GROWTH AND YIELD RESPONSES OF *Glycine max* and *Phaseolus vulgaris* TO MODE OF NITROGEN NUTRITION AND TEMPERATURE CHANGES WITH ELEVATION

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF
THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN AGRONOMY AND SOIL SCIENCE

DECEMBER 1988

BY

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ACKNOWLEDGEMENTS

My sincere gratitude goes to Dr. Duane P. Bartholomew, Dr. B. Ben Bohlool and Dr. Paul W. Singleton for their constant guidance, constructive criticisms and spirited support during this research. I am especially appreciative of the help given by Dr. Singleton and Dr. Bartholomew in the preparation of the manuscript. I am grateful to Dr. James A. Silva and Dr. Francoise M. Robert for their helpful suggestions and advice.

I would like to acknowledge Kevin Keane, Rick Koglin, Goeff Haines, Brian Duffy and Thomas Walker for their assistance in the field, the NifTAL Project staff for their untiring help, Robert Abaidoo for his participation in a part of this research.

Lastly, my heartfelt thanks goes to Maria Luz Caces, my best friend.

This research was supported in part by the U.S. Agency for International Development grants DAN-1406-G-SS-4081-00 (Indo/US Initiative on Science and Technology) and DAN-0613-C-00-2064-00 (NifTAL Project), National Science Foundation/Ecology Program, and the International Atomic Energy Agency.

ABSTRACT

Efficient exploitation of the legume-*Rhizobium* symbiosis in varied environments requires an understanding of the responses of both the host and the bacteria to management and environmental variables. The objective of this research was to study the growth, development and yield responses of soybean (*Glycine max* L. Merrill) and common bean (*Phaseolus vulgaris* L.) to mode of N nutrition and temperature at sites in an elevational transect differing in mean temperatures. In the first experiment five soybean varieties and their non-nodulating isolines from four maturity groups were grown after inoculating with three *Bradyrhizobium japonicum* strains. The proportion of nodules formed by each *B. japonicum* strain was not affected significantly by temperature, soil type, or soybean genotype. Soybean grain yield and total plant N decreased with increasing elevation. At a given site, the proportion of total plant N derived from symbiotic N_2 fixation was approximately constant. Between sites, the proportion of fixed N was inversely related to soil N availability. Vegetative and total crop durations were prolonged by cool temperature. Maturity of soybean was hastened by N insufficiency. In a second experiment, inoculated and uninoculated soybean and common bean were grown with either 9, 120, or 900 kg N ha^{-1}. As a result of extended crop durations at the higher elevation, total biomass production was similar between elevations in both legumes. Grain yield of common bean was similar at the cool and warm sites; in contrast the grain yield of soybean was greatly reduced at the cool site. Yield and total plant N of both legumes increased at mineral N levels of 120 and 900 kg ha^{-1} compared to 9 kg N ha^{-1}. Significant

symbiotic N_2 fixation limited yield and total N assimilation in soybean. Common bean utilized proportionately more mineral N than soybean throughout growth and had a higher fertilizer N use efficiency than soybean during the vegetative phase. Common bean also had a lower N requirement and N assimilation was more uniform throughout growth when compared to soybean. Maximizing N derived from fixation in common bean may require altering its N assimilation characteristics.

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Chapter 1

INTRODUCTION

Legumes respond to the environment in the same way other plants do, but what makes them physiologically distinct from other plants is their ability to harbor nitrogen-fixing rhizobia in a symbiotic relationship. The establishment of the legume-*Rhizobium* symbiosis starts with the infection of the legume host by compatible rhizobia, resulting in the formation of nodules.

Both symbiotic N_2 fixation and mineral N assimilation contributes to the N requirement of legumes. The symbiosis is functional when the legume is stressed for nitrogen and, to some degree, compensates for decreased mineral N availability. However, mineral nitrogen suppresses symbiotic N_2 fixation (Allos and Bartholomew, 1959; Chen and Phillips, 1977; Dart and Wildon, 1970; Oghoghorie and Pate, 1971). While N_2 fixation is readily replaced by mineral N, evidence presented by Gibson and Pagan (1977), Manhart and Wong (1980), and Streeter (1985) do not show any direct inhibitory effect of mineral N on N_2 fixation.

The general consensus regarding the N nutrition of legumes is that legumes require N from both symbiotic and mineral N sources for maximum yield (Bhangoo and Albritton, 1976; Harper, 1974). However, there are indications that legumes relying on mineral N produce yields similar to or greater than symbiotic plants (Kato et al. 1984; Ryle et al. 1978; Silsbury, 1977). Schweitzer and Harper (1985) found that increasing light interception by a soybean canopy using reflectors increased nitrate reductase activity in nitrate-supplied plants more than it increased nodule activity in inoculated plants, and the nitrate-supplied plants consequently produced greater dry weight. Also, Tanaka (1986)

showed that if photosynthetic activity decreased, N_2 fixation decreases more than mineral N absorption. Accordingly, Mahon and Child (1979) argued that it is difficult to increase N_2 fixation and the productivity of legumes simultaneously. Salsac et al., (1984) also concluded that plants entirely or partly dependent on mineral N had yields higher than symbiotic plants. The assimilation of mineral nitrogen does not involve the maintenance of specialized structures like root nodules as in the symbiotic plant, so it is a more energy efficient source of N. Finke et al. (1982) concluded that dependence of the plant solely on N_2 fixation is an energy expensive process.

If there is sufficient N in the soil to meet the plant's requirement, and if assimilation from the soil is not limited by other factor(s), then symbiotically fixed N will not be a major source of N for the plant. Any factor which limits the availability, uptake or assimilation of soil N should favor N_2 fixation. Low temperature is one factor which reduces the rate of soil N mineralization (Stanford et al., 1973; Cassmann and Munns, 1980), and uptake of available soil nitrogen (McDuff and Hopper, 1986; Tolley and Raper, 1985). Low temperature, therefore, may favor N_2-fixation over mineral N assimilation unless it decreases nitrogenase activity to the same degree that soil N assimilation is decreased.

Nitrogen nutrition presumably can range from complete dependence on mineral N to complete dependence on symbiotic N_2 fixation. Plants dependent on N_2 fixation or mineral N may respond differently to the environment because of the differences in their root morphology, N

assimilation process, and N nutritional level. In the case of the symbiotic plant, the response may be further complicated by specific interactions between the legume host and the rhizobial microsymbiont. The interaction between the legume host and the rhizobial microsymbiont is specific, and the compatibility of the host with any one of the competing rhizobial strains may determine whether or not a productive symbiosis will be established. Thus, any attempt to correlate legume response to environment requires careful consideration of the mode of N nutrition and the N requirement of the plant.

Developing a holistic approach to legume productivity research requires the evaluation of the legume-rhizobium system from soil, plant, and microbial perspectives in the field. Such an approach would render the results more relevant to productivity research and improve the degree of predictability of outcomes in varied environments.

Considering the complexity of environmental factors in agricultural ecosystems, the delineation of effects of single factors is often difficult. Although management variables may be made optimum and thus, eliminated as sources of variation, the effects of environmental variables such as temperature, photoperiod and irradiance are often confounded.

Temperature has direct and indirect effects on biological or biochemical processes and in legumes, these responses are readily modified by photoperiod (Salisbury, 1981). The field evaluation of temperature effects is simplified when the confounding effects of irradiance and photoperiod are minimal. Sites in an elevational

transect may permit this kind of evaluation if changes in irradiance with elevation are small.

Two experiments were conducted to study the effects of mode of N nutrition, temperature and other elevation-associated variables on the ecophysiological response of soybean and common bean. The approach taken was to study host-*Rhizobium*-environment interactions and plant genotype-temperature-mode of nitrogen nutrition interactions in soybeans adapted to a wide range of latitudes, and use the data thus obtained as the basis for a second experiment to evaluate the growth and yield responses of soybean and common bean to temperature under a range of mineral N availability conditions.

The Objectives of the experiments were:

1. Determine the effects of elevation-associated site characteristics (temperature, soil type) and soybean genotype on the competition pattern of *Bradyrhizobium japonicum* strains.
2. Determine the effects of temperature and mode of N nutrition on the phenology of a range of soybean maturity groups under similar photoperiodic regimes.
3. Assess the effects of site characteristics and soybean genotype on the contribution of N_2 fixation to plant N.
4. Evaluate the growth responses of soybean and common bean to cool and warm environments at varying levels of soil N availability.
5. Evaluate the responses of soybean and common bean to mineral N application and contrast the N assimilation patterns of the two legumes.

Chapter 2

NODULATION AND INTERSTRAIN COMPETITION BY *Bradyrhizobium japonicum* IN SOILS ALONG AN ELEVATIONAL TRANSECT

Abstract

A better understanding of the ecology of *Bradyrhizobium japonicum* in relation to its host and its environment may aid in making appropriate management decisions for soybean production in a wide range of conditions. Success of one or more of the competing rhizobial strains in forming nodules and establishing an effective symbiosis is influenced by host genotype, soil type and temperature. The effects of temperature and soil type on interstrain competition, nodulation and nitrogen assimilation in five soybean varieties belonging to maturity groups 00, IV, VI, and VIII were investigated at three sites devoid of soybean rhizobia along an elevational transect in Hawaii. The soils are classified as Humoxic Tropohumult (clayey, ferritic, isohyperthermic), Humoxic Tropohumult (clayey, oxidic, isothermic) and Entic Dystrandept (medial over loamyskeletal, isomesic) at elevations of 320, 660 and 1050 m, respectively. Sites were amended with $Ca(OH)_2$ to a pH between 5.5 and 6.0. Nutrients, except nitrogen, were provided in non-limiting amounts. Seeds were inoculated with equal numbers of *Bradyrhizobium japonicum* strains USDA 110, USDA 136b (CB 1809) and USDA 138. The mean soil/air temperatures during the experiment were 25/24, 24/22 and 22/18 C at the respective elevations. A greenhouse pot experiment was also conducted using the same soils and inoculum under a fixed temperature regime. Plant tops and nodules were sampled at 45 days after seeding. Nodule occupancy was determined by immunofluorescence. Competition patterns of the three strains were unaffected by soil type or soil

temperature. Strain USDA 110 was the best competitor, occupying on the average (5 cultivars) 81 and 64% of the nodules in the field and greenhouse experiments, respectively. Strain USDA 138 was the least successful in the field. Its nodule occupancy under greenhouse conditions, however, was significantly greater than in the field. Nodule occupancy was also related to soybean maturity group. Strain USDA 110 formed 61, 71, 88, 88, and 98% of the nodules in the field on Clay (00), Clark (IV), D68-0099 (VI), N77-4262 (VI), and Hardee (VIII), respectively. Strain USDA 136b formed very few nodules on Hardee, an Rj2 soybean variety incompatible with that strain; its nodule occupancy on all other cultivars in the field averaged about 30% (including nodules containing more than one strain). Both nodule number and weight were significantly reduced as elevation increased. Nodule number increased with increasing maturity group within a site, but nodule weight did not. Shoot dry weight and nitrogen assimilation decreased drastically with increased elevation. Nitrogen assimilation decreased from 246 mg N per plant at 320 m elevation to 26 mg N per plant at 1050 m. While soil type and temperature had no effect on strain competition, temperature had a profound influence on nodule parameters and plant growth.

Introduction

The establishment of an effective and efficient symbiosis between rhizobia and the host legume is basic to viable legume production without mineral N fertilization. The symbiosis is modified by various environmental factors. An understanding of the effects of environmental

factors on the symbiosis is therefore, essential for selecting adapted cultivars and management practices that will enhance symbiotic nitrogen fixation and yield.

Bradyrhizobium japonicum strains differ considerably in competitive ability, nodulation and nitrogen fixation (Abel and Erdman, 1964; Caldwell, 1969; Johnson and Means, 1964). The competition and nodulation characteristics of the strains have been shown to be influenced by the host genotype (Caldwell and Vest, 1968; Materon and Vincent, 1980).

Soil type may also influence the competition between strains for nodule sites. Dominance of different *B. japonicum* strains in soybean nodules from different soils have been reported (Damirgi et al., 1967; Ham et al., 1971; Johnson and Means, 1963; Keyser et al., 1984). Soil type greatly influenced the distribution of strains, with distinctly different populations of rhizobia in soybean nodules from six different soils (Damirgi et al., 1967). According to Ham et al. (1971), the presence of individual strains in soybean nodules was related to two or more of the soil properties, the most important of which was soil pH. Kosslak and Bohlool (1985) have reported that in soils devoid of native *B. japonicum*, the pattern of competition between two introduced strains remained the same regardless of soil type or soil amendments.

The recovery of specific rhizobial strains in the nodules, and nodulation and N_2 fixation are also related to temperature. Caldwell and Weber (1970) found that the dominance of different serogroups in soybean nodules was altered by planting dates, and Weber and Miller

(1972) concluded that the effect was due to differences in soil temperature. Low soil temperatures have also been shown to adversely affect both nodulation and nitrogen fixation (Lindeman and Ham, 1979; Waughman, 1977).

To understand the ecology of rhizobia in relation to the host and its environment, experiments were conducted along an elevational transect located on the island of Maui, Hawaii (part of the Maui Soil, Climate and Land Use Network: Mauinet), which provided a field laboratory of varied edaphic and climatic (primarily temperature) conditions. The purpose of the investigation reported here was to study *B. japonicum* strain competition, nodulation and early nitrogen assimilation on selected genotypes of soybean in field environments that differ in soil type, and soil and air temperatures. Other soil variables were assumed to be controlled by equalizing pH, and applying nutrients other than N at maximum levels.

Materials and Methods

Experimental design: The treatments were five soybean varieties and three sites (elevations) of planting. All varieties were inoculated with a triple-strain inoculum consisted of three *Bradyrhizobium japonicum* strains. The experimental design was a randomized complete block with three replications at each site.

Selection of sites: Three sites along an elevational transect on the slope of Mt. Haleakala, Maui, Hawaii, having similar rainfall and irradiance, were selected. The relevant characteristics of the sites are given in Table 1. All sites were free of *B. japonicum* as determined

by the most probable number (MPN) technique using growth pouches (Weaver and Frederick, 1972).

Soil amendments: The soils were limed to a pH between 5.5 to 6.0 by incorporation of $Ca(OH)_2$. Other amendments in kg ha^{-1} were 600 P as treble superphosphate, 300 K as K_2SO_4, 75 Mg as $MgSO_4$, 15 Zn as $ZnSO_4$, 2 Mo as Na_2MO_4 and 3 B as H_3BO_3.

Soybean varieties: The soybean varieties used were from four maturity groups (MG) and included Clay 00 (J.H. Orf, Agronomy and Plant Genetics, Univ. Minnesota), Clark IV (P. Cregan, USDA Nitrogen Fixation Lab., Beltsville), D68-0099 VI and N77-4262 VI, which are determinate and indeterminate selections from cv. Lee (T.E. Carter, Dept. of Crop Science, North Carolina State Univ.), and Hardee VIII (K. Hinson, Dept. of Agronomy, Univ. Florida).

Rhizobial inoculum strains: Yeast extract-mannitol (Vincent, 1970) agar slants of the serologically distinct rhizobial strains USDA 110, USDA 136b (CB 1809) and USDA 138 were obtained from the NifTAL Project collection, Paia, Hawaii (TAL strains 102, 379, and 377, respectively). Each strain was grown in yeast extract-mannitol broth at 27 C for 5 days. From each broth culture, 50 ml (approximately 5.0×10^9 cells ml^{-1}) was inoculated into 100 g gamma-irradiated peat in separate polyethylene bags and mixed thoroughly. After 14 days, plate counts were made from serial dilutions of the peat inoculants by the Miles and Misra drop plate method (Vincent, 1970). The three strains were mixed in proportions that would provide equal numbers of each strain in an inoculant that had a final population of 3.2×10^9 bacteria per g of

peat.

