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The Relationship Between Photosynthesis and the Capacity for Nitrogen Fixation in Soybean.

Eddie Paul Millhollon
Louisiana State University and Agricultural & Mechanical College

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THE RELATIONSHIP BETWEEN PHOTOSYNTHESIS AND THE CAPACITY FOR NITROGEN FIXATION IN SOYBEAN

The Louisiana State University and Agricultural and Mechanical Col. Ph.D. 1984

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THE RELATIONSHIP BETWEEN PHOTOSYNTHESIS AND THE CAPACITY FOR NITROGEN FIXATION IN SOYBEAN

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Agronomy

by
Eddie Paul Millhollon
B.S., Nicholls State University, 1977
M.S., Louisiana State University, 1980
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ABSTRACT

Diurnal trends in leaf carbohydrate partitioning and nodule activity in soybeans under natural conditions and the irradiance level required to allocate sufficient carbohydrate to obtain maximum rates of $N_2(C_2H_2)$ reduction were studied. Soybeans grown outdoors maintained constant levels of soluble carbohydrates in the leaves and constant rates of N_2 fixation and root + nodule respiration when root temperature was kept constant but shoot temperature varied. When plants were subjected to a 40-hour dark period, then exposed to 200 to 1000 $\mu E\ m^{-2}\ sec^{-1}$, 200 $\mu E\ m^{-2}\ sec^{-1}$ resulted in maximum leaf soluble carbohydrate and nodule activity. Results suggest that nodule activity is controlled by carbohydrate partitioning in the shoot and support the concept of an environment-mediated programming of carbohydrate distribution.

Carbon and nitrogen limitations to growth of symbiotically-grown soybean plants were assessed by examining growth characteristics of plants grown under low irradiance in a greenhouse and high irradiance outdoors and provided 0.0, 2.0, 6.0 or 12.0 mM NO_3. Under low irradiance, supplementing N_2 fixation with 2.0 mM NO_3 resulted in relative growth rates (RGR), leaf area ratios (LAR) and net assimilation rates (NAR) very similar to plants supplied 12.0 mM NO_3. As a result, total plant dry weight and leaf area of these two treatments were equivalent in 6-week-old plants despite a significantly lower N content in the 2.0 mM treatment.

Under high irradiance, plants supplied 6.0 or 12.0 mM NO_3 manifested greater relative growth rates and net assimilation rates during growth. Total plant dry weight and N content were also greater compared to the 0.0 and 2.0 mM treatments at six weeks. Leaf N content and area were equivalent in all treatments at this time. Results suggest that growth limitations to nodulated soybeans are primarily due to an inability to arrive at a functional balance between C and N accumulation prior to establishment of a fully functional N_2 fixation system. Once N_2 fixation is established, the increased input of N is used preferentially to increase both the photosynthetic efficiency and area of leaf tissue.

LITERATURE REVIEW

Nitrogen, the most abundant element in the earth's atmosphere, also is the single element which most commonly limits plant growth. This is because most plants are dependent upon the availability of small quantities of combined N in the soil. It is difficult to establish who was the first person to suggest that some plants may be capable of utilizing atmospheric N_2, but in 1836 Humphrey Davy wrote: "when glutinous and albuminous substances exist in plants, the azote they contain may be suspected to be derived from the atmosphere" (Stewart, 1966). From experiments conducted between 1886 and 1888, Hellriegel and Wilfarth demonstrated that only leguminous plants bearing nodules fix N_2. This was done by growing peas with or without combined N in sterile sand, non-sterile sand, and sterile sand plus soil extract. Plants grown in sterile sand did not nodulate as did some plants grown in non-sterile sand and all plants grown in sterile sand plus soil extract. Only plants bearing nodules showed growth similar to plants given combined N. They therefore postulated that the nodules were sites of N_2 fixation and were formed by soil bacteria (Fred *et al.*, 1932). In 1888, Beijerinck isolated a bacterium which caused nodule formation and named it *Bacillus radicola* which was later renamed *Rhizobium leguminosarum*. He was the first to suggest a symbiotic relationship between the bacteria and the host legume.

The simultaneous decrease in world food supplies and energy sources for industrial manufacture of N fertilizer has spurred a renewed interest in the *Rhizobium* /legume symbiosis. Besides having the unique ability to assimilate or fix atmospheric N_2, legumes, especially soybeans (*Glycine max* {L.} Merr.), have one of the highest seed protein contents known. Unfortunately, the average yield of soybeans (approximately 1610 Kg/ha) is relatively low compared to other agronomic crops. However, Boyer (1982) suggests that there is a large genetic potential for increased production since yields as high as 7,390 Kg/ha have been obtained. Yield differences between soybeans and other crops are largely due to the higher N requirement of the former (Sinclair and de Wit, 1976) and the large energy requirements of N_2 fixation (Ryle *et al.* , 1979). Harper (1974) compared yields between soybeans completely dependent on N_2 fixation or supplied abundant combined N and found yields of the latter to be twice as great. It therefore appears that the N_2 fixing system is inadequate to meet the N demands for growth. This realization has resulted in recent attempts to increase the amount of N_2 fixed by improving the bacterial symbiont (Maier and Brill, 1978). Improvement in the efficiency of biological N_2 fixation requires a knowledge of all physiological and environmental factors that limit the N_2 fixing process under field conditions.

In 1926, Leonard wrote that if "the photosynthetic function is modified by lack of light, insufficient carbon dioxide, or a deficiency in chlorophyll, it is reasonable to expect that N_2 fixation resulting from the activities of the nodule bacteria may be limited in a degree corresponding somewhat to the extent of the modification of the factors concerned" (Leonard, 1926). Thus, it was realized that there was a relationship between photosynthesis and N_2 fixation over half a century ago. This relationship has since been demonstrated to be an interdependence between the two processes; photosynthesis supplying energy for N_2 reduction and acceptor molecules for transport of reduced product and N_2 fixation supplying nitrogenous compounds necessary for photosynthesis (Bethlenfalvay *et al.*, 1978; Hardy and Havelka, 1976; Lawn *et al.*, 1974; Wilson, 1935).

Symbiotic N_2 fixation is an energy demanding process. The nitrogenase catalyzed reduction of N_2 requires two molecules of ATP for each electron transferred to N_2 or twelve ATP for complete reduction to NH_4. The Δ G for the reaction is approximately -136 Kcal/mole of N_2 reduced (Schubert, 1982). The question of whether or not the energy requirement for assimilating N_2 is greater than for assimilating combined N has been the subject of recent investigations. Finke *et al.* (1982) found that the root system of N_2 fixing soybeans respired 25% of their daily C input while plants supplied nitrate respired 16%. This increased loss of C was not accompanied by increased photosynthetic rates because rates of both plants were similar. Ryle *et al.* (1979) compared rates of photosynthesis, shoot respiration and root respiration in soybean, cowpea (*Vigna* unguiculata {L.} Walp), and white clover (Trifolium repens L.) either completely dependent on fixation of N_2 or supplied with abundant NO_3-N. They found no effect on photosynthesis or shoot respiration. Plants fixing N_2, however, respired 11-13% more fixed C each day than plants utilizing nitrate. Comparing growth coefficients of subterraneum clover (Trifolium subterraneum L.) dependent on N_2 fixation or supplied combined N, Silsbury (1977) concluded that the energy requirement of the former was much greater.

Studies by Finke et al. (1982) and Ryle et al. (1979), demonstrated that increased respiratory activity due to N_2 fixation was not accompanied by increased photosynthetic activity when compared to plants supplied combined N. This suggests that the latter were able to partition more fixed C into plant growth. Indeed, Pate et al. (1979) showed that white lupin (Lupinus alba L.) dependent on symbiotically fixed N_2 converted 57% of its net photosynthate to dry matter while plants supplied NO_3 converted 69% to dry matter. They attributed the difference to a greater energy expenditure for N_2 than NO_3 assimilation. Finke et al. (1982) demonstrated that soybeans dependent on N_2 fixation retained 8 to 12% less photosynthate as dry matter compared to nitrate supplied plants. Minchen and Pate (1973) determined that the nodules of Pisum sativum commanded 32% of the net photosynthate; 16% of which was used in growth, 37% in respiration and 47% to return reduced N to the shoot.

The amount of photosynthate available to the nodules is a major factor influencing N_2 fixation. Numerous studies have shown that factors which increase or decrease the supply of photosynthate to the N_2 fixing apparatus result in concomitant respective increases or decreases in N_2 fixation. Streeter (1973) obtained a 75% increase in apparent $N_2(C_2H_2)$ fixation after grafting an additional shoot to a soybean plant. Lawn and Brun (1974) also showed an increase or decrease in apparent N_2 fixation following a respective increase or decrease in the source/sink ratio in soybeans. Increasing carbon exchange rates by CO_2 enrichment or O_2 depletion of the atmosphere surrounding legumes has been shown to result in increased rates of apparent N_2 fixation (Hardy and Havelka, 1976; Phillips *et al.*, 1976; Quebedeaux *et al.*, 1975). These results suggest that the full potential of the N_2 fixing system is not normally expressed due to inadequate photosynthetic activity.

The limitations placed on N_2 fixation by the daily photosynthetic activity of the shoot are said to be reflected in observed diurnal variations in root/nodule activity. In the field, N_2 fixation has been reported to be closely correlated with solar radiation with activity declining significantly during darkness (Bergesen, 1970; Hardy *et al.*, 1968; Magee and Burris, 1972; Ruegg and Alston, 1978). In controlled environment studies, maximum activity has been observed near the end of a fixed light period with rates again declining significantly during the dark period (Gersen *et al.*, 1978; Mederski and Streeter, 1977;

Bethlenfalvay and Phillips, 1977). Such diurnal variations in root/nodule activity suggest that N_2 fixation relies upon a current supply of photosynthate (as opposed to that resulting from starch degradation) and does not utilize stored carbohydrate during the dark periods of the diurnal cycle.

Results suggest that plants do not regulate partitioning of photosynthate to the nodules, i.e. the amount of photosynthate which reaches the nodules is proportional to the amount produced. There are, however, reports which conflict with this idea. Williams *et al.* (1982) increased the carbon exchange rate of 2, 3 and 4 week-old soybeans by 87, 84 and 76% respectively by increasing growth chamber CO_2 concentration from 320 to 1000 µl/l. There was no noticeable effect on root/nodule activity over a ten hour period. Finn and Brun (1982) obtained similar results in 4-week old soybeans over a 36 hour period. Sheehy *et al.* (1980) increased the carbon exchange rate of soybeans over four-fold and failed to show any increase in root/nodule activity. In addition to these reports, diurnal root/nodule activity has been shown to remain fairly constant during 24 hour light/dark cycles (Fishbeck *et al.*, 1973; Haystead *et al.*, 1979; Schweitzer and Harper, 1980; Williams *et al.*, 1982). These results would seem to indicate that photosynthesis *per se* is not the limiting factor in N_2 fixation, but that some other variable may serve to regulate or control nodule function..

The products of photosynthesis are either translocated out of the chloroplast or retained there for use in starch synthesis

(Silvius *et al.*, 1979). Chatterton and Silvius (1979) demonstrated that starch accumulation in the chloroplast during the photosynthetic period is a programmed response influenced by the energy demands during the diurnal non-photosynthetic period. Soybeans were grown under two different light regimes: a.) a 14-hour photoperiod at 64 nE cm^{-2} s^{-1} and b.) a photoperiod comprised of 7 hours at 64 nE cm^{-2} s^{-1} followed by 7 hours at 1 nE cm^{-2} s^{-1}. The time of exposure at 64 nE cm^{-2} s^{-1} was termed the photosynthetic period. Plants grown in a 14-hour photosynthetic period partitioned 60% of the daily accumulated photosynthate into starch while plants grown under a 7-hour photosynthetic period partitioned 90% to this pool to sustain the supply of photosynthate during the longer dark period.

Plants have also been shown to acclimate to the total daily integrated photosynthetic photon flux density (PPFD) maintained during growth (Hofstra and Hesketh, 1975; Nobel, 1976). Chabot *et al.* (1979) found that both leaf structure and apparent photosynthesis in *Fragaria virginiana* were similar in plants subjected to the same total daily integrated PPFD even though peak PPFD was different in the two treatments. When total daily quanta varied, however, significant differences in apparent photosynthesis, leaf thickness, specific leaf weight, mesophyll cell volume and Ames/A ratio were measured. Partitioning of photosynthate is also influenced by prior acclimation to total daily integrated PPFD maintained during the photosynthetic period. When soybeans were grown under 12 hour photoperiods at either 600 or 950 µM m^{-2} s^{-1}, the amount of starch accumulation was the same in both treatments (Silvius *et al.* , 1979). The additional photosynthate formed at the higher irradiance was exported as sucrose as indicated by increased translocation rates. If plants grown at 600 µM m^{-2} s^{-1} were transferred to 950 µM m^{-2} s^{-1}, starch accumulation in the leaves increased significantly, but translocation rates did not. These results did not change two days after exposure to the higher irradiance photoperiod. Sheikholeslam *et al.* (1975) compared partitioning of photosynthate in peas (*Pisum sativum* L.) grown under 200, 500 or 800 µE m^{-2} s^{-1}. Plants grown at the higher irradiance partitioned more assimilate to the nodules. When plants grown at 500 µE m^{-2} s^{-1} were exposed to 200 or 800 µE m^{-2} s^{-1} for 10 hours, partitioning to the nodules remained unchanged. These results suggest that acclimation to a specific irradiance environment is fundamental in regulating distribution of photosynthetic products potentially available for use as energy in N_2 fixation.

Another environmental parameter capable of regulating photosynthate partitioning and N_2 fixation is temperature. Waughman (1977) examined temperature effects on nitrogenase activity in five legume species and found activity to be temperature sensitive. Although response varied between species, activity generally increased with increasing temperature up to an optimum after which it declined. Nitrogenase activity in soybeans had an optimum temperature of 30 C. Increasing temperature beyond this resulted in significant decline in activity. Sloger *et al.* (1975) compared the relationship between $N_2(C_2H_2)$ reduction in soybean and both soil and ambient temperature throughout a growing season. Average specific activity of the nodules was significantly correlated with average daily ambient temperature and cumulative daily solar radiation, but not with average soil temperature. Schweitzer and Harper (1980) demonstrated that diurnal variations in temperature, not light, were responsible for observed diurnal differences in root/nodule activity. Soybean plants maintained at 18 C showed no diurnal variation in root/nodule activity, while plants maintained at alternating 27 C day:18 C night temperatures showed a significant decrease in activity at the lower temperature. The temperature of the shoot appeared to be responsible for the observed activity, for when the root zone was maintained at 18 C, there was a significant decrease in root/nodule activity when the shoot temperature was lowered from 27 C to 18 C. Eckart and Raguse (1982) also found acetylene reduction activity to respond more to fluctuations in temperature than light and suggested that temperature buffered N_2 fixation against short-term changes in photosynthate supply. It is interesting to note that many investigations into the diurnal activity of N_2 fixation resulted in conclusions implicating light as the responsible environmental variable even though temperature is often closely correlated with light.

Results which demonstrate a close relationship between carbohydrate availability and N_2 fixation have resulted in the general assumption that N_2 fixation is primarily C limited. This concept may be oversimplified when one considers that because photosynthesis and N_2 fixation are interdependent, carbohydrate production may be a function of N availability. Bethlenfalvay *et al.* (1978) showed a 10-fold increase in the carbon exchange rate of 26-day-old peas in response to increasing the supply of NH_4^+ from 0 to 16 mM. DeJong and Phillips (1981) inoculated peas with *Rhizobium* strains with varying ability to fix N_2. As plant N increased due to the increased efficiency of the respective strain, so did photosynthetic efficiency (CO_2 fixation).

Williams *et al.* (1981) suggest that the question of whether symbiotic legumes are primarily C or N limited is analytically complex. It may be simplified by considering mature plants and developing seedlings separately. During the early development of the symbiotic legume, N is supplied from stored reserves in the cotyledons. As this supply of N is depleted, the plant enters a stage referred to as the "nitrogen hunger period" by Fred *et al.* (1938), which occurs before the nodules are capable of meeting the N demands for growth. In soybeans, N_2 fixation may not begin until three to five weeks after planting (Hardy *et al.*, 1971). Mahon and Child (1979) compared relative growth rates in peas dependent on N_2 fixation or supplied NH_4NO_3. During the early stages of growth, NH_4NO_3 increased relative growth rates. They attributed this response to a relief of the period of N stress. During later stages of growth, relative growth rates were increased also, a result which they attributed to an increased partitioning of assimilate into shoot development. Williams et al. (1981) compared dry weights of developing soybeans provided with 0.0, 1.0, or 8.0 mM NH_4NO_3 and 320 or 1000 µl/l CO_2. After 22 days, plants grown under 320 µl/l CO_2 and 8mM NH_4NO_3 had 252% and 100% greater dry weight than plants supplied 0.0 or 1.0 mM NH_4NO_3, respectively. Comparing dry weights between the two CO_2 treatments showed increases of 51%, 49% and 64% for the respective 0.0, 1.0 and 8.0 mM NH_4NO_3 treatments. Dry weight accumulation was therefore limited more by N availability than carbohydrate supply during this early stage of development. Comparing these results with those which demonstrate C limitations, it may be concluded that symbiotic legumes are primarily N limited prior to development of functioning nodules and C limited as the energy demands of N_2 fixation become significant. With this in mind, Williams et al. (1981) suggested that any attempts to enhance N_2 fixation must consider both periods of growth.

Attempts to overcome the period of N stress and supplement N_2 fixation by supplying combined N have been met with mixed results. In general, the addition of combined N inhibits the infection process, nodule development and N_2 fixation (Allos and Bartholomew, 1959; Beard and Hoover, 1971; Gibson, 1974; Harper and Cooper, 1971; Munns, 1968; Norman and Krampitz, 1946;

Weber, 1966). This antagonistic response is due to the fact that supplemental combined N tends to replace rather than contribute to N_2 fixation. There are reports, however, that small amounts of combined N actually promote nodule development and N_2 fixation. Eaglesham et al. (1983) obtained four fold increases in nodule weight and six fold increases in acetylene reduction in response to application of 36 mg N/plant to soybean. Bethlenfalvay et al. (1978) more than doubled acetylene reduction activity by adding 2mM NH_4 to peas. Williams et al. (1981) showed a similar increase in soybeans supplemented with 2 mM NO_3.

The area of N_2 fixation research currently receiving the most attention concerns the possibilities for developing more efficient relationships between rhizobia and the host legume. Progress in this area has primarily been through the development of superior strains of rhizobia. One aspect of this improvement concerns the nitrogenase enzyme. This enzyme also reduces H^+ to form H_2. It has been estimated that the production of H_2 may utilize 40 % of the energy available for N_2 fixation (Schubert and Evans, 1976). Certain strains of rhizobia have been found to possess a hydrogenase enzyme which oxidizes the H_2, thereby recapturing some of the energy lost in its formation resulting in more efficient use of carbohydrate substrate (Emerich et al. , 1979). Maier and Brill mutagenized Rhizobium japonicum through treatment with N-methyl-N′-nitro-N-nitrosoguanidine. After subculturing for several generations, individual colonies were screened for effectiveness in reducing acetylene. Out of 2500 colonies, two were found to reduce acetylene at significantly higher rates. When compared to the original wild type, soybeans inoculated with these strains had 60% greater dry weight and 100% greater N content. Maier and Brill attributed this response to earlier nodule formation since these strains apparently lacked the hydrogenase enzyme. Because these strains begin to fix N_2 earlier than the wild type, it may be assumed that they may aid in overcoming the period of N stress.

Although mutant strains of rhizobia increase N_2 fixation and plant growth under controlled environmental conditions, results concerning increased yield are not as conclusive. In the field, soil N content and indigenous rhizobia population have been shown to influence yield responses. Hanus *et al.* (1981) compared yields in soybeans inoculated with rhizobia mutants with and without the hydrogenase enzyme and failed to show significant increases. They attributed the lack of response to relatively high soil N content. Williams and Phillips (1983) compared the promotive effects on yield in soybeans by inoculating with *Rhizobium japonicum* strains 110 and a mutant of 110 (C33). Strain C33 had previously been shown to double the acetylene reduction activity when compared to strain 110 in free-living culture. In one year, strain C33 increased yields by 210 Kg/ha relative to 110. The next year, this mutant increased yields by 420 Kg/ha. Williams and Phillips attributed the greater promotive effects demonstrated in the second year to lower soil N that particular year.

When legumes are inoculated with mutant strains of rhizobia, these strains must compete with indigenous strains in the soil for nodulation sites. Therefore, the beneficial effects of the mutant strain may not be exhibited. Abel and Erdman (1964) could only show yield increases in soybean inoculated with *R. japonicum* strain 110 when fields were void of indigenous strains. Dunigan *et al.* (1984) inoculated soybeans with *R. japonicum* strain 110 over a period of seven years and determined the number of inoculated bacteria which actually produced nodules during each year. For the first four years, recovery of 110 from nodules ranged from 0-17%. This increased to 29-33% in the fifth year and 54% by the seventh. Thus, under soil conditions in which more efficient rhizobia strains must compete with indigenous strains, promotive effects may not be noticed for several years after initial introduction.

The following two manuscripts were prepared for presentation to the American Society of Plant Physiologists for publication in *Plant Physiology*.

MANUSCRIPT 1

Carbohydrate Partitioning and the Capacity of Apparent Nitrogen Fixation of Soybeans Grown Outdoors

E. P. Millhollon and L. E. Williams

Department of Agronomy
Louisiana State University, Baton Rouge, LA 70803

ABSTRACT

Diurnal trends in leaf carbohydrate partitioning and nodule activity in soybeans under natural conditions and the irradiance level required to allocate sufficient carbohydrate to obtain maximum rates of $N_2(C_2H_2)$ reduction were studied.
Soybeans grown outdoors maintained constant levels of soluble carbohydrates in the leaves and constant rates of N_2 fixation and root + nodule respiration when root temperature was kept constant but shoot temperature varied. When plants were subjected to a 40-hour dark period, then exposed to 200 to 1000 $\mu E\ m^{-2}\ sec^{-1}$, 200 $\mu E\ m^{-2}\ sec^{-1}$ resulted in maximum leaf soluble carbohydrate and nodule activity. Results suggest that nodule activity is controlled by carbohydrate partitioning in the shoot and support the concept of an environment-mediated programming of carbohydrate distribution.

INTRODUCTION

Symbiotic nitrogen fixation in legumes requires significant input of carbon substrates to provide energy for N_2 fixation and acceptor molecules for subsequent transport of reduced N. Due to this large C requirement, symbiotic N_2 fixation is closely coupled to photosynthate production and is frequently said to be limited by rates of photosynthesis. This concept is supported by experiments which show that factors known to increase photosynthesis, such as increased irradiance level, CO_2 enrichment and lowered partial pressures of O_2, result in increased rates of symbiotic N_2 fixation (7,13,14).

There are reports, however, that indicate N_2 fixation may not be directly limited by photosynthetic activity. This is supported by data showing no diurnal variation in nodule activity and a lack of response to short term increases in photosynthate production (5,6,9,15,24). In recent studies, CO_2 enrichment resulted in increased rates of leaf carbon exchange in soybeans (24) and significant increases in foliar starch content. However, neither the concentration of leaf soluble sugars nor root nodule activity increased (5,24). Thus, it would appear that the increased photosynthate production was merely channeled into starch and therefore was not made available for increases in N_2 fixation.

