 DAE in 1993 and at 22 DAE in 1991, legumes partitioned a greater proportion of their total DM to leaves. Increased allocation of DM to leaves during early vegetative stages resulted in greater leaf area. The N1 treatment maintained throughout higher leaf area crop growth and resulted in greater DM accumulation by plants less dependent on N_2 fixation.

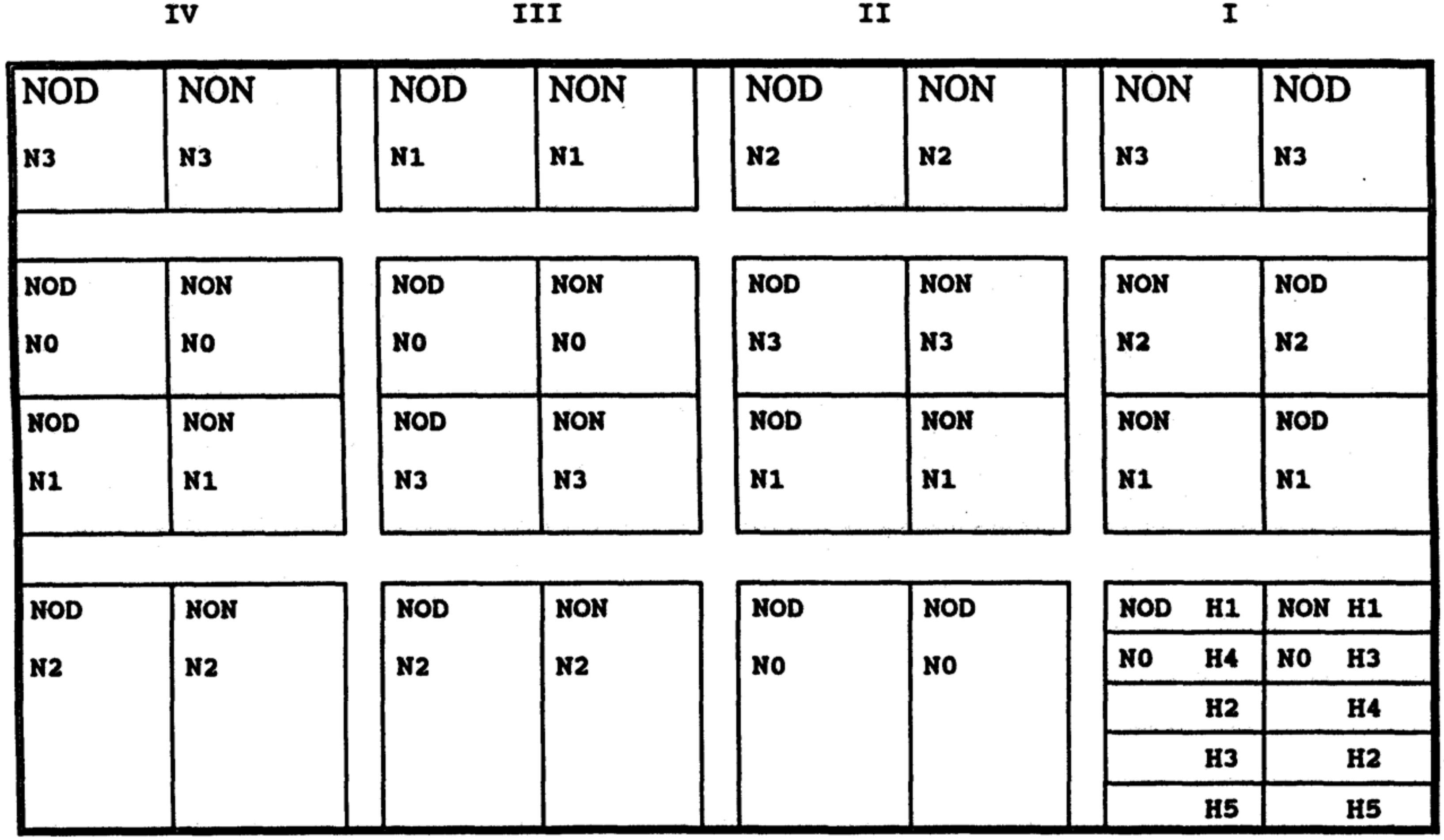
Across species and in both years, soybean plants in N1 plots had half the nodule weight of N0 plants. Thus, as N_2 fixation (and nodule weight) was suppressed, N accumulation increased by 17% compared to plants more dependent on N_2 fixation. At 10 DAE, root growth of all species in N1 plots was lower than those in N0 plots. Less root and nodule weight, at 10 DAE, in plants less dependent on N_2 fixation may account for the increased partitioning of plant DM to leaves during the early vegetative stages. For the other harvests, plants less dependent on N_2 fixation had a greater root weight than plants more dependent on N_2 fixation. Therefore increased efficiency of N accumulation per unit root weight, and increased root surface area due to greater root weight were the main factors accounting for an increase in total plant N.