Seed inoculation procedure: Gum arabic (40 g per 100 ml water) was applied to seeds at the rate of 3 ml 100 g^{-1} seed. The seeds were then coated with approximately 14 mg of the prepared peat inoculum per seed. The seeds were air dried and kept at 4 C overnight. Viable counts of rhizobia at planting averaged 1.5 x 10^{7} per seed.

Plant culture: All the sites were planted on 29 July 1985. Seeds were sown in 4-row plots measuring 4 m x 2.4 m. Rows were 60 cm apart and plants in the rows were spaced to obtain a final population of 400,000 plant ha^{-1}. The fields were irrigated the day of planting to a moisture tension of 0.03 MPa (field capacity) and maintained near that tension over the course of the experiment with the aid of tensiometers.

Weather data collection.: Temperature data were recorded by Campbell Scientific CR-21 microloggers (Campbell Scientific, Inc., Logan, UT) equipped with sensing probes for temperature (Fenwal thermistor; Fenwal Electronics, Milford, MA). Rainfall was recorded using a Sierra tipping bucket raingage (Model RG2501 tipping bucket raingage; Sierra/Misco Inc., Berkeley, CA) connected to the CR-21 micrologger.

Greenhouse experiment: An experiment was conducted in a greenhouse to separate the effect of soil type from that of temperature differences associated with site on rhizobial interstrain competition and nodulation in soybean. The experimental design was a randomized complete block with the five soybean varieties used in the field study and soils from the three field sites as treatments. There were three replications. Soils were amended as before. Nutrients applied in mg kg^{-1} of soil were

300 P, 150 K, 37.5 Mg, 7.5 Zn, 1 Mo, and 1.5 B. Seven seeds for each variety coated with inoculum prepared as above were planted in 3 liter pots filled with 2 kg of soil (oven dry basis). The pots were watered daily to a tension of 0.03 MPa with the aid of tensiometers. The plants were thinned to two per pot after 10 days. The average soil temperature in the pots was 27 C.

Sampling procedures: In the field experiment, twelve plants per replication were harvested at random from the two outer rows of each plot 45 days after planting. The plant tops were cut at the soil surface, oven dried at 70 C, weighed, ground, and the nitrogen content was determined by H_2SO_4 digestion followed by indophenol blue method (Keeny and Nelson, 1982). The roots were excavated and the nodules were collected after washing the roots free of soil, counted, dried at 70 C in a forced air oven, and weighed. Nodules from the plants grown in the greenhouse were also collected and dried 45 days after planting.

Strain identification: Occupancy of nodules by the three *Bradyrhizobium* sp. strains was determined by immunofluorescence using oven dried nodules (Somasegaran et al., 1983). At least 5%, but no fewer than 24 nodules, of the composite sample from each replication for the field experiment and 12 nodules per pot for the greenhouse experiment were selected at random. The nodules were placed in the wells of microtiter plates and rehydrated in the refrigerator overnight with a few drops of sterile water. The nodules were then crushed, smears were prepared and heat fixed on glass slides. The smears were treated with gelatin-rhodamine isothiocyanate to control background fluorescence and

non-specific staining (Bohlool and Schmidt, 1968) and after drying, they were stained with strain- specific fluorescent antibodies (Schmidt et al., 1968). The slides were observed under epifluorescence microscopy for positive reactions. Positive reaction for more than one strain in the same nodule was counted as mixed infection.

Data analysis: Data from the three sites were subjected to a combined analysis (McIntosh, 1983). Nodule and plant growth data as well as arcsine transformations of percent nodule occupancy from both experiments were subjected to analysis of variance using SAS (SAS Institute. 1982. SAS user's guide: Statistics. 1982 ed. SAS Institute Inc., Cary, NC) procedures.

Results

There were significant differences in the nodule occupancy by the strains used (Table 2). USDA 110 was the most competitive strain in both experiments; it formed 81% and 64% of nodules in the field and greenhouse, respectively. Strain USDA 138, which formed few nodules in the field, occupied the same number of nodules as USDA 136b in the greenhouse experiment. Both USDA 138 and USDA 136b were present in higher proportions in the greenhouse study than the field. However, there was no significant effect of site or soil on nodule occupancy in the two experiments.

Soybean varieties significantly differed in strain occupancy under both field and greenhouse conditions (Table 3). USDA 110 formed between 61% and 98% of the nodules in the field and 55% to 86% in the greenhouse study depending on cultivar. There was a positive correlation between

maturity group and the proportion of nodules formed by USDA 110. Where nodulation by USDA 110 was reduced, increased nodulation by USDA 138 in the pot and by USDA 136b in the field was observed.

Nodule number and dry weight were significantly greater at the 320 m site and both parameters decreased with increasing elevation for all of the varieties (Table 4). Nodule weight declined more with increasing elevation than did nodule number. Mean nodule number at Olinda was 42% of that at Kuiaha while nodule mass at Olinda was only 27% of that at Kuiaha. In general, late maturing varieties formed more nodules than early ones with the exception of Hardee. However, nodule dry weight did not increase with maturity group. Nodule number and nodule weight were not significantly affected by soils from the three sites when the same varieties were grown in pots under a constant temperature regime (Table 5).

Shoot dry weight (Table 6) and total shoot nitrogen assimilation (Table 7) decreased with increasing elevation. There were no differences between varieties in shoot dry weight within each site. Total nitrogen content did not vary with varieties except at Kuiaha where late maturing cultivars (VI and VIII) assimilated less N at 45 days after planting than MG 00 and IV.

Discussion

In this study, the effect of soil type and temperature on nodulation and early growth of a wide range of soybean maturity groups was investigated. By introducing equal numbers of three *B. japonicum* strains into soils devoid of soybean rhizobia, the effects of soil type,

soil temperature and host genotype on interstrain competition for nodule sites were evaluated.

Differences in nodule occupancy by the three strains indicate that they have inherent differences in competitive ability. Strain USDA 110, the predominant strain in this study , has previously been reported to be a superior competitor (Johnson and Means, 1964; Caldwell, 1969). Kosslak and Bohlool (1985) found USDA 110 to be highly competitive against USDA 123 on soybeans grown in vermiculite and in Hawaiian soils devoid of *B. japonicum*. However, in soils from the north central region of the U.S., strains belonging to the USDA 123 serogroup outcompeted USDA 110 (and others) and occupied the majority of the nodules on soybean. Even though the ranking of the strains in field and greenhouse nodules was similar, higher recoveries of USDA 138 from nodules of plants from the greenhouse study indicate that USDA 110 was less competitive in the pot environment. The slightly higher soil temperature (27 C) in the greenhouse than the 320 m site was not a likely cause of this shift.

Soil type did not affect the percent recovery of strains from either field or greenhouse nodules. Since there was no difference between soils in strain recovery in the greenhouse where all the soils were under the same temperature regime, we conclude that competitive attributes of strains are independent of soil type and, within a reasonable range, of soil temperature. This contradicts the view that interstrain competition is very much dependent on soil type (Damirgi et al., 1967; Johnson and Means, 1963) and soil temperature (Kvien and Ham,

1985; Weber and Miller, 1972). However, it may be noted that, in contrast to the above reports, no indigenous rhizobia were present in the soils of the present experiment. The results of this experiment agree with those of Kamicker and Brill (1986) who reported that competition patterns by indigenous strains were not related to soil type.

The varietal differences in strain recovery were related to soybean MG. Physiological development of the plant is not a contributing factor since plants within a MG were at the same growth stage in the three soils in the greenhouse but were at different stages of development in the field where decreasing temperature delayed development. Hardee was an exception to other varieties in that very few nodules where occupied by USDA 136b and USDA 138. This result may be partly explained by the fact that USDA 136b is incompatible with Hardee which carries the gene Rj2 (Materon and Vincent, 1980).

The reduction in nodule number and mass may be the direct consequence of decreasing soil temperatures as well as low temperature effect on plant growth. Decreased nodulation of soybeans at soil temperatures below 24 C has been reported as early as in 1921 by Jones and Tisdale. Gibson (1967) observed considerable delay in nodulation and a general decline in the rate of nodule formation by *Trifolium subterraneum* when root temperatures were below 22 C. Enhanced plant development and increased nodulation in soybeans with increasing temperatures within the range of 15 to 25 C have been reported previously (Kvien and Ham, 1985; Matthews and Hayes, 1982).

Despite a large decrease in nodule number between sites, the three strains occupied the same percentage of nodules in all sites. It has been suggested that infection of roots by rhizobia is less sensitive to low temperatures than nodule formation (Gibson, 1967; Kumarasinghe and Nutman, 1979). The results of this study indicate that competition among rhizobial strains for nodule sites is not related to the final number of nodules formed.

Large differences in shoot dry weight and nitrogen assimilation between sites may be attributed to the effects of soil and air temperatures on nodulation and plant growth. Plant growth was severely restricted by a 5 C lower average temperature at the highest site. There was also considerable delay in plant development with decreasing temperatures. At the lowest site, all varieties were beyond the flowering stage at 45 days with the early varieties Clay and Clark already forming seeds. At the highest site, plants with the exception of Clay and Clark had not flowered. Plant growth was directly reduced by the low average temperature at the high cool site, but cool temperature could have had an indirect effect by reducing nitrogen fixation and soil nitrogen absorption. Soil temperatures of 24 to 27 C are reported to be optimum for nitrogen fixation and nitrate absorption (Kuo and Boersma, 1971; Rufty et al., 1981) in soybeans. The temperature at the lowest site was in the vicinity of the reported optimum. The high nitrogen assimilation and growth at that site was consistent with the findings of others.

The present experiments identified USDA 110 as a better competitor

than USDA 136b and USDA 138 for nodule occupancy regardless of soil type, soil temperature or MG of soybean. Nodulation and plant growth at 45 days after planting were drastically reduced by a 5 C decline in both soil and aerial temperatures. None of the nodulation and plant growth parameters were related to occupancy of strains in the nodules. Results indicate that strains superior in competitiveness can be identified for a broad range of host genotypes, soil types, and temperature.

Table 1. Soil and site characteristics of three locations in an elevational transect on the island of Maui, Hawaii.

Site (Soil series)	Soil classification	Elevation	Total rainfall during crop growth	Average temperature during crop growth	
				Soil	Air
		m	mm	C	
Kuiaha (Haiku series)	Clayey, ferritic, isohyperthermic Humoxic Tropohumult	320	139	25.3	22.8
Haleakala (Makawao series)	Clayey, oxidic, isothermic Humoxic Tropohumult	660	112	22.9	20.9
Olinda (Olinda series)	Medial over loamy-skeletal, isomesic Entic Dystrandept	1050	122	20.7	18.2

Table 2. Proportion of nodules formed by *B. japonicum* strains from five soybean varieties grown in the field and greenhouse.[1]

Site	Nodules occupied by: USDA 110 Field	USDA 110 Pot	USDA 136b Field	USDA 136b Pot	USDA 138 Field	USDA 138 Pot
	- - - - - - - - - - % - - - - - - - - - -					
Kuiaha	80.3	62.9	25.8	28.7	3.9	37.4
Haleakala	80.3	61.8	26.9	35.2	4.2	33.6
Olinda	82.0	67.8	30.0	37.4	4.2	31.4
Mean	80.8A[2]	64.2a	27.6B	33.8b	4.1C	34.2b

1 Values are means of 5 cultivars; Nodules with more than one strain are counted for each strain testing positive.

2 Data followed by the same type letter for either field or pot are not significantly different (P=0.05) by Duncan's multiple range test.

Table 3. Proportion of nodules formed by *B. japonicum* strains from five soybean varieties grown in the field and the greenhouse[1].

Variety	Nodules formed by USDA 110		USDA 136b		USDA 138	
	Field	Pot	Field	Pot	Field	Pot
Clay	60.7a[2]	55.1b	40.7a	38.0b	7.4a	47.9a
Clark	70.8c	57.8b	31.9b	28.9b	8.8a	39.8ab
D68-0099	87.5b	61.4b	26.9b	38.9b	0.5b	37.1b
N77-4262	87.5b	60.5b	30.1b	52.4a	2.8b	33.4b
Hardee	97.7a	85.8a	8.3c	10.8c	0.9b	12.7c

1 Values are means of three soils. Nodules with more than one strain are counted for each strain testing positive.

2 Data followed by the same letter within a column are not significantly different (P=0.05) by Duncan's multiple range test.

Table 4. Number and dry weight of nodules from five soybean varieties at 45 days after planting at three different elevations.

Site	Variety					Mean
	Clay	Clark	D69-0099	N77-4262	Hardee	
	- - - - Number plant^{-1} - - - -					
	- - - - (mg plant^{-1}) - - - - -					
Kuiaha	43	40	60	65	57	53A[1]
	(289)	(278)	(294)	(236)	(249)	(269A)
Haleakala	20	32	31	35	28	29B
	(180)	(216)	(137)	(134)	(146)	(162B)
Olinda	18	24	24	28	18	22B
	(75)	(76)	(73)	(73)	(66)	(73C)
Mean	27d[1]	32cd	38ab	43a	34bc	
	(181a)	(190a)	(168ab)	(147b)	(154b)	

1 Data followed by the same letter within a row or a column are not significantly different (P=0.05) by Duncan's multiple range test.

Table 5. Number and dry weight of nodules from five soybean varieties at 45 days after planting in three different soils in the greenhouse.

Soil series (Site)	Variety					Mean
	Clay	Clark	D69-0099	N77-4262	Hardee	
	- - - - Number plant^{-1} - - - -					
	- - - - (mg plant^{-1}) - - - -					
Haiku (Kuiaha)	27 (74)	30 (102)	34 (100)	45 (114)	42 (112)	35 (100)
Makawao (Haleakala)	31 (91)	45 (93)	35 (91)	29 (86)	35 (96)	35 (92)
Olinda (Olinda)	20 (87)	27 (93)	46 (91)	35 (102)	36 (113)	33 (97)
Mean	26b[1] (84b)	34a (96ab)	39a (94ab)	37a (101a)	38a (107a)	

1 Data followed by the same letter within a row are not significantly different (P=0.05) by Duncan's multiple range

test.

Table 6. Shoot dry weight of five soybean varieties at 45 days after planting at three different elevations.

Site	Variety					
	Clay	Clark	D68-0099	N77-4262	Hardee	Mean
	- - - - - - g plant^{-1} - - - - - - -					
Kuiaha	7.7	7.7	7.8	6.3	7.0	7.3A[1]
Haleakala	4.8	5.6	5.6	5.6	5.9	5.5B
Olinda	1.0	1.1	1.0	1.0	0.9	1.0C
Mean	4.5	4.8	4.8	4.3	4.6	

1 Data followed by the same letter within the column are not significantly different (P=0.05) by Duncan's multiple range test.

Table 7. Shoot nitrogen content of five soybean varieties grown at three different elevations at 45 days after planting.

Site	Variety					
	Clay	Clark	D68-0099	N77-4262	Hardee	Mean
	- - - - mg N plant^{-1} - - - - -					
Kuiaha	294a[1]	290a	232b	195b	218b	246A[1]
Haleakala	182a	198a	181a	178a	198a	187B
Olinda	26a	28a	25a	25a	25a	26C
Mean	167a	171a	146ab	133b	147ab	

1 Data followed by the same lower case letter within a row or same upper case letter within the column are not significantly different (P=0.05) by Duncan's multiple range test.