Control of partitioning of photosynthate between reserve and mobile forms appears to be an environment-mediated response. Chatterton *et al.* (2) demonstrated that the amount of photosynthate partitioned into starch is proportional to the length of the photosynthetic period or, perhaps more importantly, to the energy requirements during the non-photosynthetic period. Silvius *et al.* (18) found that acclimation to a specific irradiance environment also regulates partitioning. Soybeans acclimated to either a moderate or high irradiance environment exhibited similar starch accumulation rates, but plants acclimated to the higher irradiance had significantly greater rates of translocation and carbon exchange. If the plants acclimated to moderate irradiance were transferred to the high irradiance level, rates of carbon exchange and starch accumulation increased, but translocation rates did not.

The irradiance environment of plants grown in the field is complex. Instantaneous photosynthetic photon flux density (PPFD) changes constantly depending upon solar angle and intermittent cloud cover. As a result, total integrated PPFD varies daily. The studies by Chatterton *et al.* (2) and Silvius *et al.* (18) indicate that acclimation to a particular environment is fundamental in regulating the partitioning of photosynthate. Such control could be especially significant in the case of the nodulated legume dependent on the availability of carbohydrate to meet the energy demands of N_2 fixation. The question of how the symbiotic legume acclimates to such a complex environment and what influence this has on the capabilities of the N_2 fixing apparatus is unknown. The purpose of this study was to determine how a symbiotic legume adjusts to its natural environment and what role such adjustments play in regulating photosynthate partitioning and N_2 fixation.

MATERIALS AND METHODS

Soybeans (Glycine max {L.} Merr. cv. Clark) were germinated in the dark at 25 C. Three days after imbibition, seedlings were inoculated with a slurry of Rhizobium japonicum USDA strain 110 and transferred to 13 cm diameter pots containing vermiculite. Pots were sealable for separate measurement and control of root and shoot functions. Plants were then placed on platforms outdoors where they remained throughout the experimental period. A nutrient solution modified to contain 2 mM KNO_3 (23) and distilled water were used alternately for daily watering of plants.

To determine effects on plants grown under different irradiance environments, experiments were conducted during the late Spring and the early Fall in Baton Rouge, Louisiana. At each time, diurnal changes in photosynthate partitioning in the leaves and root + nodule activity were determined in 35-day-old plants. Two temperature treatments were imposed during each diurnal study. During the late Spring, the root zone of one set of four replicate plants was kept at 25 ± 2 C, while that of another set was allowed to vary with ambient air temperature. During the early Fall, either both the shoot and root or just the root zone were kept at 25 ± 2 C. Temperature control was accomplished by placing either the pot or the entire plant in a plexiglass chamber equipped with a heat exchanger coupled to a water bath. When the entire plant was placed in a chamber, shoot carbon exchange rates during the day were determined using differential infrared gas analysis in an open system (24).

Diurnal activity of the roots and nodules was determined in a manner similar to that described by Sheehy *et al.* (16). Respiration was monitored by passing air at a constant flow rate through the sealed pots to an automatic gas sampling system. This system consisted of solenoid valves operated by a cam timer which sampled air in each pot every three minutes. A complete cycle was thirty minutes in duration. Carbon dioxide concentration was determined using differential infrared gas analysis. Irradiance (photosynthetically active radiation) was measured at the plant canopy top using a LI-COR Model 185B quantum radiometer. Temperature, irradiance and output from the infrared gas analyzer were recorded every minute with a data logger. At approximately four-hour intervals during the diurnal period, air flow through the pots was interrupted, and the reduction of acetylene to ethylene over a twenty-minute period was determined.

Plants were harvested at four-hour intervals in order to determine diurnal changes in nonstructural carbohydrate composition in the leaves. Leaves were separated from the rest of the plant and oven dried at 75 C for 48 hours. Leaf tissue was finely ground and a subsample analyzed for starch and soluble sugar content using the method described by Upmeyer and Koller (21).

The irradiance level required to allocate carbohydrate sufficient to produce maximum root-nodule activity was determined by first extending the normal dark period for forty hours to deplete carbohydrate reserves. Plants were then exposed to stepped increases in irradiance provided by 1000-watt-metal-halide lamps. During exposure, respiration of the roots and nodules was monitored. Root + nodule respiration reached a maximum approximately 3-4 hours after the lights were turned on regardless of irradiance level. After 10 hours, plants were assayed for acetylene reduction activity, and the leaves removed and analyzed for starch and soluble sugar content.

RESULTS

Experimental data from measurements conducted on soybeans grown in containers outdoors showed that there was no diurnal variation in root + nodule respiration and apparent N_2 fixation when the root system was maintained at a constant temperature (Figs. 1 and 2). Irradiance levels and ambient air temperatures varied considerably both times the experiment was conducted. There was a diurnal pattern of leaf starch accumulation with a maximum concentration measured at 1800 h, but only a slight variation in leaf soluble sugar content occurred either day. Root zone temperature varied between 19 and 37 C during the course of the day (Fig. 1). Root + nodule respiration had a Q_{10} of approximately 2 from 0800 h to 1400 h when root zone temperature increased from 20 to 35 C. Subsequently, respiration dropped almost three-fold to 3.5 mg CO_2 plant $^{-1}h^{-1}$ while the root-zone temperature increased to 37 C. There was a significant decrease in apparent N_2 fixation after the pot temperature had increased to above 35 C and then decreased to 30 C.

The response of whole plant apparent photosynthesis to irradiance level was measured on soybeans previous to the Fall diurnal study (Fig. 3). Irradiance levels were varied by the use of shade screens. Light saturation of apparent photosynthesis occurred at 600 µE $m^{-2}s^{-1}$ under these growth conditions.

The whole plant carbon exchange rate at 200 µE $m^{-2}s^{-1}$ was almost 50% of the light saturated values.

In order to assess potential regulating effects of irradiance levels on apparent N_2 fixation of soybeans, plants were subjected to an extended dark period of 40 h after the normal photoperiod to deplete stored carbohydrates (Fig. 4). There was no significant change in acetylene reduction until 14 h into the extended dark treatment. This approximately corresponds to the time when the normal photoperiod would have begun outdoors. There was no further decrease in apparent N_2 fixation with an additional 20 hours of darkness.

The response of nodule functioning to various levels of carbohydrate depleted soybeans is shown in Figures 5 and 6. An irradiance level of 200 µE $m^{-2}s^{-1}$ significantly increased the rate of apparent N_2 fixation. There was no further increase in nodule activity at the higher irradiance levels on either date. Leaf starch content increased with increasing irradiance levels up to 600 µE $m^{-2}s^{-1}$. Starch content in leaves after 10 hours at the higher irradiance levels was similar to the maximum value measured during both diurnal studies (Figs. 1 and 2).

DISCUSSION

Nodules of soybeans grown outdoors apparently can function at a constant rate when the temperature of the root system remains constant. This occurred despite changes in irradiance levels and shoot temperature throughout the day. These results are similar to several controlled-environment studies (6,24). Other data (15,19) suggest , however, that the acetylene reduction activity of nodules also responds to shoot temperature, perhaps resulting from temperature effects on vein loading and carbohydrate translocation from shoot to nodules. Vein loading and translocation in the phloem of wheat plants, however, has been shown to be largely unaffected by temperatures from 20 to 40 C (22). Similar translocation-temperature response curves also have been shown with bean plants (20,10). In the present study, ambient air temperatures were within limits that probably would not significantly decrease the export of carbohydrates out of the leaves or other storage organ (Figs. 1 and 2).

Optimum activity of apparent N_2 fixation in nodulated soybeans occurs at root temperatures between 20 and 30 C (4,8). There was no significant decrease in apparent N_2 fixation when root zone temperature reached 35 C (Fig. 1). Subsequent to this measurement at 1400 h, there was a significant decrease in both root + nodule respiration and acetylene reduction. The decrease in root + nodule respiration from 1400 to 1600 h occurred without a concomitant decrease in root zone temperature. It is unknown whether continued high root zone temperature and/or other related variables such as plant water status were responsible for both decreases.

The pattern of nonstructural carbohydrate content throughout the day (Figs. 1 and 2) resembled that found in soybeans grown under controlled environmental condition (3,21). Maximum starch content measured at 1800 h both days was similar to maximum values obtained after carbohydrate depleted plants had been held at a constant irradiance level for 10 hours (Figs. 5 and 6). This was probably due to the similarities in length of the normal photoperiod both days (approximately 11-12 h, Figs. 1 and 2) and the time used for the constant irradiance experiments. Chatterton and Silvius (2) have shown that the rate of starch accumulation in fully expanded soybean leaves was a function of the duration of the daily photosynthetic period. They also reported that lowering the irradiance level did not change the partitioning of photosynthate as long as the duration of the photosynthetic period remained the same (3). In this study, reduction in irradiance level below the light saturation level of 600 $\mu E\ m^{-2}s^{-1}$ (Fig. 3) resulted in a significant decrease in starch content when compared after 10 h in the light (Fig. 6). Differences in results between the two studies may have been due to differences in tissues sampled (all leaves on a plant vs. only fully expanded leaves) and sinks present (nodulated vs. non-nodulated plants).

Results from growth chamber studies in which nodulated legumes were transferred from one irradiance regime to another suggest that acclimation to a particular irradiance environment is fundamental in regulating the supply of potential energy sources for N_2 fixation (17,23). The question of concern here was how does a symbiotic legume acclimate to a natural, variable irradiance and temperature environment. One possible mechanism that may be used by plants to regulate or adapt to variable light regimes is the process of photosynthesis. Total daily PPFD (1,11) or total daily CO_2 uptake by the plant (12) appear to have the greatest influence over adaptive processes of the leaf and its photosynthetic apparatus which in turn could be the stimuli for adaptation of other physiological responses within the plant.

Results from this study indicate that soybeans grown outdoors are adapted to maintain constant maximum diurnal nodule activity. The fact that exposure to low irradiance produced maximum nodule activity supports a recent report from Sheehy *et al.* (16) wherein it was shown that carbon exchange rates as low as 10 mg CO_2 plant $^{-1}$h^{-1} were sufficient to obtain maximum acetylene reduction following a 40 h dark treatment. Thus, it would appear that the adaptive processes of soybeans are structured to withstand periods of stress which may occur during prolonged periods of inclement weather. This raises the question of whether or not these adaptive processes can be altered to allow full exploitation of the environment and increased yield.

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FIGURE LEGENDS

Fig. 1. Comparison of apparent N_2 fixation and root + nodule respiration in soybean with and without controlled root temperature to diurnal trends in leaf carbohydrate partitioning, ambient air temperature and solar radiation.

Fig. 2. Comparison of apparent N_2 fixation and root + nodule respiration in shoot and root temperature controlled or root temperature controlled soybeans to diurnal trends in leaf carbohydrate partitioning, ambient air temperature and solar radiation.

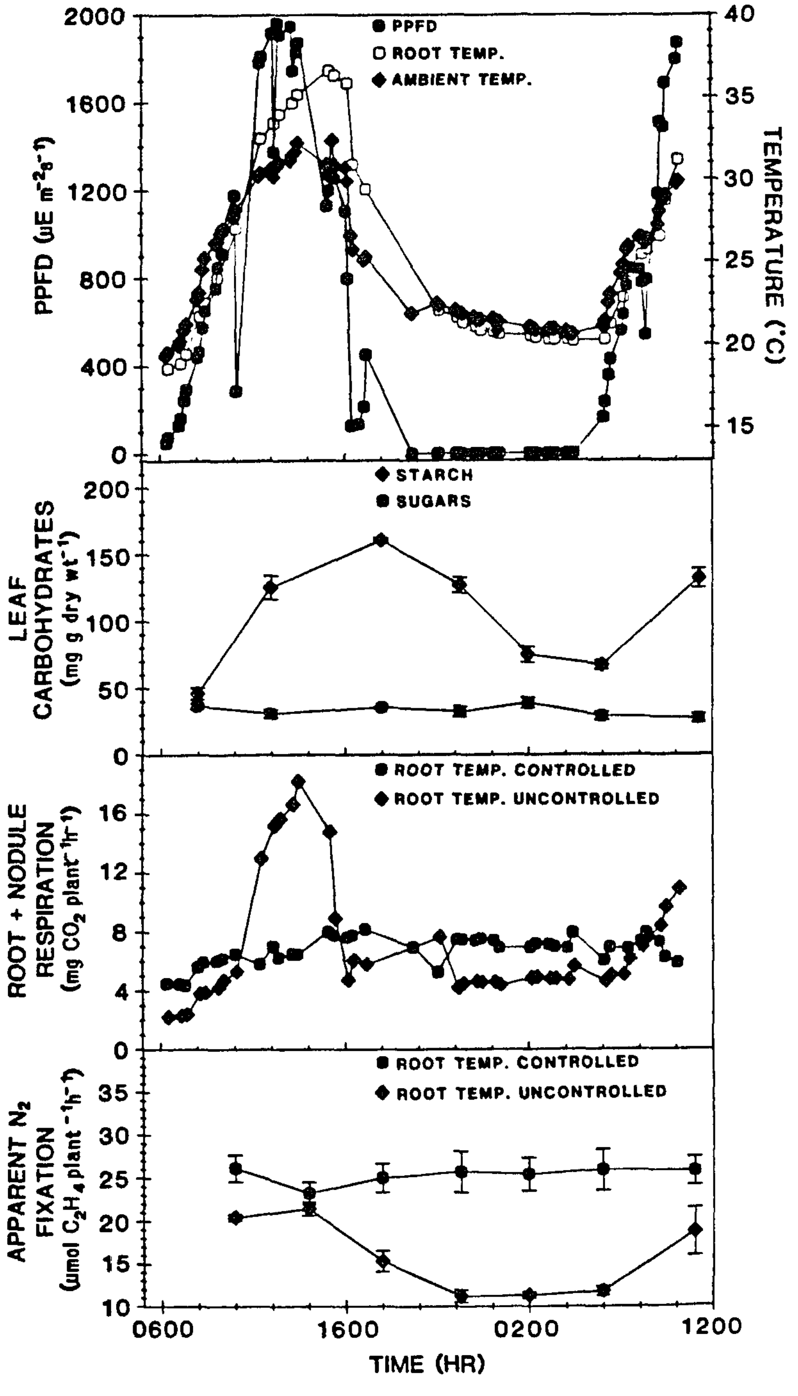
Fig. 3. Response of apparent photosynthetic rates of plants represented in Figure 2 to irradiance.
$y=47.9(1-e^{(-0.004x)})$; $R^2=0.96$

Fig. 4. Apparent N_2 fixation of plants represented in Figure 2 during a 40-hour extension of the normal dark period. Each point represents the mean ± SE of four plants.

Fig. 5. Response of apparent N_2 fixation and leaf carbohydrate partitioning to increased irradiance levels following a 40-hour extension of the normal dark period of plants represented in Figure 1. Each point represents the mean ± SE of four plants.

Fig. 6. Response of apparent N_2 fixation and carbohydrate partitioning to increased irradiance levels following a 40-hour extension of the normal dark period of plants represented in Figure 2. Each point represents the mean ± SE of four plants.

Fig. 1



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1600
1200
800
400
0
PPFD (μE m^{-2}s^{-1})
● PPFD
○ ROOT TEMP.
◆ AMBIENT TEMP.
40
35
30
25
20
15
TEMPERATURE (°C)
200
150
100
50
0
LEAF CARBOHYDRATES (mg g dry wt^{-1})
◆ STARCH
● SUGARS
16
12
8
4
0
ROOT + NODULE RESPIRATION (mg CO$_2$ plant^{-1}h^{-1})
● ROOT TEMP. CONTROLLED
◆ ROOT TEMP. UNCONTROLLED
35
30
25
20
15
10
APPARENT N$_2$ FIXATION (μmol C$_2$H$_4$ plant^{-1}h^{-1})
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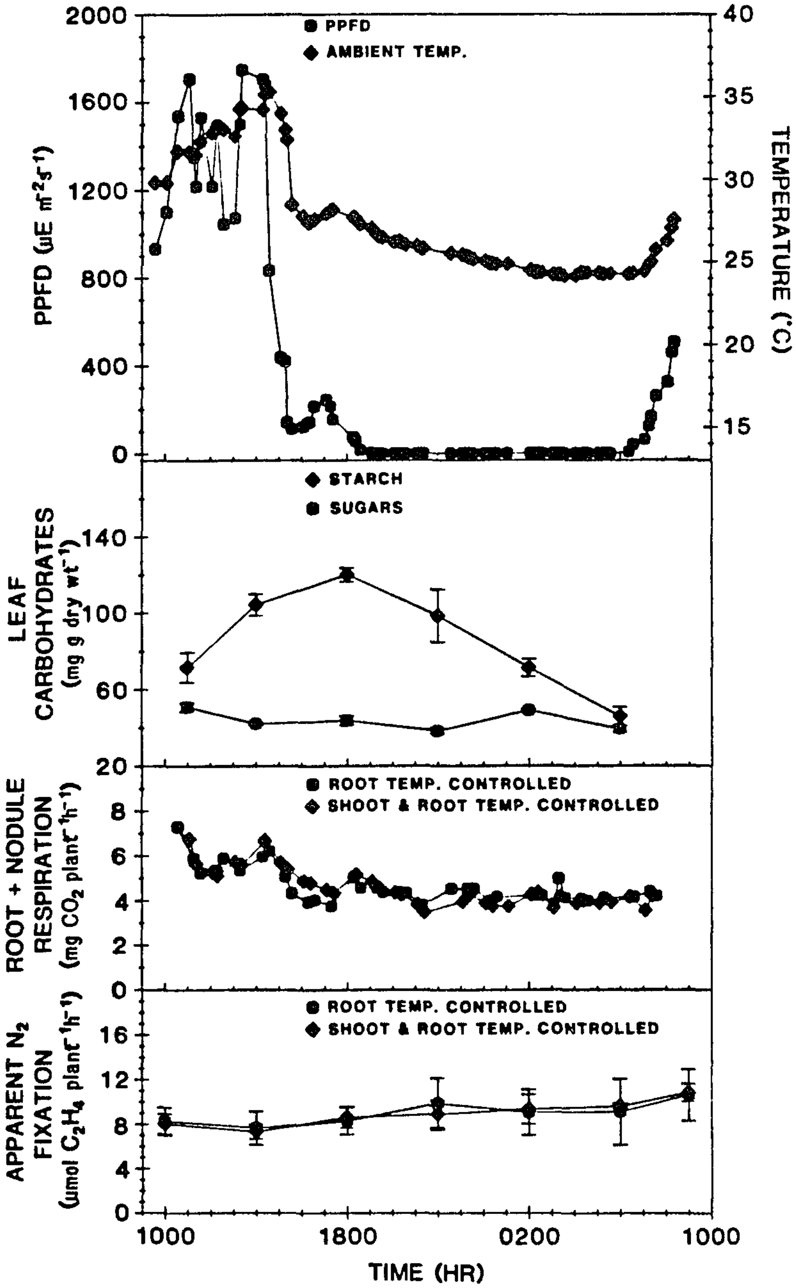
◆ ROOT TEMP. UNCONTROLLED
0600
1600
0200
1200
TIME (HR)

Fig. 2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1200
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PPFD (μE m⁻²s⁻¹)
● PPFD
◆ AMBIENT TEMP.
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35
30
25
20
15
TEMPERATURE (°C)
◆ STARCH
● SUGARS
LEAF CARBOHYDRATES (mg g dry wt⁻¹)
140
100
60
20
● ROOT TEMP. CONTROLLED
◆ SHOOT & ROOT TEMP. CONTROLLED
ROOT + NODULE RESPIRATION (mg CO₂ plant⁻¹h⁻¹)
8
6
4
2
0
● ROOT TEMP. CONTROLLED
◆ SHOOT & ROOT TEMP. CONTROLLED
APPARENT N₂ FIXATION (μmol C₂H₄ plant⁻¹h⁻¹)
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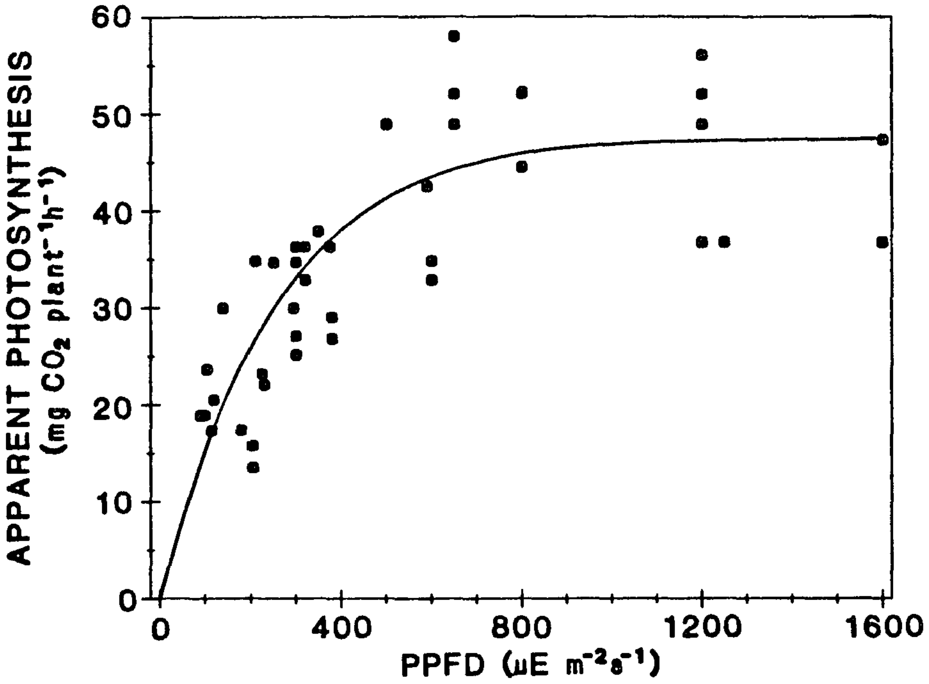
12
8
4
0
1000
1800
0200
1000
TIME (HR)