Increased biomass, yield, root weight, root surface area and uptake of N, by N-fertilized plants of all three species demonstrates that growth of legumes more dependent on N_2 fixation can be limited by N assimilation. The effect of available soil N on legume growth indicates that legume simulation models should be sensitive to available soil N.

CROPGRO is a combination of three legume models: PNUTGRO (a peanut model), SOYGRO (a soybean model) and BEANGRO (a common bean model). The model does not simulate a generic legume. Selection of species and then cultivar files will input genetic information unique for the cultivar being simulated. In addition to genetic parameters the model requires input of the following: daily weather, initial soil N and moisture content, water and N application schedule and selection of a soil file containing parameters for the soil on which the crop was grown. Simulations of legume growth by CROPGRO is responsive to soil N. CROPGRO simulates N assimilation from three sources for legume growth: soil N uptake, remobilization and N_2 fixation.

A review of several CROPGRO simulations showed that the model estimates 19% more N accumulation with an application of N. There was a decline in simulated N_2 fixation and nodule weight with N application, similar to field data. Simulated shoot and root DM accumulation, unlike field data, was unaffected or lower when N was applied. Grain yield, however, increased with N application primarily as result of greater harvest index (HI) compared to the unfertilized plots. No change in HI was observed with N application in the three field grown legumes. Thus, CROPGRO needs to be modified to increase DM production with an increase in N availability and accumulation. CROPGRO coefficients need to be changed so that leaf area and subsequent DM accumulation increase with N availability. Greater leaf area, and leaf weight, should be due to more partitioning of DM to leaves with an application of N starting from the early vegetative stages. Greater yield should be simulated as a result of greater DM accumulation with the same harvest index as legumes simulated at native levels of soil N.

Appendix 1 Experimental design for the field experiment conducted in 1991. Split-split-block, each level was randomly assigned: Soybean nodulating (NOD) and non-nodulating (NON) isolines were assigned to main plots; nitrogen treatments (N0, N1, N2 and N3) were assigned to sub-plots; harvests (H) were sub-sub-plots.



IV		III		II		I	
NOD N3	NON N3	NOD N1	NON N1	NOD N2	NON N2	NON N3	NOD N3
NOD N0	NON N0	NOD N0	NON N0	NOD N3	NON N3	NON N2	NOD N2
NOD N1	NON N1	NOD N3	NON N3	NOD N1	NON N1	NON N1	NOD N1
NOD N2	NON N2	NOD N2	NON N2	NOD N0	NOD N0	NOD H1 N0 H4 H2 H3 H5	NON H1 N0 H3 H4 H2 H5

Appendix 2 Experimental design for the field trial conducted in 1993. Split-split-split-plot, each level was randomly assigned: species were main plots; inoculation (I) sub-plots; nitrogen (N) treatments sub-sub-plots; harvests (H) were sub-sub-sub-plots.

.... block III ....

GM UN0	GM UN+	AH IN0	AH IN+	PV IN0	PV IN+
GM IN+	GM IN0	AH UN+	AH UN0	PV UN+	PV UN0

.... block I ....

AH UN0	AH UN+	PV IN+	PV IN0	GM IN+	GM IN0
AH IN0	AH IN+	PV UN+	PV UN0	GM UN0	GM UN+

.... block IV ....

AH UN0	AH UN+	GM UN+	GM UN0	PV IN0	PV IN+
H2 H4 H1 H3	AH IN0	GM IN+	GM IN0	PV UN+	PV UN0

.... block II ....