Chapter 3

EFFECT OF TEMPERATURE AND MATURITY GROUP ON PHENOLOGY OF FIELD GROWN SOYBEAN

Abstract

The environmental adaptation of soybean (*Glycine max* (L.) Merrill) genotypes depends in part on the effects of temperature and photoperiod on the durations of phenological phases. The effects of temperature and nitrogen nutrition on soybean phenology was studied at field sites with elevations of 320, 660, and 1050 m on the island of Maui, Hawaii. The mean maximum/minimum air temperatures (C) at the three elevations during the experiment were 27/20, 25/17, and 23/14 respectively. Photoperiod decreased from 13 h at planting to 11 h at the last harvest. Four determinate nodulating soybean varieties representing the maturity groups 00 (Clay), IV (Clark), VI (D68-0099) and VIII (Hardee), one indeterminate variety of group VI (N77-4262) and their non-nodulating isolines were planted at each site on the same day. pH was equalized and non-limiting amounts of all nutrients except nitrogen were applied. Soybean maturity was delayed with decreasing temperature mainly due to extended vegetative growth periods averaging 23% and 64% longer at the 660 m and 1050 m sites compared to the lowest site. Flowering and physiological maturity at the highest site were delayed on average by 22 and 25 days respectively, relative to development at the lowest site. The effect of site (temperature) on the duration of the seed-fill phase was minimal. Within each site, higher maturity groups had progressively longer vegetative phases presumably due to photoperiodic effects. The indeterminate line N77-4262 had a longer period of vegetative growth than did the corresponding determinate line D68-0099. The data

demonstrated that growing degree day calculations aid in the accurate prediction of phenological events only when photoperiodic effects were minimum. The rate of seed dry matter accumulation (kg $ha^{-1}day^{-1}$) decreased with increasing elevation, but did not show any consistent trends among maturity groups. Non-nodulating isolines flowered later and matured earlier than their nodulating counterparts due to nitrogen stress. The results indicated that low temperature prolonged vegetative and total growth duration of soybean, but had a minimal effect on duration of seed-fill under similar N nutritional levels. The extended vegetative growth period may be a mechanism with which the soybean plant co-responds for decreased rates of dry matter production under low temperature.

Introduction

The adaptation of soybean genotypes to environment depends in part on how temperature and photoperiod affect ontogeny. While rate of growth is strongly influenced by temperature, rate of development and the duration of phenological phases in soybean are determined by both temperature and photoperiod.

Most reports concerning the effects of temperature on phenological development of soybean either used planting date as a variable (Lawn and Byth, 1973; Major et al., 1975a) or employed controlled environments (Hadley et al. 1984; van Schaik and Probst, 1958). Since the induction of flowering in soybean is photoperiod sensitive and most soybean genotypes are quantitative short-day plants (Hadley et al. 1984; Lawn and Byth, 1973), the effects of temperature on soybean development in

the field are often confounded with the effects of photoperiod which change with planting date and latitude. Even though controlled environment experiments may be useful in avoiding complex interactions between photoperiod and temperature, development of plants under such conditions may not be typical of what occurs in the field.

Hodges and French (1985) compiled data for soybean which showed that temperature was important during early growth and development. Once flowering occurred, both temperature and photoperiod influenced development. According to Lawn and Byth (1973) low temperature effects on phasic development of soybean were apparent only when photoperiodic effects were minimum. Major et al., (1975a) reported that although later maturity groups flowered after the early ones, there were no differences among maturity groups I through V in sensitivity to temperature at a constant photoperiod of 14h.

An elevational transect located on the island of Maui, Hawaii, (part of the Maui Soil, Climate and Land Use Network: MauiNet) provided an opportunity to investigate the effect of temperature (elevation) on phenological development of soybean. The effect of photoperiod was minimized by planting at the different sites on the same day. The present experiment was undertaken to evaluate the effect of temperature on phenology of nodulating and non-nodulating isolines of four maturity groups of soybean.

Materials and Methods

Experimental design: Nodulating (nod) and non-nodulating (non-nod) isolines of five varieties of soybean representing four maturity groups

(00, IV, VI, VIII) were grown at elevations of 320, 660, and 1050 m. The experimental design was a randomized complete block with three replications at each site.

Field and plant culture: The sources from which seeds of soybean varieties (nod isolines) were obtained were given in Chapter 2. The non-nod isoline of each variety used in the present study was also received from the same source as that of the nod isoline. Procedures followed for soil amendment, rhizobial inoculation, plant culture and temperature data collection were described in Chapter 2.

Sampling procedures: Phenological observations were made on a 1.0 m section of row in each plot. Plants were considered to have reached a particular phenological stage when fifty percent of the plants in the 1.0 m row reached that stage. Growth stage characterization was based on the system developed by Fehr et al., (1971).

Three meters of row from the center of the two central rows were harvested at physiological maturity. Non-nod isolines were harvested at the same time as their fixing counterparts. Plants were cut at the soil surface and fresh weights for each plot were measured. A fresh weight subsample (15 to 25 plants) was then taken for dry weight determination. Samples were dried at 70 C and separated into grain and stover, which were ground separately and digested in H_2SO_4 after pre-treatment with H_2O_2 (Parkinson and Allen,1975). Ammonium was determined in the digests by the indophenol blue method (Keeny and Nelson, 1982).

Durations of vegetative and seed-filling phases were calculated, respectively, as days from planting to first flower (R1) and days to

physiological maturity (R7) minus days to beginning seed (R5). Rate of seed dry matter accumulation was determined by dividing grain yield ha^{-1} by seed-fill duration. Growing degree days (Major et al., 1975b) were calculated using a base temperature of 7.8 C (Hadley et al., 1984).

Data analysis: The data from the three sites were combined (McIntosh, 1983) and subjected to analysis of variance using SAS (SAS Institute. 1982. SAS user's guide: Statistics. 1982 ed. SAS Institute, Inc., Cary, NC) procedures.

Results

Mean maximum and minimum temperatures between the sites at 320 and 1050 m differed by about 4 C and 6 C, respectively, during the course of the experiment (Table 1). The decline in temperature from the start to the end of the experiment at a site was gradual and small (Figure 1). The photoperiod decreased from 13 h at planting to 11 h at the end of the experiment (Figure 1).

There were significant differences between sites and varieties in days to first flower (R1) and physiological maturity (R7) (Table 2). Both phenological events were delayed considerably at the higher elevations compared to the lowest elevation. The effect of increasing elevation was progressively greater on higher maturity groups. Flowering of Clay was only 12 days later at 1050 m than at 320 m while Hardee flowered 31 days later at the highest site compared to the lowest site. Similarly, Hardee flowered 15 days later than Clay at the 320 m site, but was 34 days later than Clay at 1050 m. Phenological development was consistent with maturity group number. Lower maturity

groups flowered and matured more rapidly than higher maturity groups. Thus, within a site, each event was recorded first and last, respectively, for Clay and Hardee. The indeterminate maturity group VI variety N77 required a significantly greater number of days than the determinate VI variety D68 to reach both R1 and R7.

Growing degree days (GDD) required for vegetative growth for all varieties increased slightly with increasing elevation (Table 3). Because the seed filling period was approximately constant across the sites, GDD for the total crop duration actually decreased with increasing elevation. Late maturing varieties required a greater number of GDD to complete vegetative growth and reach maturity at all sites.

The differences in duration of the seed-filling phase were much smaller than differences in durations of vegetative and total crop growth. Varieties differed significantly in duration of seed-filling (stage R5 to R7) (Table 4). Differences between sites in seed-fill duration, although significant, were small. Clay, the earliest maturing variety, had the shortest seed-filling duration. The other maturity groups were inconsistent in this regard. When averaged across sites, Clark and Hardee had the longest seed-fill period (Table 4) while N77 had the highest rate of seed dry matter accumulation (Table 5). The rate of seed dry matter accumulation declined significantly with increasing elevation and was lowest for the two early varieties at the 1050 m site.

Non-nod isolines flowered later and matured earlier than their nodulating counterparts. When averaged over sites, the isolines of Clay

and Clark were similar in days to R1 (Table 6), but non-nod isolines of other varieties were late in flowering. Nod isolines had significantly higher concentrations of N in both the shoot and the grain (Table 7) than did the non-nods. The yield of non-nods were substantially lower than the nods.

Discussion

The delay in the occurrence of first flower (R1) and physiological maturity (R7) (Table 2) due to increasing elevation was assumed to be due to a 5 C decline in mean daily temperature from the lowest to the highest site. The increased number of days to flowering at the higher elevation, however, did not result in a similar increase in days to physiological maturity. A delay in flowering of soybean due to low temperature has been reported by many investigators (Hadley et al., 1984; Hesketh et al., 1973; Steinberg and Garner, 1936; van Schaik and Probst, 1958). According to Hadley et al., (1984) the reciprocals of the days taken to flower (rate of progress towards flowering) by soybean were linear functions of mean diurnal temperature in photoperiods shorter than the critical daylength. Assuming that the effect of the prevailing photoperiod on flowering was the same at the three sites, differences between sites in days to flowering could be due to differences in temperature. The consistent phenological responses of varieties to increasing elevation (Table 2) indicates that temperature was the dominant effect. However, critical day lengths for soybean are shorter in cooler temperatures and when photoperiods are longer than the critical value, the rate of progress towards flowering is a linear

function of both temperature and photoperiod with no interaction between the two (Hadley et al., 1984). Thus, under a constant temperature regime and inductive photoperiods, soybean genotypes would flower about the same time regardless of their maturity group classification. In our experiment, flowering in order of progressively increasing maturity group number indicated that photoperiods may have been non-inductive for the higher maturity groups at the beginning of the experiment. The plants at the highest elevation may have been subjected relatively more to non-inductive photoperiods than those at the lowest elevation, further delaying flowering at that site. The overall effect was to increase the relative crop duration for successive maturity group numbers at higher elevations. Since Clay, maturity group 00, might be considered as relatively insensitive to photoperiod (Major et al., 1975a; Criswell and Hume, 1972), 12 and 43% delays in flowering at the intermediate and highest sites respectively may have been due entirely to lower temperatures.

The large increases in GDD required for vegetative development with increasing elevation for the higher maturity groups indicates the unsuitability of GDD as a predictor of phenological events in soybean under non-inductive photoperiods. The present data also agree with the conclusion of Major et al., (1975b) that thermal unit methods, including GDD, only accurately predicted development of early maturing soybean varieties, i.e., varieties which were less sensitive to photoperiod.

The data indicate that the effects of temperature on the seed-filling duration was minimal under similar N nutritional levels.

The finding of Hesketh et al., (1973) that reproductive duration was relatively unaffected by temperatures in the range of 22 to 30 C may have been due to similar seed fill durations. Varietal differences in durations of seed-filling were associated with differences in grain yield within a site; however, no consistency among sites was observed in this respect. On the other hand, a closer association between grain yield (can be calculated from Tables 4 & 5) and rate of seed dry matter accumulation (Table 5) was apparent. The above observation is in contrast to reports by others (Dunphy et al., 1979; Egli, 1975; Hanway and Weber, 1971) that grain yield is strongly related to seed-fill duration with the rate of seed dry matter accumulation being a constant.

There was significant reduction in grain yield (can be calculated from Tables 4 & 5) of all varieties due to decreasing temperature. In general, yield increased with increasing duration of vegetative growth at each site, indicating a positive relationship between vegetative dry matter production and grain yield. Therefore, delay in phenological development up to the beginning seed stage (R5) due to decreasing temperature may be an adaptation by the plant to compensate for decreased rates of growth.

The differences in the response to temperature of nod and non-nod isolines indicates how nitrogen nutrition can alter soybean phenological development. The net result of delayed flowering and hastened maturity by the non-nods was shortened reproductive and seed-fill durations. The significantly lower shoot and grain N concentrations of non-nods than nods (Table 7) indicated N insufficiency in non-nod plants. The early

maturity of non-nods was apparently due to nitrogen insufficiency. The cause of the delayed flowering of the non-nod isolines is unknown although Huxley et al., (1976) reported that while the onset of flowering was substantially delayed by lower night temperature, low nitrogen levels also delayed flowering in soybean cv. TK5. Therefore, nitrogen nutrition may be another important consideration in comparing soybean phenology across temperature regimes.

The effects of temperature on phenological development of soybean under field conditions were demonstrated in this experiment. The results indicate that low temperature delayed flowering in soybean regardless of sensitivity to photoperiod. The extended vegetative growth due to decreasing temperature may be an adaptation by the soybean plant to compensate for decreased rates of growth. Duration of seed-fill was relatively unaffected by temperature under similar N nutritional levels, but seed dry matter accumulation per day per unit land area was substantially decreased by cool temperature. Non-nodulating isolines, in general, were late in flowering but early in maturity and their development was related to the degree of N insufficiency at the respective sites.

Table 1. Mean maximum and minimum temperatures during growth of five soybean varieties at three elevations on the island of Maui, Hawaii.

Variety	Maturity Group	Temperature (C)					
		Max	Min	Max	Min	Max	Min
		320 m		660 m		1050 m	
Clay	00	26.8[1]	19.8	25.7	17.1	23.1	14.2
Clark	IV	26.5	19.8	25.5	17.1	22.9	14.0
D68	VI	26.5	19.8	25.3	17.0	22.9	14.0
N77	VI	26.4	19.8	25.3	17.0	22.5	13.7
Hardee	VIII	26.2	19.6	25.3	16.8	22.5	13.7

1 Mean temperatures from planting to maturity.

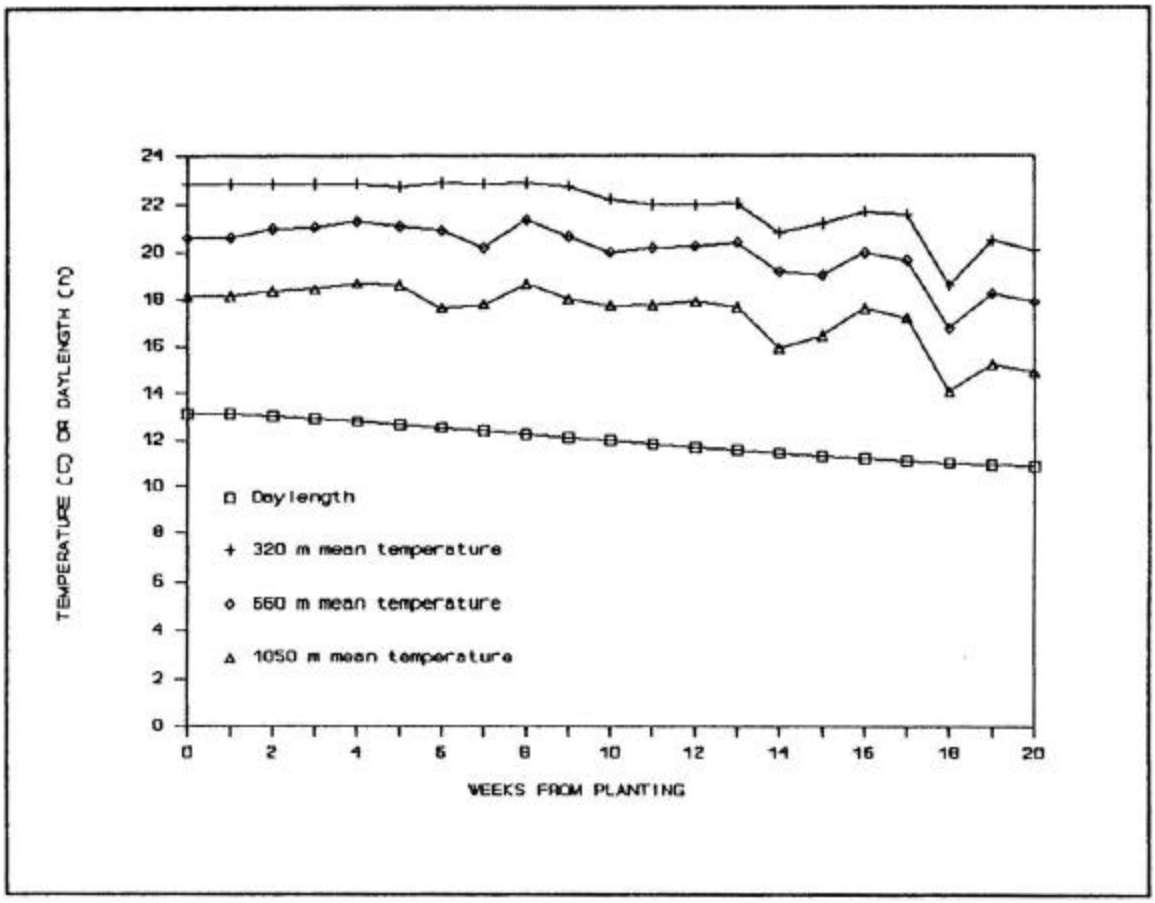


Figure 1. Weekly mean temperatures and daylengths during the growth of five soybean varieties at three elevations on the island of Maui, Hawaii.