Fig. 3


APPARENT PHOTOSYNTHESIS
(mg CO$_2$ plant^{-1}h^{-1})
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50
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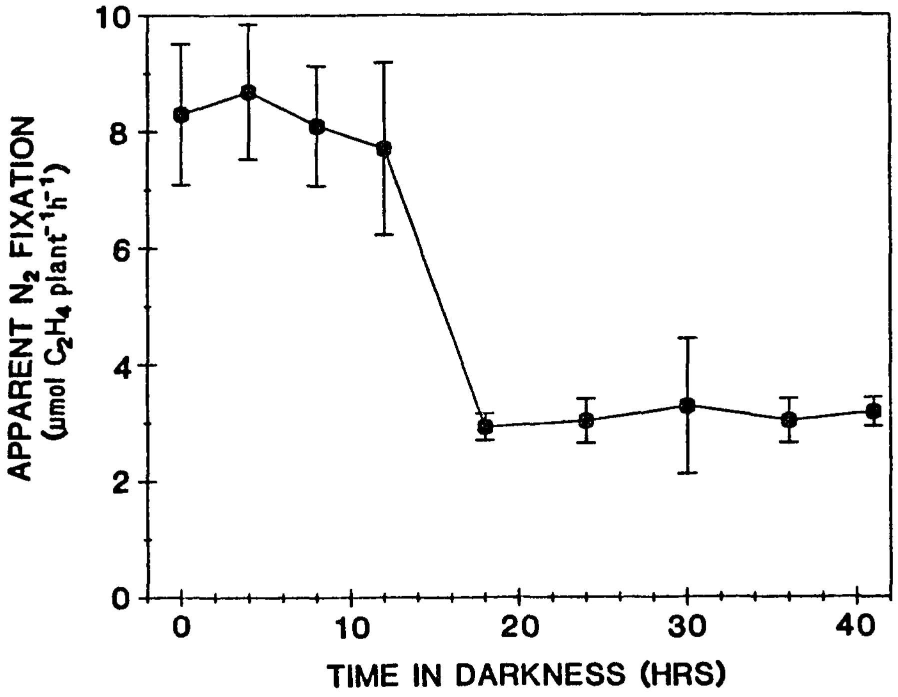
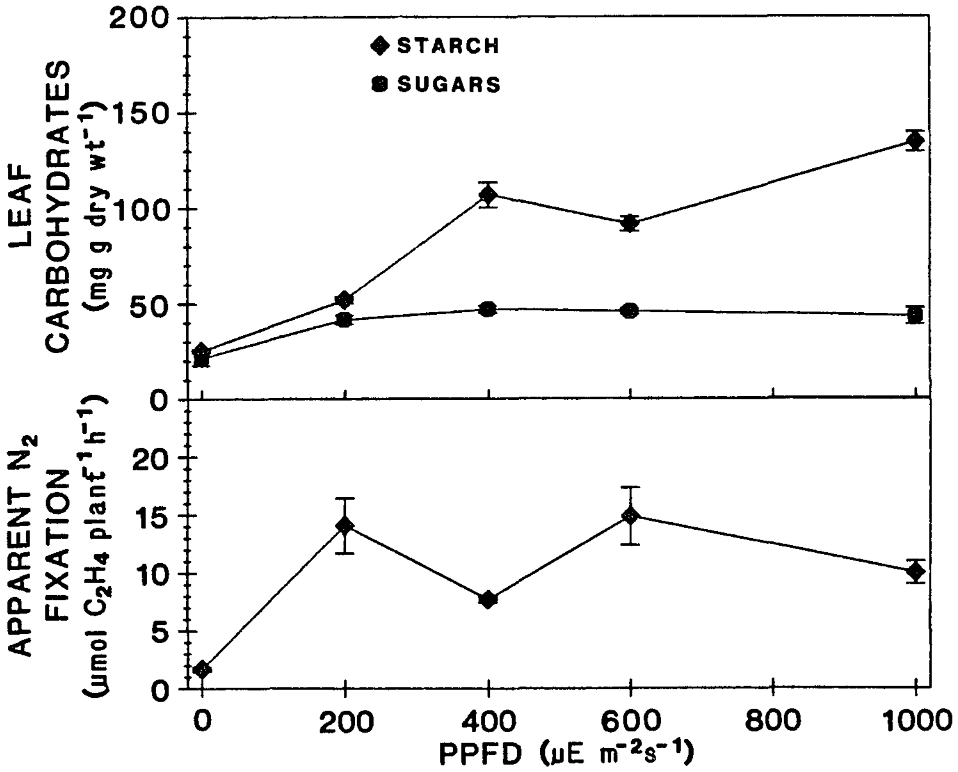
400
800
1200
1600
PPFD (µE m^{-2}s^{-1})

Fig. 4



10
8
6
4
2
0
0
10
20
30
40
APPARENT N_2 FIXATION
(µmol C_2H_4 plant^{-1}h^{-1})
TIME IN DARKNESS (HRS)

Fig. 5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● SUGARS
LEAF CARBOHYDRATES (mg g dry wt^{-1})
200
150
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0
APPARENT N_2 FIXATION (µmol C_2H_4 plant^{-1} h^{-1})
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15
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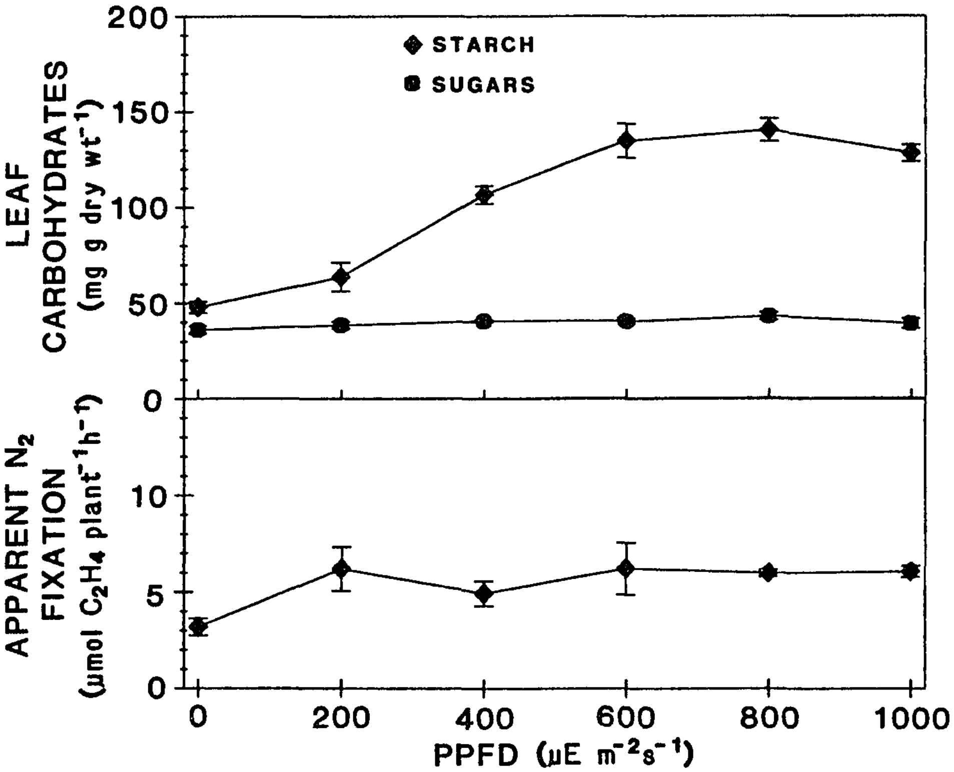
0
0
200
400
600
800
1000
PPFD (µE m^{-2}s^{-1})

Fig. 6


200
150
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10
5
0
LEAF CARBOHYDRATES
(mg g dry wt^{-1})
◆ STARCH
● SUGARS
APPARENT N_2 FIXATION
(µmol C_2H_4 plant^{-1}h^{-1})
0
200
400
600
800
1000
PPFD (µE m^{-2}s^{-1})

MANUSCRIPT 2

Carbon and Nitrogen Limitations to Growth of Soybeans Under Variable Environmental Conditions

E. P. Millhollon, L. E. Williams and E. P. Dunigan

Department of Agronomy
Louisiana State University, Baton Rouge, LA 70803

ABSTRACT

Carbon and nitrogen limitations to growth of symbiotically-grown soybean plants were assessed by examining growth characteristics of plants grown under low irradiance in a greenhouse and high irradiance outdoors and provided 0.0, 2.0, 6.0 or 12.0 mM NO_3. Under low irradiance, supplementing N_2 fixation with 2.0 mM NO_3 resulted in relative growth rates (RGR), leaf area ratios (LAR) and net assimilation rates (NAR) very similar to plants supplied 12.0 mM NO_3. As a result, total plant dry weight and leaf area of these two treatments were equivalent in 6-week-old plants despite a significantly lower N content in the 2.0 mM treatment.

Under high irradiance, plants supplied 6.0 and 12.0 mM NO_3 manifested greater relative growth rates and net assimilation rates during growth. Total plant dry weight and N content were also greater compared to the 0.0 and 2.0 mM treatments at six weeks. Leaf N content and area were equivalent in all treatments at this time. Results suggest that growth limitations to nodulated soybeans are primarily due to an inability to arrive at a functional balance between C and N accumulation prior to establishment of a fully functional N_2 fixation system. Once N_2 fixation is established, the increased input of N is used to increase both the photosynthetic efficiency and area of leaf tissue.

INTRODUCTION

The average yield of soybeans (approximately 1610 Kg/ha) is relatively low compared to other agronomic crops. Reports of record yields as high as 7,390 Kg/ha suggest that the genetic potential for higher productivity is present.

The reasons for the average lower productivity are unclear. It is known that growth of a plant is subject to an interdependence among the activities of various organs and the interdependence between photosynthesis and N_2 fixation in nodulated legumes is well documented. Several reports (e.g. 1,9,13) suggest that the large energy requirements of N_2 fixation result in C limitations to growth. Finke *et al* .(4) estimated that 25% of the daily C input was respired from the root systems of soybeans entirely dependent on N_2 fixation, versus a 16% loss in NO_3 grown plants. As a result, they concluded that N_2 fixing soybeans retained up to 12% less C as dry matter. There are, however, reports which indicate that the energy requirements of N_2 fixation are similar to requirements for assimilation and reduction of NO_3 (6,11).

In addition to reported C limitations, legume seedlings, grown under conditions of low soil N, typically enter a period of N-limited growth (10,17). This occurs after N reserves in the cotyledons are depleted and before N_2 fixation is capable of meeting the N demands for photosynthesis and growth. During this period, the developing legume must satisfy equally important demands for C and N input by constructing both photosynthetic and N_2 fixing tissue. The method by which the legume controls the partitioning of photosynthate to meet these demands under variable environmental conditions and what burden this places on the overall growth and productivity of the plant is not known.

The purpose of the present study was to assess C and N limitations of field grown soybean plants during this critical stage of development. This was accomplished by determining changes in dry matter accumulation and N distribution in soybean plants grown under low as well as natural insolation, and either completely or partially dependent on N_2 fixation.

MATERIALS AND METHODS

Plant Material and Growing Conditions

Soybeans (*Glycine max* [L.] Merr. cv. Clark) were germinated in the dark at 25 C. Three days after imbibition, seedling were inoculated with a slurry of *Rhizobium japonicum* USDA strain 110 and transferred to 13 cm diameter pots containing vermiculite. Plants were reinoculated seven days later.

Experiments were conducted at two different times of the year under two different light regimes. The first study was made on plants grown in the greenhouse during the Winter of 1983 in Baton Rouge, Louisiana. The average daily integrated photosynthetic photon flux density (PPFD) in the photosynthetically active range received at the plant canopy top during the experimental period was 8.6 E m^{-2} day^{-1}. The maximum peak instantaneous PPFD reached during this period was 1040 μE $m^{-2}s^{-1}$. Average daily ambient temperature was 22 C. Minimum and maximum temperatures were 18 and 29 C, respectively. In the second study, both Clark soybeans and the non-nodulating isogenic line L73-1054 were grown outside on platforms where average daily integrated PPFD was 31.3 E m^{-2} day^{-1}. Average, minimum and maximum daily ambient temperatures during this period were 26, 16, and 39 C, respectively. Beginning two weeks after imbibition, plants were watered every other day with a complete nutrient solution modified to contain either 0.0, 2.0, 6.0 or 12.0 mM NO_3 (15)

and on alternate days with distilled water. The non-nodulating isoline was supplied with the latter three concentrations of NO_3.

Harvest Procedure and Growth Analysis

Harvesting began two weeks after seed imbibition. All harvests were conducted at approximately 9 A.M. central standard time each day. Four plants from each treatment were harvested three times a week until plants were 45 days old. Following each harvest, apparent N_2 fixation was determined on detached root systems using the acetylene reduction assay (7). Total leaf area of the harvested plants was determined using a Licor model 3000 area meter. Dry weights of leaves,stems, roots and nodules were obtained separately after drying in a forced air oven at 75 C for 48 h. Organic N content was determined by Kjeldahl analysis (2).

Growth analysis functions were calculated as described by Hunt and Parsons (8). In this method, the polynomial (up to the third order) which best fits the logarithms of the dry weight (W) and leaf area (A) on time (T) is determined by least squares analysis. This method offers the advantage of allowing determination of general trends in growth characteristics. Relative growth rate (RGR), leaf area ratio (LAR) and net assimilation rate (NAR) are then determined as follows:

$$RGR=d(\ln W)/dt = (1/W)(dW/dt)$$

$$LAR=A/W$$

$$NAR=RGR/LAR$$

RESULTS

Greenhouse Study, Low Irradiance

Changes in leaf area ratio, net assimilation rate and relative growth rate of plants grown in the greenhouse at low irradiance and supplied either 0.0, 2.0, 6.0 or 12.0 mM NO_3 are shown in Figure 1. The most notable differences are between plants supplied any level of NO_3 and plants entirely dependent on N from seed reserves or symbiotic N_2 fixation. While relative growth rates of all plants receiving NO_3 increased for approximately fifteen days then declined, the 0.0 mM treatment maintained a constant relative growth rate. Changes in relative growth rate can be attributed to any factor which affects either the net efficiency (net assimilation rate) or the size (leaf area ratio) of the assimilatory apparatus. Examination of both of these variables shows that there was very little effect of NO_3 concentration on leaf area ratio throughout the harvesting period. The net assimilation rate of the 0.0 mM treatment, however, declined at a faster rate than the other treatments and remained lower until approximately day 25 when rates were increasing at the same time rates of the other treatments were decreasing. The constant relative growth rate of this treatment was therefore largely due to reciprocal changes in leaf area ratio and net assimilation rate.

Data showing changes in total and individual plant part dry weight and N content, leaf area and acetylene reduction activity at low irradiance are presented in Tables 1 and 2. By the thirteenth day of harvest, plants receiving no supplemental N had significantly ($P \leq 0.05$) lower N content compared to plants receiving 6.0 or 12.0 mM NO_3. The effects of this N deficiency were first noted in the lower leaf area on that day followed by lower total plant dry weight on day 18 (Table 1).

By day 27, there were no significant differences in total plant dry weight or leaf area between plants receiving 2.0 mM NO_3 and plants receiving 12.0 mM NO_3 (Table 1). This was despite significant differences in total plant N between these two treatments. Total plant dry weight and leaf area were significantly ($P \leq 0.05$) less in the 0.0 and 6.0 mM NO_3 treatment compared to the 2.0 and 12.0 mM treatments.

Outdoor Study, High Irradiance

Growth function regression lines of soybeans grown outdoors and supplemented with either 0.0, 2.0, 6.0 or 12.0 mM NO_3 are shown in Figure 2. Trends in relative growth rate were considerably different compared to low irradiance plants grown in the greenhouse. For the first fifteen days of harvest, the relative growth rates of all treatments were declining. Plants either solely dependent on N_2 fixation or supplemented with 2.0 mM NO_3 declined at a much greater rate than plants supplemented with 6.0 or 12.0 mM NO_3. Relative growth rates of plants receiving 6.0 or 12.0 mM NO_3 continued to decline, but rates of the 0.0 and 2.0 mM treatments increased.

Examination of the components of relative growth rate, net assimilation rate and leaf area ratio, shows that trends in relative growth rate of the 0.0 and 2.0 mM NO_3 treatments were largely due to similar trends in net assimilation rate.

Growth functions of non-nodulating soybeans are shown in Figure 3. Changes in relative growth rate, leaf area ratio and net assimilation rate of plants receiving 6.0 or 12.0 mM NO_3 were very similar to nodulated plants receiving the same treatment. The 2.0 mM NO_3 treatment, however, did not show the decreasing then increasing changes in relative growth rate and net assimilation rate which were apparent in nodulated plants receiving that concentration of N.

Nine days after harvesting began, significant differences ($P \leq 0.05$) in N content (Table 3) were apparent between nodulated plants entirely dependent on N_2 fixation or supplemented with 2.0 mM NO_3 and plants supplemented with 12.0 mM NO_3. At this time, non-nodulating soybeans receiving 2.0 mM NO_3 were also significantly ($P \leq 0.05$) N deficient compared to plants supplemented with 6.0 or 12.0 mM NO_3 (Table 4). The time at which significant differences in dry weight and leaf area were evident in nodulated plants coincided with the time at which these differences occurred in non-nodulating plants (Tables 5 and 6). However, whereas differences in dry weight, leaf area (Table 6) and N content (Table 4) between the 2.0 mM and 12.0 mM non-nodulating treatments continued to increase until the final harvest, differences between nodulated plants receiving the same NO_3 treatments increased until day 18, then began to decrease (Table 5). By day 28, although total plant dry weight and N content of nodulated plants were significantly ($P \leq 0.05$) less in the 2 mM treatment compared to the 12.0 mM treatment, there were no differences in leaf N content or leaf area at this time (Tables 3,5).

DISCUSSION

Although many reports have indicated that growth limitations of a symbiotic legume are primarily related to a necessity to partition carbohydrate to construct root nodules (5) and supply energy for N_2 fixation (11), results from the present study support growth chamber studies in demonstrating that growth is primarily limited by N availability (10,16).

Supplying 2.0 mM NO_3 to nodulated soybeans grown under low insolation was sufficient to obtain growth characteristics very similar to plants supplied 12.0 mM NO_3 (Fig. 1). As a result , there were no differences in final total plant dry weight or leaf area between these two treatments. This was despite significant differences in total plant as well as individual plant part N content. Raper et al. (12) have recently shown that growth of soybeans and cotton is subject to an ability to arrive at a functional balance between carbohydrate supplied from the leaves and N supplied from the root system. Our results support this concept and suggest that under these low light conditions, supplementing symbiotic N_2 fixation with 2.0 mM NO_3 was sufficient to allow the plant to arrive at such a balance. Although 12.0 mM NO_3 did result in significant increases in N content, the photosynthetic efficiency (net assimilation rate) of this treatment did not vary considerably from plants receiving 2.0 mM NO_3 (Fig. 1). Thus it would appear that, due to the low light conditions, plants supplied 12.0 mM NO_3 were unable to increase carbohydrate input in proportion to N input.

In contrast, growth functions of nodulated soybeans grown outdoors under normal insolation and supplied 2.0 mM NO_3 differed considerably from plants supplied 12.0 mM NO_3. Plants either entirely dependent on N_2 fixation or supplemented with 2.0 mM NO_3 showed decreasing relative growth rates until approximately the fifteenth day of harvest when rates began to increase. Relative growth rates of nodulated plants receiving 6.0 or 12.0 mM NO_3 continually decreased as did rates of non-nodulating plants receiving these treatments. Changes in the relative growth rates of the 0.0 and 2.0 mM treatments corresponded to similar changes in net assimilation rates, suggesting that growth of these plants was largely regulated by photosynthetic efficiency. DeJong and Phillips have recently shown a positive correlation between photosynthetic efficiency and foliar N content (2). Comparing net assimilation rates and leaf N content of all four treatments on the fourteenth day of harvest shows that differences in net assimilation rate did indeed correspond to differences in leaf N content.

The time at which relative growth rates and net assimilation rates of the 0.0 and 2.0 mM NO_3 treatments began to increase corresponded well with the time at which these plants were reducing acetylene at significantly ($P \leq 0.05$) greater rates than the 6.0 and 12.0 mM NO_3 treatments (approximately day 18, Fig. 2, Table 3.). Once N_2 fixation was well established, the increasing availability of N to the 0.0 and 2.0 mM NO_3 treatments was apparently preferentially allocated to the leaves. As a result, although total plant dry weight and N content were significantly ($P \leq 0.05$) less in these treatments compared to the 6.0 and 12.0 mM treatments, there were no differences in leaf N content or leaf area. Thus the increasing net assimilation rates of the 0.0 and 2.0 mM treatments between the fourteenth and twenty-eighth day of harvest were apparently due to increases in both the quality and quantity of assimilatory tissue.

Results from this study indicate that even though nodulated soybeans grown under low soil N conditions may allocate substantial photosynthate for nodule production and activity, limitations to growth are primarily related to N deficiencies which occur prior to establishment of a fully functional N_2 fixing apparatus. Results also support the concept that growth of soybeans is restricted by a need to arrive at a functional balance between C and N assimilation. We conclude that significant improvements in soybean yield may be possible by increasing the availability of N to the plant during the critical early stages of growth.

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Table 1. Changes in plant dry weight and leaf area of soybeans grown in the greenhouse at low irradiance and supplied four levels of nitrate. Harvesting began two weeks after the seeds were imbibed.

Plant Part	Nitrate Treatment (mMoles)	Days From First Harvest						
		0	4	9	13	18	23	27
		------------------dry weight (grams)----------------						
Total Plant	0	0.15	0.20	0.36	0.44	0.74	1.15	1.62
	2	0.18	0.21	0.39	0.59	0.98	1.48	2.06
	6	0.17	0.23	0.40	0.59	1.03	1.61	1.83
	12	0.17	0.26	0.39	0.60	1.11	1.72	2.21
	LSD(.05)	0.21						
Leaves	0	0.11	0.12	0.19	0.22	0.38	0.63	0.85
	2	0.11	0.11	0.20	0.30	0.52	0.80	1.06
	6	0.10	0.13	0.21	0.30	0.55	0.88	0.92
	12	0.11	0.14	0.21	0.32	0.61	0.97	1.12
	LSD(.05)	0.11						
Stem	0	0.01	0.02	0.06	0.08	0.14	0.23	0.35
	2	0.02	0.03	0.07	0.12	0.21	0.32	0.46
	6	0.02	0.03	0.08	0.13	0.23	0.36	0.46
	12	0.02	0.04	0.08	0.13	0.26	0.41	0.56
	LSD(.05)	0.06						
Roots	0	0.03	0.06	0.10	0.12	0.18	0.22	0.31
	2	0.05	0.07	0.11	0.15	0.21	0.30	0.43
	6	0.05	0.07	0.11	0.15	0.23	0.33	0.39
	12	0.04	0.08	0.10	0.15	0.23	0.32	0.48
	LSD(.05)	0.05						
Nodules	0	-	-	0.01	0.02	0.04	0.07	0.11
	2	-	-	0.01	0.02	0.04	0.06	0.11
	6	-	-	-	0.01	0.02	0.04	0.06
	12	-	-	-	-	0.01	0.02	0.05
	LSD(.05)	0.02						
Leaf Area		----------------------cm^2/plant---------------------						
	0	12.14	21.93	71.16	109.99	179.10	221.64	266.19
	2	10.75	28.41	82.81	153.82	237.90	289.26	338.98
	6	11.64	27.45	88.31	152.09	250.10	285.18	293.54
	12	14.90	33.97	91.22	152.95	281.08	293.67	357.80
	LSD(.05)	35.08						

Table 2. Changes in nitrogen accumulation and apparent N_2 fixation of soybeans grown in the greenhouse at low irradiance and supplied four levels of nitrate. Harvesting began two weeks after the seeds were imbibed.