AH UN0	AH UN+	GM IN+	GM IN0	PV UN+	PV UN0
AH IN0	AH IN+	GM UN+	GM UN0	PV IN+	PV IN0

AH- *Arachis hypogaea*
GM- *Glycine max*
PV- *Phaseolus vulgaris*

Appendix 3. Photosynthesis of the most recent fully expanded soybean leaf at R3 (31 and 32 days after emergence) grown in Maui, Hawaii in 1991.

N levels kg N ha^{-1}	Nodulating	Non-nodulating
	μM CO_2 m^{-2} s^{-1}	
0	19.9	14.2
256	17.9	20.3
430	19.7	18.7
856	17.1	18.1
F-test	NS	NS

Appendix 4 The effect of applied nitrogen on unit leaf rate (Hunt, 1978) of nodulating soybeans at different growth stages grown in Maui, Hawaii 1991.

N level	Days after emergence			
	10-22	22-30	30-39	39-51
		$g\ m^{-2}\ d^{-1}$		
N091¶	5.84	5.01	4.73	3.89
N191	6.88	5.08	4.13	3.44
N291	5.90	5.08	4.47	3.20
F-test	NS	NS	NS	NS

¶ N0, N1, N2 refer to an application of 0, 256 and 439 kg N ha^{-1}, respectively.

Appendix 5 The analysis of variance table for the determination of the effects of nitrogen application on the percent of nitrogen derived from the atmosphere (%Ndfa), shoot weight and fraction of dry matter allocated to leaves (DMLF) for field experiments conducted in 1991. The experimental design was a split-split-block: nodulating and non-nodulating isolines of soybean (*Glycine max* cv. Clark) were assigned to main plots; nitrogen treatments (0, 256, 430 and 856 kg N ha^{-1}) were assigned to sub-plots; harvest were sub-sub- plots. Only the nodulating soybeans were analyzed.

	%Ndfa			ln Shoot weight			DMLF		
Source	d.f.	M.S.	P	d.f.	M.S.	P	d.f.	M.S.	P
Rep	3	1100		3	0.65		3	0.04	
N	3	8674	0.0001	3	0.76	0.0014	2	0.39	0.0044
Ea	9	326		9	0.06		6	0.03	
H	4	827	0.0115	4	36.78	0.0001	1	22.84	0.0001
Eb	12	48		12	0.01		3	0.02	
N*H	8	1087		12	0.01	0.7544	2	0.17	0.1256
Ec	24	202	0.0006	36	0.49		6	0.06	

Appendix 6 The analysis of variance table for the determination of the effects of nitrogen application and time on the percent of nitrogen derived from the atmosphere (%ndfa), shoot weight and fraction of dry matter allocated to leaves (DMLF)for field experiments conducted in 1993. The experimental design was a split-split-split-plot, each treatment level was randomly assigned: species (S; peanut, soybean and common bean) were randomly to main plots; inoculation was assigned to sub-plots; nitrogen treatments (N) to sub-sub-plots; harvests (H) were sub-sub-sub-plots. Only the inoculated treatments were included in the analysis presented.

	%Ndfa			Shoot weight			DMLF		
source	d.f.	M.S.	P	d.f.	M.S.	P	d.f.	M.S.	P
Rep	3	782.21		3	0.03		3	0.67	
S	2	1273.85	0.0671	2	0.78	0.0001	2	80.04	0.0001
Ea	6	326.04		6	0.01		6	0.50	
N	1	3295.49	0.0299	1	0.35	0.0004	1	2.29	0.0064
N*S	2	392.99	0.5511	2	0.02	0.2036	2	0.39	0.1651
Eb	9	471.98		9	0.01		9	0.62	
H	2	3885.32	0.0072	2	70.51	0.0001	2	56.90	0.0001
Ec	6	264.87		6	0.01		6	1.28	
H*S	4	701.56	0.0591	4	0.22	0.0001	4	16.76	0.0001
H*N	2	162.04	0.5581	2	0.02	0.0766	2	3.10	0.0067
H*N*S	4	219.76	0.5308	4	0.002	0.7941	4	1.14	0.0938
Ed	28	272.07		30	0.001		29	0.52	

Appendix 7 The analysis of variance table for the determination of the effects of nitrogen application and time on root weight, root surface area (RSA) and the uptake of N per gram dry matter below ground per day (NE)for field experiments conducted in 1991. The experimental design was a split-split-block: nodulating and non-nodulating isolines of soybean (*Glycine max* cv. Clark) were assigned to main plots; nitrogen treatments (0, 256, 430 and 856 kg N ha^{-1}) were assigned to sub-plots; harvest were sub-sub- plots. Only the nodulating soybeans were analyzed.

