THE UNIVERSITY OF MANITOBA

THE EFFECT OF NITROGEN AND MOISTURE AVAILABILITY ON GROWTH AND SYMBIOTIC NITROGEN FIXATION IN LENTILS (*Lens culinaris*)

by

Frances Lynne Walley

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

MASTER OF SCIENCE

Department of Soil Science

Winnipeg, Manitoba

 February, 1986

ISBN 0-315-33903-9

THE EFFECT OF NITROGEN AND MOISTURE AVAILABILITY ON GROWTH AND SYMBIOTIC NITROGEN FIXATION IN LENTILS (*LENS CULINARIS*)

BY

FRANCES LYNNE WALLEY

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ABSTRACT

Field and growth chamber studies were conducted to evaluate the effect of various soil conditions, including nitrogen and moisture availability on yield and potential symbiotic nitrogen fixation of lentils. Application of fertilizer N at rates ranging from 0-200 kg $N.ha^{-1}$ and 0-360 ppm N in field and growth chamber experiments, respectively, resulted in dry matter and seed yield increases. Nitrogen stress, simulated by the addition of barley straw to the soil, limited lentil yields. Results evidence the fact that lentils are not capable of symbiotically fixing enough nitrogen to meet optimum plant growth requirements.

Moisture availability was also found to be an important factor in attaining high dry matter and seed yield of lentils. Yields were significantly reduced by the application of moisture stress and were notably influenced by the physiological stage at which stress was applied.

The effect of nitrogen availability on symbiotic nitrogen fixation was evaluated. Quantity of nitrogen symbiotically fixed in lentils was estimated using the 'A' Value method, the ^{15}N Assisted Difference method and the Classical Difference method. Nonnodulating soybeans served as the reference crop. Although these methods estimated similar quantities of nitrogen fixed under controlled conditions, the N balance techniques proved unreliable under field conditions.

Increasing increments of applied N were shown to reduce symbiotic N fixation. Nitrogen stress delayed the onset of symbiotic fixation and thus reduced the total quantity fixed.

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to: Dr. R.J. Soper, Professor, Department of Soil Science, under whose immediate supervision this investigation was conducted, for valuable advice and for helpful criticism of this manuscript; Dr. C.M. Cho, Professor, Department of Soil Science, for valuable advice, for assistance and equipment relating to ^{15}N methodology and for serving on the examining committee; Dr. P. McVetty, Professor, Department of Plant Science, for serving on the examining committee; Ms. V. Huzel and Mr. J. Rogers, for assistance, instruction and technical advice relating to laboratory procedures; Mr. G. Morden, for assistance with ^{15}N analysis and, in particular, for mass spectrometer analysis; Norma, for interest, support and, above all, unending faith.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF TABLES (continued)

LIST OF TABLES (continued)

LIST OF TABLES (continued)

LIST OF TABLES (continued)

LIST OF FIGURES

1. INTRODUCTION

Lentils (*Lens culinaris*) are a high protein seed crop used primarily for human consumption. Recently introduced into western Canada, conclusive information regarding the production of lentils is limited. Seed yield estimates ranging from 900 kg ha^{-1} (Slinkard, 1982) to 2,242 kg ha^{-1} (Manitoba Agriculture, 1980) have been proposed.

Lentils are a member of the *Leguminosae* family and, in association with the appropriate *Rhizobium*, are capable of meeting some of their nitrogen requirements through symbiotic nitrogen fixation. Unfortunately, very little information is available regarding lentil-*Rhizobium* symbiosis and, of that available, little is applicable to the western Canadian agro-climate.

In light of the growing interest in alternative cropping systems, it is likely that lentil production will continue to increase in Manitoba. Consequently, there is a need to obtain information on lentil production and nutrition. It may be desirable to retain as much nitrogen fixation as possible when producing lentils. Thus there is an additional need to determine the quantity of nitrogen which lentils are capable of fixing and identify factors which may affect fixation.

The objectives of this study were: 1) to determine the effect of various soil conditions including nitrogen and moisture availability on nitrogen fixation and yield of lentils, and 2) to evaluate methods of estimating symbiotic nitrogen fixation in lentils.

2. LITERATURE REVIEW

2.1 SYMBIOTIC NITROGEN FIXATION AND YIELD OF LENTILS

Lentils (Lens culinaris) are a recently introduced crop in western Canada and presently little conclusive information is available regarding production potential. Seed yield estimates ranging from 900 kg ha^{-1} (Slinkard, 1982) to 2,242 kg ha^{-1} (Manitoba Agriculture, 1980) have been proposed.

Summerfield (1981) reported that lentils are capable of achieving a mean seed protein content of twenty-five percent. Thus, it can be expected that lentils will exert a large demand on available nitrogen sources. Saxena (1981) suggested that a lentil crop yielding 2,000 kg ha^{-1} may take up approximately 100 kg N ha^{-1}.

Lentils are a member of the Leguminosae family and, in association with the appropriate Rhizobium, are capable of meeting some of their nitrogen requirements through symbiotic nitrogen fixation. Unfortunately, very little information is available regarding lentil-Rhizobium symbiosis and, of that available, little is applicable to the western Canadian agro-climate. Studies of nitrogen nutrition of lentils grown in Egypt indicated that more than eighty-five percent of the total nitrogen requirement of the crop may be met through nitrogen fixation (Rizk, 1966). Rennie (1984) reported that lentils grown in southern Alberta are capable of fixing 150 kg N ha^{-1}. Unfortunately, total nitrogen uptake and seed yields were not reported. Summerfield (1981) reported estimates of nitrogen fixation ranging between 35 and

75 kg N ha^{-1}. Again, corresponding total nitrogen uptake and seed yields were not reported. In light of the paucity of information available regarding the nitrogen nutritional status of lentils, it is difficult to estimate the contribution nitrogen fixation makes to total N.

Many authors have studied the effect of various environmental factors on symbiotic nitrogen fixation in grain legumes but few have reported on lentil-Rhizobium symbiosis. As Islam (1981) noted, this is due simply to a lack of information. However, much can be elucidated from data pertaining to similar legume-Rhizobium symbiotic relationships.

2.1.1 Effect of Nitrogen Application

In non-leguminous crops, application of nitrogen fertilizer generally leads to an increase in dry matter yields. This effect is complicated in leguminous crops capable of fixing atmospheric nitrogen by the effect of inorganic N on the nitrogen fixing system. Allos and Bartholomew (1955) reported studies with soybeans, peanuts, alfalfa, lespedeza, Ladino clover and birdsfoot trefoil. In all cases the presence of available inorganic nitrogen diminished symbiotic fixation. The specific influence on fixation of various levels of inorganic nitrogen varied among the legumes studied. McAuliffe et al. (1958) found that nitrogen fixation in both Ladino clover and alfalfa was reduced by nitrogen application, with the degree of reduction being definitely related to the amount of nitrogen applied. Richards and Soper (1979) reported that in fababeans, the reduction in nitrogen fixation

associated with nitrogen application was described by a significant linear inverse relationship, indicating that fababeans preferentially feed from combined inorganic nitrogen rather than symbiotically fix their needs.

The inhibitory effect of inorganic nitrogen on legume-Rhizobium symbiosis has been attributed to many mechanisms. Munns (1968) observed that if the growth medium contained nitrate, Medicago sativa had fewer root hairs, and fewer root hairs curled in response to inoculation with Rhizobium. Munns considered root hair production and curling to be a necessary prelude to infection and nodule development. Dart (1977) suggested that all stages of infection are sensitive to inorganic nitrogen, including thread growth. He found that fewer threads were formed, more were aborted and they appeared disorganized when R. meliloti was exposed to nitrate. Semu and Hume (1979) found that high levels of soil N availability, as well as applications of fertilizer N, decreased fixation in soybeans, (Glycine max L.) mainly by decreasing nodule numbers and size. Similarly, Hill-Cottingham and Lloyd-Jones (1980) reported that nodule weight in fababeans (Vicia faba (L.)) was depressed by nitrate and that there was a corresponding decrease in atmospheric nitrogen fixed. Chen and Phillips (1977) found that N fixation in Pisum sativum L. was reduced by NH_4^+ and NO_3^- ions, and ascribed this reduction to earlier nodule senescence.

Wong (1980) suggested that nitrate has many effects on the development and nitrogen fixing activity of lentil root nodules. He found that lentils grown in nitrogen free nutrient solution had six times as many nodules per plant and that nodules weighed more than three times as

much as nodules of lentils grown in a nitrate containing solution. Furthermore, it was found that nitrate also inhibited N fixing (C_2H_2 reduction) activity of lentil nodules. Wong observed that the inhibitory effects of nitrate were reduced by adding sugars to the growth medium, suggesting that the internal carbohydrate to nitrogen ratio governs nodule formation and nitrogen fixation.

Although the addition of combined nitrogen at high levels commonly inhibits development and function of legume root nodules, exceptions exist, suggesting the use of nitrogen fertilizers to stimulate nitrogen fixation. Dart and Wilson (1970) reported that urea applied at seeding at a rate of 112 ppm stimulated fixation in cowpeas (Vigna unguiculata L.) by twenty-nine percent above controls receiving no nitrogen. Agboola (1978) also studied cowpeas and concluded that increased fixation was related to concomitant increases in nodule numbers. Conversely Eaglesham et al. (1983) attributed increased fixation in cowpeas to increases in both nodule weights and nitrogenase activity. It has been postulated that the positive effects of applied nitrogen on cowpea symbiosis are explained in terms of an alleviation of the "N hunger" stage of growth during early development subsequent to cotyledon consumption and prior to the onset of significant nodule fixation (Pate and Dart, 1961). According to Allos and Bartholomew (1959), total fixation is closely related to the amount of growth. Thus, if alleviation of the N hunger stage leads to larger, more vigorous plant, there may be a tendency for plants to fix more nitrogen.

Hatfield et al. (1974) reported similar findings when studying soybeans. They found that nodule development was greatest when soybeans

received nitrogen for two weeks as compared to plants receiving no nitrogen. They concluded that a time lag in development of nodules for N fixation resulted in reduced growth and may have been responsible for limiting maximum nitrogen fixation and yield potential.