Table 2. Days to first flower (R1) and physiological maturity (R7) of nodulating isolines of five soybean varieties grown at three elevations.

	First flower			Physiological maturity		
Variety	320 m	660 m	1050 m	320 m	660 m	1050 m
	----------days----------					
Clay (00)[1]	27.7E	31.0E	39.7E	73.0E	77.7E	93.0E[2]
Clark (IV)	30.0D	35.0D	46.0D	87.0D	92.0D	107.0D
D68 (VI)	33.7C	41.3C	55.7C	90.0C	101.0C	112.0C
N77 (VI)	38.0B	49.0B	66.0B	94.0B	105.0B	128.0B
Hardee(VIII)	42.3A	54.3A	73.7A	106.0A	117.0A	133.3A
Mean	34.3c[2]	42.1b	56.2a	90.0c	98.5b	114.7a

1 Maturity grouping.

2 Means followed by the same upper case letter within a column or lower case letter within a row for each category are not significantly different (P=0.05) by Duncan's multiple range test.

Table 3. Growing degree days accumulated during vegetative growth and up to physiological maturity for nodulating isolines of five soybean varieties grown at three elevations.

	Vegetative phase			Total crop duration		
Varieties	320 m	660 m	1050 m	320 m	660 m	1050 m
	----------Growing degree days[1]----------					
Clay	432	426	444	1131	1060	1009
Clark	464	481	501	1335	1242	1140
D68	523	564	616	1382	1348	1193
N77	588	667	723	1438	1402	1318
Hardee	651	740	811	1600	1550	1370

1 Calculated using a base temperature of 7.8 C.

Table 4. Duration of seed-filling[1] of nodulating isolines of five soybean varieties grown at three elevations.

Varieties	Elevation (m) 320	660	1050	Mean
	----------days----------			
Clay	36.0	35.0	38.0	36.3D[2]
Clark	46.7	43.3	46.0	45.3A
D68	42.3	45.0	40.3	42.6B
N77	39.0	39.3	44.3	40.9C
Hardee	47.3	46.0	43.3	45.6A
Mean	42.3a[2]	41.7b	42.4a	

1 Days from beginning seed (R5) to physiological maturity (R7).
2 Means followed by the same upper case letter within a column and lower case letter within a row are not significantly different (P=0.05) by Duncan's multiple range test.

Table 5. Rate of seed dry matter accumulation[1] of nodulating isolines of five soybean varieties grown at three elevations.

Varieties	Elevation (m) 320	660	1050	Mean
	kg ha^{-1} day^{-1}			
Clay	89.5	80.2	13.6	61.1D[2]
Clark	94.8	70.7	29.5	65.0C
D68	101.0	82.7	47.1	76.9B
N77	90.5	89.6	58.2	79.4A
Hardee	102.1	78.3	43.3	74.6B
Mean	95.6a[2]	80.3b	38.4c	

1 Determined by dividing grain yield ha-1 by duration of seed fill.

2 Means followed by the same upper case letter within a column and lower case letter within a row are not significantly different (P=0.05) by Duncan's multiple range test.

Table 6. Mean days to first flower and physiological maturity for nodulating (nod) and non-nodulating (non-nod) isolines of five soybean varieties grown at two elevations.[1]

	First flower (R1)		Physiological maturity (R7)	
Varieties	Nod	Non-nod	Nod	Non-nod
	days			
Clay	32.8a[2]	32.6a	75.3a	70.5b
Clark	37.0a	36.9a	89.5a	85.2b
D68	43.6b	46.6a	95.5a	90.5b
N77	51.0b	52.4a	99.5a	96.0b
Hardee	56.8b	62.1a	111.5a	106.3b

1 Data averaged for the Kuiaha (32 m) and Haleakala (660 m) sites.

2 Means followed by the same letter within a row for each category are not significantly different (P=0.05) by Duncan's multiple range test.

Table 7. Mean grain and shoot nitrogen concentrations of nodulating (nod) and non-nodulating (non-nod) isolines and grain yield of non-nods of five soybean varieties grown at three elevations.

	Nitrogen concentration				
	Shoot		Grain		Non-nod
Varieties	Nod	Non-nod	Nod	Non-nod	Grain yield
	----------%----------				kg ha^{-1} $\quad$ %[1]
Clay	1.14a[2]	0.49b	6.46a	4.21b	826 $\quad$ 37.9
Clark	1.24a	0.46b	6.24a	4.20b	970 $\quad$ 32.9
D68	0.99a	0.44b	6.17a	3.52b	1262 $\quad$ 38.3
N77	1.08a	0.36b	6.22a	4.05b	1253 $\quad$ 39.0
Hardee	1.20a	0.59b	6.21a	4.80b	1046 $\quad$ 30.4

1 As a percentage of the grain yield of the nodulating isoline.
2 Means followed by the same letter within a row for either shoot or grain are not significantly different(P=0.05) by Duncan's multiple range test.

Chapter 4

YIELD, SOIL N UPTAKE AND N_2 FIXATION BY SOYBEANS FROM FOUR MATURITY GROUPS GROWN AT THREE ELEVATIONS

Abstract

The exploitation of the soybean-*Bradyrhizobium japonicum* symbiosis in varied environments requires an understanding of factors that may affect fixed and soil N assimilation. Temperature affects both soybean maturity and N requirement, and soil N availability. Five soybean varieties belonging to four maturity groups (00, IV, VI, and VIII) and their respective non-nodulating isolines were planted on the same day at three sites along an elevational transect in Hawaii to study the effect of temperature on yield, soil N uptake and N_2 fixation. The mean soil/air temperatures (C) during the experiment were 24/23, 23/20, and 20/18 at 320, 660, and 1050 m elevations, respectively. The seeds were inoculated at planting with *B. japonicum*. Plants were harvested at physiological maturity. Average dry matter yield and total N at the highest site were only 48 and 41%, respectively, of those at the lowest site. Dry matter yield and total N of early maturing varieties were relatively more reduced by low temperature than were later maturing varieties. Within each site, late maturing varieties produced greater yield and assimilated more N than the early maturing varieties. Nitrogen fixation contributed 80%, 66%, and 97% to total plant N at the lowest, intermediate and highest sites, respectively. Differences between sites in the proportion of N from fixation were due to differences in the availability and uptake of soil N. Although total N assimilation differed with variety, in some cases by more than 400%, the proportion of N derived from fixation was similar within a site.

Nitrogen assimilation per day was similar among varieties at a site. Since the different maturity groups had similar average N assimilation rates per day within a site, and since soil N mineralization rate per day at a site can be assumed to be constant, the proportion of total N derived from N_2 fixation is necessarily constant. Data from *in vitro* soil N mineralization and a greenhouse pot test indicated that low temperature may have decreased root growth and uptake of soil N or soil N availability more than it reduced N_2 fixation.

Introduction

Soybean plants can acquire a substantial portion of their N requirement through N_2 fixation from symbiotic association with *Bradyrhizobium japonicum*. Both management and environmental variables affect the symbiosis. Any factor that affects the availability and plant uptake of soil N could directly influence N_2 fixation and soybean yield. An understanding those factors that may differentially affect fixed and soil N assimilation could help enhance exploitation of the symbiosis.

Optimum temperature promotes growth, and symbiotic and soil N assimilation by soybean and other legumes. Increasing root temperature within the range of 15 to 27 C has been shown to increase growth and dry matter production in soybeans (Earley and Cartter, 1945; Matthews and Hayes, 1982; Trang and Giddens, 1980). Lowering both root and shoot temperature from 25 to 10 C decreased photosynthesis in soybean more than reducing shoot temperature alone (Taylor and Rowley, 1971). Nodule activity was reduced to the same extent if shoots or roots and shoots of

soybean were exposed to 18 C temperature (Schweitzer and Harper, 1980). Harding and Sheehy (1980) showed that leaf growth of lucerne (*Medicago sativa*) was correlated with air temperature, while N_2 fixation was correlated with root temperature. Temperature also affects soil N mineralization (Stanford et al., 1973; Cassman and Munns, 1980) and root-uptake of mineralized N (Tolley and Raper, 1985). Therefore, temperature may directly and indirectly affect growth of soybean through effects on photosynthesis, N uptake and N_2 fixation. Raper, et al., (1977) suggested that plants respond to temperature in such a way that N assimilation and photosynthate supply are in balance.

Due to the strong photoperiod sensitivity of soybeans (Borthwick and Parker, 1939), assessment of temperature effects on productivity under field conditions is often difficult. In the soybean producing regions of North America, average temperatures are, to some extent, a function of latitude, which confounds the temperature variable with photoperiod and irradiance.

The present investigation was undertaken to evaluate the effect of temperature on growth, phenology, (Chapter 3), yield, soil N uptake and N_2 fixation by soybean varieties from four maturity groups. Results reported here include yield, soil N uptake and N_2 fixation. By planting several varieties from a range of maturity groups at field sites along an elevational transect within the Maui Soil, Climate and Land Use Network: Mauinet, it was possible to minimize the effects of photoperiod and irradiance on soybean growth and development.

Materials and Methods

The experimental design, field and plant culture procedures, weather data collection and sampling procedures for yield and total N were described in Chapter 3. Only those procedures specific to this study are described here. Weather data for this study included irradiance measurements which were recorded by Campbell Scientific CR-21 micrologger (Campbell Scientific, Inc., Logan, UT) equipped with sensing probes for irradiance (Li-Cor LI 200ZC silicon pyranometer; Li-Cor, Inc., Lincoln, NE).

Total N and fixed N calculations: Total plant N was calculated by multiplying the percent tissue N concentration by total plant dry weight. The amount of N fixed was determined by subtracting the total plant N in the non-nod isoline from its nod counterpart.

***In vitro* soil N mineralization**: Soils from the three sites were sampled at planting to a depth of 20 cm to determine N mineralization potential of the soil under constant conditions. Samples of approximately 20 g air-dry soil were equilibrated on a pressure plate at 0.03 MPa for initial analysis and incubation (Cassman and Munns, 1980). Triplicate samples were extracted with 200 ml of 2 M KCl before and after incubation at 25 C in 250 ml flasks for two weeks. Flasks were covered with aluminum foil that was pierced with two pinholes. Water loss was negligible during incubation. Nitrate and NH_4^+-N were determined from the filtered extracts by cadmium reduction and indophenol blue methods, respectively (Keeny and Nelson, 1982).

Greenhouse experiment: Clark non-nod soybean was planted in pots (two plants pot^{-1}, three replications) containing 4 kg soil (oven-dry basis)

from each of the three sites to measure plant uptake of N from the soils under a constant temperature regime. Soils were maintained at 0.03 MPa tension. The mean soil temperature during the experiment was 27 C. At 45 days from planting, plants were cut at the soil surface and analyzed for total N as described previously.

Data analysis: The data from the three sites were combined (McIntosh, 1983) and subjected to analysis of variance using SAS procedures (SAS Institute Inc., Box 8000, Cary, North Carolina 27511-8000).

Results

Irradiance at the 660 m and 1050 m sites was 80 and 75% of that at the 320 m site (Table 1). Mean soil and air temperatures declined by about 5 C from the 1050 m to 320 m.

There were significant differences in grain and stover yields among sites and varieties (Table 2). Average grain yield and stover yields at the highest site were only 41 and 51%, respectively, of those at the lowest site. Yields were reduced at higher elevations to a greater extent in the early maturing varieties, Clay and Clark, than in later maturing varieties. In general, grain yields increased with maturity group number at a site. Variety N77-4262, an indeterminate maturity group VI entree was less affected by low temperatures than other varieties. Hardee produced the highest average yield.

Total N assimilation (Table 3) and amounts of N fixed (Table 4) decreased with increasing elevation (Table 3 and 4). The effect of elevation was relatively greater on the early maturing variety, Clay. Total N and fixed N increased with increasing maturity group number.

Varietal differences in the rate of N assimilation within a site were relatively small compared to the effect of temperature (Table 5).

The relative contributions from symbiotic N_2 fixation and soil N uptake to total N were significantly different between sites (Table 6), but relatively constant for all varieties within a site except for N77-4262 at the 320 m site. The greatest soil N uptake was observed at the Haleakala site (660 m) and the result was consistent with *in vitro* mineralization measurements (Table 7). Soil N uptake at Olinda (1050 m) was negligible. While actual soil N uptake was comparable to the estimated amounts of mineralized N at the lowest site, it was reduced to a half and to less than a tenth at the intermediate and the highest sites, respectively. The rate of plant N uptake in the greenhouse experiment from the soils of the two higher sites were similar to estimated rates of mineralization (Table 8).

Discussion

By planting soybean at different elevations in the same latitude, any interactive effects between photoperiod and temperature on plant growth and development were minimized. Thus, the site effects on grain yield, symbiotic N_2 fixation, and soil N uptake of soybean genotypes were assumed to be due to differences in temperature. It is unlikely that the 25% difference in irradiance between sites accounted for the reduction in yield and total plant N observed in this experiment. Despite similar irradiances at the 660 and 1050 m elevations, there were large differences in yield and N assimilation between these two sites. Since nutrients other than N and water were assumed to be non-limiting,

the reduction in yield was attributed primarily to decreasing temperature with increasing elevation. Low temperatures may have reduced both photosynthesis and nutrient assimilation.

Seed yield was positively correlated with total N assimilation and the amount of nitrogen fixed. Crop durations increased with maturity group number as well as with decreasing temperature (Chapter 3). Increased crop duration was mainly due to an extended vegetative phase and was positively correlated with seed yield within a site. Thus, the increase in yield and total N with increasing maturity group number was due to increasing dry matter resulting from prolonged growth. Though total N and yield increased with maturity group, the rate of N assimilation (kg N ha^{-1} d^{-1}) was not related to maturity differences between cultivars within a site. The large reductions in N assimilation per day due to decreasing temperature demonstrates the effect of temperature on crop growth and N requirement, and in turn on N assimilation, as suggested by Raper et al., (1977). Yield and N assimilation of short term varieties were reduced more by low temperature than longer maturing varieties, probably due to the short vegetative durations.

The fact that 97% of the plant N requirement of nod isolines at Olinda was met by symbiotic fixation and that variety N77-4262 fixed similar amounts of N at all sites suggests that the effect of temperature was far greater on plant growth than on the process of symbiotic N_2 fixation. The similar concentrations of N in stover across sites (1.1-1.2 %) and grain (6.2-6.3 %) of nod isolines also indicates

that the observed decrease in yield was not primarily due to low temperature limiting symbiotic N_2 fixation but due to the direct effect of temperature on overall plant growth.

The proportions of N derived from fixation (Table 6) within a site were similar despite large differences in total N assimilation between maturity groups. Since the different maturity groups had similar average N assimilation rates per day within a site and soil N mineralization rate per day at a site was assumed to be constant, the proportion of total N derived from N_2 fixation necessarily was constant. This is reasonable since soil N availability and uptake are functions of soil and temperature, and, within a site, temperature was relatively constant for the different maturity groups.

Soil N uptake at Olinda was negligible as determined by the total N of the non-nod isolines (Table 7). The lab mineralization study indicated that under controlled conditions, the N mineralization rate of the Olinda soil was similar to that of Kuiaha. The greenhouse pot test at a higher mean soil temperature than that in the field indicated that under favorable temperature, non-nod plants grown in Olinda soil could assimilate substantial amounts of N (Table 8).

The nod isolines at Olinda, with little available soil N, assimilated substantial amounts of N, and the tissue N concentrations of nod isolines did not differ between sites. How low soil N availability affected N_2 fixation at Olinda cannot be determined from this experiment. Presumably, the cool temperature mainly reduced photosynthesis and thus the requirement for N in the case of the nod

isolines.