	Ln root weight			Ln RSA			NE		
Source	d.f.	M.S.	P	d.f.	M.S.	P	d.f.	M.S.	P
Rep	3	0.02		3	0.04		3	2.73	
N	2	0.11	0.0484	2	0.23	0.0587	2	24.01	0.0008
Ea	6	0.02		6	0.05		6	0.81	
H	1	13.86	0.001	1	11.18	0.0001	1	29.22	0.0035
Eb	3	0.02		3	0.01		3	0.41	
N*H	2	0.05	0.0617	2	0.01	0.7036	2	6.62	0.0492
Ec	6	0.01		6	0.03		6	1.28	

Appendix 8 The analysis of variance table for the determination of the effects of nitrogen application and time on root weight, root surface area (RSA) and the uptake of N per gram of below ground dry matter per day (NE) for field experiments conducted in 1993. The experimental design was a split-split-split-plot, each treatment level was randomly assigned: species (S; peanut, soybean and common bean) were randomly to main plots; inoculation was assigned to sub-plots; nitrogen treatments (N) to sub-sub-plots; harvests (H) were sub-sub-sub-plots. Only the inoculated treatments were included in the analysis presented.

	Ln root weight			Ln RSA			NE		
source	d.f.	M.S.	P	d.f.	M.S.	P	d.f.	M.S.	P
Rep	3	0.06		3	0.08		3	0.23	
S	2	1.36	0.0003	2	0.37	0.0172	2	2.64	0.0277
Ea	6	0.68		6	0.09		6	0.47	
N	1	0.10	0.4014	1	0.12	0.3506	1	6.19	0.0001
N*S	2	0.04	0.9593	2	0.03	0.9886	2	0.36	0.0463
Eb	9	0.11		9	0.11		9	0.12	
H	2	35.54	0.0001	2	38.43	0.0001	2	49.45	0.0001
Ec	6	0.06		6	0.07		6	0.06	
H*S	4	0.57	0.001	4	0.40	0.0005	4	1.19	0.0001
H*N	2	0.24	0.007	2	0.44	0.0024	2	3.52	0.0001
H*N*S	4	0.03	0.3569	4	0.05	0.5257	4	0.49	0.0021
Ed	29	0.03		29	0.06		38	0.09	

Appendix 9 The effect of nitrogen application and time on the weights of:leaves, stems, cotyledons, and pods of non-nodulating soybean grown in Maui, Hawaii in 1991.

Variable	N levels	Days after emergence				
		10	22	30	39	51
				kg ha^{-1}		
Leaf	N0¶	51	238	509	912	1067
	N1	68	462	970	1454	1693
	N2	61	454	962	1539	2037
	N3	66	437	925	1648	2053
F-test	N	**	***	***	***	***
Stem	N0	23	137	453	945	1121
	N1	29	281	911	1883	2581
	N2	26	291	861	1905	2971
	N3	27	267	780	1942	2844
F-test	N	*	***	***	***	***
Cotyledon	N0	19.8	14.7	-#	-	-
	N1	24.5	15.6	-	-	-
	N2	22.1	14.2	-	-	-
	N3	22.8	15.5	-	-	-
F-test	N	**	NS			
Pod	N0	-	-	-	57	600
	N1	-	-	-	98	1024
	N2	-	-	-	994	1023
	N3	-	-	-	118	1155
F-test	N				**	**

¶ N0, N1, N2, N3 refer to an application of 0. 256, 430 and 856 kg N ha^{-1}, respectively.

Not determined or not measurable.

Appendix 10 The effect of nitrogen application and time on the nitrogen concentration of:leaves, stems, cotyledons, and pods of non-nodulating soybean grown in Maui, Hawaii, in 1991.