Plant Part	Nitrate Treatment (mMoles)	Days From First Harvest						
		0	4	9	13	18	23	27
		----------------------------mg N---------------------------						
Total Plant	0	11.62	11.44	13.82	9.60	14.96	20.84	32.34
	2	11.62	11.11	14.22	15.77	22.57	27.73	37.32
	6	11.78	13.30	16.23	18.54	27.31	35.53	40.00
	12	11.87	15.74	17.27	22.53	34.53	42.03	55.87
	LSD(.05)	7.32						
Leaves	0	7.88	7.95	9.91	6.74	10.51	15.08	22.78
	2	7.82	7.16	9.38	11.09	16.08	19.12	24.43
	6	7.44	8.40	10.59	13.13	19.00	25.02	25.82
	12	7.52	9.96	10.84	14.66	23.48	26.16	36.52
	LSD(.05)	4.69						
Stems	0	1.17	0.81	1.52	1.05	1.72	2.66	4.77
	2	1.16	1.42	1.77	1.95	3.09	3.92	5.79
	6	1.45	1.60	2.24	2.33	3.99	5.56	6.66
	12	1.53	1.90	2.81	3.22	5.45	8.46	9.09
	LSD(.05)	1.39						
Roots + Nodules	0	2.57	2.68	2.39	1.81	2.73	3.10	4.79
	2	2.64	2.53	3.07	2.73	3.40	4.69	7.10
	6	2.89	3.30	3.40	3.08	4.32	4.95	7.52
	12	2.82	3.88	3.62	4.65	5.40	7.41	10.26
	LSD(.05)	1.21						
Apparent N_2 Fixation		--------------µmoles ethylene/plant/hour------------						
	0	-	-	1.28	4.00	7.09	12.21	18.66
	2	-	-	0.69	2.93	7.52	10.56	13.97
	6	-	-	0.27	1.02	3.3.1	5.37	6.51
	12	-	-	0.27	0.11	1.44	0.96	3.95
	LSD(.05)	2.37						

Table 3. Changes in nitrogen accumulation and apparent N_2 fixation of soybeans grown outdoors at high irradiance and supplied four levels of nitrate. Harvesting began two weeks after the seeds were imbibed.

Plant Part	Nitrate Treatment (mMoles)	Days From First Harvest						
		0	4	9	14	18	23	28
		mg N						
Total Plant	0	12.48	13.32	11.68	14.02	23.44	49.33	98.04
	2	12.59	12.28	13.84	16.66	23.60	51.30	91.81
	6	8.12	10.51	21.31	31.70	47.27	67.54	107.06
	12	11.60	16.23	29.76	45.02	75.91	93.72	124.21
	LSD(.05)	13.33						
Leaves	0	8.33	8.65	7.15	7.35	15.82	33.53	63.33
	2	7.26	8.14	8.60	13.16	15.67	35.86	61.30
	6	5.02	6.37	14.45	20.98	32.21	42.16	68.50
	12	6.81	10.27	19.42	28.47	44.08	54.85	65.88
	LSD(.05)	8.98						
Stems	0	1.54	1.70	1.40	3.83	3.44	7.84	19.37
	2	2.35	1.60	1.68	2.27	3.18	7.08	15.81
	6	1.14	1.56	2.56	4.95	7.62	11.43	19.08
	12	1.93	3.04	4.58	8.94	17.76	22.36	34.36
	LSD(.05)	3.35						
Roots + Nodules	0	2.61	2.97	3.13	3.79	4.18	7.97	15.34
	2	2.98	2.95	3.55	4.52	4.75	8.36	14.69
	6	1.96	2.58	4.30	5.77	7.45	13.95	19.48
	12	2.86	2.92	5.76	7.62	14.07	16.51	23.97
	LSD(.05)	2.86						
Apparent N_2 Fixation		µmoles ethylene/plant/hour						
	0	-	0.10	3.87	6.21	9.29	24.52	41.16
	2	-	0.09	1.97	4.75	5.57	21.27	41.64
	6	-	0.10	1.40	3.70	4.71	6.33	21.66
	12	-	0.14	0.62	0.74	0.65	1.04	1.83
	LSD(.05)	6.04						

Table 4. Changes in nitrogen accumulation of non-nodulated soybeans grown outdoors at high irradiance and supplied four levels of nitrate. Harvesting began two weeks after the seeds were imbibed.

Plant Part	Nitrate Treatment (mMoles)	Days From First Harvest						
		0	4	9	14	18	23	28
		mg N						
Total Plant	2	7.36	6.24	8.01	8.48	15.90	18.36	23.11
	6	6.42	7.77	12.69	19.60	21.72	37.56	62.20
	12	6.44	8.51	17.60	31.33	47.36	75.32	92.40
	LSD(.05)	8.22						
Leaves	2	4.61	3.62	4.69	4.45	8.88	10.80	13.06
	6	3.46	4.12	7.32	12.06	17.72	23.02	37.15
	12	3.74	5.00	11.60	19.65	29.62	45.20	53.25
	LSD(.05)	5.29						
Stems	2	1.03	1.02	0.83	0.88	1.76	2.03	3.22
	6	1.48	1.21	1.78	2.43	3.53	5.05	10.28
	12	1.18	1.29	2.53	5.54	9.32	14.11	25.49
	LSD(.05)	2.19						
Roots	2	1.72	1.85	2.50	3.15	5.26	5.52	6.84
	6	1.48	2.43	3.59	5.11	4.89	9.49	10.28
	12	1.52	2.22	3.48	6.13	8.42	16.01	20.03
	LSD(.05)	2.85						

Table 5. Changes in plant dry weight and leaf area of soybeans grown outdoors at high irradiance and supplied four levels of nitrate. Harvesting began two weeks after the seeds were imbibed.

Plant Part	Nitrate Treatment (mMoles)	Days From First Harvest						
		0	4	9	14	18	23	28
		dry weight (grams)						
Total Plant	0	0.25	0.41	0.76	0.99	1.25	2.48	4.23
	2	0.28	0.37	0.75	1.11	1.32	2.37	3.86
	6	0.20	0.32	0.89	1.48	2.00	3.47	5.69
	12	0.24	0.43	0.97	1.61	2.73	3.86	5.98
	LSD(.05)	0.65						
Leaves	0	0.14	0.21	0.32	0.37	0.53	1.04	1.80
	2	0.14	0.19	0.33	0.41	0.55	1.01	1.57
	6	0.11	0.16	0.43	0.62	0.89	1.36	2.34
	12	0.13	0.24	0.49	0.71	1.26	1.59	2.15
	LSD(.05)	0.26						
Stem	0	0.03	0.06	0.14	0.20	0.27	0.61	1.13
	2	0.04	0.06	0.14	0.23	0.29	0.58	1.06
	6	0.02	0.05	0.18	0.37	0.54	1.01	1.47
	12	0.03	0.07	0.20	0.42	0.77	1.23	1.61
	LSD(.05)	0.18						
Roots	0	0.08	0.14	0.26	0.35	0.36	0.63	0.99
	2	0.10	0.12	0.25	0.40	0.41	0.64	0.99
	6	0.07	0.11	0.26	0.45	0.49	1.01	1.73
	12	0.09	0.12	0.26	0.46	0.68	1.02	1.19
	LSD(.05)	0.20						
Nodules	0	-	-	0.04	0.07	0.09	0.20	0.31
	2	-	-	0.03	0.07	0.07	0.14	0.24
	6	-	-	0.02	0.04	0.08	0.09	0.15
	12	-	-	0.02	0.02	0.02	0.02	0.03
	LSD(.05)	0.04						
Leaf Area		cm^2/plant						
	0	18.80	50.25	87.84	115.63	165.23	255.50	525.12
	2	24.90	46.25	94.41	134.09	180.54	349.01	545.06
	6	19.59	39.16	124.53	194.01	291.55	406.51	585.67
	12	15.10	49.43	138.86	203.28	279.04	440.26	536.73
	LSD(.05)	61.67						

Table 6. Changes in plant dry weight and leaf area of non-nodulated soybeans grown outdoors at high irradiance and supplied four levels of nitrate. Harvesting began two weeks after the seeds were imbibed.

Plant Part	Nitrate Treatment (mMoles)	Days From First Harvest						
		0	4	9	14	18	23	28
		dry weight (grams)						
Total Plant	2	0.19	0.26	0.46	0.64	0.91	1.43	1.66
	6	0.16	0.29	0.50	1.00	1.21	2.33	3.73
	12	0.18	0.28	0.56	0.99	1.53	3.46	3.57
	LSD(.05)	0.66						
Leaves	2	0.10	0.12	0.18	0.23	0.38	0.59	0.71
	6	0.09	0.13	0.22	0.42	0.57	0.93	1.54
	12	0.10	0.14	0.29	0.46	0.75	1.46	1.60
	LSD(.05)	0.22						
Stems	2	0.02	0.03	0.06	0.11	0.17	0.29	0.36
	6	0.02	0.03	0.08	0.19	0.27	0.59	1.02
	12	0.02	0.03	0.10	0.23	0.40	0.93	1.04
	LSD(.05)	0.15						
Roots	2	0.07	0.11	0.19	0.30	0.36	0.55	0.59
	6	0.05	0.13	0.20	0.39	0.37	0.81	1.17
	12	0.06	0.11	0.17	0.30	0.38	1.07	0.93
	LSD(.05)	0.35						
Leaf Area		cm^2/plant						
	2	13.56	30.60	55.19	81.53	121.92	191.04	222.14
	6	14.29	32.33	72.53	148.44	197.36	307.51	453.07
	12	12.04	37.13	96.06	150.87	238.86	430.15	414.86
	LSD(.05)	57.10						

FIGURE LEGENDS

Fig. 1. Estimated progress curves representing leaf area ratios (A), net assimilation rates (B), and relative growth rates (C) of soybeans grown under an average daily irradiance of 8.6 E m^{-2} s^{-1} and completely dependent on nitrogen fixation (←-------→), or supplemented with 2 mM NO_3 (←—— ——→), 6 mM NO_3 (←————→), or 12 mM NO_3 (←—— - ——→).

Fig. 2. Estimated progress curves representing leaf area ratios (A), net assimilation rates (B), and relative growth rates (C) of soybeans grown under an average daily irradiance of 31.3 E m^{-2} s^{-1}day and completely dependent on nitrogen fixation (←-------→), or supplemented with 2 mM NO_3 (←—— ——→), 6 mM NO_3 (←————→), or 12 mM NO_3 (←—— - ——→).

Fig. 3. Estimated progress curves representing leaf area ratios (A), net assimilation rates (B), and relative growth rates (C) of non-nodulating isogenic line L73-1054 Clark soybeans grown under an average daily irradiance of 31.3 E m^{-2} s^{-1} and supplied 2 mM NO_3 (←-------→), 6 mM NO_3 (←—— ——→), or 12 mM NO_3 (←————→).

Fig. 1

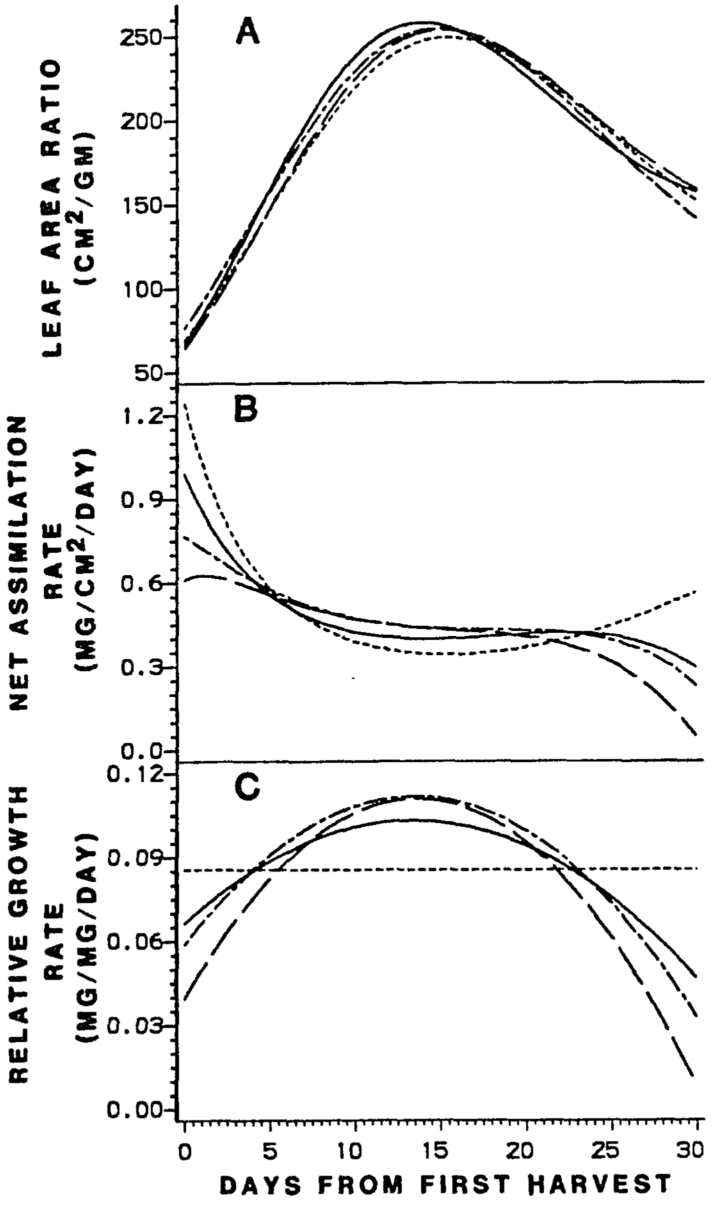

A
LEAF AREA RATIO (CM^2/GM)
250
200
150
100
50
B
NET ASSIMILATION RATE (MG/CM^2/DAY)
1.2
0.9
0.6
0.3
0.0
C
RELATIVE GROWTH RATE (MG/MG/DAY)
0.12
0.09
0.06
0.03
0.00
0
5
10
15
20
25
30
DAYS FROM FIRST HARVEST

Fig. 2

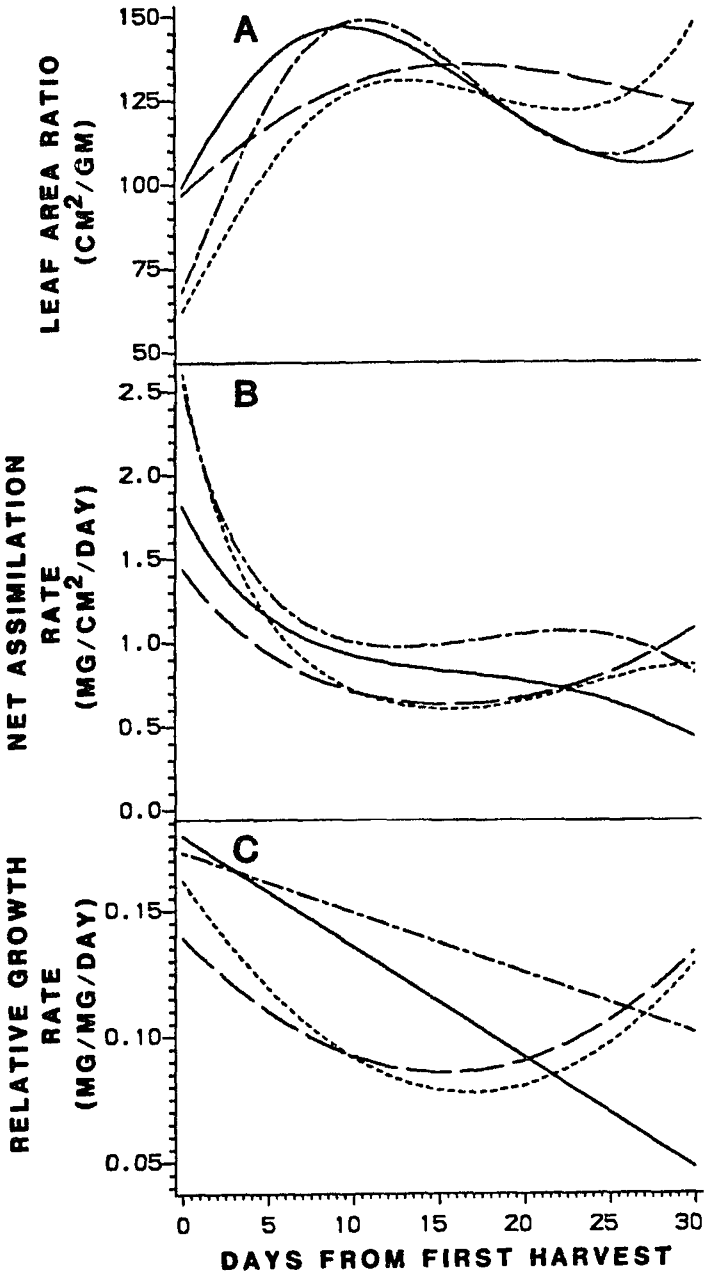
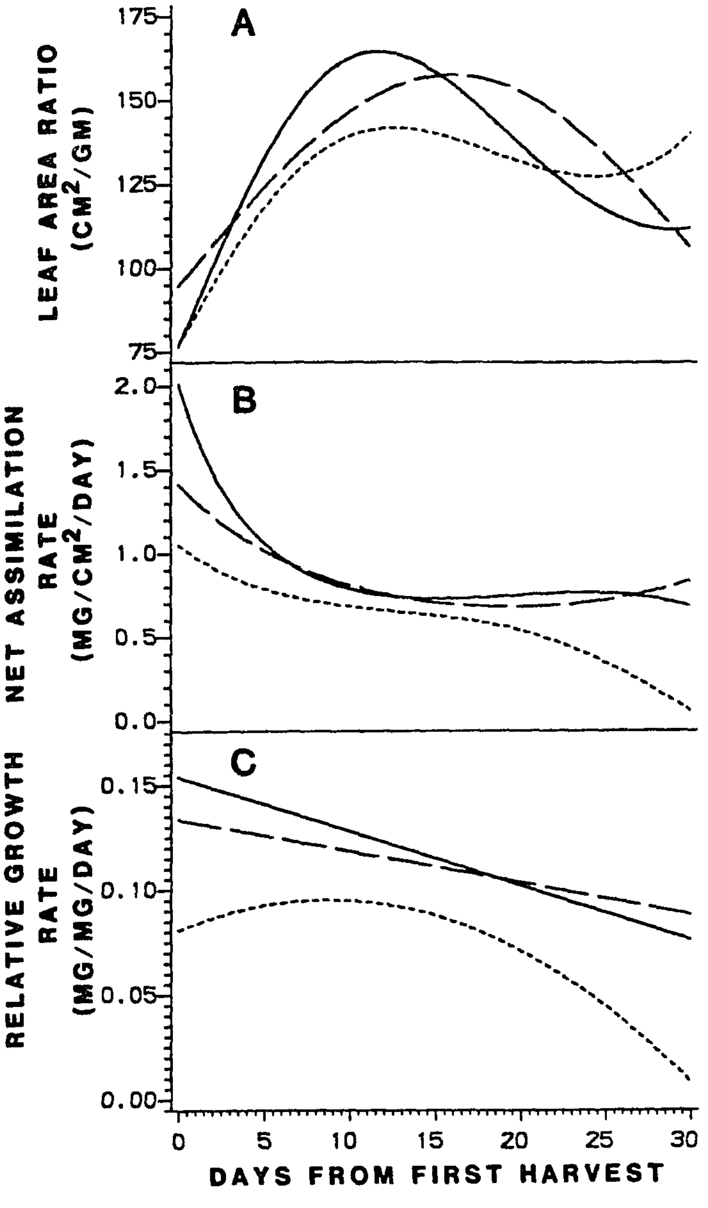

A
B
C
LEAF AREA RATIO (CM^2/GM)
150
125
100
75
50
NET ASSIMILATION RATE (MG/CM^2/DAY)
2.5
2.0
1.5
1.0
0.5
0.0
RELATIVE GROWTH RATE (MG/MG/DAY)
0.15
0.10
0.05
0
5
10
15
20
25
30
DAYS FROM FIRST HARVEST

Fig. 3


A
LEAF AREA RATIO (CM²/GM)
175
150
125
100
75
B
NET ASSIMILATION RATE (MG/CM²/DAY)
2.0
1.5
1.0
0.5
0.0
C
RELATIVE GROWTH RATE (MG/MG/DAY)
0.15
0.10
0.05
0.00
0
5
10
15
20
25
30
DAYS FROM FIRST HARVEST

SUMMARY

Physiological and environmental limitations to nitrogen fixation and growth of soybeans were investigated. Under natural conditions of variable solar radiation and ambient air temperature, 35-day-old plants with constant root zone temperature maintained constant levels of foliar soluble sugars and constant rates of root + nodule respiration and acetylene reduction over a 24-hour period. These results were interpreted to indicate that a constant supply of photosynthate was being partitioned to the nodules. When root zone temperature was allowed to vary with ambient air temperature, nodule activity also varied suggesting that reports of diurnal variation in nodule activity may have been due to diurnal variations in root zone temperature.

Following the diurnal study, the normal dark period of the plants was extended to 40 hours to deplete levels of stored carbohydrates. Plants were then exposed to increases in irradiance provided by metal-halide lamps. It was found that exposure to 200 $\mu E\ m^{-2}s^{-1}$ enabled plants to produce sufficient carbohydrate to obtain maximum rates of acetylene reduction. This irradiance level was approximately one-third of the light saturation level for photosynthesis. It was suggested that plants were acclimated to produce sufficient carbohydrate for maximum nodule activity at low irradiance levels with additional photosynthate being partitioned into starch for maintenance

during non-photosynthetic periods. It was also suggested that photosynthesis *per se* does not limit nitrogen fixation, but that acclimation to a particular environment determines the amount of photosynthate partitioned for nitrogen fixation.

In a separate study, carbon and nitrogen limitations to growth of symbiotically grown soybeans were assessed using mathematical growth analysis techniques. Comparisons were made between plants grown under low insolation in a greenhouse during the Winter and outdoors during the Spring. Plants were either entirely dependent on symbiotically fixed N_2, or supplemented with 2.0 mM, 6.0 mM or 12.0 mM NO_3. Beginning two weeks after seeds were imbibed, dry weight and N content of plant parts, total leaf area and rates of C_2H_2 reduction were determined at frequent intervals. Dry weight and leaf area data were used to calculate relative growth rates (RGR), net assimilation rates (NAR) and leaf area ratios (LAR).

Supplementing N_2 fixation with 2.0 mM NO_3 resulted in maintenance of growth functions which were very similar to plants supplied 6.0 or 12.0 mM NO_3. RGR and LAR of these treatments increased for two weeks, then declined. Plants solely dependent on N_2 fixation, however, maintained similar LAR, but RGR remained constant. Approximately two weeks after harvesting began, plants dependent on fixed N_2 were significantly N-deficient relative to all plants receiving NO_3. The effects of the N deficiency were first noted in significantly lower leaf area followed by significantly lower total plant dry weight. At the final harvest, total plant dry weight and leaf area were equivalent to plants supplemented with 2.0 mM or 12.0 mM NO_3.

RGR of all treatments grown outdoors declined for the first two weeks with rates of the 0.0 mM and 2.0 mM NO_3 treatments declining at a much greater rate than plants supplemented with 6.0 mM or 12.0 mM NO_3. During the final two weeks of harvest, RGR of the 6.0 mM and 12.0 mM NO_3 treatments continued to decline, but rates of the 0.0 mM and 2.0 mM NO_3 treatments increased. This period corresponded with the time during which the 0.0 and 2.0 mM NO_3 treatments had significantly greater rates of acetylene reduction compared to the 6.0 and 12.0 mM NO_3 treatments. Examination of the components of RGR, NAR and LAR, showed that trends in RGR of the 0.0 mM and 2.0 mM NO_3 treatments were largely due to similar trends in NAR. Significant N deficiencies were evident in the 0.0 mM and 2.0 mM NO_3 treatments nine days after harvesting began. By the final harvest, the 0.0 mM and 2.0 mM NO_3 treatments were deficient in total plant N and dry matter compared to the 12.0 mM NO_3 treatment. There were no differences in leaf N content or leaf area indicating that once N_2 was established, N was preferentially allocated to the leaves.