Considering the complexity of the interplay of factors that may affect soil N availability and plant uptake, there are two reasonable temperature-related explanations for the low soil N uptake observed. During field preparation soil N might have been immobilized due to incorporation of residues from the previous grass vegetation, and immobilization may have been prolonged due to low soil temperatures. However, the primary tillage operations were carried out 4 weeks before planting at all sites, yet more soil N was available at the Haleakala site even though the soil temperature there was lower than that at the Kuiaha site. Another possibility might have been that soil N assimilation and N_2 fixation by soybean are differentially tolerant to low temperature, such that root growth and uptake of soil N was reduced more than N_2 fixation. Rufty et al., (1981) reported that root growth and nitrate absorption were less at 18 C than at 24 C. In contrast, soybean nodules have been reported to have maximum activity over the range of 15 and 30 C (Pankhurst and Sprent, 1976). Thus, in this experiment, N_2 fixation may not have been reduced by low temperature to the same extent as was mineral N assimilation.

The different behavior of N77-4262 is of interest. Although yield and N assimilation of N77-4262 were relatively low at the lowest site, they were less affected by temperature than other varieties. This variety fixed similar amounts of N at the lowest and highest sites. The results indicate that this indeterminate variety may be suited for a wide range of temperature regimes at this latitude.

The results demonstrate the dependence of soybean growth and N assimilation on temperature. Decreasing temperatures caused greater restrictions on plant growth and dry matter production than on the functioning of symbiotic N_2 fixation. The relative contribution of symbiotic N_2 fixation was a function of soil N availability at a site regardless of crop duration and total N assimilation by different varieties. N assimilation per unit time was relatively constant for maturity groups within a site despite large differences in total N assimilation.

Table 1. Characteristics of three sites in an elevational transect on the island of Maui, Hawaii.

Site (Elev., m)	Soil classification	Rainfall[1]	Temperature[1]		Irradiance[1]
			Soil	Air	
		mm	C		W m^{-2}
Kuiaha (320)	Clayey, ferritic, isohyperthermic Humoxic Tropohumult	415	24.4	22.6	262
Haleakala (660)	Clayey, oxidic, isothermic Humoxic Tropohumult	407	22.6	20.6	211
Olinda (1050)	Medial over loamy-skeletal, isomesic	678	19.6	17.8	194

1 All data averaged across the crop duration of all five varieties at a site

Table 2. Grain and stover yields of five nodulating soybean varieties grown at three different elevations under the same photoperiodic regime.

Site (elevation, m)	Varieties: Clay VI	Clark VI	D68-0099 VIII	N77-4262 IV	Hardee 00[1]	Mean
	kg ha^{-1}					
Grain yield						
Kuiaha (320)	3223	4424	4273	3530	4833	4057A[2]
Haleakala (660)	2803	3066	3721	3526	3601	3344B
Olinda (1050)	517	1357	1903	2580	1877	1647C
Mean	2181d[2]	2949c	3299ab	3212b	3437a	
Stover Yield						
Kuiaha	3105	3859	3661	3265	4117	3601A
Haleakala	2891	3381	3763	3829	4060	3585A
Olinda	585	1389	2088	2496	2534	1819B
Mean	2194d	2877c	3171b	3197b	3570a	

1 Indicates maturity group designation of the variety.
2 Data followed by the same letter within the column or row are not significantly different (P=0.05) by Duncan's multiple range test.

Table 3. Total nitrogen assimilation by five nodulating soybean varieties grown at three different elevations.

Site (elevation, m)	Varieties: Clay 00[1]	Clark IV	D68-0099 VI	N77-4262 VI	Hardee VIII	Mean
	kg N ha^{-1}					
Kuiaha (320)	236	317	316	260	349	295A[2]
Haleakala (660)	199	246	271	258	276	250B
Olinda (1050)	44	99	128	186	144	120C
Mean	160d[2]	221c	238b	234bc	256a	

1 Indicates maturity group designation of the variety.
2 Data followed by the same letter within the column or row are not significantly different (P=0.05) by Duncan's multiple range test.

Table 4. Symbiotically fixed nitrogen by five soybean varieties grown at three different elevations.

Site (elevation, m)	Varieties: Clay 00[1]	Clark IV	D68-0099 VI	N77-4262 VI	Hardee VIII	Mean
	----------kg N ha^{-1}----------					
Kuiaha (320)	199	258	268	180	281	237A[2]
Haleakala (660)	122	175	176	169	188	166B
Olinda (1050)	42	97	125	183	139	117C
Mean	121c[2]	177b	190ab	177b	203a	

1 Indicates maturity group designation of the variety.
2 Data followed by the same letter within the column or row are not significantly different (P=0.05) by Duncan's multiple range test.

Table 5. Average rate of nitrogen assimilation by five soybean varieties grown at three different elevations.

Site (elevation, m)	Varieties: Clay 00[1]	Clark IV	D68-0099 VI	N77-4262 VI	Hardee VIII	Mean	
	kg N ha^{-1} day^{-1}						
Kuiaha (320)	3.23	3.64	3.51	2.76	3.29	3.29A[2]	Haleakala (660)
2.56	2.67	2.68	2.45	2.36	2.55B		
Olinda (1050)	0.48	0.93	1.14	1.45	1.08	1.02C	
Mean	2.09b[2]	2.41a	2.44a	2.22b	2.24b		

1 Indicates maturity group designation of the variety.
2 Data followed by the same letter within the column or row are not significantly different (P=0.05) by Duncan's multiple range test.

Table 6. Percent of total nitrogen derived from symbiotic fixation by five soybean varieties grown at three elevations.

Site (elevation, m)	Varieties: Clay 00[1]	Clark IV	D68-0099 VI	N77-4262 VI	Hardee VIII	Mean
	----------%----------					
Kuiaha (320)	85	82	85	70	80	80B[2]
Haleakala (660)	62	71	65	66	68	66C
Olinda (1050)	95	98	98	98	96	97A
Mean	81ab[2]	84a	83a	78b	82ab	

1 Indicates maturity group designation of the variety.

2 Data followed by the same letter within the column or row are not significantly different (P=0.05) by Duncan's multiple range test.

Table 7. Comparison of estimated amounts of mineralized N and actual soil nitrogen uptake by five soybean varieties at three elevations.[1]

Varieties	Kuiaha, 320 m		Haleakala, 660 m		Olinda, 1050 m			
	Mineralizable nitrogen	Soil nitrogen uptake	Mineralizable nitrogen	Soil nitrogen uptake	Mineralizable nitrogen	Soil nitrogen uptake		
	kg N ha^{-1}							
Clay	46.8	37.0	145.1	76.7	37.1	2.2	Clark	56.0
	58.6	169.3	70.4	41.9	2.3	D68-0099	57.7	47.7
	184.4	95.3	43.7	2.7	N77-4262	59.8	78.6	191.0	88.7
	48.5	4.8	Hardee	66.1	67.9	211.8	87.5	50.4	4.8

1 Mineralizable nitrogen estimated from the soil incubation study (25 C, 0.03 MPa) and adjusted for soil temperature and the duration of crop growth. [For details of calculation, see Stanford et al. (1973, 1974)]. Actual soil N uptake is the N content of non-nodulating plants.

Table 8. Nitrogen uptake by Clark non-nodulating plants grown in three soils in the greenhouse at a mean soil temperature of 27 C.

Soils	Estimated rate of nitrogen mineralization[1]	Rate of nitrogen uptake by shoot[2]
	-------ug N g $soil^{-1}$ day^{-1}--------	
Kuiaha	0.39	1.02
Haleakala	1.29	1.31
Olinda	0.34	0.47

1 Estimated from soil incubation study (25 C, 0.03 MPa).
2 Calculated from the N uptake from 4 kg of soil (oven dry basis) by two plants after 45 days of growth.

Chapter 5

GROWTH RESPONSES OF FIELD GROWN SOYBEAN AND COMMON BEAN TO TEMPERATURE AT VARYING LEVELS OF N NUTRITION

Abstract

An understanding of the adaptation of legumes to varied environments is a prerequisite for selecting appropriate species or cultivar for maximum yield in a new environment. To assess this adaptation in soybean (*Glycine max* (L.) Merrill) and common bean (*Phaseolus vulgaris* L.) to temperature at varying levels of N nutrition, these two legumes were evaluated. The two species were grown at elevations of 320 m and 1050 m with applied N levels 9, 120 and 900 kg N ha^{-1}. The mean soil/air temperatures (C) during the experiment were 25/23 and 21/19 at the 320 and 1050 m elevations, respectively. Measurements of growth were made at end bloom (R2/R3), pod fill (R5/R6) and physiological maturity (R7). Higher elevation (low temperature) extended the vegetative growth period of both species an average of 44% and total crop duration 38%; low temperature had a relatively greater effect on soybean than on common bean. Application of 900 kg N ha^{-1} delayed the average days to R7 of soybean from 98 to 102 and common bean from 85 to 87. Average crop growth rate decreased by 26% at the 1050 m site compared to the 320 m site, but each legume attained similar dry weights at the two sites because the duration of growth was prolonged at the 1050 m site. Grain yield of soybean was higher than common bean at the 320 m site; the reverse was the case at the 1050 m site. Grain yield of soybean decreased by 30% at the 1050 m site due to a reduction in harvest index. In contrast to soybean, the grain yield of common bean was similar at the two elevations. Application of 900 kg N ha^{-1}

increased the grain yield an average of 22% primarily by increasing the crop growth rate and total dry matter production. During vegetative growth, common bean accumulated 46% more dry weight than soybean due to a 34 % higher crop growth rate and a longer vegetative period than soybean. The crop growth rate during the early reproductive phase of soybean (13.9 g m^{-2} d^{-1}) was 31% faster than common bean, yet the average growth rates of the two legumes over the crop duration were not significantly different. Total dry matter of soybean was 9% greater than common bean at R7, primarily due to a longer crop duration. Soybean had increased leaf production (leaf area and leaf weight ratio) and substantially reduced unit leaf rate compared to common bean at the 1050 m site. Nitrogen application increased the leaf area and crop growth rate of both legumes. The results indicate that extended growth duration and increased leaf production were the primary ways by which soybean and common bean compensated for reduced rate of growth at the higher elevation. The growth and yield of common bean was less affected by cool temperature than soybean.

Introduction

Understanding the effects of varied environments on growth and development of soybean (*Glycine max* (L.) Merrill) and common bean (*Phaseolus vulgaris* L.) may be helpful in selecting the appropriate species or cultivar for maximum grain yield in a new environment. Grain yield is determined by the total dry matter production and the proportion of total dry matter partitioned to reproductive tissue. Total dry matter is a function of the rate and duration of growth. Both

the rate and the duration of growth as well as partitioning of dry matter within the plant are influenced by environmental variables.

Temperature affects growth, development, and yield of legumes. Low temperature decreases their growth rate by decreasing the rate of photosynthesis through effects on structural and functional characteristics of leaves (Austin and Maclean, 1972; Gourdon and Planchon, 1982;). Simultaneously, low temperature extends the duration of growth by delaying transitions to successive growth phases in the life cycle of soybean and other legumes (Chapter 3; Hadley et al., 1984). A decrease in the growth rate of legumes associated with low temperature is partially offset by an increase in growth duration, so that the net effect of low temperature on total yield is reduced (Chapter 3; Shibles, 1980; Summerfield and Wien, 1980). In addition to the effect on the total dry matter production, temperature also regulates assimilate partitioning; low temperature during the reproductive phase can reduce yield of soybean (Holmberg, 1973). Low night temperature restricts pod formation (Hume and Jackson, 1981) and seed growth rate (Seddigh and Jolliff, 1984) by soybean.

Among the nutrient variables, nitrogen is especially important for legumes because of their high N requirement and tissue N concentration. Photosynthesis and dry matter accumulation by soybean and common bean are positively correlated with tissue N concentration (Lugg and Sinclair, 1981; Ojima, et al., 1965; Ryle and Hesketh, 1969). A reduction in N supply decreases the growth rate of new tissue more than the rate of photosynthesis in older leaves (Raper and Peedin, 1978).

Nitrogen stress also affects partitioning of N to seeds. Nitrogen limits the partitioning of dry matter and N to seeds relatively more than photosynthesis in soybean (Crafts-Brandner et al. 1984; Streeter, 1978).

The nitrogen requirement of legumes is met from both symbiotic and mineral N sources. The plant N status as influenced by symbiosis and mineral N availability have been ignored in studies of growth and development of legumes. Experiments conducted in 1985 (Chapter 3) showed that non-nodulated soybean plants grown under moderate levels of soil N matured earlier and yielded less than their nodulated counterparts due to N stress, regardless of the temperature regime. The high requirement of seeds for N, at a time when current assimilation of N from mineral and atmospheric sources is declining (Wych and Rains, 1979; Thibodeau and Jaworski, 1975), requires considerable remobilization of N from vegetative tissues to maintain seed filling in legumes (Sinclair and de Wit, 1976). Temperature and mineral N supply regulate both current assimilation and remobilization from vegetative tissues of N to seed in legumes.

It is clear that the effects of temperature and mineral N supply on growth, development and N nutrition are aspects of adaptability of legumes to environment. The purpose of the present study was to evaluate the response of soybean and common bean to temperature differences associated with elevation under varying levels of N nutrition.

Materials and Methods

Experimental design: The treatments were elevations of 320 and 1050 m, inoculated and uninoculated soybean, *Glycine max* (L) Merrill, and common bean, *Phaseolus vulgaris* (L), and nitrogen levels of 9, 120, and 900 kg N ha^{-1}. The experimental design was a randomized complete block with three replications at each elevation. At each site, the treatments were arranged in a split-split plot with soybean and common bean assigned to main plots, inoculation to sub plots and the three levels of nitrogen to sub-sub plots.

Field and plant culture: The characteristics of the two sites on the island of Maui, Hawaii are described in Table 1. The sites were tilled and lime was incorporated four weeks before planting to attain a soil pH between 5.5 to 6.0. Nutrients except nitrogen added before planting in kg ha^{-1} were 600 P; 300 K; 75 Mg; 25 Zn; 3 B; and 2 Mo.

Seeds of common bean, *P. vulgaris* cv. Brazil 2 (Centro Internacional de Agricultura Tropical) and soybean, *G. max* cv. Clark were planted at the 1050 m site on July 8, 1987 and at the 320 m site on July 15, 1987. The seeds for the inoculated treatment were coated with peat inoculants containing *Rhizobium leguminosarum* biovar *phaseoli* strains TAL 182, CIAT 632 and CIAT 899 for common bean and *Bradyrhizobium japonicum* strains USDA 110, USDA 136b, and USDA 138 for soybean. Seeds were sown in rows 60 cm apart in plots 9.5 by 3.0 m to attain a final population of 400,000 plants ha^{-1}. Plots were watered to field capacity on the day of planting and reirrigated when tensiometer readings were approximately 0.02 MPa. Nitrogen as ammonium sulfate was applied in equal amounts after planting and at growth stages end bloom

(R2/R3) and pod fill (R5/R6).

Plant sampling procedures: Measurements of growth and yield were made by harvesting plants approximately at growth stages end bloom, pod fill and physiological maturity (R7). Plant fresh weight was estimated by harvesting all plants at the soil surface from a 2.4 by 1.8 m area. Dry matter content of whole plants was established from a 10 to 15 plant sub sample after drying at 60 C. The sub samples at R7 were threshed for seed yield. Ground samples were digested by the salicylic acid modification of the Kjeldahl procedure (Keeney and Nelson, 1982) and total nitrogen in the digests was measured by the indophenol blue method (Keeney and Nelson, 1982).

Leaf area was measured with a Licor LI-3100 leaf area meter on leaves removed from 6 plants selected at random from each harvest. Leaves and stems were then dried (60 C) and weighed separately.

Growth analysis: Mean crop growth rate (CGR) was calculated by dividing dry matter accumulated by days between harvests. Leaf area index (LAI) was expressed as the leaf area per unit of land area. Leaf weight ratio (LWR) was calculated by dividing leaf dry weight by the total shoot dry weight. Approximate values of unit leaf rate (ULR) for each growth phase were calculated by the equation: ULR = Y / LAD, where Y is the dry matter increment during a given growth period and LAD is the leaf area duration for the same growth period (Hunt, 1982). LAD was calculated by the formula: LAD = (L1 + L2) (T2-T1) / 2, where L1 and L2 are the LAI at the beginning and end of the growth period and T1 and T2 are the number of days from the beginning and end of the growth period (Hunt, 1982).