Variable	N levels	Days after emergence				
		10	22	30	39	51
				%		
Leaf	N0¶	4.31	4.47	3.87	2.88	5.23
	N1	6.49	6.44	6.34	5.50	5.70
	N2	6.87	6.85	6.76	6.65	5.98
F-test	N	***	***	***	***	***
Stem	N0	2.48	1.99	1.61	1.05	0.73
	N1	4.72	3.92	3.26	2.08	1.78
	N2	5.34	4.50	3.89	3.56	2.92
F-test	N	***	***	***	***	***
Cotyledon	N0	4.22	2.20	-≠	-	-
	N1	6.59	3.91	-	-	-
	N2	7.11	4.12	-	-	-
F-test	N	***	***	-	-	-
Pod	N0	-	-	-	3.12	2.38
	N1	-	-	-	4.08	3.83
	N2	-	-	-	5.03	4.38
F-test	N				***	***

¶ N0, N1, N2, refer to an application of 0. 256, and 430 kg N ha^{-1}, respectively.

≠ Not determined or not measurable.

Appendix 11 The effect of nitrogen application and time on the weights and nitrogen concentration of: fine roots and root crowns of non-nodulating and nodulating soybean grown in Maui, Hawaii, in 1991.

Variable	N levels	Days after emergence			
		Weight		N concentration	
		22	51	22	51
		Kg ha^{-1}		%	
NON-NODULATING					
Fine Roots	N0¶	66	455	-#	-
	N1	100	410	-	-
	N2	-	322	-	-
	N3	63	-	-	-
F-test	N	*	NS		
Root crowns	N0	-	429	1.40	0.53
	N1	-	527	1.99	0.78
	N2	-	485	2.71	1.11
F-test	N		NS	***	***
NODULATING					
Fine root	N2	-	274	-	-
	N3	58	-	-	-
Root crown	N2	-	483	-	1.23
	N3	83	-	2.61	-

¶ N0, N1, N2, N3 refer to an application of 0. 256, 430 and 856 kg N ha^{-1}, respectively.

Not determined or not measurable.

Appendix 12 The effect of nitrogen application and time on above ground dry matter (DM) accumulation and leaf area index (LAI) of non-nodulating soybean grown in Maui, Hawaii, in 1991.

Variable	N levels	Days after emergence				
		10	22	30	39	51
				kg ha^{-1}		
DM.	N0¶	93	390	963	1914	2788
	N1	122	759	1881	3434	5297
	N2	108	759	1823	3539	6032
	N3	116	720	1795	3708	6051
F-test	N	**	***	***	***	***
				m^2 m^{-2}		
LAI	N0	0.17	0.82	1.83	2.59	2.45
	N1	0.21	1.44	3.91	5.43	5.55
	N2	0.18	1.49	3.64	6.51	6.58
	N3	0.21	1.35	3.44	6.50	6.70
F-test	N	*	**	***	***	**

¶ N0, N1, N2, N3 refer to an application of 0. 256, 430 and 856 kg N ha^{-1}, respectively.

Appendix 13 The effect of nitrogen application and time on above ground dry matter (DM) accumulation, the weight of leaves, stems cotyledons and pods, and leaf area index (LAI) of modulating soybean grown in Maui, Hawaii, in 1991.

Variable	N levels	Days after emergence				
		10	22	30	39	51
				kg ha^{-1}		
DM	N2¶	118	714	1747	3502	5531
	N3	115	684	1563	3420	5608
Leaf	N2	63	440	920	1466	1741
	N3	64	385	855	1525	1936
Stem	N2	30	258	823	1909	2539
	N3	28	233	708	1740	2510
Cotyledon	N2	25	15	-	-	-
	N3	19	16	-	-	-
Pod	N2	-	-	-	127	1250
	N3	-	-	-	156	1162
				m^2 m^{-2}		
LAI	N2	0.20	1.45	3.51	5.45	5.54
	N3	0.21	1.17	2.96	5.24	6.10

¶ N2, N3 refer to an application of 430 or856 kg N ha^{-1}, respectively.

Appendix 14 The effect of nitrogen application and time on the weight of plant parts of three uninoculated legumes grown in Maui, Hawaii, in 1993.

Variable	Species	N levels	Days after emergence		
			10	23	44
				kg ha^{-1}	
Leaf	Peanut	N0	82	284	1269
		N1	80	335	1573
	Soybean	N0	54	288	920
		N1	61	305	1407
	Common bean	N0	57	246	995
		N1	69	351	1506
F-test	Species (S)		**	NS	**
	Nitrogen (N)		NS	**	***
	S X N		NS	NS	NS
Stem	Peanut	N0	51	210	1507
		N1	46	254	1897
	Soybean	N0	25	211	1171
		N1	29	234	2038
	Common bean	N0	17	129	1076
		N1	19	207	1933
F-test	Species (S)		***	**	NS
	Nitrogen (N)		NS	**	***
	S X N		NS	NS	*
Cotyledon or pods at 44 DAE	Peanut	N0	24	25	166
		N1	22	20	204
	Soybean	N0	21	14	336
		N1	22	10	410
	Common bean	N0	-#	-	69
		N1	-	-	214
F-test	Species (S)		NS	**	***
	Nitrogen (N)		NS	*	**
	S X N		NS	NS	NS

¶ N0,N1 refer to an application of 0 or 275 kg N ha^{-1}.