It was suggested that results from this study indicated that growth limitations to nodulated soybeans are primarily due to an inability to arrive at a functional balance between C and N accumulation prior to establishment of a fully functional N_2 fixation system. It was also suggested that once N_2 fixation is established, the increased input of N is used to increase both the quality and quantity of leaf tissue.

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APPENDIX

Appendix Table 1 contains data for the diurnal study conducted during the late Spring of 1982. Data are coded in the following manner:

Column

A. Time (hours).

B. Temperature treatment. C and U denote controlled and uncontrolled root-zone temperature, respectively.

C. Temperature (^{o}C) of interior chamber enclosing roots and nodules.

D. Ambient temperature.

E. Irradiance ($\mu E\ m^{-2}\ s^{-1}$).

F. Mean respiration rate from roots and nodules (mg CO_2 plant^{-1} hour^{-1}).

G. Standard error of mean respiration rate.

Appendix Table 1 (continued).

A	B	C	D	E	F	G
634	C	22.77	19.33	49.42	4.48	0.57
644	U	18.57	19.53	74.65	2.21	0.13
704	C	22.93	19.96	128.39	4.45	0.53
714	U	18.88	20.21	161.51	2.28	0.12
734	C	23.26	20.88	243.48	4.36	0.57
744	U	19.44	21.23	292.15	2.38	0.09
804	C	21.07	22.74	438.91	5.60	.
814	U	21.72	23.15	465.41	3.80	0.09
834	C	25.01	24.56	572.49	5.93	0.83
844	U	22.54	25.24	649.96	3.87	0.06
904	C	26.50	26.14	751.33	5.99	0.91
914	U	24.02	26.25	850.18	4.18	0.21
934	C	27.68	26.74	913.42	6.14	1.04
944	U	25.45	26.97	1020.21	4.69	0.27
1004	C	28.58	27.75	1175.56	6.47	1.17
1014	U	27.05	28.15	285.93	5.30	0.41
1138	C	26.40	30.21	1782.14	5.81	0.62
1148	U	32.49	30.40	1810.48	12.97	1.48
1208	C	25.31	30.65	1915.49	6.98	1.62
1218	U	33.39	30.15	1372.03	15.18	2.48
1238	C	24.43	30.77	1961.11	6.22	1.64
1248	U	33.94	31.03	1907.71	15.65	1.26
1308	C	23.91	31.10	1947.46	6.47	1.55
1318	U	34.63	31.44	1744.13	16.64	0.03
1338	C	23.84	31.66	1827.53	6.45	1.34
1348	U	35.14	32.21	1873.15	18.20	1.45
1508	C	25.27	30.90	1127.69	7.98	1.31
1518	U	36.58	30.27	1197.16	14.77	0.85
1538	C	24.99	32.35	1307.06	7.75	1.33
1548	U	36.30	30.89	1252.92	8.89	0.84
1608	C	25.46	30.69	1103.85	7.62	1.18
1618	U	35.84	29.89	796.03	4.72	0.09
1638	C	24.54	26.59	125.80	7.75	1.38
1648	U	30.90	25.73	132.94	6.05	0.29
1708	C	24.32	25.10	212.89	8.15	2.75
1718	U	29.41	25.29	449.97	5.77	0.12
2108	C	27.14	22.46	0.00	5.23	0.80
2118	U	22.06	22.35	0.00	7.61	0.42
2208	C	25.33	22.06	0.00	7.44	0.50
2218	U	21.62	21.98	0.00	4.18	0.41
2238	C	25.70	21.86	0.00	7.33	0.36
2248	U	21.29	21.83	0.00	4.48	0.56
2308	C	26.03	21.66	0.00	7.39	0.37
2318	U	21.08	21.49	0.00	4.62	0.71
2338	C	26.21	21.45	0.00	7.52	0.22
2348	U	20.84	21.50	0.00	4.55	0.60

Appendix Table 1 (continued).

A	B	C	D	E	F	G
8	C	26.35	21.53	0.00	7.41	0.35
18	U	20.76	21.52	0.00	4.59	0.60
38	C	25.86	20.84	0.00	6.93	0.32
48	U	20.63	21.36	0.00	4.38	0.56
208	C	25.25	21.00	0.00	6.93	0.55
218	U	20.53	21.00	0.00	4.82	1.07
238	C	25.35	20.90	0.00	7.16	0.73
248	U	20.40	20.80	0.00	4.89	1.13
308	C	25.40	20.80	0.00	7.13	0.73
318	U	20.30	20.90	0.00	4.79	0.98
338	C	25.40	20.90	0.00	6.98	0.69
348	U	20.30	20.90	0.00	4.79	0.98
408	C	25.40	20.80	0.00	6.90	0.70
418	U	20.30	20.60	0.00	4.72	1.01
438	C	25.40	20.55	0.00	7.92	0.69
448	U	20.20	20.60	0.00	5.64	1.01
608	C	24.95	21.08	164.51	6.06	0.88
618	U	20.30	21.40	234.55	4.65	0.63
638	C	25.45	22.50	355.45	6.93	1.03
648	U	21.23	23.00	427.85	5.03	0.66
708	C	26.10	24.25	556.24	6.09	0.90
718	U	22.80	24.77	630.60	5.06	0.84
738	C	26.80	25.68	760.67	6.85	0.94
748	U	24.33	25.90	839.35	6.11	0.95
808	C	27.48	26.43	834.45	7.34	0.97
818	U	25.40	26.43	773.68	6.96	0.89
838	C	27.85	26.08	537.24	7.92	1.21
848	U	25.70	26.33	787.28	7.61	1.18
908	C	27.88	27.20	1179.53	7.31	1.05
918	U	26.53	28.13	1507.05	8.35	1.30
938	C	26.93	28.75	1486.08	6.27	0.66
948	U	28.70	22.20	1686.53	9.65	1.49
1008	C	25.90	29.70	1795.39	5.88	0.52
1018	U	31.17	29.87	1870.85	10.90	1.60

Appendix Table 2. Mean ± standard error of foliar starch and soluble sugar content in 35-day-old soybeans harvested during the Spring diurnal study.

Time (Hours)	Soluble Sugars (mg/g dry weight)	Starch (mg/g dry weight)
0800	36.53±1.95	45.94±4.35
1200	30.70±3.07	125.21±8.86
1800	35.23±0.78	160.40±1.72
2230	31.98±3.94	126.71±5.81
0200	38.38±3.97	74.79±5.80
0600	28.68±3.10	66.80±3.19
1130	26.90±3.35	131.76±7.18

Appendix Table 3. Mean ± standard error of acetylene reduction activity of 35-day-old soybeans during the Spring diurnal study with and without temperature controlled root zone.

Time (Hours)	Root Zone Temperature Controlled	Root Zone Temperature Uncontrolled
1000	26.10±1.56	20.40±0.35
1400	23.20±1.29	21.40±0.78
1800	25.00±1.67	15.30±1.21
2230	25.70±2.40	11.20±0.73
0200	25.40±1.93	11.30±0.36
0600	25.95±2.37	11.80±0.53
1130	25.90±1.65	18.80±2.81

Appendix Table 4. Mean ± standard error of foliar starch and soluble sugar content and acetylene reduction activity of 35-day-old soybeans grown during the late Spring and exposed to 10 hours at a specific irradiance level following 40 hours of darkness.

Irradiance Level	Soluble Sugars (mg/g dry weight)	Starch (mg/g dry weight)	$\frac{\mu\text{moles Ethylene}}{\text{plant/hour}}$
0	21.50±3.94	25.08±0.72	1.68±0.19
200	41.58±2.25	51.84±1.69	14.07±2.38
400	46.88±1.68	106.81±6.55	7.69±0.26
600	45.68±1.37	91.42±3.76	14.86±2.48
1000	42.98±4.31	134.27±5.09	10.00±0.99

Appendix Table 5 contains data for the diurnal study conducted during the early Fall of 1982. Data are coded in the following manner:

Column

A. Time (hours).

B. Temperature treatment. S/R and R denote controlled shoot and root temperature and controlled root temperature, respectively.

C. Temperature (°C) of interior chamber enclosing shoot and/or root.

D. Ambient temperature (°C).

E. Irradiance (µE m^{-2} s^{-1}).

F. Mean respiration rate from roots and nodules (mg CO_2 $plant^{-1}$ $hour^{-1}$).

G. Standard error of mean respiration rate.

Appendix Table 5 (continued).

A	B	C	D	E	F	G
1059	S/R	25.87	31.72	1537.00	7.30	0.71
1109	R	27.77	31.66	1706.75	6.75	0.76
1129	S/R	25.04	31.52	1350.00	5.87	0.48
1139	R	25.87	31.50	1217.50	5.63	0.58
1159	S/R	24.66	32.29	1530.33	5.23	0.45
1209	R	26.42	32.82	1217.25	5.27	0.46
1229	S/R	24.99	33.27	1806.67	5.36	0.40
1239	R	26.06	33.16	1262.75	5.10	0.58
1259	S/R	24.63	33.01	1045.33	5.88	0.59
1309	R	25.43	32.63	1071.75	5.73	0.66
1329	S/R	24.48	34.27	1499.00	5.34	0.54
1339	R	26.74	34.31	1747.00	5.60	0.64
1429	S/R	25.10	34.22	1704.00	5.96	.
1439	R	25.68	35.15	1680.25	6.66	0.80
1459	S/R	25.27	35.30	832.67	6.23	0.58
1509	R	24.22	33.99	437.00	5.70	0.64
1529	S/R	24.04	33.01	420.33	5.08	0.34
1539	R	24.06	32.40	141.75	5.40	0.33
1559	S/R	23.34	28.46	112.33	4.33	0.19
1609	R	23.84	27.74	120.75	4.87	0.32
1629	S/R	23.71	27.40	138.33	3.93	0.27
1639	R	25.90	27.41	141.75	4.77	0.28
1659	S/R	24.84	27.57	213.67	4.02	0.29
1709	R	24.87	27.88	244.00	4.47	0.30
1729	S/R	24.32	28.09	213.67	3.75	0.31
1739	R	24.98	28.15	156.25	4.34	0.27
1829	S/R	24.66	27.70	73.00	5.04	0.53
1839	R	24.77	27.64	56.75	5.14	0.28
1859	S/R	25.11	27.27	15.00	4.55	0.42
1909	R	24.76	27.04	3.00	4.84	0.28
1929	S/R	25.10	26.70	0.00	4.55	0.39
1939	R	24.94	26.58	0.00	4.57	0.20
1959	S/R	25.39	26.46	0.00	4.37	0.43
2009	R	24.97	26.19	0.00	4.34	0.17
2029	S/R	25.80	26.24	0.00	4.37	0.43
2039	R	24.98	26.17	0.00	4.24	0.20
2059	S/R	25.73	26.02	0.00	4.33	0.29
2109	R	24.95	25.97	0.00	3.84	0.16
2129	S/R	25.76	25.78	0.00	3.80	0.31
2139	R	24.99	25.80	0.00	3.48	0.18
2259	S/R	25.73	25.50	0.00	4.51	0.27
2309	R	25.10	25.41	0.00	3.94	0.18
2329	S/R	25.94	25.33	0.00	4.51	0.27
2339	R	25.09	25.27	0.00	4.24	0.13
2359	S/R	25.89	25.12	0.00	4.51	0.08
9	R	25.10	25.04	0.00	3.88	0.20
29	S/R	25.78	24.91	0.00	3.98	0.23
39	R	25.14	24.89	0.00	3.74	0.20

Appendix Table 5 (continued).

A	B	C	D	E	F	G
59	S/R	25.46	24.84	0.00	4.15	0.23
109	R	25.16	24.85	0.00	3.74	0.23
209	R	25.28	24.47	0.00	4.24	0.41
229	S/R	25.23	24.34	0.00	4.21	0.22
239	R	25.28	24.35	0.00	4.34	0.38
259	S/R	25.46	24.32	0.00	4.20	0.42
309	R	25.25	24.23	0.00	3.65	0.27
329	S/R	25.52	24.20	0.00	4.59	0.42
339	R	25.31	24.20	0.00	4.18	0.26
359	S/R	25.48	24.10	0.00	4.11	0.28
409	R	25.35	24.12	0.00	3.85	0.31
429	S/R	25.41	24.32	0.00	4.06	0.22
439	R	25.21	24.30	0.00	3.98	0.29
459	S/R	25.46	24.30	0.00	3.97	0.20
509	R	25.15	24.31	0.00	3.87	0.21
529	S/R	25.41	24.22	0.00	4.11	0.21
639	R	25.02	24.23	6.00	4.14	0.22
659	S/R	25.48	24.27	39.00	4.15	0.04

Appendix Table 6. Mean ± standard error of foliar starch and soluble sugar content in 35-day-old soybeans harvested during the Fall diurnal study.

Time (Hours)	Soluble Sugars (mg/g dry weight)	Starch (mg/g dry weight)
1100	50.87±2.23	71.70±7.88
1400	42.13±0.67	104.46±5.62
1800	43.73±2.45	120.10±3.55
2200	38.13±1.31	98.49±13.83
0200	48.97±0.62	71.25±4.64
0600	39.25±1.64	45.85±4.86

Appendix Table 7. Mean ± standard error of acetylene reduction activity of 35-day-old soybeans during the Fall diurnal study with either controlled shoot and root zone temperature or controlled root zone temperature.

Time (Hours)	Shoot and Root Zone Temperature Controlled	Root Zone Temperature Controlled
1000	8.26±1.25	8.03±0.90
1400	7.67±1.49	7.33±0.64
1800	8.30±1.21	8.63±0.95
2200	9.83±2.32	8.90±1.21
0200	9.06±2.05	9.34±1.32
0600	9.09±2.96	9.59±0.25
0900	10.59±2.30	10.80±0.79

Appendix Table 8. Mean ± standard error of foliar starch and soluble sugar content and acetylene reduction activity of 35-day-old soybeans grown during the early Fall and exposed to 10 hours at a specific irradiance level following 40 hours of darkness.

Irradiance Level	Soluble Sugars (mg/g dry weight)	Starch (mg/g dry weight)	μmoles Ethylene plant/hour
0	36.13±1.91	47.90±2.93	3.54±0.67
200	38.50±1.72	63.73±7.49	6.43±0.75
400	40.55±0.70	106.64±4.68	4.94±0.64
600	40.35±1.48	134.65±8.93	6.22±1.35
800	42.87±1.95	140.29±5.96	6.00±0.18
1000	39.15±2.60	128.14±4.42	6.08±0.29

Appendix Table 9. Nutrient solutions modified to contain specific concentrations of nitrate.

	Nitrate Concentration			
	0mM	2mM	6mM	12mM
Compound	Final Concentration in Solution			
KH_2PO_4	2mM	2mM	2mM	2mM
$MgSO_4$	1mM	1mM	1mM	1mM
$CaSO_4$	4mM	4mM	1mM	
K_2HPO_4	1mM			
KNO_3		2mM	2mM	2mM
$Ca(NO_3)_2$			3mM	5mM

Micronutrient Concentration in all solutions.

Compound	Final Concentration in Solution
KCL	50µM
H_3BO_3	25µM
$MnSO_4 \cdot H_2O$	5µM
$ZnSO_4 \cdot 7H_2O$	2µM
$CuSO_4 \cdot 5H_2O$	0.5µM
H_2MoO_4	0.5µM
$CoCl_2 \cdot 6H_2O$	4µM

pH adjusted to 6.8

Appendix Table 10. *Rhizobium japonicum* incubation medium.

Compound	Final Concentration in Medium
Mannitol	1.00 g/l
Yeast Extract	1.00 g/l
KH_2PO_4	0.30 g/l
Na_2HPO_4	0.30 g/l
$MgSO_4$	0.10 g/l
$CaCl_2$	0.50 g/l
H_3BO_3	10.00 mg/l
$ZnSO_4 \cdot 2H_2O$	1.00 mg/l
$FeCl_3$	1.00 mg/l
$CuSO_4 \cdot 5H_2O$	0.50 mg/l
$MnCl_2$	0.15 mg/l
$NaMoO_4 \cdot 2H_2O$	0.10 mg/l
Biotin	0.20 mg/l
Agar	15.00 g/l

pH adjusted to 6.8

Appendix Table 11 contains data for the growth analysis study conducted in the greenhouse during the Winter. The experiment began January 7, 1983, when seeds were imbibed, and continued through February 21, 1983, which was the final harvest date. Harvesting began January 22, 1983. Data are coded in the following manner:

<u>Column</u>

A. Concentration of nitrate (mMoles) in nutrient solution administered on alternate days.

B. Days from first harvest.

C. Plant Part.

D. Mean of four replicate plant part dry weights (grams).

E. Standard error of the mean plant part dry weight.

F. Mean percent nitrogen content of individual plant parts. Values listed for roots are actually a combination of roots and nodules.

G. Standard error of mean percent nitrogen content.

H. Mean total leaf area (cm^2).

I. Standard error of mean total leaf area.

Appendix Table 11 (continued).

A	B	C	D	E	F	G	H	I
0	0	LEAVES	0.11	0.03	7.27	0.21	12.14	2.03
0	0	ROOTS	0.03	0.00	7.42	0.43	12.14	2.03
0	0	STEM	0.01	0.00	9.49	1.06	12.14	2.03
0	2	LEAVES	0.13	0.00	.	.	22.39	2.80
0	2	ROOTS	0.06	0.01	.	.	22.39	2.80
0	2	STEM	0.02	0.00	.	.	22.39	2.80
0	4	LEAVES	0.12	0.00	6.58	0.50	21.93	3.06
0	4	NODULES	0.00	0.00	.	.	21.93	3.06
0	4	ROOTS	0.07	0.01	4.11	0.34	21.93	3.06
0	4	STEM	0.03	0.00	5.36	0.76	21.93	3.06
0	6	LEAVES	0.14	0.01	.	.	38.70	5.00
0	6	NODULES	0.00	0.00	.	.	38.70	5.00
0	6	ROOTS	0.07	0.01	.	.	38.70	5.00
0	6	STEM	0.03	0.01	.	.	38.70	5.00
0	9	LEAVES	0.19	0.00	5.28	0.42	71.16	3.15
0	9	NODULES	0.01	0.00	.	.	71.16	3.15
0	9	ROOTS	0.10	0.01	2.52	0.19	71.16	3.15
0	9	STEM	0.06	0.00	2.39	0.25	71.16	3.15
0	11	LEAVES	0.23	0.01	.	.	106.82	5.54
0	11	NODULES	0.01	0.00	.	.	106.82	5.54
0	11	ROOTS	0.12	0.01	.	.	106.82	5.54
0	11	STEM	0.07	0.01	.	.	106.82	5.54
0	13	LEAVES	0.22	0.02	2.96	0.19	109.99	9.75
0	13	NODULES	0.02	0.00	.	.	109.99	9.75
0	13	ROOTS	0.12	0.01	1.45	0.08	109.99	9.75
0	13	STEM	0.08	0.01	1.26	0.02	109.99	9.75
0	16	LEAVES	0.35	0.01	.	.	193.88	4.74
0	16	NODULES	0.04	0.00	.	.	193.88	4.74
0	16	ROOTS	0.16	0.01	.	.	193.88	4.74
0	16	STEM	0.14	0.00	.	.	193.88	4.74
0	18	LEAVES	0.38	0.04	2.80	0.27	179.10	16.24
0	18	NODULES	0.04	0.01	.	.	179.10	16.24
0	18	ROOTS	0.18	0.01	1.52	0.20	179.10	16.24
0	18	STEM	0.14	0.01	1.20	0.17	179.10	16.24
0	20	LEAVES	0.52	0.02	.	.	225.81	7.34
0	20	NODULES	0.06	0.00	.	.	225.81	7.34
0	20	ROOTS	0.22	0.01	.	.	225.81	7.34
0	20	STEM	0.20	0.01	.	.	225.81	7.34
0	23	LEAVES	0.63	0.03	2.39	0.07	221.64	5.97
0	23	NODULES	0.07	0.00	.	.	221.64	5.97
0	23	ROOTS	0.22	0.01	1.42	0.05	221.64	5.97
0	23	STEM	0.23	0.01	1.16	0.11	221.64	5.97
0	25	LEAVES	0.62	0.05	.	.	210.58	17.57
0	25	NODULES	0.08	0.01	.	.	210.58	17.57
0	25	ROOTS	0.21	0.02	.	.	210.58	17.57
0	25	STEM	0.25	0.03	.	.	210.58	17.57

Appendix Table 11 (continued).

A	B	C	D	E	F	G	H	I
0	27	LEAVES	0.85	0.15	2.76	0.16	266.19	37.21
0	27	NODULES	0.11	0.02	.	.	266.19	37.21
0	27	ROOTS	0.31	0.05	1.55	0.05	266.19	37.21
0	27	STEM	0.35	0.07	1.36	0.05	266.19	37.21
0	30	LEAVES	1.00	0.06	3.81	0.06	367.11	15.65
0	30	NODULES	0.16	0.01	.	.	367.11	15.65
0	30	ROOTS	0.42	0.02	1.55	0.04	367.11	15.65
0	30	STEM	0.49	0.03	1.83	0.08	367.11	15.65
2	0	LEAVES	0.11	0.01	7.20	0.15	10.75	1.33
2	0	ROOTS	0.05	0.00	5.06	1.07	10.75	1.33
2	0	STEM	0.02	0.00	6.78	1.32	10.75	1.33
2	2	LEAVES	0.13	0.01	.	.	23.22	2.83
2	2	ROOTS	0.06	0.01	.	.	23.22	2.83
2	2	STEM	0.03	0.00	.	.	23.22	2.83
2	4	LEAVES	0.11	0.01	6.27	0.44	28.41	4.43
2	4	NODULES	0.00	0.00	.	.	28.41	4.43
2	4	ROOTS	0.07	0.00	3.72	0.34	28.41	4.43
2	4	STEM	0.03	0.00	4.61	0.34	28.41	4.43
2	6	LEAVES	0.12	0.01	.	.	36.71	1.20
2	6	NODULES	0.00	0.00	.	.	36.71	1.20
2	6	ROOTS	0.06	0.00	.	.	36.71	1.20
2	6	STEM	0.03	0.00	.	.	36.71	1.20
2	9	LEAVES	0.20	0.01	4.65	0.10	82.81	2.35
2	9	NODULES	0.01	0.00	.	.	82.81	2.35
2	9	ROOTS	0.11	0.00	2.94	0.31	82.81	2.35
2	9	STEM	0.07	0.00	2.52	0.07	82.81	2.35
2	11	LEAVES	0.24	0.01	.	.	107.74	8.78
2	11	NODULES	0.01	0.00	.	.	107.74	8.78
2	11	ROOTS	0.12	0.01	.	.	107.74	8.78
2	11	STEM	0.09	0.01	.	.	107.74	8.78
2	13	LEAVES	0.30	0.01	3.64	0.11	153.82	9.16
2	13	NODULES	0.02	0.00	.	.	153.82	9.16
2	13	ROOTS	0.15	0.01	1.77	0.05	153.82	9.16
2	13	STEM	0.12	0.01	1.59	0.06	153.82	9.16
2	16	LEAVES	0.35	0.01	.	.	200.30	8.06
2	16	NODULES	0.03	0.00	.	.	200.30	8.06
2	16	ROOTS	0.18	0.00	.	.	200.30	8.06
2	16	STEM	0.15	0.00	.	.	200.30	8.06
2	18	LEAVES	0.52	0.01	3.08	0.14	237.90	4.66
2	18	NODULES	0.04	0.00	.	.	237.90	4.66
2	18	ROOTS	0.21	0.00	1.61	0.01	237.90	4.66
2	18	STEM	0.21	0.00	1.46	0.03	237.90	4.66
2	20	LEAVES	0.56	0.02	.	.	245.20	8.44
2	20	NODULES	0.05	0.00	.	.	245.20	8.44
2	20	ROOTS	0.25	0.01	.	.	245.20	8.44
2	20	STEM	0.23	0.01	.	.	245.20	8.44

Appendix Table 11 (continued).