Yield responses of nitrogen fixing legumes to N fertilizer applied at planting are usually taken to indicate that N fixation is less than optimal. Rizk (1966), observing that lentils could fix up to eighty-five percent of their N requirements, suggested that little or no increase in yield can be anticipated with N fertilizer addition. He found that soil and symbiotically fixed N sources were generally sufficient to meet lentil nitrogen requirements. This contention was supported by Chowdhury et al. (1974), Sekhon et al. (1977) and Ojha et al. (1977), all of whom independently reported that the addition of 25 kg N ha^{-1} did not lead to increases in lentil yield. However, although yield increases are generally taken to indicate insufficient N fixation, it does not follow that lack of response necessarily indicates that fixation is optimal for lentil N requirements. Small dressings of fertilizer nitrogen may lessen fixation of atmospheric N so that there is no net gain in the nitrogen available to the plant. McEwan (1970) has suggested that in order to determine whether yield is limited by fixation, nitrogen must be applied in amounts exceeding those usually fixed.

2.1.2 Effect of Water Stress

Lentils have an indeterminate flowering habit and it has been suggested that some drought stress is required in the latter part of the

flowering process in order to encourage maturation of younger pods, thereby increasing seed yields (Slinkard and Drew, 1982). Cowpeas also exhibit an indeterminate flowering habit and although the phenomenum of hastened maturity due to water stress have been observed in this species (Lawn, 1982), concomitant yield increases have not been observed (Hiler et al., 1972; Turk et al., 1980; Turk and Hall, 1980). Technical difficulties involved in establishing and maintaining prescribed moisture conditions as well as different legume species studied could be responsible for these conflicting observations. It is apparent, however, that further research pertaining to the response of lentils to moisture stress is required.

In assessing the effect of drought stress on lentil production the influence of soil moisture on nodule formation and symbiotic fixation, and the subsequent effect on yield, should be examined. Reductions in soil water availability have been shown to adversely affect nitrogen fixation in numerous crop species (Sprent, 1971, 1972a, 1972b, 1976; Kuo and Boersma, 1971; Engin and Sprent, 1973).

Sprent (1970) examined the effects of water stress on detached soybean nodules and reported that nodule water contents down to eighty percent of turgid weight resulted in depressed but recoverable rates of nitrogen fixation. Below eighty percent water content, acetylene and nitrogen reducing activities ceased, respiratory rates became very low and gross structural changes occurred. Rehydration of nodules did not stimulate recovery of nitrogen fixation. It was concluded that water supply had a major effect on the amount of N fixed in the field, particularily in plants with nodules near the soil surface.

Bennett and Albrecht (1984) studied the effect of water stress on nitrogen fixation by intact soybean plants. They found that nitrogen fixation was significantly reduced after ten days of withholding water and activities declined to essentially zero as stress became progressively more severe.

Reductions in nitrogen fixation due to an imposed drought stress have been ascribed to several mechanisms. Pate et al. (1969) suggested that lower rates of water movement out of the nodule during water stress may restrict export of products of nitrogen fixation, thus limiting fixation through a feedback mechanism. However, Sprent (1970) noted that should such a feedback mechanism exist, it might be expected that water stress would affect reduction of nitrogen but not acetylene, since the products of acetylene reduction are gaseous and are not directly dependent on water for its removal. Several studies have indicated that moisture stress leads to decreased acetylene reduction activity (Sprent, 1970, 1971, 1972, 1976; Pankhurst and Sprent, 1975). Alternatively, Sprent (1971) suggested that the inhibitory effect of water stress may be related to anatomical alterations within the nodules. Sprent observed that plasmodesmatal connections between infected and uninfected cells within soybean nodules ruptured in response to severe moisture stress. Sprent postulated that uninfected cells within the nodules play a vital role in nodule metabolism and possibly also in transporting material to the cortex for subsequent export from the nodule. Thus breakage of plasmodesmatal connections between infected and uninfected cells led to cessation of nodule functioning. Sprent (1972) later suggested that the collapse of vacuolate cells of the nodule cortex in

response to even mild water stress may result in changes in metabolism, transport and, ultimately, nitrogen fixation.

Pankhurst and Sprent (1975) found that seventy-five percent of the nitrogen fixing activity and fifty percent of the respiratory activity of detached soybean nodules was lost when nodules were moderately stressed. Increasing the oxygen partial pressure from 10^4 to 10^5 Pa completely restored nitrogen fixation and respiration. This led Pankhurst and Sprent to conclude that in moderately stressed nodules, the actual activity of the bacteroids may not be directly impaired by water stress, but rather that the apparent loss of activity in these nodules may be due to lack of O_2. They suggested that water stress may impose a physical barrier to gaseous diffusion, noting that the loss of water from plant cells renders them more impermeable to gaseous diffusion. Alternatively, they suggested that water stress may alter the affinity of nodule leghaemoglobin for O_2. Thus the apparent oxygen diffusion barrier may lie, at least in part, in less effective transport of O_2 by leghaemoglobin to the bacteroids.

In addition to the direct effects water stress may have on nodule functioning, Sprent (1972) postulated that these effects may be aggravated by reduced photosynthate production by wilted leaves. Huang et al. (1975a, 1975b) supplied evidence indicating that reductions in photosynthesis by soybeans during water stress led to reductions in nitrogen fixation. In addition to decreased photosynthate production, Kuo and Boersma (1971) suggested that decreases in nitrogen fixation with increasing soil water suction could be attributed to decreased translocation of carbohydrates to the root nodules. This contention is

supported by Hartt (1967) who showed that reduced water supply depressed translocation of ^{14}C-labelled photosynthate.

It is evident that moisture availability will affect symbiotic nitrogen fixation in leguminous crops. Although several independent mechanisms have been proposed by which fixation is adversely affected by moisture stress, it is likely that these mechanisms operate simultaneously leading to decreased atmospheric nitrogen supply (Bennett and Albrecht, 1984). Thus, although drought stress may lead to lentil seed yield increases by facilitating the maturation of younger pods, a concomitant decrease in nitrogen fixation is likely to occur.

2.2 METHODS OF MEASURING SYMBIOTIC NITROGEN FIXATION

Evaluation of the effects of specific interactions between legume host, Rhizobium and the environment necessitates effective quantification of nitrogen fixation. In a recent review, Herridge (1982) outlined techniques available and commonly used to measure nitrogen fixation including nitrogen balance and ^{15}N dilution methods, and the acetylene reduction assay.

In utilizing nitrogen balance methods, nitrogen uptake by the test legume is referred to uptake by a nonfixing reference crop. This method has also been referred to as the "difference" method (Williams et al., 1977) and "special controls for legumes" (Hardy and Holsten, 1977). The method consists of comparing the total nitrogen content of the legume test crop to the total nitrogen content of a nonfixing reference crop grown under identical conditions. The nitrogen increment is considered

to represent nitrogen derived from fixation.

Three approaches to the use of nitrogen balance are available. One approach measures the difference in total nitrogen between inoculated and uninoculated plants of the same variety. Another utilizes the difference between nitrogen uptake by a nodulated and nonnodulated near isogenic lines (Weber, 1966). The final method compares uptake of nitrogen by a legume and a nonlegume (Bell and Nutman, 1971). Regardless of the reference crop chosen, a number of assumptions are implied by the technique, eg., equivalency of root nitrogen content, soil zone explored, and seasonal patterns of soil N uptake (Williams et al., 1977, Vasilas and Ham, 1984). Rennie (1982) contended that such assumptions can be met only through the use of ineffectively inoculated or uninoculated test legume plants as the reference crop. However, he reported that in soils having indigenous populations of Rhizobium, it may be difficult to ensure that ineffectively inoculated or uninoculated legumes do not become nodulated. The use of a nonnodulating isoline as the nonfixing control plant overcomes the problem of inadvertent nodulation. However, nodulating and nonnodulating soybean isolines have been shown to differ significantly in nitrogen fertilizer utilization at various growth stages (Deibert et al., 1979) as well as nitrate reductase activity (Liv and Hadley, 1971). Rennie (1982), studied the use of a nonnodulating soybean isoline in determining nitrogen fixation in soybeans, and concluded that it was an inappropriate nonfixing control plant because it tended to over-estimate nitrogen fixation.

Hardy and Holsten (1977) indicated that although the use of nitrogen balance methods in determining nitrogen fixation offers the

advantage of simplicity, they do not provide absolute quantitative measurements and may not even provide valid relative comparisons between sites and varieties. In agreement, Rennie et al. (1978) suggested that, at best, nitrogen balance methods provide only qualitative indications of nitrogen fixed and thus care should be taken in the interpretation of results.

The classical nitrogen balance method has been modified somewhat through the use of ^{15}N labelled fertilizer and has been referred to as the ^{15}N Assisted Difference method (Richards and Soper, 1979). Richards and Soper (1979) added identical quantities of ^{15}N labelled fertilizer to inoculated fababeans (Vicia faba L. var. Minor) and barley (Hordeum vulgare L. var. Conquest). Symbiotic nitrogen fixation was then calculated in the following manner:

$$S = P - B - F - {}^{15}N$$

where S = quantity of N symbiotically fixed by test legume

P = total test legume shoot N

B = contribution of soil N as measured by reference nonlegume

F = contribution of seed N

^{15}N = contribution of N fertilizer, as measured by tracer ^{15}N.

Many authors described the use of ^{15}N isotope dilution techniques applied to the estimation of nitrogen fixation (Ham, 1973; Hardy and Holsten, 1977; Rennie et al., 1978; Herridge, 1982; Rennie and

Rennie, 1983). Of these ^{15}N isotope dilution techniques, a specialized dilution technique has been described by Fried and Broeshart (1975) in which 'A_n' values of a legume and a nonfixing control are utilized.

The 'A_n' value is based on the 'A' value concept originally proposed by Fried and Dean (1952). They proposed that a plant confronted with two sources of a nutrient will obtain the nutrient from each source in direct proportion to the amounts available. Thus, determination of the available soil nutrient can be made in terms of an added fertilizer standard, provided that the nutrient in the plant derived from the standard is determined. The use of ^{15}N labelled fertilizer facilitates determination of fertilizer derived nitrogen. Fried and Dean (1952) proposed the following mathematical expression of this relationship:

$$A = \frac{B(1-y)}{y}$$

where A = amount of nutrient available in the soil

B = amount of nutrient in the standard

y = proportion of nutrient in the plant from the standard

Assuming that a plant confronted by two sources of nutrient will take up the nutrient from each source in proportion to the amounts available, it follows that the 'A' value should not be affected by the rate of fertilizer standard applied (Fried and Broeshart, 1975). This contention has been supported by a number of workers (Legg and Allison, 1959; Hunter and Carter, 1965; Legg and Stanford, 1967; Smith and Legg, 1971), indicating that the 'A' value is indeed normally independent of

rate of nitrogen application. In contrast, Broadbent (1970) has reported that as fertilizer levels increase, the 'A' value may decrease significantly, remain constant, or increase more than fifty percent. He concluded that the usefulness of the 'A' value is dependent on careful definition of experimental conditions, specifically the rate of standard applied and method of placement.