Not determined or not measurable.

Appendix 15 The effect of nitrogen application and time on nitrogen concentration of plant parts of three uninoculated legumes grown in Maui, Hawaii, in 1993.

Variable	Species	N levels	Days after emergence		
			10	23	44
				%	
Leaf	Peanut	N0	5.30	3.61	4.50
		N1	6.13	4.18	4.98
	Soybean	N0	5.99	4.83	3.61
		N1	6.54	4.96	5.70
	Common bean	N0	5.36	1.25	3.70
		N1	6.31	1.74	4.57
F-test	Species (S)		**	***	*
	Nitrogen (N)		***	**	***
	S X N		NS	NS	**
Stem	Peanut	N0	3.14	1.99	1.83
		N1	3.84	2.13	2.14
	Soybean	N0	4.07	1.70	0.95
		N1	4.90	1.84	1.83
	Common bean	N0	3.19	1.25	1.18
		N1	4.98	1.74	1.54
F-test	Species (S)		***	**	***
	Nitrogen (N)		***	*	***
	S X N		**	NS	***
Cotyledon or pods at 44 DAE	Peanut	N0	2.26	1.84	4.65
		N1	2.57	2.02	4.56
	Soybean	N0	5.61	1.65	2.99
		N1	6.37	1.79	4.01
	Common bean	N0	-#	-	4.18
		N1	-	-	4.56
F-test	Species (S)		***	**	***
	Nitrogen (N)		**	*	**
	S X N		NS	NS	**

¶ N0,N1 refer to an application of 0 or 275 kg N ha^{-1}.
# Not determined or not measurable.

Appendix 16 The effect of nitrogen application and time on the weight of fine roots and root crowns of three uninoculated legumes grown in Maui, Hawaii, in 1993.

Variable	Species	N levels	Days after emergence		
			10	23	44
				kg ha^{-1}	
Fine roots	Peanut	N0	18	91	258
		N1	25	133	292
	Soybean	N0	25	179	446
		N1	29	182	416
	Common bean	N0	12	103	323
		N1	9	146	409
F-test	Species (S)		NS	**	**
	Nitrogen (N)		NS	*	NS
	S X N		NS	NS	NS
Root crowns	Peanut	N0	22	30	62
		N1	21	32	73
	Soybean	N0	25	121	276
		N1	29	128	323
	Common bean	N0	14	55	169
		N1	15	69	252
F-test	Species (S)		***	***	***
	Nitrogen (N)		NS	NS	**
	S X N		NS	NS	NS

¶ N0,N1 refer to an application of 0 or 275 kg N ha^{-1}.

Appendix 17 The effect of nitrogen application and time on the nitrogen concentration of fine roots and root crowns of three uninoculated legumes grown in Maui, Hawaii, in 1993.

Variable	Species	N levels	Days after emergence		
			10	23	44
				%	
Fine roots	Peanut	N0	3.61	2.64	3.65
		N1	3.61	2.66	3.71
	Soybean	N0	3.77	2.87	3.55
		N1	3.77	2.97	3.66
	Common bean	N0	4.94	3.06	4.11
		N1	4.94	6.21	3.87
F-test	Species (S)		–§	***	*
	Nitrogen (N)		–	NS	NS
	S X N		–	NS	NS
Root crowns	Peanut	N0	3.26	2.20	1.77
		N1	3.57	2.29	1.84
	Soybean	N0	3.10	1.31	0.89
		N1	3.71	1.47	1.04
	Common bean	N0	2.60	1.38	1.06
		N1	3.64	1.65	1.21
F-test	Species (S)		**	***	***
	Nitrogen (N)		***	*	**
	S X N		**	NS	NS

¶ N0,N1 refer to an application of 0 or 275 kg N ha^{-1}.