A	B	C	D	E	F	G	H	I
2	23	LEAVES	0.80	0.06	2.39	0.11	289.26	20.06
2	23	NODULES	0.06	0.01	.	.	289.26	20.06
2	23	ROOTS	0.30	0.01	1.54	0.05	289.26	20.06
2	23	STEM	0.32	0.03	1.21	0.04	289.26	20.06
2	25	LEAVES	0.90	0.04	.	.	302.20	12.59
2	25	NODULES	0.08	0.00	.	.	302.20	12.59
2	25	ROOTS	0.34	0.02	.	.	302.20	12.59
2	25	STEM	0.39	0.02	.	.	302.20	12.59
2	27	LEAVES	1.06	0.03	2.30	0.23	338.98	8.24
2	27	NODULES	0.11	0.01	.	.	338.98	8.24
2	27	ROOTS	0.43	0.02	1.64	0.03	338.98	8.24
2	27	STEM	0.46	0.01	1.25	0.03	338.98	8.24
2	30	LEAVES	1.05	0.10	3.26	0.11	362.63	22.65
2	30	NODULES	0.07	0.03	.	.	362.63	22.65
2	30	ROOTS	0.45	0.02	1.72	0.04	362.63	22.65
2	30	STEM	0.52	0.06	1.43	0.07	362.63	22.65
6	0	LEAVES	0.10	0.02	7.30	0.41	11.64	1.71
6	0	ROOTS	0.05	0.01	6.34	0.49	11.64	1.71
6	0	STEM	0.02	0.00	9.09	0.69	11.64	1.71
6	2	LEAVES	0.13	0.01	.	.	23.22	2.45
6	2	ROOTS	0.06	0.01	.	.	23.22	2.45
6	2	STEM	0.03	0.00	.	.	23.22	2.45
6	4	LEAVES	0.13	0.01	6.67	0.23	27.45	4.06
6	4	NODULES	0.00	0.00	.	.	27.45	4.06
6	4	ROOTS	0.07	0.00	4.48	0.39	27.45	4.06
6	4	STEM	0.03	0.00	5.31	0.49	27.45	4.06
6	6	LEAVES	0.16	0.01	.	.	49.78	3.28
6	6	NODULES	0.00	0.00	.	.	49.78	3.28
6	6	ROOTS	0.08	0.00	.	.	49.78	3.28
6	6	STEM	0.04	0.00	.	.	49.78	3.28
6	9	LEAVES	0.21	0.01	5.14	0.28	88.31	8.76
6	9	NODULES	0.00	0.00	.	.	88.31	8.76
6	9	ROOTS	0.11	0.01	3.05	0.14	88.31	8.76
6	9	STEM	0.08	0.01	2.99	0.21	88.31	8.76
6	11	LEAVES	0.23	0.02	.	.	103.27	12.25
6	11	NODULES	0.00	0.00	.	.	103.27	12.25
6	11	ROOTS	0.10	0.01	.	.	103.27	12.25
6	11	STEM	0.09	0.01	.	.	103.27	12.25
6	13	LEAVES	0.30	0.03	4.31	0.30	152.09	14.46
6	13	NODULES	0.01	0.00	.	.	152.09	14.46
6	13	ROOTS	0.15	0.01	2.10	0.09	152.09	14.46
6	13	STEM	0.13	0.02	1.86	0.13	152.09	14.46
6	16	LEAVES	0.33	0.04	.	.	189.67	18.80
6	16	NODULES	0.01	0.00	.	.	189.67	18.80
6	16	ROOTS	0.17	0.02	.	.	189.67	18.80
6	16	STEM	0.14	0.02	.	.	189.67	18.80

Appendix Table 11 (continued).

A	B	C	D	E	F	G	H	I
6	18	LEAVES	0.55	0.02	3.44	0.10	250.10	7.68
6	18	NODULES	0.02	0.00	.	.	250.10	7.68
6	18	ROOTS	0.23	0.00	1.91	0.06	250.10	7.68
6	18	STEM	0.23	0.01	1.73	0.06	250.10	7.68
6	20	LEAVES	0.52	0.02	.	.	238.38	4.86
6	20	NODULES	0.02	0.01	.	.	238.38	4.86
6	20	ROOTS	0.24	0.02	.	.	238.38	4.86
6	20	STEM	0.24	0.01	.	.	238.38	4.86
6	23	LEAVES	0.88	0.06	2.85	0.11	285.18	18.25
6	23	NODULES	0.04	0.00	.	.	285.18	18.25
6	23	ROOTS	0.33	0.02	1.49	0.18	285.18	18.25
6	23	STEM	0.36	0.02	1.55	0.24	285.18	18.25
6	25	LEAVES	1.00	0.07	.	.	307.75	16.78
6	25	NODULES	0.05	0.00	.	.	307.75	16.78
6	25	ROOTS	0.39	0.04	.	.	307.75	16.78
6	25	STEM	0.47	0.03	.	.	307.75	16.78
6	27	LEAVES	0.92	0.07	2.84	0.09	293.54	23.03
6	27	NODULES	0.06	0.01	.	.	293.54	23.03
6	27	ROOTS	0.39	0.02	1.93	0.18	293.54	23.03
6	27	STEM	0.46	0.04	1.54	0.33	293.54	23.03
6	30	LEAVES	1.07	0.02	3.40	0.12	380.57	6.74
6	30	NODULES	0.09	0.01	.	.	380.57	6.74
6	30	ROOTS	0.53	0.01	1.85	0.06	380.57	6.74
6	30	STEM	0.61	0.05	1.29	0.13	380.57	6.74
12	0	LEAVES	0.11	0.01	6.80	0.57	14.90	1.27
12	0	ROOTS	0.04	0.00	6.64	0.62	14.90	1.27
12	0	STEM	0.02	0.00	7.38	0.79	14.90	1.27
12	2		.	.	.	.	19.20	1.69
12	4	LEAVES	0.14	0.00	6.88	0.15	33.97	1.43
12	4	NODULES	0.00	0.00	.	.	33.97	1.43
12	4	ROOTS	0.07	0.00	4.77	0.15	33.97	1.43
12	4	STEM	0.04	0.00	4.91	0.15	33.97	1.43
12	6	LEAVES	0.15	0.01	.	.	46.66	1.71
12	6	NODULES	0.00	0.00	.	.	46.66	1.71
12	6	ROOTS	0.07	0.00	.	.	46.66	1.71
12	6	STEM	0.04	0.00	.	.	46.66	1.71
12	9	LEAVES	0.21	0.01	5.11	0.21	91.22	4.90
12	9	NODULES	0.00	0.00	.	.	91.22	4.90
12	9	ROOTS	0.10	0.00	3.49	0.43	91.22	4.90
12	9	STEM	0.08	0.01	3.64	0.29	91.22	4.90
12	11	LEAVES	0.26	0.01	.	.	110.40	5.28
12	11	NODULES	0.00	0.00	.	.	110.40	5.28
12	11	ROOTS	0.11	0.00	.	.	110.40	5.28
12	11	STEM	0.09	0.01	.	.	110.40	5.28
12	13	LEAVES	0.32	0.01	4.53	0.23	152.95	6.72
12	13	NODULES	0.00	0.00	.	.	152.95	6.72
12	13	ROOTS	0.15	0.01	3.23	0.42	152.95	6.72
12	13	STEM	0.13	0.01	2.39	0.25	152.95	6.72

Appendix Table 11 (continued).

A	B	C	D	E	F	G	H	I
12	16	LEAVES	0.42	0.02	.	.	232.74	9.20
12	16	NODULES	0.01	0.00	.	.	232.74	9.20
12	16	ROOTS	0.18	0.01	.	.	232.74	9.20
12	16	STEM	0.18	0.00	.	.	232.74	9.20
12	18	LEAVES	0.61	0.02	3.85	0.17	281.08	12.82
12	18	NODULES	0.01	0.00	.	.	281.08	12.82
12	18	ROOTS	0.23	0.01	2.32	0.04	281.08	12.82
12	18	STEM	0.26	0.01	2.10	0.04	281.08	12.82
12	20	LEAVES	0.73	0.02	.	.	304.61	7.97
12	20	NODULES	0.02	0.00	.	.	304.61	7.97
12	20	ROOTS	0.28	0.00	.	.	304.61	7.97
12	20	STEM	0.34	0.01	.	.	304.61	7.97
12	23	LEAVES	0.97	0.06	2.69	0.07	293.67	21.22
12	23	NODULES	0.02	0.01	.	.	293.67	21.22
12	23	ROOTS	0.32	0.03	2.35	0.10	293.67	21.22
12	23	STEM	0.41	0.03	2.06	0.12	293.67	21.22
12	25	LEAVES	0.98	0.04	.	.	314.89	12.78
12	25	NODULES	0.02	0.01	.	.	314.89	12.78
12	25	ROOTS	0.36	0.01	.	.	314.89	12.78
12	25	STEM	0.46	0.02	.	.	314.89	12.78
12	27	LEAVES	1.12	0.09	3.28	0.04	357.80	21.29
12	27	NODULES	0.05	0.01	.	.	357.80	21.29
12	27	ROOTS	0.48	0.03	2.16	0.05	357.80	21.29
12	27	STEM	0.56	0.04	1.64	0.04	357.80	21.29
12	30	LEAVES	1.36	0.08	3.58	0.06	427.88	34.30
12	30	NODULES	0.06	0.02	.	.	427.88	34.30
12	30	ROOTS	0.66	0.08	2.20	0.14	427.88	34.30
12	30	STEM	0.67	0.05	2.05	0.24	427.88	34.30

Appendix Table 12 contains data for the growth analysis study conducted outdoors during the Spring and Summer. The experiment began May 9, 1984, when seeds were imbibed, and continued through June 24, 1983, which was the final harvest date. Harvesting began May 23, 1983. Data are coded in the following manner:

Column

A. Concentration of nitrate (mMoles) in nutrient solution administered on alternate days. The abbreviation NN denotes the non-nodulating treatment.

B. Days from first harvest.

C. Plant Part.

D. Mean of four replicate plant part dry weights (grams).

E. Standard error of the mean plant part dry weight.

F. Mean percent nitrogen content of individual plant parts. Values listed for roots are actually a combination of roots and nodules.

G. Standard error of mean percent nitrogen content.

H. Mean total leaf area (cm^2).

I. Standard error of mean total leaf area.

Appendix Table 12 (continued).

A	B	C	D	E	F	G	H	I
0	0	LEAVES	0.14	0.02	5.71	0.19	18.80	4.96
0	0	ROOTS	0.08	0.01	3.51	0.39	18.80	4.96
0	0	STEMS	0.03	0.01	6.56	0.89	18.80	4.96
0	2	LEAVES	0.14	0.01	.	.	19.45	5.87
0	2	NODULES	0.00	0.00	.	.	19.45	5.87
0	2	ROOTS	0.09	0.00	.	.	19.45	5.87
0	2	STEMS	0.04	0.00	.	.	19.45	5.87
0	4	LEAVES	0.21	0.01	4.22	0.04	50.25	2.70
0	4	NODULES	0.00	0.00	.	.	50.25	2.70
0	4	ROOTS	0.14	0.01	2.11	0.05	50.25	2.70
0	4	STEMS	0.06	0.00	2.71	0.04	50.25	2.70
0	7	LEAVES	0.27	0.02	.	.	79.73	3.83
0	7	NODULES	0.02	0.00	.	.	79.73	3.83
0	7	ROOTS	0.20	0.01	.	.	79.73	3.83
0	7	STEMS	0.11	0.01	.	.	79.73	3.83
0	9	LEAVES	0.32	0.02	2.24	0.18	87.84	8.31
0	9	NODULES	0.04	0.00	.	.	87.84	8.31
0	9	ROOTS	0.26	0.01	1.21	0.06	87.84	8.31
0	9	STEMS	0.14	0.01	1.00	0.07	87.84	8.31
0	11	LEAVES	0.39	0.02	.	.	111.21	6.15
0	11	NODULES	0.05	0.01	.	.	111.21	6.15
0	11	ROOTS	0.32	0.01	.	.	111.21	6.15
0	11	STEMS	0.17	0.01	.	.	111.21	6.15
0	14	LEAVES	0.37	0.00	2.01	0.61	115.63	4.79
0	14	NODULES	0.07	0.00	.	.	115.63	4.79
0	14	ROOTS	0.35	0.02	1.15	0.09	115.63	4.79
0	14	STEMS	0.20	0.01	1.94	0.47	115.63	4.79
0	16	LEAVES	0.58	0.03	.	.	176.02	11.23
0	16	NODULES	0.10	0.01	.	.	176.02	11.23
0	16	ROOTS	0.40	0.03	.	.	176.02	11.23
0	16	STEMS	0.27	0.01	.	.	176.02	11.23
0	18	LEAVES	0.53	0.08	2.91	0.13	165.23	21.87
0	18	NODULES	0.09	0.01	.	.	165.23	21.87
0	18	ROOTS	0.36	0.05	1.12	0.09	165.23	21.87
0	18	STEMS	0.27	0.04	1.26	0.08	165.23	21.87
0	21	LEAVES	0.81	0.06	.	.	201.14	14.41
0	21	NODULES	0.15	0.02	.	.	201.14	14.41
0	21	ROOTS	0.56	0.04	.	.	201.14	14.41
0	21	STEMS	0.41	0.03	.	.	201.14	14.41
0	23	LEAVES	1.04	0.03	3.21	0.28	255.50	11.35
0	23	NODULES	0.20	0.01	.	.	255.50	11.35
0	23	ROOTS	0.63	0.07	1.27	0.04	255.50	11.35
0	23	STEMS	0.61	0.02	1.29	0.08	255.50	11.35
0	25	LEAVES	1.23	0.08	.	.	404.51	35.12
0	25	NODULES	0.20	0.02	.	.	404.51	35.12
0	25	ROOTS	0.64	0.04	.	.	404.51	35.12
0	25	STEMS	0.71	0.10	.	.	404.51	35.12

Appendix Table 12 (continued).

A	B	C	D	E	F	G	H	I
0	28	LEAVES	1.80	0.21	3.53	0.10	525.12	49.28
0	28	NODULES	0.31	0.03	.	.	525.12	49.28
0	28	ROOTS	0.99	0.13	1.54	0.04	525.12	49.28
0	28	STEMS	1.13	0.16	1.70	0.11	525.12	49.28
0	30	LEAVES	1.94	0.28	.	.	577.01	65.01
0	30	NODULES	0.32	0.05	.	.	577.01	65.01
0	30	ROOTS	0.99	0.19	.	.	577.01	65.01
0	30	STEMS	1.22	0.21	.	.	577.01	65.01
2	0	LEAVES	0.14	0.01	5.14	0.18	24.90	0.79
2	0	ROOTS	0.10	0.01	3.14	0.07	24.90	0.79
2	0	STEMS	0.04	0.00	6.76	1.12	24.90	0.79
2	2	LEAVES	0.15	0.01	.	.	32.02	1.12
2	2	NODULES	0.00	0.00	.	.	32.02	1.12
2	2	ROOTS	0.11	0.01	.	.	32.02	1.12
2	2	STEMS	0.05	0.00	.	.	32.02	1.12
2	4	LEAVES	0.19	0.02	4.27	0.26	46.25	3.65
2	4	NODULES	0.00	0.00	.	.	46.25	3.65
2	4	ROOTS	0.12	0.01	2.43	0.13	46.25	3.65
2	4	STEMS	0.06	0.01	2.89	0.22	46.25	3.65
2	7	LEAVES	0.28	0.02	.	.	76.31	2.92
2	7	ROOTS	0.20	0.01	.	.	76.31	2.92
2	7	STEMS	0.11	0.01	.	.	76.31	2.92
2	9	LEAVES	0.33	0.00	2.62	0.07	94.41	3.45
2	9	NODULES	0.03	0.00	.	.	94.41	3.45
2	9	ROOTS	0.25	0.02	1.42	0.01	94.41	3.45
2	9	STEMS	0.14	0.00	1.17	0.04	94.41	3.45
2	11	LEAVES	0.38	0.04	.	.	114.95	11.42
2	11	NODULES	0.05	0.00	.	.	114.95	11.42
2	11	ROOTS	0.29	0.04	.	.	114.95	11.42
2	11	STEMS	0.18	0.03	.	.	114.95	11.42
2	14	LEAVES	0.41	0.04	3.03	0.28	134.09	8.38
2	14	NODULES	0.07	0.01	.	.	134.09	8.38
2	14	ROOTS	0.40	0.02	1.13	0.07	134.09	8.38
2	14	STEMS	0.23	0.02	0.97	0.14	134.09	8.38
2	16	LEAVES	0.55	0.06	.	.	175.29	15.97
2	16	NODULES	0.07	0.01	.	.	175.29	15.97
2	16	ROOTS	0.44	0.04	.	.	175.29	15.97
2	16	STEMS	0.28	0.03	.	.	175.29	15.97
2	18	LEAVES	0.55	0.10	2.83	0.29	180.54	24.06
2	18	NODULES	0.07	0.01	.	.	180.54	24.06
2	18	ROOTS	0.41	0.06	1.16	0.04	180.54	24.06
2	18	STEMS	0.29	0.04	1.06	0.14	180.54	24.06
2	21	LEAVES	0.94	0.03	.	.	304.06	17.66
2	21	NODULES	0.14	0.01	.	.	304.06	17.66
2	21	ROOTS	0.75	0.03	.	.	304.06	17.66
2	21	STEMS	0.57	0.01	.	.	304.06	17.66

Appendix Table 12 (continued).

A	B	C	D	E	F	G	H	I
2	23	LEAVES	1.01	0.02	3.57	0.14	349.01	3.34
2	23	NODULES	0.14	0.02	.	.	349.01	3.34
2	23	ROOTS	0.64	0.02	1.30	0.04	349.01	3.34
2	23	STEMS	0.58	0.01	1.22	0.11	349.01	3.34
2	25	LEAVES	1.26	0.04	.	.	386.63	9.75
2	25	NODULES	0.19	0.01	.	.	386.63	9.75
2	25	ROOTS	0.84	0.00	.	.	386.63	9.75
2	25	STEMS	0.79	0.01	.	.	386.63	9.75
2	28	LEAVES	1.57	0.20	3.85	0.29	545.06	72.36
2	28	NODULES	0.24	0.02	.	.	545.06	72.36
2	28	ROOTS	0.99	0.08	1.48	0.04	545.06	72.36
2	28	STEMS	1.06	0.13	1.44	0.15	545.06	72.36
2	30	LEAVES	2.42	0.22	.	.	635.75	25.24
2	30	NODULES	0.35	0.05	.	.	635.75	25.24
2	30	ROOTS	1.41	0.10	.	.	635.75	25.24
2	30	STEMS	1.54	0.11	.	.	635.75	25.24
2NN	0	LEAVES	0.10	0.01	4.22	0.38	13.56	1.20
2NN	0	ROOTS	0.07	0.00	2.56	0.17	13.56	1.20
2NN	0	STEMS	0.02	0.00	5.22	0.29	13.56	1.20
2NN	2	LEAVES	0.10	0.01	.	.	22.21	1.91
2NN	2	ROOTS	0.09	0.01	.	.	22.21	1.91
2NN	2	STEMS	0.02	0.00	.	.	22.21	1.91
2NN	4	LEAVES	0.12	0.01	3.05	0.18	30.60	2.52
2NN	4	ROOTS	0.11	0.01	1.63	0.10	30.60	2.52
2NN	4	STEMS	0.03	0.00	3.36	0.22	30.60	2.52
2NN	7	LEAVES	0.16	0.01	.	.	52.76	3.06
2NN	7	ROOTS	0.15	0.02	.	.	52.76	3.06
2NN	7	STEMS	0.05	0.00	.	.	52.76	3.06
2NN	9	LEAVES	0.18	0.01	2.53	0.11	55.19	2.76
2NN	9	ROOTS	0.19	0.00	1.33	0.05	55.19	2.76
2NN	9	STEMS	0.06	0.00	1.29	0.06	55.19	2.76
2NN	11	LEAVES	0.27	0.02	.	.	82.59	7.10
2NN	11	ROOTS	0.22	0.02	.	.	82.59	7.10
2NN	11	STEMS	0.11	0.01	.	.	82.59	7.10
2NN	14	LEAVES	0.23	0.01	1.93	0.08	81.53	0.47
2NN	14	ROOTS	0.30	0.01	7.78	6.74	81.53	0.47
2NN	14	STEMS	0.11	0.01	0.79	0.02	81.53	0.47
2NN	16	LEAVES	0.30	0.01	.	.	94.82	3.00
2NN	16	ROOTS	0.29	0.03	.	.	94.82	3.00
2NN	16	STEMS	0.12	0.00	.	.	94.82	3.00
2NN	18	LEAVES	0.38	0.03	2.36	0.07	121.92	7.37
2NN	18	ROOTS	0.36	0.04	1.46	0.19	121.92	7.37
2NN	18	STEMS	0.17	0.01	1.03	0.04	121.92	7.37
2NN	21	LEAVES	0.54	0.02	.	.	175.35	12.97
2NN	21	ROOTS	0.50	0.04	.	.	175.35	12.97
2NN	21	STEMS	0.29	0.03	.	.	175.35	12.97

Appendix Table 12 (continued).