Soil sampling: Extractable soil NH_3-N and NO_3-N were determined from soil samples collected to a depth of 20 cm 5 days after planting. Soil suspensions (1 part air dry soil: 7 parts 2M KCl) were agitated for one hour on a mechanical shaker. NH_3-N and NO_3-N were determined from filtered KCl extracts by the indophenol blue and the cadmium reduction methods, respectively (Keeny and Nelson, 1982).

Data analysis: The data from the inoculated segment of the experiment were subjected to analysis of variance procedures. Data from the uninoculated segment of the experiment were discarded because of nodulation observed in the uninoculated controls of common bean (see Appendix Table A-1).

Results and Discussion

The effects of temperature and N nutrition on growth and grain yield of soybean and common bean were studied at elevations of 320 m and 1050 m which provided approximately a 4 C differential in both soil and air temperatures (Table 1). The irradiance at the 1050 m site was 23% lower than the 320 m site, but the total solar radiation received during the total crop duration was highest at the higher site (Table 1). The difference in the irradiance between the sites was assumed to be insignificant for reasons discussed elsewhere (Chapter 4).

Only main effect means are presented and discussed unless significant interactions were encountered in the analysis of variance.

The vegetative and total durations were extended at the high cool site, with a relatively greater effect on soybean than common bean (Table 2). Although common bean flowered later than soybean, it matured

earlier resulting in shorter reproductive and total durations compared to soybean. Flowering and maturity of soybean were also delayed by cool temperatures in the 1985 experiment (Chapter 3) and similar results have been obtained by others (Hadley et al., 1984; Hesketh et al., 1973). Days to flowering increased relatively more at the 1050 m site than reproductive or total duration for both legumes, a result similar to that obtained in 1985 (Chapter 3).

Nitrogen application did not affect days to flowering but slightly delayed maturity of both legumes. Maturity of soybean was delayed by 5% by application of 900 kg N ha^{-1} compared to a 2% delay in common bean. This result is in agreement with the conclusion reached in Chapter 3 that N-deficient non-nodulating soybean plants matured earlier than their nodulating counterparts (Chapter 3).

The effect of elevation and N nutrition on growth and development will ultimately affect the grain yield of legumes. Grain yield is dependent on vegetative growth (source) and reproductive growth (sink) (Summerfield and Wien, 1980) as well as on the proportion of total dry matter partitioned to seed. Grain yields of soybean and common bean in the present experiment were differentially affected by the site environment (Table 3). While soybean had higher grain yield than common bean at the 320 m site, the reverse was true at the 1050 m site. The maximum grain yield of soybean at the highest site was 39% lower than that at the 320 m site. Unlike soybean, grain yield of common bean was similar between elevations. Grain yield of both legumes generally increased with N application, but the effect of N on soybean yield at

the 1050 m site was marginal.

Harvest indices of the two species were comparable at the 320 m site (Table 4). While dry matter partitioning to grain yield in common bean was relatively unaffected by low temperature, soybean proved to be quite sensitive. Despite similar total dry weights between elevations for both legumes (Table 5), grain yield of soybean decreased due to drastic reduction in harvest index at the higher site. Low temperature during the reproductive phase is reported to adversely affect pod and seed growth in soybean (Holmberg, 1973; Hume and Jackson, 1980; Seddigh and Jolliff, 1984). According to Thorne (1982), low temperature reduces the translocation of photosynthate into soybean seed. The substantial decrease in the whole plant tissue N concentration of soybean at the 1050 m site (Table 4) might have exacerbated the effect of low temperature on harvest index (Crafts-Brandner et al., 1984; Streeter, 1978).

For a given harvest index, increases in grain yield may be realized by increased total dry matter yield. Dry matter yield is a function of the duration of crop growth and crop growth rate. Total dry weights at the different growth stages (Table 5) between elevations were not different except at the pod fill stage. Crop growth rate during all three growth phases was significantly lower at the 1050 m site than at the 320 m site (Table 6). Similar final dry weights for the two legumes at both sites indicate that the prolonged duration of growth (Table 2) compensated for the reduced rates of growth at the higher elevation. Compensation for low temperature inhibition of growth has been

demonstrated for soybean (Chapter 3; Shibles, 1980; Summerfield and Wien, 1980). The results of this experiment indicates a similar mechanism for common bean.

The pattern of growth of the two legumes was unaffected by temperature. Dry weight accumulation by common bean during vegetative phase was 46% greater than soybean (Table 6) due to a 34% increase in crop growth rate and an 18% longer vegetative phase. The crop growth rate of soybean during the early reproductive phase was faster than that of common bean. The final dry weight of soybean was 9% greater than common bean. Although total dry matter of soybean was greater than for common bean at maturity (Table 5), the difference between the two legumes was not proportional to the 17% greater crop duration of soybean, mainly due to the fact that the crop growth rate of soybean declined substantially during the late reproductive phase. Despite the differences in crop growth rates between soybean and common bean during different phases of growth, average crop growth rates of the two legumes for the entire crop were similar. Therefore, total crop period accounted for most of the differences in final dry weight between soybean and common bean.

Crop growth rate increased with increase in N applied (Table 6) and the differences effectively accounted for the observed differences in dry matter accumulation (Table 5). Crop duration was also extended by N application (Table 2) accounting in part for the increased dry weight.

A reduced photosynthetic rate at low temperature may be

compensated for by increased dry matter allocation to leaves. Increased leaf production for soybean was observed at the 1050 m site (Table 7) where leaf area index and leaf weight ratio were substantially higher than at the 320 m site, especially at pod fill. In contrast, leaf area index and leaf weight ratio of common bean were similar at the 320 and 1050 m sites, an indication of its tolerance to cool temperature. Nitrogen application increased leaf area (see Appendix Table A-6) with no differential effect between the two legumes. The increased partitioning of dry weight into leaves observed at the cool site agrees with the observation of Wann and Raper (1984) that plants respond to changes in temperature by altering the partitioning of dry matter among organs. A higher leaf area retained for a longer period of time at the higher site may increase the total canopy photosynthesis and therefore, net dry matter accumulation.

Data in Table 8 indicate that the unit leaf rate, a measure of net photosynthesis per unit leaf area, was generally lower at the 1050 m site; unit leaf rate of soybean was relatively more affected than common bean. Both legumes had similar unit leaf rates during the vegetative phase, but soybean had significantly less leaf area and so had a lower crop growth rate and less dry weight accumulation than common bean. The greatest difference in unit leaf rate of soybean at the two elevations was during the early reproductive phase when the leaf area was also the greatest. The unit leaf rate during the early reproductive phase was substantially greater at the 320 m site for soybean than common bean, but both legumes had similar rates at the 1050 m site. In contrast to

soybean, the unit leaf rate of common bean was relatively unchanged between growth phases. Unit leaf rate was unaffected by N application (see Appendix Table A-7) and therefore, differences in crop growth rate were primarily due to changes in leaf area with N application.

Conclusions: The responses of soybean and common bean to temperature and N application were evaluated in terms of growth and phenological development. Reduced rates of growth by both legumes at the higher elevation was offset primarily by prolonged crop duration. Greater partitioning of dry weight into leaves was an additional mechanism by which soybean responded to low temperature. In spite of equivalent dry weight production among cool and warm sites, grain yield of soybean was reduced at the cool site due to lowered harvest index. Growth and yield of common bean was more tolerant to low temperature than soybean

Table 1. Environmental characteristics at two sites in an elevational transect on the island of Maui, Hawaii.

Site	Elevation	Soil classification	Extractable Soil N[1]	Temperature[2]		Solar radiation	
				Soil	Air	Irradiance[2]	Total[3]
	m		g N kg^{-1}	-- C --		W m^{-2}	MJ
Kuiaha	320	Humoxic Tropohumult	0.05	25	23	243	1640
Olinda	1050	Entic Dystrandept	0.10	21	19	186	1770

1 KCl extractable NH_3^+ and NO_3^- at five days after planting in plots receiving 3 kg N ha^{-1} as ^{15}N tracer.
2 Average for the experimental period.
3 Total solar radiation for the experimental period.

Table 2. Days to first flower (R1) and physiological maturity (R7) of inoculated soybean and common bean grown at two elevations with three levels of N.

Nitrogen applied[1]	R1		R7	
	Soybean	Bean	Soybean	Bean
kg N ha^{-1}	- - - - - - days		- - - - - -	
	320 m elevation			
9	28	35	79	73
120	28	35	84	74
900	28	35	84	75
	1050 m elevation			
9	43	48	116	97
120	43	48	119	98
900	43	48	120	98

1 Total applied split into equal applications at planting, end bloom and pod fill.

Table 3. Grain yield of soybean and common bean as affected by elevation and N application.

N applied[1]	Elevation (m)			
	320 m		1050 m	
	Soybean	Common bean	Soybean	Common bean
kg N ha^{-1}	- - - kg ha^{-1} - - -			
9	2801	2865	2369	2814
120	3317	2928	2601	2981
900	4260	3315	2307	3293

Analysis of variance

Source	df	Probability of significance by F-test
Elevation (E)	1	0.003
Legume (L)	1	0.310
Nitrogen (N)	2	0.002
E x L	1	0.003
E x N	2	0.031
L x N	2	0.596
E x L x N	2	0.032

1 Total applied split into equal applications at planting, end bloom and pod fill.

Table 4. Effect of elevation by legume species interaction on harvest index and whole plant tissue N concentration at physiological maturity.

Legume species	Elevation (m) 320	1050
	Harvest index (HI)	
Soybean	0.48	0.36
Common bean	0.49	0.45
	Tissue N concentration (TNC)	
	- - g N kg^{-1} - -	
Soybean	38	32
Common bean	26	25

Analysis of variance

Source	df	Probability of significance by F-test HI	TNC
Elevation (E)	1	0.002	<0.001
Legume (L)	1	0.004	<0.001
E x L	2	0.009	<0.001

Table 5. Main effects of elevation, legume species, and N level on total dry weights at different growth stages.

Treatment variable	Growth stage		
	End bloom[1]	Pod fill[2]	Maturity[3]
	- - - - - - - kg ha^{-1} - - - - - - -		
Elevation			
m			
320	2018	5087	6641
1050	1947	4463	6777
Legume			
Soybean	1614	4925	6983
Common bean	2351	4626	6435
Nitrogen level[4]			
kg N ha^{-1}			
9	1806	4533	6140
120	1997	4814	6674
900	2143	4980	7313

Analysis of variance

Source	df	Probability of significance by F-test		
Elev. (E)	1	0.599	0.001	0.673
Leg. (L)	1	0.001	0.223	0.041
Nitr. (N)	2	0.015	0.016	0.002

1 End bloom (R2/R3) harvest was 35 and 51 days after planting (DAP) for soybean and 41 and 55 DAP for common bean at 320 and 1050 m sites, respectively.
2 Pod fill (R5/R6) harvest was at 58 and 76 DAP for soybean and 62 and 77 DAP for common bean at 320 and 1050 m sites, respectively.
3 Harvest was at physiological maturity (R7).
4 Total applied split into equal applications at planting, end bloom and pod fill.

Table 6. Main effects of elevation, legume species, and N levels on mean crop growth rate during different phases.

Treatment variable	Growth phase			
	Vegetative[1]	Early repr.[2]	Late repr.[3]	Crop cycle[4]
	- - - - - g m^{-2} d^{-1} - - - - -			
Elevation				
m				
320	5.2	13.9	8.8	8.5
1050	3.7	10.6	8.4	6.3
Legume				
Soybean	3.8	13.9	6.6	7.2
Common bean	5.1	10.6	10.7	7.6
Nitrogen level[5]				
kg N ha^{-1}				
9	4.1	12.0	7.3	6.9
120	4.5	12.3	8.5	7.3
900	4.8	12.4	10.1	8.0

Analysis of variance

Source	df	Probability of significance by F-test			
Elev. (E)	1	0.006	0.001	0.758	0.001
Leg. (L)	1	0.004	0.014	0.003	0.152
Nitr. (N)	2	0.008	0.750	0.252	0.012

1 Vegetative phase is the period from planting to end bloom (R2/R3).
2 Early reproductive phase is the period from end bloom (R2/R3) to pod fill (R5/R6).
3 Late reproductive phase is the period from pod fill (R5/R6) to physiological maturity (R7).
4 Crop cycle is the period from planting to physiological maturity.
5 Total applied split into equal applications at planting, end bloom and pod fill.

Table 7. Effect of elevation by legume species interaction on leaf characteristics at different growth stages.

Legume	End bloom[1]		Pod fill[2]		Maturity[3]	
	320 m	1050 m	320 m	1050 m	320 m	1050 m
			Leaf Area Index (LAI)			
Soybean	2.3	3.6	3.9	5.3	1.8	2.5
Common bean	3.7	3.8	3.6	3.6	1.4	1.7
			Leaf Weight Ratio (LWR)			
Soybean	0.48	0.52	0.25	0.37	0.09	0.16
Common bean	0.42	0.49	0.21	0.24	0.08	0.08

Source	df	Analysis of variance: Probability of significance by F-test					
		End bloom		Pod fill		Maturity	
		LAI	LWR	LAI	LWR	LAI	LWR
Elev. (E)	1	0.015	<0.001	<0.001	0.004	0.100	0.002
Legume (L)	1	0.037	<0.001	0.012	<0.001	0.045	0.002
E x L	1	0.070	<0.025	0.036	0.002	0.389	0.007

1 End bloom (R2/R3) harvest was 35 and 51 days after planting (DAP) for soybean and 41 and 55 DAP for common bean at 320 and 1050 m sites, respectively.

2 Pod fill (R5/R6) harvest was at 58 and 76 DAP for soybean and 62 and 77 DAP for common bean at 320 and 1050 m sites, respectively.

3 Maturity harvest was at physiological maturity (R7).

Table 8. Effect of elevation and legume species on unit leaf rate during different growth phases.

Legume	Vegetative[1]		Early repr.[2]		Late repr.[3]	
	320 m	1050 m	320 m	1050 m	320 m	1050 m
	- - - - - - - g m^{-2} d^{-1} - - - - - - -					
Soybean	3.65	1.92	5.15	2.65	2.82	1.34
Common bean	3.33	2.10	3.25	2.60	3.85	4.37

Analysis of variance

Source	df	Probability of significance by F-test		
		End bloom	Pod fill	Maturity
Elev. (E)	1	<0.001	<0.001	0.229
Legume (L)	1	0.543	0.002	0.005
E x L	1	0.094	0.003	0.050

1 End bloom (R2/R3) harvest was 35 and 51 days after planting (DAP) for soybean and 41 and 55 DAP for common bean at 320 and 1050 m sites, respectively.

2 Pod fill (R5/R6) harvest was at 58 and 76 DAP for soybean and 62 and 77 DAP for common bean at 320 and 1050 m sites, respectively.