The measurement of the amount of nitrogen symbiotically fixed by a legume crop as proposed by Fried and Broeshart (1975) involves simultaneous determinations of the 'A' values of the test legume and a nonfixing control crop. Nitrogen fertilizer labelled with ^{15}N is applied at a low rate to the legume in order to minimize interference with nitrogen fixation, but at a normal rate to the nonfixing crop such that an adequate nitrogen supply exists. The 'A' value for the legume crop represents both symbiotically fixed nitrogen and soil nitrogen whereas the 'A' value for the nonfixing control represents soil nitrogen alone. The quantity of fixed nitrogen is calculated by multiplying the difference in 'A' values between the test legume and the nonfixing reference crop with the percent utilization of fertilizer nitrogen by the legume crop.

In determining nitrogen fixed by a legume using the 'A' value, or any other technique which utilizes ^{15}N enriched fertilizer, consideration must be given to the nonuniformity in labelling of plant material (Rennie et al., 1978). Rennie et al. (1978) reported that estimates of the amount of nitrogen fixed can be significantly altered by the selection of plant parts used for the calculations. They reported that natural abundance of ^{15}N in various plant parts

frequently varies significantly and suggested that a more correct approach on calculating N fixation would be to calculate a mean atmosphere percent ^{15}N excess for both grain and straw.

Isotopic discrimination by plants is also of concern when using ^{15}N labelled fertilizer (Rennie et al., 1978). That the nitrogen fixing system does not discriminate between ^{14}N and ^{15}N is a basic assumption in the use of ^{15}N for determination of nitrogen fixation (Hauck and Bremner, 1976). Delwiche and Steyne (1970) investigated nitrogen fixation by Azobacter vinelandii and reported a slight discrimination against fixation of the ^{15}N molecule. In contrast, Rennie et al. (1976) reported that nitrogen fixation estimates from ^{15}N values for Vicia faba were unaffected by isotopic discrimination. Hauck and Bremner (1976) stated that for most studies, slight differences in the behavior of ^{14}N and ^{15}N in biological systems can be considered negligible.

A further method used in estimating nitrogen fixation is the acetylene reduction assay. The nature of nitrogenase which enables it to not only reduce N_2 to NH_3 but also C_2H_2 to C_2H_4 led to the development of this technique (Hardy et al., 1968). As described by Havelka et al. (1982), the acetylene reduction assay involves the exposure of a nitrogen fixing system to an atmosphere containing acetylene. Following incubation, the atmosphere is sampled and analysed for ethylene by gas chromatographic separation of acetylene and ethylene coupled by their assay with a flame ionization detector.

The acetylene reduction assay is an indirect method of determining nitrogen fixation in that an alternate electron acceptor is used to

measure nitrogenase activity. Thus, a conversion factor must be used to express acetylene reduced in nitrogen equivalents. The reduction of either three mol of C_2H_2 to C_2H_4 (1) or one mol N_2 to ammonia (2) requires six electrons.

$$C_2H_2 + 2H^+ + 2e^- \quad C_2H_4 \qquad (1)$$

$$N_2 + 6H^+ + 6e^- \quad 2NH_3 \qquad (2)$$

Thus, when comparing potential nitrogen fixation to acetylene reduced, a theoretical conversion factor of three is required.

Although some researchers have obtained good correlations to the theoretical value of 3.0 (Hardy et al. 1971), wide variations have been reported (Rennie et al., 1978). Bergerson (1970) compared acetylene reduction and $^{15}N_2$ uptake by detached soybean nodules and found ratios of acetylene reduced to nitrogen fixed ranging from 2.7 to 4.2.

Rennie et al. (1978) have given a number of reasons why C_2H_2/N_2 ratios can be expected to vary. Of these reasons, they suggested the most important is likely that, subsequent to reduction, nitrogen is assimilated for protein synthesis whereas acetylene measures only nitrogenase activity and makes no contribution to the microrganism's metabolism. Bergerson (1970) noted that although acetylene is isoelectronic with nitrogen and has similar molecular dimensions the large disparity in water solubilties greatly affects experimentally determined reaction rates. Ae and Nishi (1983) investigated the reduction of N_2 and found that not all the electrons are consumed in the reduction of N_2; some are used for the reduction of H^+ and subsequent evolution of H_2 gas into the atmosphere. This does not occur in the presence of C_2H_2. Furthermore, Ae and Nishi (1983) reported that

some legumes possess uptake hydrogenase activity which dissociates molecular H_2 to H^+ and e^-. This electron can then be utilized by nitrogenase. They reported that the conversion factor for estimating the amount of fixed N_2 from C_2H_2 reduction could vary between three and six, depending on hydrogenase activity of the root nodules. Finally, Bergerson (1970) noted that due to the unstable nature of the nitrogenase enzyme system, failure to match environmental conditions in the acetylene reduction assay with those of the nitrogen fixing system in nature may have a differential effect on the C_2H_2/N_2 ratio.

Subsequent to the determination of acetylene reduced, symbiotic nitrogen fixation can be calculated using the equation reported by Hardy and Holsten (1977):

$$gN_2(C_2H_2)\text{fixed}/(\text{hr sample})=28(e-b-i)/\text{scrv}/t/f$$

where

e,b,i, and s = peak height, or area, for C_2H_2 in analyzed sample of 50 ul from, respectively (i) experimental sample incubated with C_2H_2 (ii) experimental sample preincubated in the absence of C_2H_2 (for C_2H_4 background) (iii) incubation chamber with C_2H_2 but without sample (for C_2H_4 impurity) (iv) C_2H_4 standard.

c = concentration of ethylene in standard expressed as moles x litre^{-1} at STP.

r = ratio of peak height of internal standard in incubation chamber without sample to peak height in experimental incubation chamber with sample.

v = volume of incubation chamber in liters at STP.

t = time of incubation in hours.

f = conversion factor for moles C_2H_2 reduced to moles N_2 fixed.

28 = molecular weight of N_2.

Hardy et al. (1973) noted several advantages of the acetylene reduction assay. They reported that the acetylene reduction assay is 10^2 to 10^4 times as sensitive as ^{15}N methods. Furthermore, ethylene gas samples are easy to retrieve and directly analyzed without further chemical treatment. Ethylene gas is stable facilitating storage. Finally, the assay process is relatively portable, allowing for field determinations to be made.

Despite apparent advantages, Rennie et al. (1978) indicated that the acetylene reduction technique has several limitations. As previously noted, the theoretical conversion value of three is not valid in all cases. Thus, Herridge (1982) suggested that it is of upmost importance to establish a quantitative relationship between acetylene reduced and nitrogen fixed for the particular legume-Rhizobium association under study using carefully standardized procedures. Extrapolation to total nitrogen fixed over the growing season is questionable as acetylene reduction is a short-term kinetic measurement, strongly influenced by the existence of large diurnal and seasonal variations in rate of enzyme activity (Rennie et al., 1979). Finally, difficulty is associated with recovering and assaying total root nodule systems.

A number of techniques are presently available to study nitrogen

fixation in legumes. However, of the methods discussed, each is not without limitations. It is of upmost importance that care be taken when using any technique and that equal care be taken in the interpretation and reporting of experimental results.

3. MATERIAL AND METHODS

3.1 GROWTH CHAMBER EXPERIMENTS

Soils. The soil used in each of the three growth chamber experiments was the Ap horizon of a St. Claude Series loamy fine sand (Ehrlich et al., 1957), which is a Rego Black Chernozem, carbonated phase, in the Canadian System of Soil Classification. The soil was collected in September, 1983 and was stored in an air dry condition. In preparation for each experiment, the soil was sieved to pass through a 2 mm mesh sieve and thoroughly mixed. A summary of chemical and physical characteristics of the soil is given in Table 1.

3.1.1 Growth Chamber Experiment A

An experiment with lentils (*Lens culinaris* var. Eston) and nonnodulating soybeans (*Glycine max* (L.), clay maturity type) was conducted in the growth chamber to investigate the effect of nitrogen addition and availability on nitrogen uptake, dry matter yield, seed yield, harvest index and nitrogen fixation in lentils. Nonnodulating soybeans served as a reference crop and were used to estimate uptake and quantity of available soil nitrogen.

Experimental Design and Procedure. A completely randomized experiment consisting of 10 treatments and three replicates was conducted. Treatments are outlined in Table 2.

Nitrogen, supplied as reagent grade urea, was applied to both the test crop and the reference crop at rates of 0, 30, 90 and 360 ppm. In

Table 1. Summary of physical and chemical characteristics of soil used in growth chamber experiments.

Soil Characteristics	
Series	St. Claude
Subgroup	Rego Black Chernozem, carbonated phase
Textural Class	loamy fine sand
pH	7.2
Salinity (dS m^{-1})	0.3
Nitrate - nitrogen (ppm)	12.6
$NaHCO_3$ extractable PO_4-P (ppm)	6.6
NH_4OAC extractable K (ppm)	47
Sulphate-sulphur (ppm)	8.6
Carbonate content	high
Field capacity (%)	23
Permanent wilting point (%)	7.33

Table 2. Treatments used in Growth Chamber Experiment A for lentils and nonnodulating soybeans.

Treatment Number	Nitrogen Applied (ppm)	Type of Plant
1	0 (1)	lentil
2	0	lentil
3	30	lentil
4	90	lentil
5	360 (2)	lentil
6	0 (1)	nonnodulating soybean
7	0	nonnodulating soybean
8	30	nonnodulating soybean
9	90	nonnodulating soybean
10	360 (2)	nonnodulating soybean

(1) Treatment includes the addition of 1% barley straw (60 grams).

(2) Split into 4 applications of 90 ppm N each, applied at seeding and at 37, 48 and 69 days after seeding.

addition, a treatment in which barley straw containing 0.63 percent nitrogen was mixed with the soil was included in the experiment. To ensure an adequate supply of nutrients other than nitrogen, all treatments received 100 ppm P as KH_2PO_4, 200 ppm K as KH_2PO_4 and K_2SO_4, 10ppm Zn as $ZnSO_4$ $7H_2O$, 5 ppm Cu as $CuSO_4$ $5H_2O$ and 37 ppm S from the latter three carriers. These rates were calculated on an air dry soil basis.