A	B	C	D	E	F	G	H	I
2NN	23	LEAVES	0.59	0.01	1.82	0.08	191.04	14.95
2NN	23	ROOTS	0.55	0.03	1.01	0.03	191.04	14.95
2NN	23	STEMS	0.29	0.01	0.70	0.03	191.04	14.95
2NN	25	LEAVES	0.61	0.03	.	.	202.12	13.59
2NN	25	ROOTS	0.55	0.02	.	.	202.12	13.59
2NN	25	STEMS	0.31	0.04	.	.	202.12	13.59
2NN	28	LEAVES	0.71	0.05	1.84	0.04	222.14	10.97
2NN	28	ROOTS	0.59	0.05	1.16	0.05	222.14	10.97
2NN	28	STEMS	0.36	0.05	0.88	0.05	222.14	10.97
2NN	30	LEAVES	0.74	0.02	.	.	226.47	12.31
2NN	30	ROOTS	0.59	0.03	.	.	226.47	12.31
2NN	30	STEMS	0.38	0.02	.	.	226.47	12.31
6	0	LEAVES	0.11	0.01	4.72	0.37	19.59	2.24
6	0	ROOTS	0.07	0.01	2.81	0.20	19.59	2.24
6	0	STEMS	0.02	0.00	4.92	0.10	19.59	2.24
6	2	LEAVES	0.13	0.02	.	.	32.16	4.18
6	2	NODULES	0.00	0.00	.	.	32.16	4.18
6	2	ROOTS	0.09	0.01	.	.	32.16	4.18
6	2	STEMS	0.04	0.01	.	.	32.16	4.18
6	4	LEAVES	0.16	0.01	3.95	0.46	39.16	2.40
6	4	NODULES	0.00	0.00	.	.	39.16	2.40
6	4	ROOTS	0.11	0.01	2.39	0.12	39.16	2.40
6	4	STEMS	0.05	0.00	3.36	0.25	39.16	2.40
6	7	LEAVES	0.27	0.01	.	.	98.48	2.13
6	7	NODULES	0.01	0.00	.	.	98.48	2.13
6	7	ROOTS	0.20	0.01	.	.	98.48	2.13
6	7	STEMS	0.13	0.00	.	.	98.48	2.13
6	9	LEAVES	0.43	0.02	3.35	0.06	124.53	6.57
6	9	NODULES	0.02	0.00	.	.	124.53	6.57
6	9	ROOTS	0.26	0.01	1.65	0.12	124.53	6.57
6	9	STEMS	0.18	0.01	1.44	0.10	124.53	6.57
6	11	LEAVES	0.54	0.03	.	.	152.16	8.00
6	11	NODULES	0.03	0.00	.	.	152.16	8.00
6	11	ROOTS	0.35	0.01	.	.	152.16	8.00
6	11	STEMS	0.26	0.02	.	.	152.16	8.00
6	14	LEAVES	0.62	0.06	3.42	0.21	194.01	17.70
6	14	NODULES	0.04	0.01	.	.	194.01	17.70
6	14	ROOTS	0.45	0.06	1.31	0.10	194.01	17.70
6	14	STEMS	0.37	0.04	1.49	0.42	194.01	17.70
6	16	LEAVES	0.83	0.04	.	.	239.52	11.72
6	16	NODULES	0.06	0.01	.	.	239.52	11.72
6	16	ROOTS	0.53	0.03	.	.	239.52	11.72
6	16	STEMS	0.49	0.03	.	.	239.52	11.72
6	18	LEAVES	0.89	0.08	3.57	0.12	291.55	5.61
6	18	NODULES	0.08	0.02	.	.	291.55	5.61
6	18	ROOTS	0.49	0.06	1.57	0.06	291.55	5.61
6	18	STEMS	0.54	0.03	1.45	0.09	291.55	5.61

Appendix Table 12 (continued).

A	B	C	D	E	F	G	H	I
6	21	LEAVES	1.27	0.11	.	.	365.99	26.91
6	21	NODULES	0.08	0.01	.	.	365.99	26.91
6	21	ROOTS	0.86	0.05	.	.	365.99	26.91
6	21	STEMS	0.79	0.05	.	.	365.99	26.91
6	23	LEAVES	1.36	0.07	3.10	0.09	406.51	12.90
6	23	NODULES	0.09	0.02	.	.	406.51	12.90
6	23	ROOTS	1.01	0.11	1.41	0.08	406.51	12.90
6	23	STEMS	1.01	0.05	1.15	0.11	406.51	12.90
6	25	LEAVES	1.58	0.15	.	.	420.17	34.82
6	25	NODULES	0.10	0.02	.	.	420.17	34.82
6	25	ROOTS	1.14	0.13	.	.	420.17	34.82
6	25	STEMS	1.08	0.08	.	.	420.17	34.82
6	28	LEAVES	2.34	0.14	2.95	0.19	585.67	26.14
6	28	NODULES	0.15	0.03	.	.	585.67	26.14
6	28	ROOTS	1.47	0.17	1.33	0.07	585.67	26.14
6	28	STEMS	1.73	0.14	1.12	0.08	585.67	26.14
6	30	LEAVES	2.43	0.04	.	.	590.42	19.22
6	30	NODULES	0.17	0.03	.	.	590.42	19.22
6	30	ROOTS	1.40	0.10	.	.	590.42	19.22
6	30	STEMS	1.81	0.06	.	.	590.42	19.22
6NN	0	LEAVES	0.09	0.01	3.85	0.33	14.29	1.68
6NN	0	ROOTS	0.05	0.01	2.72	0.14	14.29	1.68
6NN	0	STEMS	0.02	0.00	8.27	2.39	14.29	1.68
6NN	2	LEAVES	0.09	0.01	.	.	21.80	2.67
6NN	2	ROOTS	0.08	0.01	.	.	21.80	2.67
6NN	2	STEMS	0.02	0.00	.	.	21.80	2.67
6NN	4	LEAVES	0.13	0.00	3.22	0.15	32.33	2.74
6NN	4	ROOTS	0.13	0.01	1.86	0.10	32.33	2.74
6NN	4	STEMS	0.03	0.00	3.69	0.38	32.33	2.74
6NN	7	LEAVES	0.18	0.01	.	.	60.85	5.22
6NN	7	ROOTS	0.17	0.01	.	.	60.85	5.22
6NN	7	STEMS	0.06	0.01	.	.	60.85	5.22
6NN	9	LEAVES	0.22	0.01	3.35	0.23	72.53	4.00
6NN	9	ROOTS	0.20	0.01	1.77	0.07	72.53	4.00
6NN	9	STEMS	0.08	0.01	2.17	0.18	72.53	4.00
6NN	11	LEAVES	0.27	0.00	.	.	92.24	1.79
6NN	11	ROOTS	0.19	0.02	.	.	92.24	1.79
6NN	11	STEMS	0.10	0.00	.	.	92.24	1.79
6NN	14	LEAVES	0.42	0.02	.	.	148.44	2.65
6NN	14	ROOTS	0.39	0.02	1.31	0.01	148.44	2.65
6NN	14	STEMS	0.19	0.01	1.26	0.04	148.44	2.65
6NN	16	LEAVES	0.48	0.02	.	.	155.67	7.53
6NN	16	ROOTS	0.34	0.04	.	.	155.67	7.53
6NN	16	STEMS	0.22	0.02	.	.	155.67	7.53
6NN	18	LEAVES	0.57	0.04	3.34	0.07	197.36	13.18
6NN	18	ROOTS	0.37	0.04	1.34	0.07	197.36	13.18
6NN	18	STEMS	0.27	0.03	1.34	0.15	197.36	13.18

Appendix Table 12. (continued).

A	B	C	D	E	F	G	H	I
6NN	21	LEAVES	0.87	0.05	.	.	283.16	19.64
6NN	21	ROOTS	0.69	0.04	.	.	283.16	19.64
6NN	21	STEMS	0.50	0.05	.	.	283.16	19.64
6NN	23	LEAVES	0.93	0.02	2.49	0.12	307.51	10.45
6NN	23	ROOTS	0.81	0.04	1.17	0.05	307.51	10.45
6NN	23	STEMS	0.59	0.02	0.86	0.06	307.51	10.45
6NN	25	LEAVES	1.21	0.13	.	.	403.80	36.49
6NN	25	ROOTS	1.09	0.12	.	.	403.80	36.49
6NN	25	STEMS	0.72	0.14	.	.	403.80	36.49
6NN	28	LEAVES	1.54	0.22	2.47	0.14	453.07	51.92
6NN	28	ROOTS	1.17	0.17	1.28	0.06	453.07	51.92
6NN	28	STEMS	1.02	0.18	1.02	0.04	453.07	51.92
6NN	30	LEAVES	1.74	0.08	.	.	463.75	21.54
6NN	30	ROOTS	1.39	0.06	.	.	463.75	21.54
6NN	30	STEMS	1.15	0.04	.	.	463.75	21.54
12	0	LEAVES	0.13	0.01	5.38	0.60	15.10	2.12
12	0	ROOTS	0.09	0.00	3.34	0.12	15.10	2.12
12	0	STEMS	0.03	0.00	6.50	0.85	15.10	2.12
12	2	LEAVES	0.14	0.00	.	.	30.17	1.72
12	2	NODULES	0.00	0.00	.	.	30.17	1.72
12	2	ROOTS	0.09	0.01	.	.	30.17	1.72
12	2	STEMS	0.04	0.00	.	.	30.17	1.72
12	4	LEAVES	0.24	0.02	4.35	0.12	49.43	4.17
12	4	NODULES	0.00	0.00	.	.	49.43	4.17
12	4	ROOTS	0.12	0.01	2.37	0.10	49.43	4.17
12	4	STEMS	0.07	0.00	4.54	1.00	49.43	4.17
12	7	LEAVES	0.36	0.02	.	.	107.44	6.92
12	7	NODULES	0.01	0.00	.	.	107.44	6.92
12	7	ROOTS	0.20	0.02	.	.	107.44	6.92
12	7	STEMS	0.13	0.01	.	.	107.44	6.92
12	9	LEAVES	0.49	0.02	3.99	0.13	138.86	4.47
12	9	NODULES	0.02	0.00	.	.	138.86	4.47
12	9	ROOTS	0.26	0.01	2.22	0.06	138.86	4.47
12	9	STEMS	0.20	0.01	2.27	0.08	138.86	4.47
12	11	LEAVES	0.62	0.01	.	.	179.39	7.15
12	11	NODULES	0.02	0.00	.	.	179.39	7.15
12	11	ROOTS	0.33	0.03	.	.	179.39	7.15
12	11	STEMS	0.28	0.02	.	.	179.39	7.15
12	14	LEAVES	0.71	0.03	4.04	0.14	203.28	11.78
12	14	NODULES	0.02	0.00	.	.	203.28	11.78
12	14	ROOTS	0.46	0.04	1.65	0.09	203.28	11.78
12	14	STEMS	0.42	0.03	2.16	0.14	203.28	11.78
12	16	LEAVES	0.94	0.08	.	.	250.34	22.18
12	16	NODULES	0.02	0.01	.	.	250.34	22.18
12	16	ROOTS	0.53	0.04	.	.	250.34	22.18
12	16	STEMS	0.56	0.05	.	.	250.34	22.18

Appendix Table 12 (continued).

A	B	C	D	E	F	G	H	I
12	18	LEAVES	1.26	0.11	3.54	0.36	279.04	36.53
12	18	NODULES	0.02	0.01	.	.	279.04	36.53
12	18	ROOTS	0.68	0.05	2.07	0.14	279.04	36.53
12	18	STEMS	0.77	0.04	2.34	0.26	279.04	36.53
12	21	LEAVES	1.43	0.07	.	.	355.12	23.76
12	21	NODULES	0.02	0.01	.	.	355.12	23.76
12	21	ROOTS	0.80	0.12	.	.	355.12	23.76
12	21	STEMS	0.91	0.05	.	.	355.12	23.76
12	23	LEAVES	1.59	0.06	3.45	0.08	440.26	18.58
12	23	NODULES	0.02	0.00	.	.	440.26	18.58
12	23	ROOTS	1.02	0.07	1.62	0.02	440.26	18.58
12	23	STEMS	1.23	0.04	1.81	0.07	440.26	18.58
12	25	LEAVES	1.99	0.02	.	.	458.49	33.52
12	25	NODULES	0.04	0.00	.	.	458.49	33.52
12	25	ROOTS	1.16	0.04	.	.	458.49	33.52
12	25	STEMS	1.38	0.12	.	.	458.49	33.52
12	28	LEAVES	2.15	0.20	3.10	0.20	536.73	35.96
12	28	NODULES	0.03	0.01	.	.	536.73	35.96
12	28	ROOTS	1.19	0.18	2.04	0.08	536.73	35.96
12	28	STEMS	1.61	0.15	2.20	0.28	536.73	35.96
12	30	LEAVES	2.73	0.29	.	.	597.56	48.94
12	30	NODULES	0.03	0.01	.	.	597.56	48.94
12	30	ROOTS	1.58	0.23	.	.	597.56	48.94
12	30	STEMS	1.93	0.16	.	.	597.56	48.94
12NN	0	LEAVES	0.10	0.01	3.81	0.39	12.04	2.67
12NN	0	ROOTS	0.06	0.01	2.67	0.25	12.04	2.67
12NN	0	STEMS	0.02	0.00	6.71	1.34	12.04	2.67
12NN	2	LEAVES	0.10	0.00	.	.	23.51	1.49
12NN	2	ROOTS	0.08	0.01	.	.	23.51	1.49
12NN	2	STEMS	0.02	0.00	.	.	23.51	1.49
12NN	4	LEAVES	0.14	0.00	3.66	0.15	37.13	0.93
12NN	4	ROOTS	0.11	0.01	2.13	0.16	37.13	0.93
12NN	4	STEMS	0.03	0.00	3.95	0.45	37.13	0.93
12NN	7	LEAVES	0.22	0.01	.	.	70.42	3.84
12NN	7	ROOTS	0.16	0.01	.	.	70.42	3.84
12NN	7	STEMS	0.07	0.00	.	.	70.42	3.84
12NN	9	LEAVES	0.29	0.02	3.97	0.07	96.06	7.72
12NN	9	ROOTS	0.17	0.02	2.08	0.09	96.06	7.72
12NN	9	STEMS	0.10	0.01	2.55	0.15	96.06	7.72
12NN	11	LEAVES	0.40	0.03	.	.	124.54	2.47
12NN	11	ROOTS	0.22	0.01	.	.	124.54	2.47
12NN	11	STEMS	0.16	0.01	.	.	124.54	2.47
12NN	14	LEAVES	0.46	0.04	4.27	0.04	150.87	10.94
12NN	14	ROOTS	0.30	0.02	2.04	0.09	150.87	10.94
12NN	14	STEMS	0.23	0.02	2.43	0.09	150.87	10.94
12NN	16	LEAVES	0.65	0.03	.	.	182.43	8.64
12NN	16	ROOTS	0.41	0.04	.	.	182.43	8.64
12NN	16	STEMS	0.37	0.03	.	.	182.43	8.64

Appendix Table 12 (continued).

A	B	C	D	E	F	G	H	I
12NN	18	LEAVES	0.75	0.09	3.96	0.08	238.86	31.04
12NN	18	ROOTS	0.38	0.06	2.22	0.15	238.86	31.04
12NN	18	STEMS	0.40	0.05	2.38	0.15	238.86	31.04
12NN	21	LEAVES	1.14	0.10	.	.	331.24	38.82
12NN	21	ROOTS	0.74	0.12	.	.	331.24	38.82
12NN	21	STEMS	0.70	0.05	.	.	331.24	38.82
12NN	23	LEAVES	1.46	0.14	3.12	0.14	430.15	38.37
12NN	23	ROOTS	1.07	0.18	1.53	0.12	430.15	38.37
12NN	23	STEMS	0.93	0.07	1.58	0.29	430.15	38.37
12NN	25	LEAVES	1.50	0.20	.	.	423.04	49.59
12NN	25	ROOTS	0.81	0.17	.	.	423.04	49.59
12NN	25	STEMS	0.99	0.11	.	.	423.04	49.59
12NN	28	LEAVES	1.60	0.22	3.40	0.23	414.84	47.08
12NN	28	ROOTS	0.93	0.18	2.26	0.22	414.84	47.08
12NN	28	STEMS	1.04	0.13	2.75	0.12	414.84	47.08
12NN	30	LEAVES	2.33	0.21	.	.	593.43	44.84
12NN	30	ROOTS	1.86	0.68	.	.	593.43	44.84
12NN	30	STEMS	1.60	0.13	.	.	593.43	44.84

Appendix Tables 13 and 14 contain acetylene reduction values for plants harvested from the greenhouse during the Winter (Table 13) or from outside during the Spring and Summer (Table 14). Data are coded in the following manner:

Column

A. Concentration of nitrate (mMoles) in nutrient solution administered on alternate days.

B. Days from first harvest.

C. Mean acetylene reduction activity (uMoles C_2H_4/plant/hour).

D. Standard error of mean acetylene reduction activity.

Appendix Table 13.

A	B	C	D	A	B	C	D
0	6	0.85	0.46	6	6	0.00	0.00
0	9	1.28	0.18	6	9	0.27	0.10
0	11	1.60	0.51	6	11	0.32	0.14
0	13	4.00	0.40	6	13	1.02	0.29
0	16	4.85	0.29	6	16	1.44	0.24
0	18	7.09	1.46	6	18	3.31	0.56
0	20	7.62	0.51	6	20	3.15	0.98
0	23	12.21	0.56	6	23	5.37	0.46
0	25	14.18	2.68	6	25	7.68	0.97
0	27	18.66	2.09	6	27	6.51	1.05
0	30	6.83	1.77	6	30	3.42	1.71
2	6	0.00	0.00	12	6	0.00	0.00
2	9	0.69	0.24	12	9	0.00	0.00
2	11	1.30	0.40	12	11	0.11	0.06
2	13	2.93	0.24	12	13	0.11	0.06
2	16	2.51	0.57	12	16	0.48	0.18
2	18	7.52	0.59	12	18	1.44	0.20
2	23	10.56	1.95	12	20	1.55	0.18
2	25	13.95	1.29	12	23	0.96	0.54
2	27	13.97	1.46	12	25	1.39	0.48
2	30	5.02	1.08	12	27	3.95	0.86

Appendix Table 14 (continued).

A	B	C	D
0	4	0.10	0.03
0	7	1.57	0.30
0	9	3.87	0.84
0	11	5.72	1.55
0	14	6.21	0.88
0	16	7.11	1.47
0	18	9.29	0.70
0	21	20.30	2.24
0	23	24.52	1.49
0	25	27.78	2.03
0	28	41.16	6.27
0	30	33.78	4.52
2	4	0.09	0.06
2	7	1.55	0.57
2	9	1.97	0.21
2	11	3.87	0.87
2	14	4.75	1.78
2	16	8.11	0.92
2	18	5.57	0.69
2	21	19.27	2.78
2	23	21.27	1.44
2	25	28.61	0.91
2	28	41.64	6.04
2	30	39.71	4.67
6	4	0.10	0.04
6	7	0.31	0.06
6	9	1.40	0.45
6	11	2.09	0.64
6	14	3.70	0.57
6	16	3.72	0.78
6	18	4.71	1.85
6	21	7.07	2.70
6	23	6.33	1.52
6	25	8.43	3.21
6	28	21.66	4.12
6	30	17.05	4.71
12	4	0.14	0.05
12	7	0.42	0.12
12	9	0.62	0.13
12	11	0.47	0.12
12	14	0.74	0.24
12	16	0.62	0.17
12	18	0.65	0.25
12	21	0.62	0.24
12	23	1.04	0.24
12	25	1.94	0.28
12	28	1.85	0.47
12	30	1.67	0.48

Appendix Tables 15 and 16 contain total daily integrated photosynthetic photon flux densities (PPFD) received during the harvesting period for studies conducted in the greenhouse (Table 15) or outdoors (Table 16). Data are coded in the following manner:

Column

A. Days from first harvest. Harvesting began two weeks after seeds were imbibed.

B. Total daily integrated PPFD (µE/m^2/day)

Appendix Table 15.

A	B	A	B
0	5.85	16	12.12
1	9.98	17	11.05
2	9.45	18	7.88
3	5.05	19	5.69
4	3.91	20	9.66
5	10.21	21	7.71
6	7.88	22	13.60
7	12.07	23	14.02
8	5.90	24	12.59
9	2.51	25	3.46
10	12.73	26	13.34
11	13.14	27	12.35
12	7.66	28	14.73
13	2.00	29	16.94
14	3.90	30	5.57
15	12.12		

Appendix Table 16.

A	B	A	B
0	40.10	16	41.65
1	41.39	17	42.30
2	42.24	18	40.88
3	38.74	19	35.13
4	36.20	20	31.00
5	37.80	21	33.54
6	37.44	22	17.16
7	28.84	23	25.48
8	39.01	24	11.86
9	38.28	25	12.36
10	38.07	26	33.87
11	35.25	27	32.98
12	21.41	28	16.63
13	11.94	29	18.02
14	18.52	30	21.81
15	39.55		

Appendix Table 17. Analysis of variance for the regression of the natural log of total leaf area on time (days) for nodulated soybeans grown in the greenhouse and entirely dependent on N_2 fixation.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	3	61.04489299	20.34829766	352.80
ERROR	52	2.99920142	0.05767695	PR > F
CORRECTED TOTAL	55	64.04409440		0.0001

R-SQUARE	C.V.	ROOT MSE	DEP. VAR. MEAN
0.953170	5.2626	0.24016026	4.563535691

SOURCE	DF	TYPE I SS	F VALUE	PR > F
LINEAR	1	55.69040898	965.62	0.0001
QUADRATIC	1	5.12896128	88.93	0.0001
CUBIC	1	0.22184203	3.85	0.0552

PARAMETER	ESTIMATE	T FOR HO: PARAMETER=0	PR > |T|	STD ERROR OF ESTIMATE
INTERCEPT	2.38316311	24.55	0.0001	0.09705697
LINEAR	0.27696888	9.24	0.0001	0.02996393
QUADRATIC	-0.00858659	-3.59	0.0007	0.00239279
CUBIC	-0.00010316	1.96	0.0552	0.00005260

Appendix Table 18. Analysis of variance for the regression of the natural log of total leaf area on time (days) for nodulated soybeans grown in the greenhouse and supplied 2mM NO_3^-.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	2	69.62529624	34.81264812	1149.96
ERROR	53	1.60445794	0.03027279	PR > F
CORRECTED TOTAL	55	71.22975419		0.0001

R-SQUARE	C.V.	ROOT MSE	DEP. VAR.MEAN
0.977475	3.6993	0.17399078	4.70338152

SOURCE	DF	TYPE I SS	F VALUE	PR > F
LINEAR	1	62.68761840	2070.76	0.0001
QUADRATIC	1	6.93767785	229.17	0.0001

PARAMETER	ESTIMATE	T FOR HO: PARAMETER=0	PR > |T|	STD ERROR OF ESTIMATE
INTERCEPT	2.44883087	41.76	0.0001	0.05863581
LINEAR	0.24948598	26.75	0.0001	0.00932504
QUADRATIC	-0.00461371	-15.14	0.0001	0.00030477

Appendix Table 19. Analysis of variance for the regression of the natural log of total leaf area on time (days) for nodulated soybeans grown in the greenhouse and supplied 6mM NO$_3^-$.

```
SOURCE            DF     SUM OF SQUARES     MEAN SQUARE   F VALUE
MODEL              3      65.32350982       21.77450327    630.79
ERROR             52       1.79502381        0.03451969    PR > F
CORRECTED TOTAL   55      67.11853363                      0.0001

R-SQUARE       C.V.         ROOT MSE          DEP. VAR. MEAN
0.973256      3.9389        0.18579475        4.71695174

SOURCE            DF     TYPE I SS     F VALUE     PR > F

LINEAR            1      58.59743817  1697.51     0.0001
QUADRATIC         1       6.52540475   189.03     0.0001
CUBIC             1       0.20066690     5.81     0.0195

                                  T FOR HO:     PR > |T|    STD ERROR
OF
PARAMETER           ESTIMATE      PARAMETER=0               ESTIMATE

INTERCEPT           2.43546770    32.44         0.0001      0.07508601
LINEAR              0.29208941    12.60         0.0001      0.02318094
QUADRATIC          -0.00886815    -4.79         0.0001      0.00185113
CUBIC               9.8115293E-05  2.41         0.0195      0.00004069
```