3 Maturity harvest was at physiological maturity (R7).

Chapter 6

NITROGEN ASSIMILATION AND N_2 FIXATION IN SOYBEAN AND COMMON BEAN AS AFFECTED BY ELEVATION AND MINERAL N AVAILABILITY

Abstract

The relationships between N assimilation, mineral N availability, and N_2 fixation in soybean (*Glycine max* L.) and common bean (*Phaseolus vulgaris* L.) grown under two temperature regimes were investigated. Soybean and common bean were grown at elevations of 320 and 1050 m where mean soil/air temperatures during the experiment were 25/23 and 21/19 C, respectively. Ammonium sulfate with an excess of ^{15}N-isotope was used to provide mineral N levels of 9, 120, and 900 kg N ha^{-1}. Nitrogen assimilation increased as applied mineral N increased for both species at both sites. With 900 kg N ha^{-1}, N assimilation by both species increased by 25% compared to treatments receiving 9 kg N ha^{-1}. With 900 kg N ha^{-1}, N assimilation by soybean at the 320 m site was 320 kg N ha^{-1} which was 67% higher than the maximum N assimilation by common bean. Total N of soybean at the 1050 m site was 31% less than that at the 320 m site; total N of common bean was similar at the two sites. Total N assimilation at the 1050 m site by both species was reduced far less than the reduction in the rate of N assimilation, because crop duration was greatly extended. The relative increases in N assimilation rates with increased mineral N in both species were generally greater during vegetative and late reproductive growth phases compared to the early reproductive phase. Nitrogen assimilation was most rapid during the early reproductive phase of soybean while for common bean it was relatively uniform throughout growth. The early reproductive phase of soybean accounted for 56% of total plant N assimilation but only 24% of

total crop duration. N_2 fixation by soybean followed a pattern similar to that of N assimilation; the period of maximum N assimilation coincided with the period of peak N_2 fixation. Common bean, relied relatively more on mineral N assimilation than soybean during the vegetative phase partly due to greater fertilizer N use efficiency. Common bean can grow better in low N soil than soybean, given the typical lag time in the development of the symbiotic N_2 fixing system. Symbiotic N_2 fixation may limit plant N assimilation in both legumes.

Introduction

The N requirement of legumes can be met by mineral N assimilation or symbiotic N_2 fixation, or both. However, symbiotic N_2 fixation is not a major source of N during the early and late phases of growth. Symbiotic N_2 fixation begins only after nodule formation, which is preceded by the colonization of the rhizosphere and the infection of legume roots by rhizobia (Hardy et al., 1971; Vincent, 1974). Dinitrogen fixation generally reaches a peak at early pod fill and then declines during the late reproductive phase (Lawn and Brun, 1974a; Latimore et al., 1977; Thibodeau and Jaworski, 1975). Therefore, mineral N is a major source of N for legumes during both the early and late growth phases.

The interaction between genetic yield potential and environment determines the N requirement of a legume. The contributions from symbiosis and mineral N to total plant N are determined by the legume N requirement and the soil N availability in a given environment. When soil N uptake is less than the legume N requirement, N_2 fixation is

promoted. The N_2 fixation potential is approximately equal to the aggregate of deficits in mineral N uptake per day over the legume life cycle. The daily N requirement of the legume is dependent on its growth rate and developmental stage. Therefore, N_2 fixation also depends on the ontogeny of the legume, its efficiency of mineral N uptake, soil N availability and yield potential.

Temperature is a major environmental variable that regulates yield potential, plant N requirement and soil N availability. Temperature regulates crop growth rate and crop development and, therefore, the rate of N assimilation. Experiments conducted in 1985 (Chapter 3) indicated that the reduction in total plant N in soybean due to decreased rates of growth and N assimilation under low temperature was partially compensated for by an extended crop duration. Temperature also regulates N uptake directly (Rufty et al., 1981; Tolley and Raper, 1985). Since temperature indirectly regulates plant N requirement by regulating growth and directly regulates mineral N uptake, it also regulates the N_2 fixation potential.

Mineral N application in sufficient amounts replaces N_2 fixation (Allos and Bartholomew, 1959). However, N_2 fixation compensates to a degree for decreased mineral N availability, and therefore, there is a paradox in interpreting the response of legumes to mineral N application. Significant yield and total plant N increases are not always obtained in legumes in response to N fertilization (Bhangoo and Albritton, 1976; Cassman et al., 1981; Diebert et al., 1979; Lawn and Brun, 1974b; Piha and Munns, 1987; Summerfield et al., 1977). It has

been suggested that both mineral and fixed N are required for maximum yield and N assimilation in legumes (Bhangoo and Albritton, 1976; Franco et al., 1979; Harper, 1974). However, considering the high energy requirement for the development, maintenance and functioning of the symbiotic system in comparison to mineral N assimilation (Finke et al., 1982; Pate et al., 1979; Ryle et al., 1978 & 1979), the yield potential of legumes relying totally on N_2 fixation may be limited. Soybean grown in solution culture supplied with mineral N assimilates substantially greater amounts of N than do nodulated plants (Jones et al., 1981; Lathwell and Evans, 1951; Wych and Rains, 1979). Similar results have not been reported from field experiments. It appears that legumes have a greater yield potential than can be supported by the symbiosis. Attaining the genetic yield potential may require maintaining an adequate fertilizer N regimen throughout growth.

Legumes differ substantially in their N requirements. While comparisons between soybean and common bean under identical growing conditions are rare (Piha and Munns, 1987), the data for soybean (Chapter 4; Cassman et al., 1981; Herridge et al., 1984;) and common bean (Rennie and Kemp, 1983; Westerman et al., 1981) show that soybean has a higher N requirement than common bean. The higher N requirement of soybean is mainly due to a greater seed N concentration than common bean rather than greater dry matter accumulation or seed yield. Because of the contrasting N requirements of these two legumes, soil N availability that is sufficient to meet the N requirement of common bean may be inadequate to meet the soybean N requirement without significant

N_2 fixation. Accordingly, soybean meets a major portion of its N requirement through symbiosis in soils of low to moderate N availability (Chapter 4) while many reports indicate very little or no N_2 fixation in common bean (El-Nadi et al., 1972; Graham, 1981) except under very low soil N conditions (Piha and Munns, 1987; Rennie and Kemp, 1983; Westerman et al., 1981).

It is clear that in order to maximize the N derived from fixation in legumes, it is important to understand the patterns of N assimilation for the two crops and how those patterns may be influenced by the interactions between environment, legume N requirement, and soil N availability. The contrasting N requirements and N_2 fixation potentials of soybean and common bean provide an opportunity to identify N assimilation traits that are useful in plant breeding programs aimed at increasing N_2 fixation. In the present experiment, the effects of elevation (temperature) and N regimes on the relationships between yield potential, N requirement, soil N uptake, and N_2 fixation by soybean and common bean were studied.

Materials and Methods

Common bean (*Phaseolus vulgaris* L.) and soybean (*Glycine max* (L.) Merrill) were grown with and without inoculation at elevations of 320 and 1050 m on the island of Maui with applied nitrogen levels of 9, 120 and 900 kg N ha^{-1} as ammonium sulfate. The experimental design, site characteristics, field and plant culture, and sampling procedures for determining plant dry matter yield were described in detail previously in Chapter 5. Only those procedures which were specific to this

investigation are described below.

N and ^{15}N application: The total N applied was split into thirds with one-third applied just after planting, one-third at end bloom (R2/R3), and one-third at pod fill (R5/R6) stages. At each stage, aqueous solutions of ^{15}N enriched ammonium sulfate were sprayed on 1.35 x 3.0 m microplots within each 9.5 x 3.0 m whole plot. The aqueous ammonium sulfate was enriched to provide 30 mg ^{15}N m^{-2} in each microplot at each application. Aqueous solutions of natural ^{15}N abundance ammonium sulfate was sprayed on the remaining area of the whole plots.

Plant sampling: At growth stages end bloom, pod fill and physiological maturity (R7), 10 to 15 plants were harvested from the center three rows of the five-row microplots by cutting them at the soil surface. Whole-plant fresh and dry weights were determined on the microplot subsamples. The plants were dried to a constant weight at 60 C. The dried microplot subsamples were ground and digested in H_2SO_4 with 1% (w:v) salicylic acid to convert tissue N, including nitrate, into ammonium. Ammonium-N in digest subsamples was measured by the indophenol blue method (Keeney and Nelson, 1982). Kjeldahl digests were steam distilled with 13 N NaOH and the distillates were collected in 0.02 N H_2SO_4. The distillates were then acidified to a pH of 4.5 to 5.0 and concentrated by evaporating on a hot plate under medium heat for mass spectroscopic analysis of ^{15}N (Bergersen, 1980).

Sampling and extraction of soil N: Soil samples from the ^{15}N microplots were collected to a depth of 20 cm (4 samples per microplot) at 5 days after planting, end bloom, pod fill and physiological maturity.

Extractable soil NH_3-N and NO_3-N were determined as described in Chapter 5.

^{15}N calculations: The amount of plant N derived from the atmosphere and fertilizer and the fertilizer use efficiency were calculated by equations developed for ^{15}N dilution techniques (International Atomic Energy Agency, 1983). The atom% ^{15}N excess was calculated with reference to the natural ^{15}N abundance of air (0.3663 atom% ^{15}N). The non-nodulating isoline served as the non-N_2-fixing reference plant for soybean. N_2 fixation could not be determined for common bean because some nodulation was observed in the uninoculated controls (see Appendix Table A-1). The non-nodulating isoline of soybean was judged to be an unsatisfactory reference crop for common bean because of differences between the two legumes in total mineral N uptake and the pattern of N assimilation.

Statistical analysis: The data from the inoculated segment of the experiment were subjected to analysis of variance.

Results and Discussion

The mean air temperatures during the experiment were 24 and 19 C at the 320 and 1050 m elevations, respectively (Chapter 5). The extractable N (NH_4^+ and NO_3^-) at planting was 0.05 and 0.10 g kg^{-1} soil at the 320 and 1050 m sites, respectively (Chapter 5).

In the present experiment, total N assimilation for soybean decreased significantly with increased elevation while N assimilation of common bean was relatively unaffected (Table 1). Nitrogen assimilation by both legumes increased significantly with increasing applied mineral

N. The results indicate that up to 900 kg N ha^{-1}, N assimilation by both soybean and common bean was limited by mineral N availability. Nitrogen assimilation for both legumes increased by 25% for plants fertilized with 900 kg N ha^{-1} compared to plants receiving only 9 kg N ha^{-1}. Although the elevation by N interaction was not significant, the increase in total N of soybean in response to mineral N application was less pronounced at the 1050 m site. The data demonstrate that maximum N assimilation by soybean and common bean can only be realized when sufficient mineral N is applied. Reports on total plant N increases in the field due to mineral N applications have been inconsistent and the total N of non-N_2 fixing plants has seldom exceeded that of an N_2-fixing counterpart (Bhangoo and Albritton, 1976; Cassman et al., 1981; Diebert et al., 1979; Harper, 1974; Lawn and Brun, 1974b; Piha and Munns, 1987; Weber, 1966; Westerman et al., 1981). The total amount of mineral N applied or the application regimen used in other experiments may have been inadequate to maintain sufficient N in the soil solution to meet plant N assimilation potential. High levels of N in the root zone may increase crop yield by permitting a plant to partition relatively more dry matter to shoots than to roots (Cassman et al., 1980; Tolley-Henry and Raper 1986; Vessey and Layzell, 1987) compared to plants that rely on symbiotic N_2 fixation. In this study, N_2 fixation by soybean declined with N application, but was still appreciable at 900 kg N ha^{-1} at the warm site (Table 2). It appears that, unless fertilizer use efficiency can be improved, more than 900 kg ha^{-1} mineral N needs to be applied to completely suppress N_2 fixation and obtain maximum N

assimilation by soybean under warm growing conditions.

Mineral N application increased the total N assimilated by increasing the average rate of N assimilation by both species (Table 3). The average rate of N assimilation over the duration of the crop was 25% higher at 900 kg N ha^{-1} than it was at 9 kg N ha^{-1}; however, the same response was not observed at all growth phases. The increase in the rate of N assimilation with increasing N applied was generally greatest during the vegetative and late reproductive phases. The rate of N assimilation did not increase with applied N during the early reproductive phase except for common bean at the 320 m site. Since the N_2 fixation by soybean was limited during the vegetative and the late reproductive phases (Table 2), mineral N availability during the early and late growth periods is critical to attain maximum N assimilation.

Low temperature reduces rate of growth and N assimilation, but this effect can be compensated for by extended crop duration. The results indicate that the rate of N assimilation at the 1050 m site was reduced by as much as 53% in soybean and 24% in common bean compared to the 320 m site (Table 3). Yet, no reduction in total N assimilation was observed at the 1050 m site in common bean and the reduction in soybean was only 31% compared to the 320 m site (Table 1). Therefore, total N assimilated over the crop cycle was less affected by low temperature than the rate at which N was assimilated.

The relative N assimilation in relation to the relative duration of different growth phases of soybean and common bean is displayed in Table 4. Soybean and common bean were found to have distinct N

assimilation patterns over time. On average, 50% of the total N assimilated by common bean occurred during vegetative growth, the period of which accounted for 56% of its total crop duration, indicating that growth and N assimilation were proportional. The above relationship between N assimilation and growth phase duration was continued up to physiological maturity of common bean. In contrast, only 28% of the total N was assimilated by soybean during its vegetative phase, a period that accounted for 44% of its total crop duration. The slow rate of N assimilation during the vegetative phase of soybean was followed by a period of rapid N assimilation in the early reproductive phase when 56% of the total N was assimilated in 24% of its total crop duration. The differences in the pattern of N assimilation between soybean and common bean in the present experiment generally prevailed across sites, however, the patterns were less pronounced at the 1050 m site. The pattern of N assimilation observed in the present experiment may be characteristic to these legumes.

The relationship between growth phase and N assimilation for these legumes seems to influence their N_2 fixation potentials. The relative contributions of symbiotic N_2 fixation and mineral N assimilation to total plant N are determined by the N requirement of the legume, the soil N availability and the efficiency of mineral N uptake. Under a given level of soil N availability, the relative importance of the two modes of N nutrition is determined by the N assimilation characteristics of the legume. The lower per day N requirement of common bean may limit N_2 fixation compared to soybean under conditions of equal mineral N

availability. The relationship between the time course of N assimilation by the legume and the ontogeny of the symbiotic N_2 fixing system may also have an influence on the amount of N_2 fixed. The very rapid N assimilation during the relatively short early reproductive phase of soybean was also the time when N_2 fixation reached a maximum (Table 2). Additionally, isotope analysis indicated that the early N requirement of common bean was mostly met by soil N assimilation. The fertilizer N use efficiency of common bean was about two times higher than soybean at end bloom, but was similar to soybean both at pod fill and physiological maturity (Table 5). Consistent with the higher fertilizer N use efficiency of common bean during vegetative growth, the extractable soil N was significantly lower in plots of common bean than soybean at end bloom (Table 6). The extractable soil N in plots of both legumes were similar at pod fill, but was lower under soybean than common bean at physiological maturity. Therefore, common bean had a higher reliance on mineral N uptake than soybean during vegetative growth and utilized proportionately more mineral N throughout growth. The higher mineral N uptake efficiency of common bean during vegetative growth when the symbiosis is still establishing gives the plant an advantage over soybean under low soil N.

Conclusions: Attaining maximum N assimilation by common bean and soybean in the field requires greater mineral N levels applied at more frequent intervals than has been a common practice in other experiments. The data presented here show that significant N_2 fixation limits total N assimilation by soybean. The pattern of N assimilation in soybean was

characterized by low N assimilation during vegetative growth, followed by rapid assimilation during the early reproductive phase. Nitrogen assimilation by common bean was relatively uniform throughout the growth cycle. Nitrogen assimilation by soybean followed a pattern similar to the ontogeny of the symbiotic N_2-fixing system; the period of maximum N assimilation coincided with the period of peak N_2 fixation. Common bean, instead relies more on mineral N assimilation due to higher mineral N use efficiency than soybean, especially during the vegetative phase. Maximizing N derived from fixation in common bean may require altering its N assimilation characteristics.

Table 1. Elevation by legume by N level interaction on N assimilation at physiological maturity.

N applied	Elevation (m)			
	320		1050	
	Soybean	Common bean	Soybean	Common bean
(kg ha^{-1})[1]	- - - - - kg N ha^{-1} - - - - -			
9	241	142	209	149
120	252	147	229	170
900	320	191	220	192

Analysis of variance

Source	df	Probability of significance by F-test
Elevation (E)	1	0.067
Legume (L)	1	<0.001
Nitrogen (N)	2	<0.001
E x L	1	0.010
E x N	2	0.011
L x N	2	0.983
E x L x N	2	<0.083

1 Total applied split into equal applications at planting, end bloom and pod fill.

Table 2. Effect of elevation and N level and growth stage on N_2 fixation[1] by soybean at different growth stages.