In preparing the treatments, 6 kg of air dry soil was spread on a plastic sheet. Potassium phosphate was dissolved in distilled deionized water and 20 ml of the resulting solution was pipetted into a hand held spray bottle. This was further diluted with less than 10 ml of distilled deionized water, sprayed onto the soil as a fine mist and then thoroughly mixed. Thirty milliliters of a second solution containing potassium sulfate, zinc sulfate and copper sulfate was applied in the same method.

In an attempt to avoid urea toxicity damage to the plants, the application of 360 ppm nitrogen was made through successive additions of 90 ppm nitrogen applied at seeding and at 37, 48 and 69 days after seeding. The latter three additions of nitrogen were made by pouring a urea solution onto the soil surface, followed by additions of distilled water. Where urea was applied at seeding, a solution containing urea dissolved in distilled deionized water was applied at a rate of 5 ml and 15 ml, corresponding to 30 and 90 ppm N, respectively. The urea solution was applied to the soil in the method described for the other nutrients.

For treatments receiving barley straw, 60 grams of finely chopped barley straw containing 0.63 percent nitrogen was added to the soil and mixed throughout.

Following nutrient addition, the soil was placed in individual plastic pots lined with plastic liners. The experiment began 20 January, 1984. Ten lentil seeds, inoculated with Nitragin Corporation[1] 'C' culture (Rhizobium leguminosarum) in a slurry at many times the recommended rate of 190 grams inoculum per 47 kilograms seed, were sown at a depth of 2.5 cm into each of 15 pots. Six nonnodulating soybean seeds were sown at a depth of 2.5 cm into each of the remaining 15 pots. The pots were then brought to two-thirds field capacity by weight through the addition of distilled water. Following germination and emergence the soil was maintained at field capacity by adding distilled water on a daily basis for the duration of the experiment.

Pots were placed in a single growth chamber in which there was a 16 hour daylength and eight hour night photoperiod regime. The light source was Sylvannia cool whites supplemented with 10 percent incandescent. The photosynthetically active radiation was measured to be 550 microeinsteins m^{-2} sec^{-1} at plant canopy height. Temperature in the growth chamber varied between a daytime temperature of 22°C and a night time temperature of 15°C. Humidity was maintained at 80 percent during the night and 50 percent during the day.

Following emergence, the lentils were thinned to four plants per pot and the nonnodulating soybeans to two plants per pot. Detailed observation of the plants were made and recorded on a daily basis.

Plants were grown for 95 days after seeding at which time lentils had reached maturity. Due to the indeterminate flowering habit of

---

[1] Supplier: The Nitragin Company, Milwaukee, Wisconsin, U.S.A., 53219.

lentils it was necessary to define maturity as the stage at which mature seeds were apparent on the plant and seed drop was noted. The aerial portion of the plants were harvested and mixed with any senescent leaves collected prior to maturity. Lentils were separated by hand into seed and straw portions. The plant material was then oven dried for 48 hours in a forced air oven at 60°C. Oven dry weights were determined and plant samples were ground in a Wiley mill to pass through a two millimeter sieve. Nitrogen analysis was carried out on the plant material. Nitrogen in the plant material is reported on an oven dry basis.

3.1.2 Growth Chamber Experiment B

A second growth chamber experiment with lentils was conducted simultaneously with Growth Chamber Experiment A to investigate the effect of water stress, applied at different physiological stages, on nitrogen accumulation, dry matter and seed yield, and harvest index. No direct estimation of the quantity of nitrogen fixed was attempted.

Experimental Design and Procedure. A completely randomized experiment consisting of four treatments and three replicates was conducted using lentils as the test crop. The treatments, which consisted of simulating a moisture stress condition at three distinct physiological stages of lentils, are outlined in Table 3.

All treatments received nitrogen supplied as reagent grade urea, at a rate of 30 ppm N. A relatively low rate of nitrogen was applied in an attempt to minimize the deleterious effects of added fertilizer nitrogen on symbiotic fixation yet supply sufficient nitrogen for

Table 3. Treatments used in Growth Chamber Experiment B for lentils.

Treatment Number	Physiological Stage When Moisture Stress was Applied
1	No moisture stress applied
2	Pre-bud
3	Flowering
4	Early pod fill

seedling establishment. In addition, all treatments received a basal application of 100 ppm P as KH_2PO_4, 200 pm K as KH_2PO_4 and K_2SO_4, 10 ppm Zn as $ZnSO_4$ $7H_2O$, 5 ppm Cu as $CuSO_4$ $5H_2O$ and 37 ppm S from the latter three carriers. All fertilizer rates were calculated on an air dry soil basis. Fertilizers were applied to the soil in the method described in Section 3.1.1 for Growth Chamber Experiment A.

Following nutrient addition, the soil was placed in individual plastic pots lined with plastic liners. The experiment began on 20 January, 1984. Ten lentil seeds, inoculated with Nitragin Corporation 'C' Culture (Rhizobium leguminosarum) using the slurry method at many times the recommended rate of 190 grams inoculum per 47 kilograms seed, were sown at a depth of 2.5 centimeters into each of the twelve pots. The pots were then brought up to two-thirds field capacity by weight through the addition of distilled water. Following germination and emergence, the pots were then brought up to field capacity through the addition of distilled water.

All pots were then placed in a single growth chamber in which there was a 16 hour daylength and 8 hour night photoperiod regime. The light source was Sylvannia cool whites supplemented with 10 percent incandescent. The photosynthetically active radiation was measured to be 550 microeinsteins m^{-2} sec^{-1} at plant canopy height. Temperature in the growth chamber varied between a daytime temperature of 22°C and a night time temperature of 15°C. Humidity was maintained at 80 percent during the night and 50 percent during the day.

Following emergence the lentils were thinned to four plants per pot. Detailed observations including visible signs of stress and developmental stage of the lentils were made on a daily basis and recorded.

Pots were weighed on a daily basis and the soil was restored to field capacity through the addition of distilled water as required. For pots receiving Treatment 1, which was intended to simulate a situation in which no moisture stress was experienced by the plants, field capacity was maintained until maturity. During growth stages in which heavy water use was noted, maintainance of field capacity was achieved by adding distilled water twice daily or as required. Moisture stress, applied to Treatments 2, 3 and 4 at pre-bud, flowering and early podfill stages of growth, respectively, was simulated by withdrawing the daily additions of water to the pots at the appropriate growth stages. No water was added to the pots until the plants had begun to visibly wilt. When wilting was noted, field capacity was restored by adding distilled water. Having restored the soil to field capacity, water was again withheld and the plants were allowed to wilt. This cyclic pattern of wilting and watering was maintained until maturity.

Plants were grown for 95 days after seeding at which time lentils had reached maturity. The aerial portion of the plants were harvested and mixed with any senescent leaves collected prior to maturity. Lentils were separated by hand into seed and straw components. The plant material was then oven dried for 48 hours in a forced air oven at 60°C. Oven dry weights were determined and plant samples were ground in a Wiley mill to pass through a two millimeter sieve. Nitrogen

analysis was carried out on the plant material. Nitrogen in the plant material is reported on an oven dry basis.

3.1.3 Growth Chamber Experiment C

An experiment with lentils and nonnodulating soybeans was conducted in a growth chamber to investigate the effect of nitrogen addition and availability on nitrogen uptake dry matter yield, harvest index and nitrogen fixation in lentils as a function of time. Nonnodulating soybeans were used to measure uptake and quantity of available soil nitrogen.

Experimental Design and Procedure. A completely randomized experiment consisting of six treatments and three replicates was conducted using lentils as the test crop and nonnodulating soybeans as the reference crop. The experiment was replicated six times in its entirety in order to accommodate six harvests taken at different times throughout the duration of the experiment. Treatments are outlined in Table 4.

Nitrogen, supplied as reagent grade urea, was applied to both the test crop and the reference crop at rates of 0, 30 and 200 ppm. Urea applied at a rate of 30 ppm was labelled with 2.33 atom percent ^{15}N excess. Urea applied at a rate of 200 ppm was labelled with 2.09 atom percent ^{15}N excess. Where no nitrogen was applied, barley straw containing 0.69 percent nitrogen was incorporated into the soil at a rate of one percent. To ensure an adequate supply of other nutrients, all treatments received 100 ppm P as KH_2PO_4, 200 ppm K as KH_2PO_4 and KH_2SO_4, 10 ppm Zn as $ZnSO_4\ 7H_2O$, 5 ppm Cu as $CuSO_4\ 5H_2O$ and 37 ppm S from the latter three carriers. These rates were calculated on

Table 4. Treatment descriptions for lentils and nonodulating soybeans used in Growth Chamber Experiment C.

Treatment Number	Nitrogen Applied (ppm)	Type of Plant
1	0 (1)	lentil
2	30	lentil
3	200	lentil
4	0 (1)	nonnodulating soybean
5	30	nonnodulating soybean
6	200	nonnodulating soybean

(1) Treatment includes the addition of 1% barley straw (60 grams).

an air dry soil basis.

In preparing the treatments, sieved soil was loaded in lots of 21 kilograms each into a large capacity cement mixer. Potassium phosphate was dissolved in deionized distilled water and 70 millilitres of the resulting solution was placed in a hand held spray bottle. The solution was further diluted by not more than 50 millilitres of distilled water and sprayed onto the soil as a fine spray while the soil was being mixed in the cement mixer. A second solution contained potassium sulphate, zinc sulphate and copper sulphate dissolved in deionized distilled water. One hundred and five millilitres of this solution was placed in a hand held sprayer, further diluted with not more than 30 millilitres of distilled water and applied to the soil in the manner described for potassium phosphate.

Six thousand grams of the treated soil was then spread on a plastic sheet. For treatments receiving urea, the soil was weighed and divided in half on a weight basis. Urea, to be applied at a rate of 30 ppm, was dissolved in deionized distilled water and placed in a hand-held sprayer. This solution was further diluted with approximately 25 millilitres distilled water and sprayed, as a fine mist, onto the surface of the soil intended for the bottom half of the pot. The soil was thoroughly mixed by hand while the urea solution was being applied. For treatments receiving urea at a rate of 200 ppm, twenty millilitres of a second solution was placed in a hand-held spray bottle, further diluted with approximately 25 millilitres of distilled water and sprayed onto the soil intended for the bottom half of the pot in the above manner. The placement of nitrogen in the bottom half of the pot was carried out

in an attempt to minimize seedling damage caused by urea toxicity. Although urea tocicity damage was not anticipated at a rate of 30 ppm, N, the nitrogen treatment was limited to the bottom half of the pot to maintain consistency within treatments. For treatments not receiving nitrogen, 60 grams of barley straw was thoroughly mixed throughout the entire 6,000 grams soil.