Appendix Table 20. Analysis of variance for the regression of the natural log of total leaf area on time (days) for nodulated soybeans grown in the greenhouse and supplied 12mM NO$_3^-$.

```
SOURCE            DF      SUM OF SQUARES     MEAN SQUARE   F VALUE
MODEL              2       67.22323163       33.61161582   1652.54
ERROR             53        1.07798717        0.02033938   PR > F
CORRECTED TOTAL   55       68.30121881                     0.0001

R-SQUARE        C.V.         ROOT MSE          DEP. VAR.MEAN
0.984217        2.9631       0.14261620        4.81312806

SOURCE             DF     TYPE I SS     F VALUE     PR > F

LINEAR             1      60.87468857   2992.95     0.0001
QUADRATIC          1       6.34854306    312.13     0.0001

                                  T FOR HO:     PR > |T|    STD ERROR
OF
PARAMETER            ESTIMATE     PARAMETER=0               ESTIMATE

INTERCEPT            2.60875553   54.28         0.0001      0.04806241
LINEAR               0.24192964   31.65         0.0001      0.00764352
QUADRATIC           -0.00441347  -17.67         0.0001      0.00024981
```

Appendix Table 21. Analysis of variance for the linear regression of natural log of dry weight with time(days) of nodulated soybeans grown in the greenhouse and entirely dependent on nitrogen fixation.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	1	35.73435558	35.73435558	980.81
ERROR	54	1.96740831	0.03643349	PR > F
CORRECTED TOTAL	55	37.70176389		0.0001

R-SQUARE	C.V.	ROOT MSE	DEP. VAR. MEAN
0.947817	31.6565	0.19087558	-0.60295911

SOURCE	DF	TYPE I SS	F VALUE	PR > F
LINEAR	1	35.73435558	980.81	0.0001

PARAMETER	ESTIMATE	T FOR H0: PARAMETER=0	PR > |T|	STD ERROR OF ESTIMATE
INTERCEPT	-1.85118553	-39.12	0.0001	0.04731966
LINEAR	0.08566260	31.32	0.0001	0.00273526

Appendix Table 22. Analysis of variance for the regression of the natural log of total plant dry weight on time (days) for nodulated soybeans grown in the greenhouse and supplied 2mM NO_3^-.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	3	41.08739207	13.69579736	905.66
ERROR	52	0.78636650	0.01512243	PR > F
CORRECTED TOTAL	55	41.87375858		0.0001

R-SQUARE	C.V.	ROOT MSE	DEP. VAR. MEAN
0.981221	26.7624	0.12297330	-0.45949951

SOURCE	DF	TYPE I SS	F VALUE	PR > F
LINEAR	1	40.675435585	2689.74	0.0001
QUADRATIC	1	0.07252802	4.80	0.0330
CUBIC	1	0.33942847	22.45	0.0001

PARAMETER	ESTIMATE	T FOR H0: PARAMETER=0	PR > |T|	STD ERROR OF ESTIMATE
INTERCEPT	-1.72275911	-34.66	0.0001	0.04969772
LINEAR	0.03966018	2.58	0.0126	0.01534294
QUADRATIC	0.00524252	4.28	0.0001	0.00122522
CUBIC	-0.00012761	-4.74	0.0001	0.00002693

Appendix Table 23. Analysis of variance for the regression of the natural log of total plant dry weight on time (days) for nodulated soybeans grown in the greenhouse and supplied 6mM NO_3^-.

```
SOURCE           DF     SUM OF SQUARES     MEAN SQUARE    F VALUE
MODEL             3      41.51542555       13.83847518     444.02
ERROR            52       1.62065792        0.03116650     PR > F
CORRECTED TOTAL  55      43.13608347                       0.0001

R-SQUARE        C.V.        ROOT MSE         DEP. VAR. MEAN
0.962429        38.8832     0.17654036       -0.45402761

SOURCE           DF     TYPE I SS     F VALUE     PR > F

LINEAR            1     41.38318311  1327.81      0.0001
QUADRATIC         1      0.03443774     1.10      0.2980
CUBIC             1      0.09780471     3.14      0.0823

                              T FOR HO:        PR > |T|     STD ERROR OF
PARAMETER     ESTIMATE        PARAMETER=0                   ESTIMATE

INTERCEPT    -1.76990182    -24.81             0.0001       0.07134600
LINEAR        0.06653302      3.02             0.0039       0.02202631
QUADRATIC     0.00274231      1.56             0.1250       0.00175892
CUBIC        -6.8498129E-05 -1.77              0.0823       0.00003867
```

Appendix Table 24. Analysis of variance for the regression of the natural log of total plant dry weight on time (days) for nodulated soybeans grown in the greenhouse and supplied 12mM NO_3^-.

```
SOURCE           DF     SUM OF SQUARES     MEAN SQUARE    F VALUE
MODEL             3      39.19124811       13.06374937    1201.02
ERROR            48       0.52210739        0.01087724     PR > F
CORRECTED TOTAL  51      39.71335550                       0.0001

R-SQUARE        C.V.        ROOT MSE         DEP. VAR. MEAN
0.986853        38.0748     0.10429399       -0.27391856

SOURCE           DF     TYPE I SS     F VALUE     PR > F

LINEAR            1     38.94205438  3580.14      0.0001
QUADRATIC         1      0.05517083     5.07      0.0289
CUBIC             1      0.19402290    17.84      0.0001

                              T FOR HO:        PR > |T|     STD ERROR OF
PARAMETER     ESTIMATE        PARAMETER=0                   ESTIMATE

INTERCEPT    -1.73135587    -36.09             0.0001       0.04797237
LINEAR        0.05893457      4.39             0.0001       0.01341840
QUADRATIC     0.00392412      3.74             0.0005       0.00104867
CUBIC        -9.6806842E-05 -4.22              0.0001       0.00002292
```

Appendix Table 25. Analysis of variance for the regression of the natural log of total leaf area on time (days) for nodulated soybeans grown outdoors and entirely dependent on N_2 fixation.

```
SOURCE            DF     SUM OF SQUARES     MEAN SQUARE    F VALUE
MODEL              3      62.87030415      20.95676805     156.78
ERROR             52       6.95089015       0.13367096     PR > F
CORRECTED TOTAL   55      69.82119430                      0.0001

R-SQUARE       C.V.         ROOT MSE        DEP. VAR. MEAN
0.900447       7.5946       0.36561040      4.81407285

SOURCE            DF      TYPE I SS     F VALUE     PR > F

LINEAR             1      60.19676549   450.34      0.0001
QUADRATIC          1       1.25567752     9.39      0.0034
CUBIC              1       1.41786115    10.61      0.0020

                                T FOR HO:        PR > |T|   STD ERROR OF
PARAMETER     ESTIMATE          PARAMETER=0                 ESTIMATE

INTERCEPT     2.65420525         17.92           0.0001     0.14808729
LINEAR        0.30127333          6.64           0.0001     0.04537558
QUADRATIC    -0.01352224         -3.74           0.0005     0.00361659
CUBIC         0.00025774          3.26           0.0020     0.00007914
```

Appendix Table 26. Analysis of variance for the regression of the natural log of total leaf area on time (days) for nodulated soybeans grown outdoors and supplied 2mM NO_3^-.

```
SOURCE            DF     SUM OF SQUARES     MEAN SQUARE    F VALUE
MODEL              3      50.58724515      16.86241505    658.73
ERROR             51       1.30551968       0.02559843    PR > F
CORRECTED TOTAL   54      51.89276483                     0.0001

R-SQUARE       C.V.         ROOT MSE        DEP .VAR. MEAN
0.974842       3.2427       0.15999508      4.93402545

SOURCE            DF        TYPE I SS     F VALUE     PR > F

LINEAR             1        49.96458256  1951.86      0.0001
QUADRATIC          1         0.47874657    18.70      0.0001
CUBIC              1         0.14391602     5.62      0.0216

                                  T FOR HO:        PR > |T|   STD ERROR OF
PARAMETER      ESTIMATE           PARAMETER=0                 ESTIMATE

INTERCEPT      3.18930050         49.13            0.0001     0.06491276
LINEAR         0.18187944          9.07            0.0001     0.02004640
QUADRATIC     -0.00498748         -3.09            0.0032     0.00161207
CUBIC          8.4498010E-05       2.37            0.0216     0.00003564
```

Appendix Table 27. Analysis of variance for the regression of the natural log of total leaf area on time (days) for nodulated soybeans grown outdoors and supplied 6mM NO_3^-.

```
SOURCE             DF      SUM OF SQUARES     MEAN SQUARE    F VALUE
MODEL               3       60.49675076       20.16558359    789.75
ERROR              49        1.25117242        0.02553413    PR > F
CORRECTED TOTAL    52       61.74792318                      0.0001

R-SQUARE         C.V.          ROOT MSE         DEP. VAR.MEAN
0.979737         3.1548        0.15979403       5.06506404

SOURCE              DF      TYPE I SS     F VALUE     PR > F

LINEAR              1       56.52561939  2213.73     0.0001
QUADRATIC           1        3.63564252   142.38     0.0001
CUBIC               1        0.33548884    13.14     0.0007

                                T FOR HO:        PR > |T|     STD ERROR OF
PARAMETER        ESTIMATE       PARAMETER=0                   ESTIMATE

INTERCEPT        2.90904065     44.84            0.0001       0.06487199
LINEAR           0.27675186     13.75            0.0001       0.02013271
QUADRATIC       -0.00924272     -5.66            0.0001       0.00163232
CUBIC            0.00013146      3.62            0.0007       0.00003627
```

Appendix Table 28. Analysis of variance for the regression of the natural log of total leaf area on time (days) for nodulated soybeans grown outdoors and supplied 12mM NO_3^-.

```
SOURCE             DF     SUM OF SQUARES     MEAN SQUARE     F VALUE
MODEL               3      63.15394367       21.05131456      724.95
ERROR              50       1.45192097        0.02903842      PR > F
CORRECTED TOTAL    53      64.60586465                        0.0001

R-SQUARE         C.V.         ROOT MSE          DEP. VAR. MEAN
0.977526         3.3440       0.17040663        5.09595535

SOURCE             DF      TYPE I SS     F VALUE     PR > F

LINEAR             1      56.14480610  1933.47      0.0001
QUADRATIC          1       5.90219844   203.25      0.0001
CUBIC              1       1.10693914    38.12      0.0001

                                T FOR HO:        PR > |T|     STD ERROR OF
PARAMETER       ESTIMATE        PARAMETER=0                   ESTIMATE

INTERCEPT        2.72601629     39.39            0.0001       0.06920307
LINEAR           0.35287009     16.49            0.0001       0.02140287
QUADRATIC      -0.014601        -8.51            0.0001       0.00171484
CUBIC           0.00023154       6.17            0.0001       0.00003750
```

Appendix Table 29. Analysis of variance for the regression of the natural log of dry weight on time (days) of soybeans grown outdoors and entirely dependent on nitrogen fixation.

```
SOURCE           DF     SUM OF SQUARES    MEAN SQUARE    F VALUE
MODEL             3      44.54459803      14.84819934    404.05
ERROR            52       1.91090762       0.03674822    PR > F
CORRECTED TOTAL  55      46.45550565                     0.0001

R-SQUARE       C.V.          ROOT MSE          DEP. VAR MEAN
0.958866     196.4109        0.19169826        0.09760063

SOURCE           DF      TYPE I SS     F VALUE     PR > F

LINEAR           1      44.23186628    1203.65     0.0001
QUADRATIC        1       0.10502025       2.86     0.0969
CUBIC            1       0.20771150       5.65     0.0211

                                   T FOR HO:       PR > |T|     STD ERROR OF
PARAMETER     ESTIMATE             PARAMETER=0                  ESTIMATE

INTERCEPT    -1.48281677         -19.10           0.0001       0.07764570
LINEAR        0.16230444           6.82           0.0001       0.02379150
QUADRATIC    -0.00499582          -2.63           0.0111       0.00189627
CUBIC         9.8648031E-05        2.38           0.0211       0.00004149
```

Appendix Table 30. Analysis of variance for the regression of the natural log of dry weight on time (days) for soybeans grown outdoors and supplied 2mM NO_3^-.

```
SOURCE          DF     SUM OF SQUARES    MEAN SQUARE    F VALUE
MODEL            3      46.69002589      15.56334196    583.96
ERROR           51       1.35921305       0.02665124    PR > F
CORRECTED TOTAL 54      48.04923894                     0.0001

R-SQUARE       C.V.          ROOT MSE           DEP. VAR MEAN
0.971712     119.4620        0.16325206         0.13665608

SOURCE          DF       TYPE I SS     F VALUE     PR > F

LINEAR           1      46.56836915    1747.32     0.0001
QUADRATIC        1       0.00393831       0.15     0.7023
CUBIC            1       0.11771844       4.42     0.0405

                                    T FOR HO:      PR > |T|     STD ERROR OF
PARAMETER     ESTIMATE              PARAMETER=0                 ESTIMATE

INTERCEPT    -1.38999276         -21.01           0.0001       0.06616267
LINEAR        0.13933116           6.84           0.0001       0.02035770
QUADRATIC    -0.00348516          -2.14           0.0375       0.00163127
CUBIC         7.5220783E-05        2.10           0.0405       0.00003579
```

Appendix Table 31. Analysis of variance for the regression of the natural log of total plant dry weight on time (days) for nodulated soybeans grown outdoors and supplied 6mM NO_3^-.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	2	60.71850369	30.35925185	169.51
ERROR	52	9.31335781	0.17910303	PR > F
CORRECTED TOTAL	54	70.03186150		0.0001

R-SQUARE	C.V.	ROOT MSE	DEP. VAR. MEAN
0.867013	187.2329	0.42320566	0.22603172

SOURCE	DF	TYPE I SS	F VALUE	PR > F
LINEAR	1	60.02258855	335.13	0.0001
QUADRATIC	1	0.69591514	3.89	0.0001

PARAMETER	ESTIMATE	T FOR HO: PARAMETER=0	PR > |T|	STD ERROR OF ESTIMATE
INTERCEPT	-1.61255992	-11.16	0.0001	0.14445571
LINEAR	0.15628544	6.84	0.0001	0.02285985
QUADRATRIC	-0.00147637	-1.97	0.0540	0.00074898

Appendix Table 32. Analysis of variance for the regression of the natural log of total plant dry weight on time (days) for nodulated soybeans grown outdoors and supplied 12mM NO_3^-.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	2	57.95990167	28.97995083	1424.47
ERROR	51	1.03756654	0.02034444	PR > F
CORRECTED TOTAL	53	58.99746821		0.0001

R-SQUARE	C.V.	ROOT MSE	DEP. VAR. MEAN
0.982413	38.1598	0.14263394	0.37378088

SOURCE	DF	TYPE I SS	F VALUE	PR > F
LINEAR	1	56.22976629	2763.89	0.0001
QUADRATIC	1	1.73013538	85.04	0.0001

PARAMETER	ESTIMATE	T FOR HO: PARAMETER=0	PR > |T|	STD ERROR OF ESTIMATE
INTERCEPT	-1.50782980	-31.07	0.0001	0.04853315
LINEAR	0.17627256	23.25	0.0001	0.00758011
QUADRATIC	-0.00225727	-9.22	0.0001	0.00024477

Appendix Table 33. Analysis of variance for the regression of the natural log of total leaf area on time (days) for non-nodulated soybeans grown outdoors and supplied 2mM NO_3^-.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	3	41.95010475	13.98336825	566.71
ERROR	51	1.25841463	0.02467480	PR > F
CORRECTED TOTAL	54	43.20851938		0.0001

R-SQUARE	C.V.	ROOT MSE	DEP. VAR. MEAN
0.970876	3.5620	0.15708213	4.40994012

SOURCE	DF	TYPE I SS	F VALUE	PR > F
LINEAR	1	39.74394090	1610.71	0.0001
QUADRATIC	1	2.11584174	85.75	0.0001
CUBIC	1	0.09032211	3.66	0.0613

PARAMETER	ESTIMATE	T FOR H0: PARAMETER=0	PR > |T|	STD ERROR OF ESTIMATE
INTERCEPT	2.67524160	41.98	0.0001	0.06372067
LINEAR	0.19877514	10.15	0.0001	0.01958098
QUADRATIC	-0.00545639	-3.50	0.0010	0.00155679
CUBIC	6.5092210E-05	1.91	0.0613	0.00003402

Appendix Table 34. Analysis of variance for the regression of the natural log of total leaf area on time (days) for non-nodulated soybeans grown outdoors and supplied 6mM NO_3^-.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	2	68.14382400	34.07191200	1357.00
ERROR	52	1.30563326	0.02510833	PR > F
CORRECTED TOTAL	54	69.44945726		0.0001

R-SQUARE	C.V.	ROOT MSE	DEP. VAR. MEAN
0.981200	3.3153	0.15845609	4.77954089

SOURCE	DF	TYPE I SS	F VALUE	PR > F
LINEAR	1	65.62488631	2613.67	0.0001
QUADRATIC	1	2.51893769	100.32	0.0001

PARAMETER	ESTIMATE	T FOR H0: PARAMETER=0	PR > |T|	STD ERROR OF ESTIMATE
INTERCEPT	2.70056751	50.07	0.0001	0.05393781
LINEAR	0.19737888	23.21	0.0001	0.00850543
QUADRATIC	-0.00275382	-10.02	0.0001	0.00027494

Appendix Table 35. Analysis of variance for the regression of the natural log of total leaf area on time (days) for non-nodulated soybeans grown outdoors and supplied 12mM NO_3^-.

```
SOURCE            DF     SUM OF SQUARES     MEAN SQUARE    F VALUE
MODEL              3      74.30182014       24.76727338     511.58
ERROR             52       2.51749404        0.04841335     PR > F
CORRECTED TOTAL   55      76.81931418                       0.0001

R-SQUARE        C.V.          ROOT MSE          DEP. VAR. MEAN
0.967228        4.4838        0.22003033        4.90722920

SOURCE            DF      TYPE I SS     F VALUE     PR > F

LINEAR             1      69.22782408  1429.93      0.0001
QUADRATIC          1       4.59614002    94.94      0.0001
CUBIC              1       0.47785604     9.87      0.0028

                                     T FOR H0:      PR > |T|   STD ERROR OF
PARAMETER       ESTIMATE             PARAMETER=0               ESTIMATE

INTERCEPT       2.50264270           28.08          0.0001     0.08912136
LINEAR          0.30562107           11.19          0.0001     0.02730776
QUADRATIC      -0.01041145           -4.78          0.0001     0.00217652
CUBIC           0.00014963            3.14          0.0028     0.00004763
```

Appendix Table 36. Analysis of variance for the regression of the natural log of dry weight on time (days) for non-nodulated soybeans grown outdoors and supplied 2mM NO_3^-.

```
SOURCE            DF     SUM OF SQUARES     MEAN SQUARE    F VALUE
MODEL              3      30.69722209       10.23240736    620.12
ERROR             51       0.84154044        0.01650079    PR > F
CORRECTED TOTAL   54      31.53876253                      0.0001

R-SQUARE        C.V.          ROOT MSE          DEP. VAR MEAN
0.973317       31.3194        0.12845541       -0.41014607

SOURCE            DF      TYPE I SS     F VALUE     PR > F

LINEAR             1     30.13621473   1826.35      0.0001
QUADRATIC          1      0.47415650     28.74      0.0001
CUBIC              1      0.08685087      5.26      0.0259

                                     T FOR H0:     PR > |T|    STD ERROR OF
PARAMETER     ESTIMATE               PARAMETER=0               ESTIMATE

INTERCEPT    -1.66493329             -31.95        0.0001      0.05210819
LINEAR        0.08057264               5.03        0.0001      0.01601253
QUADRATIC     0.00168082               1.32        0.1926      0.00127308
CUBIC        -6.3829150E-05           -2.29        0.0259      0.00002782
```

Appendix Table 37. Analysis of variance for the regression of the natural log of total dry weight on time (days) for non-nodulated soybeans grown outdoors and supplied 6mM NO_3^-.

```
SOURCE            DF     SUM OF SQUARES     MEAN SQUARE    F VALUE
MODEL              2      61.19185310       30.59592655    1051.45
ERROR             52       1.51313297        0.02909871    PR > F
CORRECTED TOTAL   54      62.70498607                      0.0001

R-SQUARE        C.V.          ROOT MSE          DEP. VAR. MEAN
0.975869       177.2883       0.17058344        -0.09621813

SOURCE            DF      TYPE I SS     F VALUE     PR > F

LINEAR             1      61.00359397  2096.44      0.0001
QUADRATIC          1       0.18825913     6.47      0.0001

                                   T FOR HO:         PR > |T|   STD ERROR OF
PARAMETER      ESTIMATE            PARAMETER=0                  ESTIMATE

INTERCEPT     -1.84842904          -31.83            0.0001     0.05806592
LINEAR         0.13355900           14.59            0.0001     0.00915639
QUADRATIC     -0.00075284          - 2.54            0.0001     0.00029598
```

Appendix Table 38. Analysis of variance for the regression of the natural log of total dry weight on time (days) for non-nodulated soybeans grown outdoors and supplied 12mM NO_3^-.

```
SOURCE             DF     SUM OF SQUARES     MEAN SQUARE   F VALUE
MODEL               2      66.30632944       33.15316472   774.27

ERROR              53       2.26939216        0.04281872   PR > F
CORRECTED TOTAL    55      68.57572160                     0.0001

R-SQUARE         C.V.          ROOT MSE         DEP. VAR. MEAN
0.96907         384.6650       0.20692685       0.05379404

SOURCE            DF       TYPE I SS     F VALUE     PR > F

DAYS               1      65.73923599    1535.29     0.0001
DAYS*DAYS          1       0.56709344      13.24     0.0006

                                   T FOR HO:         PR > |T|   STD ERROR OF
PARAMETER      ESTIMATE            PARAMETER=0                  ESTIMATE

INTERCEPT      -1.83321181         -26.05            0.0001     0.07037378
LINEAR          0.15387266          14.00            0.0001     0.01099356
QUADRATIC      -0.00129159          -3.64            0.0006     0.00035491
```

VITA

Eddie Paul Millhollon was born in Portsmouth, Virginia, on August 2, 1953. His family moved to Tucson, Arizona in 1954. Here he attended elementary school until his family moved to Houma, Louisiana in 1960. In Houma, he graduated from Terrebonne High School in May, 1971. The author entered Nicholls State University in September, 1973 and received a B.S. degree in Biology from that institution in May, 1977.

In August, 1977, he enrolled as a graduate student at Louisiana State University in the Department of Plant Pathology and Crop Physiology and received the M.S. degree in December, 1980. Since May 1982, Eddie has been a candidate for a Ph.D. in the Department of Agronomy at Louisiana State University.

The author is married to the former Beverly Anne Hebert of Gibson, Louisiana. They have two daughters; Michelle and Linda.

EXAMINATION AND THESIS REPORT

Candidate: Eddie P. Millhollon

Major Field: Agronomy

Title of Thesis: The Relationship Between Photosynthesis and the Capacity for Nitrogen Fixation in Soybean

Approved:

Edward P Dunigan

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

J A Martin

David J. Longstreth

Date of Examination:

June 7, 1984