§ Samples were pooled across blocks.

Appendix 18 The effect of nitrogen application and time on the above ground dry matter and leaf area index (LAI) of three uninoculated legumes grown in Maui, Hawaii, in 1993.

Variable	Species	N levels	Days after emergence		
			10	23	44
				kg ha^{-1}	
DM	Peanut	N0	157	519	2942
		N1	149	609	3673
	Soybean	N0	100	513	2428
		N1	112	548	3855
	Common bean	N0	73	375	2140
		N1	88	557	3653
F-test	Species (S)		***	*	*
	Nitrogen (N)		NS	**	***
	S X N		NS	*	*
LAI	Peanut	N0	0.24	0.77	4.01
		N1	0.24	0.93	4.72
	Soybean	N0	0.23	1.06	2.99
		N1	0.25	1.10	5.32
	Common bean	N0	0.22	0.76	4.00
		N1	0.25	1.12	6.80
F-test	Species (S)				
	Nitrogen (N)				
	S X N				

¶ N0,N1 refer to an application of 0 or 275 kg N ha^{-1}.
\# Not determined or not measurable.

Appendix 19 The effect of time on total non-structural carbohydrate'(TNC) concentration in different plant parts of nodulating soybean plants grown with native soil nitrogen in Maui, Hawaii, in 1993.

N levels	Days after emergence								
	10		22			51			
	Leaf	Stem	Leaf	Stem	Root	Leaf	Stem	Root	Pod
				%					
N0	13.9	8.8	5.9	6.1	6.1	5.3	7.3	4.5	7.7
N1	6.8	6.1	6.4	6.7	5.8	4.1	5.6	1.2	12.0
N2	6.5	5.5	4.2	6.4	4.0	3.2	5.9	4.0	7.9
F-test	*	NS	NS	NS	*	NS	NS	**	*

N0, N1 and N2 refer to an application of 0, 256 and 430 kg n ha^{-1}.

TNC concentration was determined using the method described by Christiansen (1982). Finely ground oven dried samples of 100 mg were weighed out into 50 ml test tubes. To this 5 mls of water was added, stoppered and emersed in a 100°c bath for 10 minutes. After cooling, 5 mls of 0.2 M acetate buffer was added plus 1 ml of enzyme mix (distilled water was used in the blanks). The mixture was then placed in a shaking water bath (48°C) for 16 hours. On cooling the samples were filtered. From the filtrate 0.5 to 1 ml aliquotes plus 1 ml alkaline reagent were added to 50 ml test tubes and placed in a boiling water bath for 20 minutes. The mixture was cooled and 1 ml of arsenomolybdate was added and diluted, with distilled water, to 10 mls. The samples were vortexed before optical density was measured using a spectrophotometer set at 540 nm. Description of the various solutions used in this analysis are given in the following pages.

The enzyme mix contained the following: 45 mls distilled water, 5 mls of 0.1 M acetate buffer, 2.5 mls invertase concentrate, 1.25 g amyloglucosidase and 0.1 g thymol.

The alkaline reagent contained the following: 25 g anhydrous sodium carbonate, 25 g potassium sodium tartrate, 20 g sodium bicarbonate and 200 g anhydrous sodium sulfate in 700 ml distilled water. Then 6 g of cupric sulfate pentahydrate was dissolved in 40 ml distilled water followed by one drop of concentrated sulfuric acid. The two solutions were combined.

The arsenomolybdate solution contained the following: 25 g of ammonium molybdate tetrahydrate in 450 ml distilled water, then 21 mls of concentrated sulfuric acid while stirring. Then 3 g of sodium arsenate was dissolved in 25 mls of distilled water. The solutions were combined in a brown bottle and incubated at 37^0C for 24 hours.

The acetate buffer was made from the following two solutions, 0.2 M acetic acid and 0.2 M sodium acetate. For the acetic acid solution, 11.6 ml of glacial acetic acid was added to 500 ml of distilled water, cooled and water added to make up 1 liter. For the sodium acetate solution, 16.4 g of anhydrous sodium acetate was dissolved in a liter of distilled water.

For the 0.2 M buffer, 300 ml of 0.2 N acetic acid was combined with 200 ml sodium acetate and adjusted to pH 4.5 using either stock solution. The solution was kept refrigerated. To make the 0.1 M acetate buffer the 0.2 M solution was appropriately diluted.

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