Following nutrient and barley straw addition, the soil was placed in individual plastic pots lined with plastic liners. The experiment began 20 August, 1984. Ten lentil seeds, inoculated with Nitrogen Corporation 'C' culture (Rhizobium leguminosarum) in a slurry at many times the recommended rate of 190 grams inoculum per 47 kilograms of seed, were sown at a depth of 2.5 centimeters. Nonnodulating soybeans were also sown at a depth of 2.5 centimeters. The pots were then brought to two-thirds field capacity by weight through the addition of distilled water. Following germination and emergence the soil was brought up to field capacity. For the duration of the experiment, pots were weighed on a daily basis and field capacity was restored by adding distilled water.

Due to space limitations, two growth chambers were used. To avoid confounding the experiment with variations attributable to growth chamber conditions, the pots were rotated on a daily basis between growth chambers. The photoperiod regime in the growth chambers was set at a 16 hour day length and 8 hour night. The light source was Sylvannia cool whites supplemented with 10 percent incandescent. The photosynthetically active radiation was measured to be 550 microeinsteins m^{-2} sec^{-1} at plant canopy height in the first growth

chamber and 500 microeinsteins m^{-2} sec^{-1} in the second. Temperature varied between a daytime temperature of 22°C and a night time temperature of 15°C. Humidity was maintained at 100 percent during the night and 70 percent during the day.

Following emergence, the lentils were thinned to four plants per pot and the nonnodulating soybeans to two plants per pot.

Six harvests were taken from the lentils and nonnodulating soybeans with intervals of eight to twenty days between each harvest. Specific harvest dates and the corresponding developmental stage of the lentils are listed in Table 5. Sampling dates were randomly assigned to three pots within each treatment. Aerial portions were harvested and mixed with any senescent leaves collected prior to harvest. Lentils were separated by hand into seed and straw components. The root portion of the lentils were also collected. Following removal of aerial plant material pots were inverted onto a 2 millimeter screen table. Soil was carefully removed in its entirety from the pot in an attempt to minimize disturbance of the roots. The soil was then washed from the roots using gently running water, directed with a garden hose. The roots were then rinsed in distilled deionized water to remove any adhering soil particles. Degree of nodulation was noted.

The plant material was oven dried in a forced air oven for 48 hours at 60°C. Oven dry weights were determined and plant samples were ground in a Wiley mill to pass through a 2 millimeter sieve. Nitrogen in the plant material is reported on an oven dry basis.

Table 5. Date, days from seeding and developmental stage of lentils of each harvest taken in Growth Chamber Experiment C.

Harvest Number	Date	Days from Seeding	Developmental Stage of Lentils
1	13/9/84	23	Prebud
2	24/9/84	34	Bud
3	2/10/84	42	First Flower
4	15/10/84	55	Bloom
5	30/10/84	70	Pod development
6	19/11/84	90	Ripe seed

3.2 FIELD EXPERIMENTS

3.2.1 Field Experiment 1983

An experiment with lentils and nonnodulating soybeans was conducted during the summer of 1983 under field conditions to investigate the effect of applied nitrogen on nitrogen fixation and yield of lentils. Where nitrogen was applied at a rate of 30 kg N ha^{-1}, determination of nitrogen fixation was made through a series of six harvests.

Soils. The experimental site, designated Roland, was located near Roland, Manitoba (NE 1/4 12-4-5W) on a Gretna Association (Ellis and Shafer, 1943) which is classified as a gleyed Rego Black Chernozem (carbonated phase) in the Canadian System of Soil Classification. A summary of soil characteristics is given in Table 6.

Soil samples were taken in the early spring prior to seeding at depths of 0-15 cm, 15-30 cm, 30-60 cm, 60-90 cm and 90-120 cm in order to determine soil chemical and physical characteristics. Soils were sampled at the four corners of the experimental plot and in several random locations within the plot. Subsamples of soils to be used for nitrate nitrogen analysis were oven dried at 105°C for 24 hours. Soils to be used for analysis other than nitrate nitrogen were air dried. All soil samples were ground to pass through a 2 millimeter sieve.

Experimental Design and Procedure. A randomized complete block design experiment consisting of seven treatments and four replicates was conducted using lentils (*Lens culinaris* var Eston) as the test crop and nonnodulating soybeans (*Glycine max* (L.) maturity group 0) as the reference crop.

Table 6. Summary of physical and chemical characteristics of soil used in Field Experiment 1983.

Site Characteristics	
Legal Description	NE 1/4 12-4-5W
Association	Gretna
Soil Classification	Gleyed Rego Black Chernozem
Textural Class	Clay (light subphase)

Soil Characteristics	Value
pH (0-15 cm)	8.3
Carbonate Content (0-15 cm)	-
Salinity (dS m^{-1}) (0-15 cm)	0.30
Nitrate-nitrogen (kgN ha^{-1}) (0-120 cm)	50.9
$NaHCO_3$ extractable PO_4-P (ppm) (0-15 cm)	10.6
NH_4OAC exchangeable K (ppm) (0-15 cm)	298
SO_4-S (kgS ha^{-1}) (0-60 cm)	110

Treatment plots, with the exception of Treatments 2 and 6, were 1.62 metres wide, 7 metres long and contained nine seed rows at 0.18 metre spacings. Plots for Treatment 2 and Treatment 6 were 5.1 metres wide and contained 27 seed rows. The additional seed rows were included in order to accommodate a series of harvests to be taken from these treatments during the growing season. Blocks were separated by 1.5 meter roadways. Seeding was accomplished using a nine row, double disk Allis Chalmers press drill. Treatments used in this experiment are outlined in Table 7. Nitrogen was applied as urea at rates of 0, 30, 100 and 200 kg N ha^{-1} to lentils and at rates of 0, 30 and 100 kg N ha^{-1} to nonnodulating soybeans. Where nitrogen was applied at a rate of 30 kg N ha^{-1} to lentils (Treatment 2), six treatment subplots were located within each treatment plot. Treatment subplots measured 0.9 metres wide and 1.1 metres long. Within Treatment 2 subplots, a solution of reagent grade urea, labelled with 3.67 atom percent ^{15}N and dissolved in distilled water, was applied using a pressurized hand held sprayer. Establishment of treatment subplots in which ^{15}N labelled urea was applied facilitated the determination of symbiotically fixed nitrogen at six harvest dates. Similarly, where nitrogen was applied at a rate of 30 kg N ha^{-1} to nonnodulating soybeans (Treatment 6), six treatment subplots were located within each treatment plot. Reagent grade urea applied within these treatment subplots was labelled with 1.81 atom percent ^{15}N. Where nitrogen was applied at a rate of 100 kg N ha^{-1} to both lentils (Treatment 3) and nonnodulating soybeans (Treatment 7) only one treatment subplot was located within each treatment plot. Reagent grade urea applied to these

Table 7. Treatment descriptions for lentils and nonnodulating soybeans used in Field Experiment 1983.

Treatment Number	Nitrogen Applied (kg N ha^{-1})	Crop Seeded
1	0	lentil
*2	30	lentil
3	100	lentil
4	200	lentil
5	0	nonnodulating soybean
*6	30	nonnodulating soybean
7	100	nonnodulating soybean

* Indicates larger subplot area designed to accommodate six harvests.

treatment subplots was labelled with 1.73 atom percent ^{15}N. The treatment subplot facilitated a single determination of symbiotically fixed nitrogen at final harvest. Similarly, where nitrogen was applied at a rate of 200 kg N ha^{-1} to lentils (Treatment 4) a single treatment subplot was located within each treatment plot in which reagent grade urea labelled with 1.31 atom percent ^{15}N was applied. Unlabelled prilled urea (46-0-0) was hand broadcast onto the remainder of each of the treatment plots at the respective rate. Nitrogen was incorporated into the soil to a depth of 10 centimeters.

Phosphorus, supplied as triple super phosphate (monocalcium phosphate (0-46-0)) was sidebanded at a rate of 30 kg P_2O_5 ha^{-1} to all treatment plots in an attempt to ensure that this element was not limiting.

Lentils were inoculated with Nitragin Corporation[1] 'C' culture inoculum (Rhizobium leguminosarum), applied in a granular form with the seed. Higher than recommended rates of inoculum were used to ensure good nodulation.

Lentils and nonnodulating soybeans were seeded 27 May, 1983, at rates of 75 kg ha^{-1} and 85 kg ha^{-1}, respectively. Seeding depth was 2.5 centimeters for both crops. Germination and emergence was very poor and uneven due to extreme droughty conditions experienced during the early part of the growing season. In an attempt to ensure germination and seedling establishment, ^{15}N treatment subplots were irrigated by hand on June 9 and June 10. An area measuring 9 centimeters

---

[1] The Nitragin Company, Milwaukee, Wisconsin, U.S.A., 53219.

wide and 110 centimeters long within each 0 N treatment plot was also irrigated. Irrigation was successful in promoting germination and seedling establishment.

Glyphosate (Roundup), was applied at a rate of 0.95 L ha^{-1} prior to seeding. Following emergence, diclofop methyl (Hoegrass) was applied at a rate of 3.75 L ha^{-1}. Weed control was also carried out by mechanical means and by handweeding.

Six harvests of aerial dry matter were taken from the lentils and nonnodulating soybeans receiving nitrogen at a rate of 30 kg N ha^{-1} (Treatment 2 and Treatment 6) with intervals of nine to fourteen days between each harvest. Specific harvest dates and the corresponding developmental stage of the lentils are listed in Table 8. Harvests were taken from within the central rows of the ^{15}N labelled treatment subplots. Three rows, 90 centimeters long were sampled at each harvest. Lentil samples taken at the final harvest were separated into seed and straw components. Plant samples were oven dried in a forced air oven at 60°C for 48 hours and oven dry weights were determined. Plant samples were ground in their entirety in a Wiley mill to pass through a 2 millimeter sieve.