N applied	Growth stage		
	End bloom	Pod fill	Maturity
	- - -kg N ha^{-1}- - -		
$(kg\ ha^{-1})^2$	320 m elevation		
9	37	161	168
120	25	117	109
900	20	29	41
	1050 m elevation		
9	13	70	67
120	9	55	49
900	0	0	0

Analysis of variance

Source	df	Probability of significance by F-test		
Elevation (E)	1	0.003	0.001	0.007
Nitrogen (N)	2	0.124	0.001	0.003
E x N	2	0.685	0.307	0.474

1 Determined by ^{15}N-dilution using non-nodulating isoline of soybean as reference.
2 Total applied split into equal applications at planting, end

bloom and pod fill.

Table 3. Effect of elevation, N application and legume species on the rate of N assimilation during crop cycle.

N applied	Elevation (m)			
	320		1050	
	Soybean	Common bean	Soybean	Common bean
(kg ha^{-1})[1]	- - - - g m^{-2} d^{-1} - - - -			
	Vegetative phase (Veg.)[2]			
9	0.17	0.19	0.11	0.12
120	0.19	0.20	0.14	0.12
900	0.22	0.28	0.15	0.16
	Early reproductive phase (ER)[3]			
9	0.67	0.22	0.50	0.28
120	0.64	0.20	0.50	0.38
900	0.68	0.28	0.49	0.29
	Late reproductive phase (LR)[4]			
9	0.15	0.16	0.07	0.11
120	0.16	0.20	0.08	0.10
900	0.34	0.15	0.04	0.20
	Crop Cycle (CC)[5]			
9	0.31	0.20	0.18	0.15
120	0.30	0.20	0.19	0.17
900	0.38	0.25	0.18	0.19

Analysis of variance

Source	df	Probability of significance by F-test			
		Veg.	ER	LR	CC
Elevation (E)	1	0.003	0.288	<0.001	<0.001
Legume (L)	1	0.098	<0.001	0.568	0.001
Nitrogen (N)	2	<0.001	0.879	0.040	<0.001
E x L	1	0.085	0.008	0.027	0.003
E x N	2	0.219	0.206	0.409	0.010
L x N	2	0.268	0.674	0.531	0.781
E x L x N	2	0.645	0.632	0.002	0.211

1 Total applied split into equal applications at planting, end bloom and pod fill.
2 Vegetative phase is the period from planting to end bloom.
3 Early reproductive phase is the period from end bloom to pod fill.
4 Late reproductive phase is the period from pod fill to physiological maturity.
5 Crop cycle is the period from planting to physiological maturity.

Table 4. Relative N assimilation[1] during different growth phases of soybean and common bean grown at two elevations.

Growth phase	Elevation (m)			
	320		1050	
	Soybean	Common bean	Soybean	Common bean
	- - - - - - - - - % - - - - - - - - -
Vegetative	25 (43)[2]	58 (56)	31 (44)	42 (56)
Early repr.	56 (28)	30 (28)	57 (21)	41 (22)
Late repr.	19 (29)	12 (16)	12 (35)	17 (22)

Source	df	Analysis of variance Probability of significance by F-test		
		Vegetative	Early repr.	Late repr.
Elevation (E)	1	0.157 (0.055)	0.120 (<0.001)	0.297 (<0.001)
Legume (L)	1	<0.001 (<0.001)	<0.001 (<0.001)	0.654 (<0.001)
E * L	1	0.003 (0.249)	0.039 (0.003)	0.052 (0.375)

1 Amount of N assimilated during the growth phase expressed as a percentage of total N at physiological maturity.
2 Value in parenthesis is growth phase duration expressed as a percentage of total crop duration.

Table 5. Effect of legume and N levels on fertilizer N use efficiency[1] during the crop cycle.

N applied	Growth stage					
	End bloom		Pod fill		Maturity	
	Soybean	Bean	Soybean	Bean	Soybean	Bean
(kg ha^{-1})[2]	- - - - - - - % - - - - - - -					
9	7.1	14.8	31.0	34.5	35.4	37.1
120	10.4	16.0	34.3	33.0	42.7	40.1
900	10.1	18.2	19.4	17.4	18.4	13.8

Analysis of variance

Source	df	Probability of significance by F-test		
Legume (L)	1	0.001	0.971	0.437
Nitrogen (N)	2	0.033	<0.001	<0.001
L x N	2	0.510	0.128	0.355

1 Fertilizer N use efficiency is determined by using ^{15}N isotope as tracer and is the percent of applied fertilizer nitrogen assimilated by the plant.
2 Total applied split into equal applications at planting, end bloom and pod fill.

Table 6. Effect of legume and N levels on KCl-extractable soil N during the crop cycle.

N applied	Growth stage					
	End bloom		Pod fill		Maturity	
	Soybean	Bean	Soybean	Bean	Soybean	Bean
$(kg\ ha^{-1})^1$	$g\ N\ kg^{-1}$ soil					
9	0.029	0.017	0.019	0.014	0.016	0.016
120	0.026	0.025	0.023	0.022	0.015	0.019
900	0.101	0.075	0.155	0.140	0.173	0.215

Analysis of variance

Source	df	Probability of significance by F-test		
Legume (L)	1	0.001	0.971	0.437
Nitrogen (N)	2	<0.001	<0.001	<0.001
L x N	2	0.034	0.290	<0.001

1 Total applied split into equal applications at planting, end bloom and pod fill.

SUMMARY AND CONCLUSIONS

Experiments were conducted to study the growth and yield responses of soybean (*Glycine max* L. Merrill) and common bean (*Phaseolus vulgaris* L.) to mode of nitrogen nutrition and temperature. Three sites in an elevational transect (320, 660, 1050 m) differing in mean annual temperatures were used to conduct two experiments.

In the first experiment, five varieties of *G. max* belonging to four maturity groups (00, IV, VI and VIII) were planted at the three sites to determine i) the effect of temperature, soil type and host genotype on nodulation by a mixed inoculum composed of three serologically distinct *Bradyrhizobium japonicum* strains; ii) the phenology of soybean as affected by temperature, maturity group and nitrogen nutritional status; and iii) the effect of elevation (site) and maturity group on the proportion of nitrogen derived from N_2 fixation. The mean soil/air temperatures (C) during the experimental period were 24/23, 23/20, and 20/18 at 320, 660, and 1050 m elevations, respectively.

In a second experiment, inoculated and uninoculated soybean and common bean were grown with either 9, 120 or 900 kg N ha^{-1} at elevations of 320 and 1050 m. The objectives were i) to evaluate the growth responses of *G. max* and *P. vulgaris* to cool and warm temperatures under varying levels of nitrogen nutrition; and ii) to contrast the nitrogen assimilation patterns of *G. max* and *P. vulgaris*. The mean soil/air temperatures (C) during this experiment were 25/23 and 21/19 at 320 and 1050 m elevations, respectively.

In the first experiment, differences in soil, temperature, and soybean growth between sites had little effect on the pattern of nodule

occupancy by *B. japonicum* strains USDA 110, 136b and 138 inoculated in a mixture. USDA 110 occupied 81% of nodules and plant growth parameters were unrelated to differences in nodule occupancy by the strains. Average dry matter and nitrogen accumulation at the highest site were only 48 and 41% respectively of that obtained at the lowest site. Nitrogen fixation contributed 80, 66, and 97% of total plant nitrogen at the lowest, intermediate and highest sites, respectively. Although total nitrogen assimilation between varieties differed in some cases by 400%, the proportions of nitrogen derived from fixation were similar within a site. Soybean maturity was delayed with increasing elevation mainly due to extended vegetative growth periods averaging 23 and 64% longer at the 660 and 1050 m sites compared to the lowest site. The effect of elevation on the duration of the seed-fill phase was minimal, but the rate of seed dry matter accumulation (kg ha^{-1} d^{-1}) decreased with increasing elevation. Non-nodulating isolines matured earlier than their nodulating counterparts due to nitrogen insufficiency.

In the second experiment, the number of days to flowering and maturity for both soybean and common bean were delayed at 1050 m compared to the 320 m elevation; low temperature had a relatively greater effect on soybean than common bean. Average crop growth rate (kg biomass ha^{-1} d^{-1}) decreased by 26% at the 1050 m site compared to the 320 m site, but each legume attained similar dry weights among the sites due to prolonged duration of growth at the 1050 m site. Grain yield of soybean was 30% lower at the 1050 m site due to reduced harvest index. In contrast to soybean, the grain yield of common bean was similar

between elevations. Application of 900 kg N ha^{-1} increased the grain yield an average of 22% primarily by increasing the crop growth rate and total dry matter production. Common bean accumulated 46% more dry weight than soybean during vegetative growth due to a higher crop growth rate and a longer vegetative duration than soybean. Average crop growth rate for the entire growth period for the two legumes was similar; the total dry matter of soybean was 9% greater than common bean because of longer crop duration. Soybean had increased leaf production (leaf area and leaf weight ratio) and substantially reduced unit leaf rate (g m^{-2} d^{-1}) at the high elevation compared to common bean. Nitrogen application increased the leaf area and crop growth rate of both legumes. With 900 kg N ha^{-1}, N assimilation by both legumes increased by 25% compared to treatments receiving 9 kg N ha^{-1}. With 900 kg N ha^{-1}, N assimilation by soybean at the 320 m site was 320 kg N ha^{-1} which was 67% higher than the maximum N assimilation by common bean. Total N of soybean at the 1050 m site was 31% less than that at the 320 m site; total N of common bean did not differ between the two sites. Because crop duration of both species was greatly extended at the high, cool site, total N assimilation was reduced far less than the rate of N assimilation. Nitrogen assimilation was more rapid during the early reproductive phase of soybean while for common bean N assimilation was relatively uniform throughout growth. Nitrogen fixation by soybean followed a pattern similar to that of N assimilation; the period of maximum N assimilation coincided with the period of peak N_2 fixation. Common bean relied relatively more on mineral N due to greater fertilizer N use efficiency

than soybean, especially during the vegetative phase.

The results of the two experiments indicated that: i) rhizobial strains superior in competitiveness may be identified for a broad range of host genotypes and environments; ii) the proportion of nitrogen derived from symbiotic nitrogen fixation by soybean was approximately constant at a site and therefore the contribution of the symbiosis to a crop's nitrogen requirement may be predicted after characterizing soil nitrogen availability at a site; iii) low temperature prolonged the vegetative and total growth of soybean and common bean; iv) maturity of soybean was dependent on the nitrogen nutritional status of the crop and nitrogen stress hastened maturity; v) extended growth duration and increased leaf production were two primary responses which compensated for reduced rate of growth of soybean and common bean at the higher elevation; vi) growth and yield of common bean were more stable across a wider range of temperatures than was the case for soybean; vii) maximum N assimilation by soybean and common bean does not occur if there is significant N_2 fixation; and viii) nitrogen derived from N_2 fixation by common bean probably can only be maximized by genetic manipulation of its N assimilation characteristics.

APPENDIX

The following tables are part of the experiment described in Chapters 5 and 6. They were deemed to be not of sufficient importance to be incorporated into the body of the dissertation.

Table A-1. Effect of elevation and N level on nodule dry weight at end bloom (R2/R3).

N applied[1]	Soybean	Common bean	
		Uninoculated	Inoculated
kg N ha^{-1}	- - - - - - - g m^{-2} - - - - - - -		
	320m elevation		
3	8.6	4.6	6.9
40	6.7	2.7	5.7
300	3.3	0.5	0.5
	1050m elevation		
3	3.9	0.6	3.8
40	2.6	0.8	2.4
300	1.4	0.6	1.0

1 N applied at planting.

Table A-2. Days to flowering (R1) and physiological maturity (R7) of the non-nodulating isoline of Clark soybean grown at two elevations with three levels of mineral N.

Nitrogen applied[1]	R1	R7
kg N ha^{-1}	- - - -days- - - - -	
	320 m elevation	
9	28	74
120	28	79
900	28	82
	1050 m elevation	
9	43	110
120	43	118
900	43	120

1 Total N applied was split into equal applications at planting, end bloom, and pod fill.

Table A-3. Effect of N level on the harvest index of legumes grown at two elevations.

N applied[1]	Soybean		Common bean
	Nod	Non-nod	
kg N ha^{-1}	320m elevation		
9	0.47	0.34	0.51
120	0.48	0.43	0.47
900	0.50	0.50	0.50
	1050m elevation		
9	0.36	0.32	0.45
120	0.38	0.36	0.45
900	0.33	0.28	0.45

1 Total N applied was split into equal applications at planting, end bloom, and pod fill.

Table A-4. Grain yield of the non-nodulating isoline of Clark soybean grown at two elevations with three levels of mineral N.

N applied[1]	Elevation (m) 320 m	1050 m
kg N ha^{-1}	- - - kg ha^{-1} - - -	
9	1316	2030
120	2387	2464
900	4163	2074

1 Total N applied was split into equal applications at planting, end bloom and pod fill.

Table A-5. Total N assimilation by the non-nodulating isoline of Clark soybean grown at two elevations with three levels of mineral N.

N applied[1]	Elevation (m) 320 m	1050 m
kg N ha^{-1}	- - - kg ha^{-1} - - -	
9	84	165
120	152	203
900	299	232

1 Total N applied was split into equal applications at planting, end bloom and pod fill.

Table A-6. Effect of N level on the leaf area index (LAI) and leaf weight ratio (LWR) of legumes at different growth stages.

N applied[1]	End bloom[2]		Pod fill[3]		Maturity[4]	
	LAI	LWR	LAI	LWR	LAI	LWR
kg N ha^{-1}	- - - - - - - kg ha^{-1} - - - - - - - - - -					
9	3.1	0.49	3.8	0.26	1.9	0.11
120	3.4	0.47	4.2	0.26	2.0	0.11
900	3.6	0.47	4.2	0.28	1.7	0.09

1 Total N applied was split into equal applications at planting, end bloom, and pod fill.
2 End bloom (R2/R3) harvest was 35 and 51 days after planting (DAP) for soybean and 41 and 55 DAP for common bean at 320 and 1050m sites, respectively.
3 Pod fill (R5/R6) harvest was at 58 and 76 DAP for soybean and 62 and 77 DAP for common bean at 320 and 1050m sites, respectively.
4 Maturity harvest was at physiological maturity (R7).

Table A-7. Effect of N level on the unit leaf rate of legumes at different growth stages.

N applied[1]	End bloom[2]	Pod fill[3]	Maturity[4]
kg N ha^{-1}	- - - - - - - g m^{-2} d^{-1} - - - - - - -		
9	2.72	3.55	2.73
120	2.77	3.38	2.97
900	2.76	3.31	3.60

1 Total N applied was split into equal applications at planting, end bloom, and pod fill.
2 End bloom (R2/R3) harvest was 35 and 51 days after planting (DAP) for soybean and 41 and 55 DAP for common bean at 320 and 1050m sites, respectively.
3 Pod fill (R5/R6) harvest was at 58 and 76 DAP for soybean and 62 and 77 DAP for common bean at 320 and 1050m sites, respectively.
4 Maturity harvest was at physiological maturity (R7).

Table A-8. Fertilizer use efficiency of legumes grown at two elevations with three N levels.

N applied[1]	Soybean		Common bean	
	320m	1050m	320m	1050m
kg N ha^{-1}	- - - - - - % - - - - - - -			
9	26	47	25	48
120	37	49	31	49
900	21	16	15	13

1 Total N applied was split into equal applications at planting, end bloom, and pod fill.

Table A-9. KCl-extractable soil N after harvest of legumes grown at two elevations with three N levels.

N applied[1]	Soybean		Common bean	
	320m	1050m	320m	1050m
kg N ha^{-1}	- - - - - g N kg^{-1} soil - - - - -			
9	0.015	0.016	0.016	0.016
120	0.015	0.015	0.013	0.024
900	0.150	0.200	0.179	0.251

1 Total N applied was split into equal applications at planting, end bloom, and pod fill.

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