A final harvest was taken 21 August, 1983 (day 86), for all remaining lentil and nonnodulating soybean treatments. Where ^{15}N labelled nitrogen was applied, harvests were taken from within the central rows of the ^{15}N labelled treatment subplots. Three rows, 90 centimeters long were sampled at each harvest. Where nitrogen was not applied, plant samples were taken from three central 90 centimeter rows within the treatment plot. The lentil plant samples were separated into seed

Table 8. Date, days from seeding and developmental stage of lentils at each harvest taken in Field Experiment 1983.

Harvest Number	Date	Days from Seeding	Developmental Stage of Lentils
1	20/6/83	24	Prebud
2	29/6/83	33	Prebud
3	13/7/83	47	First flower
4	27/7/83	61	Early pod development
5	10/8/83	75	Pod development
6	21/8/83	86	Ripe seed

and straw components. Plant samples were oven dried, weighed and ground in their entirety to pass through a 2 millimeter sieve.

All values calculated for yield and nitrogen accumulation were determined on an oven dry basis.

3.2.2 Field Experiments 1984

The effect of nitrogen application on nitrogen fixation, nitrogen accumulation and yield of lentils was studied during the 1984 growing season under field conditions. Two experimental locations, designated Haywood and Morden, were used in this investigation. In addition, a lysimeter experiment in which lentil root development was investigated, was carried out at the Haywood site. The objective of the lysimeter experiment was to determine dry matter and nitrogen accumulation in the roots of lentils. The use of ^{15}N labelled fertilizer facilitated the determination of symbiotically fixed nitrogen. The contribution of symbiotically fixed nitrogen accumulated in the roots to total plant nitrogen was determined.

Soils. Experimental sites for the field experiments were established in the spring of 1984 at two locations in Manitoba. One site, designated Morden, was established near Morden, Manitoba (SW 1/4 35-3-5W) on an Altona series (Smith and Michalyna, 1973) which is classified as an Orthic Black Chernozem in the Canadian System of Soil Classification. A second site, designated Haywood, was established near Haywood, Manitoba (NE 1/4 25-8-6W) on an Almasippi series (Ehrlich et al. 1957), which is classified as a gleyed Rego Black Chernozem in the Canadian System of Soil Classification. A summary of soil characteristics is given in Table 9.

Table 9. Summary of physical and chemical characteristics of soils used in Field Experiments 1984.

Site Characteristics	Morden	Haywood
Legal Description	SW 1/4 35-3-5W	NE 1/4 25-5-9-6W
Soil Series	Altona	Almasippi
Soil Classification	Orthic Black Chernozem	gleyed Rego Black Chernozem
Textural Class	fine sandy loam	loamy very fine sand

Soil Characteristics	Value	Value
pH (0-15 cm)	8.2	8.1
Carbonate Content (0-15 cm)	-	-
Salinity (dS m^{-1}) (0-15 cm)	0.2	0.2
Nitrate-nitrogen(kgN ha^{-1}) (0-120 cm)	34.6	63.5
$NaHCO_3$ exchangeable PO_4-P (ppm) (0-15 cm)	5.6	5.12
NH_4OAC exchangeable (ppm) (0-15 cm)	175.5	58.7
SO_4 - S (kg S ha^{-1}) (0-60 cm)	23.8	30.8

Soil samples were taken in the early spring prior to seeding at depths of 0-15 cm, 15-30 cm, 30-60 cm, 60-90 cm and 90-120 cm in order to determine chemical and physical characteristics of the soil. Soils were sampled at the four corners of each experimental plot and in several random locations within the plots. Subsamples of soils to be used for nitrate-nitrogen analysis were oven dried at 105°C for 24 hours. Soils to be used for analysis other than nitrate-nitrogen were air dried. All soil samples were ground to pass through a 2 millimeter sieve prior to analysis.

Experimental Design and Procedure - Nitrogen Rate Study. A randomized complete block design consisting of seven treatment and four replicate was used at both experimental locations. Lentils (Lens culinaris var Eston) were used as the test crop and nonnodulating soybeans (Glycine max. (L.), Clay maturity type) served as the reference crop. Treatment plots were 3.2 meters wide and 7.0 meters long and contained 18 seed rows at 18 centimeter spacings. Blocks were separated by 1.5 meter roadways, seeded to Columbus wheat. Experimental sites were surrounded by 3.2 meter guard rows seeded to Columbus wheat. Seeding was accomplished using a nine row, double disk Allis Chalmers press drill.

Nitrogen was applied as urea at rates of 0, 30, 90, and 180 kg N ha^{-1} to lentils and at rates of 0, 30 and 90 kg N ha^{-1} to nonnodulating soybeans. Treatment subplots were established within some of the treatment plots in which ^{15}N labelled urea was applied. Application of urea labelled with ^{15}N facilitated the determination of symbiotically fixed nitrogen. Treatments used at both Morden and Haywood are outlined in Table 10.

Table 10. Treatment descriptions for lentil and nonnodulating soybeans used in Field Experiments 1984.

Treatment Number	Nitrogen Applied (kg N ha^{-1})	Crop Seeded	Number of ^{15}N labelled subplots		Atom percent ^{15}N excess in fertilizer applied to subplots
			Morden	Haywood	
1	0	lentil	0	0	-
2	30	lentil	2	5	3.39
3	90	lentil	1	2	1.73
4	180	lentil	0	1	1.98
5	0	nonnodulating soybean	0	0	-
6	30	nonnodulating soybean	2	5	1.55
7	90	nonnodulating soybean	1	1	1.73

Treatment subplots measured 90 centimeters wide and 110 centimeters long. A solution of reagent grade urea labelled with ^{15}N was dissolved in distilled water and sprayed on to lentil subplots using a hand held pressurized sprayer at rates of 30, 90 and 180 kg N ha^{-1}. The urea was labelled with 3.39, 1.73 and 1.98 atom percent ^{15}N excess for the 30, 90, and 180 kg N ha^{-1} rates of application, respectively. Similarly, urea labelled with 1.55 and 1.73 atom percent ^{15}N excess was applied to soybean subplots at rates of 30 and 90 kg N ha^{-1}, respectively. Unlabelled prilled urea (46-0-0) was hand broadcast onto the remainder of each treatment plot at the respective rate.

Phosphorus, supplied as triple super phosphate (monocalcium phosphate (0-46-0)) was hand broadcast at a rate of 100 kg P_2O_5 ha^{-1} to all treatment plots at both sites. Potassium, supplied as muriate of potash (0-0-60), was hand broadcast at a rate of 200 kg K_2O ha^{-1} at the Haywood site. Application of nutrients other than nitrogen was done to insure that these nutrients would not be limiting. Fertilizers were incorporated to a depth of 10 centimeters.

Lentils were inoculated with Nitragin Corporation[1] 'C' culture inoculum (Rhizobium leguminosarum). To ensure good nodulation, the inoculant was applied as a slurry at many times the recommended rate of 190 grams inoculum per 47 kilograms of seed.

Lentils and nonnodulating soybeans were seeded 22 May, 1984 at the Haywood site and 23 May, 1984 at the Morden site. Lentils and nonnodu-

---

[1] The Nitragin Company, Milwaukee, Wisconsin, U.S.A., 53219.

lating soybeans were seeded at rates of 50 kg ha^{-1} and 100 kg ha^{-1}, respectively.

Following emergence, diclofop methyl (Hoegrass) was applied at a rate of 3.75 L ha^{-1} at both sites. Weed control was also carried out by mechanical means and handweeding.

A series of harvests of aerial dry matter were taken from both the lentils and nonnodulating soybeans during the growing season. Where ^{15}N labelled nitrogen was applied, the number of harvests taken corresponds to the number of ^{15}N labelled subplots. Where no nitrogen was applied (Treatment 1 and Treatment 5), five harvests were taken during the growing season. Specific harvest dates and the corresponding developmental stage of the lentils are listed in Table 11. Harvests of ^{15}N enriched plant material were taken from within the central rows of the ^{15}N labelled subplots. Three rows, 90 centimeters long were sampled at each harvest. Where no ^{15}N labelled nitrogen was applied, plant samples were taken from three central 90 centimeter rows within the treatment plot. Lentil samples taken at the final harvest were separated into seed and straw components. Plant samples were oven dried in a forced air oven at 60°C for 48 hours and oven dry weights were determined. Plant samples were ground in their entirety in a Wiley mill to pass through a two millimeter sieve. All values calculated for yield and nitrogen accumulation were determined on an oven dry basis. Acetylene reduction determinations were carried out on July 10, July 19, and August 4 at both sites. A final acetylene reduction determination was done on August 10 and August 21 at Morden and Haywood, respectively. Acetylene reduction determinations were

Table 11. Date, days from seeding and developmental stage of lentils at each harvest taken in Field Experiments 1984.

	Rate of Nitrogen Fertilizer Applied (kg N ha^{-1})															
	0				30				90				180			
Developmental	Haywood		Morden		Haywood		Morden		Haywood		Morden		Haywood		Morden	
Stage of Lentils	Date	Days*	Date	Days	Date	Days	Date	Days	Date	Days	Date	Days	Date	Days	Date	Days
Bud	10/7/84	49	10/7/84	48	10/7/84	49										
Early Bloom	19/7/84	58			19/7/84	58										
Bloom	27/7/84	66	19/7/84	57	27/7/84	66	19/7/84	57	27/7/84	66	19/7/84	57	27/7/84	66	19/7/84	57
Early Pod	4/8/84	74	27/7/84	65	4/8/84	74										
Pod Develop			4/8/84	73												
Ripe Seed	21/8/84	91	10/8/84	79	10/8/84	91	10/8/84	79	21/8/84	91	10/8/84	79	21/8/84	91	10/8/84	79

* Indicates days after seeding

replicated four times within each treatment plot. Sixteen plants were selected at random from within each treatment plot. The entire plant was dug up using a spade and roots were extracted to a depth of approximately 25 centimeters. Soil adhering to the roots was removed by hand and the aerial portion of the plant was detached. Four roots were then placed in a one liter mason jar fitted with a serum stopper in the lid. The jar was sealed and 20 milliliters of acetylene was injected into the jar with a graduated 30 milliliter syringe. The jars were placed in the shade to prevent dessication of nodules and allowed to incubate for one hour. Following incubation, 20 milliliters of gas were removed from the jar. Ten milliliters of the gas sample were dispelled and the remaining 10 milliliters were injected into a 10 milliliter vacutainer.[2] Gas samples were stored in the vacutainers until analyzed on a gas chromatograph.

Experimental Design and Procedure - Lysimeter Study. A lysimeter study was conducted immediately adjacent to the Haywood field site in which lentils were grown as the test crop and nonnodulating soybeans served as a reference crop. Five harvests were taken during the field season. At each harvest date, four lysimeters containing lentils and four lysimeters containing soybeans, were harvested.

The lysimeters were hollow steel cylinders measuring 30.5 cm long and had a cross sectional area of 0.10 m^2. Lysimeters were arranged in 10 rows with 4 lysimeters in each row, and 30 centimeter spacings

---

[2] Becton Dickinson and Co. Canada Ltd., 2464 South Sheridan Way, Mississauga, Ontario, L5J 2M8.

between each lysimeter. Although ideally lysimeters should have been designated for the lentils and nonnodulating soybeans in a random fashion, the fact that the entire lysimeter was to be removed at each harvest put constraints on the experimental design and thus lentils were seeded in a block of twenty lysimeters and nonnodulating soybeans were seeded in the remaining block.

The top 10 centimeters of soil were removed from each lysimeter and spread on a plastic sheet. Urea, labelled with ^{15}N and dissolved in distilled water, was sprayed onto the soil at rate of 30 kg N ha^{-1} using a hand held sprayer. The urea was labelled with 3.30 and 1.70 atom percent ^{15}N excess for lentils and nonnodulating soybeans, respectively. Phosphorus, supplied as super triple phosphate (monocalcium phosphate (0-46-0)) was applied in a granular form at a rate of 100 kg P_2O_5 ha^{-1}. The soil was thoroughly mixed and replaced into each lysimeter. Lentils were inoculated with Nitragin Corporation 'C' culture inoculum (Rhizobium leguminosarum). The inoculant was applied as a slurry at many times the recommended rate of 190 grams inoculum per 47 kilograms seed to ensure good nodulation. Lentils and nonnodulating soybeans were seeded on 1 June, 1984. Twenty-five lentil seeds were seeded at a depth of 2.5 centimeters into each of 20 lysimeters. Fifteen nonnodulating soybean seeds were seeded at a depth of 2.5 centimeters into each of the remaining twenty lysimeters. The area surrounding the lysimeters and extending 2 meters beyond the border of the lysimeter study was seeded to lentils and soybeans.

Following germination and emergence lentils and nonnodulating soybeans were thinned to 14 and 7 plants per lysimeter, respectively.

Lysimeter harvests were taken at the same time as Haywood field experiment harvests. Thus five harvests were taken during the growing season at bud, early bloom, bloom, early pod, and ripe seed stages of lentils. At each stage, the aerial dry matter from four lentil lysimeters and four nonnodulating soybeans lysimeters was harvested. Lysimeters were then excavated and removed without disturbing soil and roots contained within.

Intact lysimeters were placed on a sheet of nylon mesh to facilitate transfer. Lysimeters were placed in large capacity containers and water was added to flood the soil. After 24 hours, intact lysimeters were transferred to a 2 mm screen table. The soil was then washed from the roots using gently running water, directed with a garden hose. The roots were then rinsed in distilled water to remove any adhering soil particles. Degree of nodulation was noted.

Plant samples were oven dried in a forced air oven at 60°C for 48 hours and oven dry weights were determined. Samples were ground in their entirety in a Wiley mill to press through a 2 millimeter sieve.

3.3 ANALYTICAL PROCEDURES

3.3.1 Soil Analysis

Soil Texture. Soil texture was estimated by hand on unground soil samples.

Soil pH. Soil pH was determined on a 1:3 soil to water paste using a standard glass calomel combination electrode.

Soil Salinity - Soil salinity was determined on a 1:3 soil to water

paste using a Fisher Combination electrode on a Radiometer conductivity meter.

Calcium Carbonate Content. An estimation of the calcium carbonate content was made on the basis of the degree of effervescence of a soil sample when treated with a 1:3 HCl to water solution.

Soil Nitrate Nitrogen. Soil nitrate nitrogen was determined by hydrazine reduction on a Technicon Auto Analyzer System using a modification of the automated colorimetric procedure of Kamphake et al. (1967).

Available Phosphorus. $NaHCO_3$ extractable phosphorus was determined by a modification of the method proposed by Olsen et al. (1954). Five grams of soil were extracted with 100 milliliters of 0.5 M $NaHCO_3$ (pH 8.5) and one gram of pretreated charcoal. The samples were shaken for 30 minutes, filtered through Whatman no. 42 paper, and the phosphorus level in the extractant determined colorimetrically by the acid molybdate - ascorbic acid method (Murphy and Riley 1962). The absorbance was read using a spectrophotometer at 885 nm.

Exchangeable Potassium. NH_4OAc extractable K was determined using a modification of the procedure described by Pratt (1965). Five grams of soil were shaken with 100 milliliters of a solution containing 1.0 M NH_4OAc and 250 ppm $LiNO_3$ for one hour. The samples were filtered and potassium content of the extractant was determined using a Perkin-Elmer model 303 atomic absorption spectrophotometer.

Sulfate Sulfur. Fifty milliliters of 0.001 M $CaCl_2$ extracting solution was added to 25 grams of soil. The solution was shaken for 30 minutes and then filtered through Whatman no. 42 paper. Calcium

chloride extractable sulfate sulfur was determined using the barium chloride turbidimetric method as described by Lazrus et al. (1966) on a Technicon Auto Analyzer II at 460 nm.

Field Capacity. Air dry soil was placed in a 20 centimeter plastic cylinder which had a 4.5 centimeter diameter. The bottom of the cylinder was covered by a wire mesh which prevented loss of soil. Water was added to the surface of the soil until the wetting front had moved to the midpoint of the soil column. The open end of the cylinder was then covered with parafilm to prevent evaporation and the soil column was left undisturbed for 48 hours. A soil sample was then taken from the center of the wetted soil and wet weight was determined. The sample was then oven dried at 105°C for 48 hours and reweighed. Field capacity was calculated on an oven dry basis.

Permanent Wilting Percentage. Moisture content of a soil sample at 1500 kPa (θ_{1500}) was determined using a pressure membrane apparatus. Permanent wilting percentage was then calculated using the equation proposed by Shaykewich (1965):

$$PWP = 0.0207 + 0.77468\ (\theta_{1500})$$

3.3.2 Plant Analysis

Total Nitrogen Content. Total nitrogen content of plant material was determined by a modified Kjeldahl-Gunning method as described by Jackson (1958). Digestion was accelerated using Special Kjeltabs S3,5 which contained 3.5 grams K_2SO_4 and 0.0035 grams Se. Nitrogen content was determined using a Tecator Kjeltec Auto 1030 Analyzer.

Excess Atom Percent ^{15}N. Nitrogen contents of ^{15}N enriched

plant material and ^{15}N enriched fertilizer standards either obtained or used in Field Experiment 1983 and Growth Chamber Experiment C were determined by a modification of the Kjeldahl-Gunning method as described by Jackson (1958). The modification consisted of using 50 milliliters of 0.1 N H_2SO_4 instead of 50 milliliters of 2% H_3BO_3 - indicator solution to trap the ammonium nitrogen liberated by distillation. The solution was then back titrated with 0.1 N NaOH. For ^{15}N enriched plant material and ^{15}N enriched fertilizer standards obtained or used in Field Experiments 1984, nitrogen content was determined using a Tecator Kjeltec Auto 1030 Analyzer, modified to accommodate the back titration.

Titrated distillate collected was acidified with one drop of concentrated H_2SO_4. Samples were then evaporated to a volume of not more than 10 milliliters and transferred into glass test tubes to await ^{15}N analysis.

Nitrogen gas was prepared from the samples by a modification of the method described by Bremner (1965). This modification consisted of discarding the use of liquid N_2 to freeze out water vapour and gaseous by-products of hypobromite oxidation. Alternatively, water vapour was removed from the sample by approximately 10 milliliters of concentrated H_2SO_4 contained in the collection tube, as suggested by C.M. Cho[1].

A Micromass 602 mass spectrometer, equipped with a Speedomax chart recorder was used in the analysis of N_2 gas for $^{14}N/^{15}N$

---

[1] C.M. Cho, Professor, Department of Soil Science, University of Manitoba, Winnipeg, Manitoba, R3T 2N2.

ratios. Ion current intensities of atomic mass unit twenty-eight ($^{14}N^{14}N$) and atomic mass unit twenty-nine ($^{14}N^{15}N$) were determined using a single collector scanning method. Peak heights were measured and atom percent ^{15}N was calculated using the equation (see Bremner (1965)):

$$(3) \quad \text{Atom Percent } ^{15}N = \frac{100}{2R+1}$$

where R is given by the ratio of ion current intensities corresponding to atomic mass unit twenty-eight and atomic mass unit twenty-nine. Thus R is represented by the following equation:

$$(4) \quad R = \frac{^{14}N^{14}N}{^{14}N^{15}N}$$

In order to determine atom percent ^{15}N excess in the enriched sample material, percent natural ^{15}N abundance was determined for samples which had not been enriched with ^{15}N. Measured natural abundance of ^{15}N using the mass spectrometer (Micromass 602) was found to be 0.382, rather than the quoted value of 0.366 (Hauck and Bremner, 1976). Thus, excess atom percent ^{15}N was calculated by subtracting 0.382 from percent ^{15}N abundance in the enriched material.

Percent Nitrogen Derived from Fertilizer (Ndff). Atom percent ^{15}N excess in the plant material is expressed in terms of the original fertilizer added. Thus:

$$\%\text{Ndff} = \frac{(\%\ ^{15}N \text{ excess in enriched plant material})}{(\%\ ^{15}N \text{ excess in enriched N fertilizer})} \times 100$$

In order to calculate the nitrogen fertilizer accumulated in the plant material, % Ndff, dry matter yield and percent nitrogen were determined. Thus:

Nitrogen Fertilizer Accumulated (kg N ha^{-1}) =

Yield (Kg ha^{-1}) x $\frac{\%N}{100}$ x $\frac{\%\ Ndff}{100}$

3.3.3 Ethylene Analysis

Gas samples prepared using the acetylene reduction assay were analyzed on a Varian Model 3700 gas chromatograph equipped with a Hewlett Packard 3390 A intergrator unit. Operational conditions for the gas chromatograph are reported in Table 12. A one milliliter gas sample was injected into the gas chromatograph using a gastight locking syringe.[1] Calibration gas[2] contained 100 ppm ethylene in nitrogen gas. Quantity of ethylene in the sample was determined by measuring the ethylene peak area of the sample relative to the peak area of the external ethylene standard.

---

1 Supplier: Scientific Glass Engineering Co. Ltd., 1-3 Baillie St., North Melbourne, Victoria, Australia.

2 Supplier: Alltech Associates, Inc. Applied Science Labs. 2051 Waukegan Road, Deerfield, Il., 60015.

Table 12. Gas Chromatograph Run Parameters.

Column Material - glass
Column Support - Poropak T
Column Dimensions - length 1.83m, external diameter 6 mm, internal diameter 2mm.
Injector Temperature - 70°C
Detector Temperature - 100°C
Oven Temperature - 40°C
Carrier Gas (N_2) Flow Rate - 20mL min^{-1}
Hydrogen Gas Flow Rate - 20mL min^{-1}
Air Flow Rate - 300mL min^{-1}
Range - 10^{-11}mV
Chart Speed - 0.5cm min^{-1}

4. RESULTS AND DISCUSSION

4.1 GROWTH CHAMBER EXPERIMENTS

4.1.1 Growth Chamber Experiment A

Lentils have an indeterminate growth habit and it has been reported that some stress is required during flowering to stimulate heavy pod set (Slinkard and Drew, 1982; Saskatchewan Agriculture, 1985). It is presently recommended in Saskatchewan that a nitrogen stress may stimulate heavy pod set and may be induced by seeding early on cereal stubble (Saskatchewan Agriculture, 1985). The objective of this experiment was to investigate the effect of nitrogen addition and availability on nitrogen uptake, dry matter yield, seed yield, harvest index and nitrogen fixation in lentils. Nitrogen stress was induced by adding barley straw to the soil. Quantity of symbiotically fixed nitrogen was estimated using the Classical Difference method. Nonnodulating soybeans served as a reference crop.

The addition of supplemental nitrogen was found to be an important factor in increasing dry matter yield of lentils (Table 13). Significant dry matter yield increases were obtained with the addition of 360 ppm N. Although not significant, the data suggest that addition of 90 ppm N also resulted in dry matter yield increases. Application of 30 ppm N did not significantly affect lentil dry matter yield. Amendment of the soil with barley straw resulted in a significant depression in lentil dry matter yield. Lentils grown in soil amended with barley straw were notably affected during early developmental stages. As early

Table 13. Effect of nitrogen availability on dry matter yield of lentil and nonnodulating soybean shoots (Growth Chamber Experiment A).

Nitrogen rate (ppm)	Dry matter yield (g pot^{-1})			
	Lentils		Nonnod soybeans	
0	59.0 b[1]	A[2]	20.0 d	B
0 (1% barley straw)	44.8 c	A	6.3 e	B
30	61.8 b	A	32.7 c	B
90	67.4 ba	A	62.4 b	A
360	72.5 a	B	118.4 a	A

1 Means followed by the same lowercase letter in each column are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

2 Means followed by the same uppercase letter in each row are not significantly different at P = 0.05 (t-test).

as ten days after seeding, lentils began to show signs of N stress, developing a deep purple discoloration on the stem and the midvein of the leaves. Eighteen days after seeding lentils grown on soil treated with barley straw had developed chlorotic lower leaves and were notably stunted in development. However, signs of recovery from early N deficiency were observed approximately twenty-seven days after seeding. At this time N stressed lentils initiated new vegetative growth and began branching. In contrast, nonnodulating soybeans did not show any signs of recovery from the N stress and thus it is possible that lentil recovery was due to the establishment and functioning of N fixing nodules. However, the severity of the initial N deficiency was such that lentils were unable to fully recover despite indications of the occurrence of N fixation and thus the initial N stress was manifested in reduced dry matter yields. Similar results were reported by Hatfield et al. (1974) who found that dry weight of inoculated soybeans grown in a nitrogen free solution was significantly lower than that of inoculated soybeans receiving N during early growth stages. They suggested that reduced growth was attributable to a time lag in the development of nitrogen fixing nodules. Similarly, Pate and Dart (1961) postulated that the positive effects of applied nitrogen on cowpea symbiosis are explained in terms of an alleviation of the "N hunger" stage of growth during early development prior to the onset of significant nodule fixation and are manifested in significant dry matter yield increases.

Supplemental nitrogen was also shown to be an important factor in increasing nonnodulating soybean dry matter yields (Table 13). Increasing increments of applied N resulted in concomitant dry matter

yield increases. Although the application of 30 ppm N did not result in increased lentil dry matter yield, a significant increase in nonnodulating soybean dry matter yield was obtained. Thus the data suggest that the application of 30 ppm N resulted in decreased fixation so that there was no net gain in the quantity of N available to the lentils. Amendment of the soil with barley straw significantly reduced nonnodulating soybean yields. Throughout the duration of the experiment these nonnodulating soybeans were visibly N deficient being both severely chlorotic and stunted, and plants did not develop beyond four trifoliate leaves.

Lentil dry matter yields were significantly greater than the corresponding nonnodulating soybean yields where N was applied at rates of 0 and 30 ppm and where barley straw was added. It is likely that increased lentil dry matter yields resulted from the additional symbiotically fixed N source.

Lentils and nonnodulating soybeans shared similar dry matter yields where N was applied at a rate of 90 ppm. A further increase in the rate of N application resulted in significantly higher nonnodulating soybean yields than corresponding lentil yields. Thus the data suggest that at the highest rate of applied N, lentil dry matter yield was limited by its maximum yield potential whereas nonnodulating soybeans were capable of continued response to applied N.

Although data suggest that application of 90 and 360 ppm N led to increased N accumulation in lentil shoots, positive N yield responses were not statistically significant (Table 14). Application of 30 ppm N did not increase N accumulation in lentil shoots, suggesting that

Table 14. Effect of nitrogen availability on nitrogen accumulation in lentil and nonnodulating soybean shoots (Growth Chamber Experiment A).

Nitrogen rate (ppm)	Nitrogen accumulation (mg pot^{-1})	
	Lentils	Nonnod soybeans
0	1393 a[1] A[2]	142 dc B
0 (1% barley straw)	1010 b A	72 d B
30	1348 a A	243 c B
90	1522 a A	457 b B
360	1537 a A	1642 a A

[1] Means followed by the same lowercase letter in each column are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

[2] Means followed by the same uppercase letter in each row are not significantly different at P = 0.05 (t-test).

fertilizer nitrogen lessened fixation of atmospheric N to the extent that there was no net gain in nitrogen available to the plant. Addition of barley straw to the soil resulted in a significant reduction in N accumulation, indicating that symbiotic N fixation alone was not sufficient to meet lentil N requirements. Thus data suggests that some nitrogen is required from either soil and (or) fertilizer N to encourage the early establishment of lentils, thereby increasing N yields.

Nitrogen accumulated in nonnodulating soybeans closely reflected dry matter yields (Table 14). Increasing increments of applied N resulted in concomitant nitrogen yield increases. Amendment of the soil with barley straw resulted in reduced N yield.

Significantly more nitrogen was accumulated by the lentils than the soybeans at all but the 360 ppm N rate of application. Thus it is suggested that at all but the 360 ppm N rate of application, symbiotically fixed N contributed to the improved N nutrition of lentils. Where N was applied at a rate of 360 ppm, nitrogen accumulation in lentils and nonnodulating soybeans was not significantly different, indicating that N fixation in lentils was inhibited by the high rate of N application.

Where nitrogen was applied at a rate of 90 ppm N, nitrogen yield of lentils was significantly greater than that of the nonnodulating soybeans, despite similar dry matter yields. Furthermore, application of N at a rate of 360 ppm did not significantly increase either dry matter or N yield of lentils. In contrast, both nonnodulating soybean parameters were significantly increased by the addition of 360 ppm N. Thus the data indicate that at 90 and 360 ppm N, lentil yield was limited by yield potential whereas nonnodulating soybean yield was limited by N

availability. Hence, it is likely that nonnodulating soybeans were able to take up all of the available N in the pot.

Although responses were not statistically significant, lentil seed yields tended to increase with increasing increments of applied N (Table 15). The lowest seed yield was obtained where soil was amended with barley straw. These results are in direct contrast to the recommendation that a nitrogen stress, induced by early seeding on cereal stubble, is required to stimulate heavy pod set (Saskatchewan Agriculture, 1985).

Similarly, nitrogen accumulation in the seed was also significantly reduced by amending the soil with barley straw (Table 15). Although seed N increased with the addition of 90 and 360 ppm N, responses were not statistically significant. The addition of 30 ppm N did not significantly affect N accumulation in the seed. Thus it is concluded that N fixation alone can not meet lentil seed N requirements.

Percent protein in the seed was not significantly affected by any treatment (Table 15). However, the lowest percentage protein was realized where lentils were grown on soil amended with barley straw.

Harvest index, which describes the ratio of seed yield to total dry matter yield, was greatest where lentils were grown in soil amended with barley straw (Table 15). Thus, under conditions of N stress, production of seed made a greater contribution to total dry matter yield as compared to the remaining treatments. Although statistically significant differences were not obtained, the data suggest that high rates of applied N may stimulate vegetative growth to a greater degree than reproductive growth.

Table 15. Effect of nitrogen availability on seed yield, nitrogen accumulation in seed, percent protein of seed and harvest index (Growth Chamber Experiment A).

Nitrogen rate (ppm)	Seed yield ($g\ pot^{-1}$)	N accumulation in seed ($mg\ pot^{-1}$)	Percent protein	Harvest index (%)
0	27.2 ab[1]	1089 a	25.0 a	46.1 b
0 (1% barley straw)	23.6 b	858 b	22.6 a	52.8 a
30	27.4 ab	1046 a	23.8 a	44.4 b
90	30.6 a	1193 a	24.3 a	45.4 b
360	31.1 a	1189 a	23.8 a	42.9 b

[1] Means followed by the same lowercase letter in each column are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

Symbiotically fixed nitrogen was determined using the Classical Difference method and is reported in Table 16. Maximum accumulation of symbiotically fixed N was realized where fertilizer N was not added to the soil. Although not statistically significant, application of fertilizer N at rates of 30 and 90 ppm N resulted in a slight reduction in the quantity of symbiotically fixed N accumulated in the lentils. Application of 360 ppm N completely inhibited symbiotic fixation. These results are in agreement with a number of studies in which diminished fixation resulted from the application of fertilizer N (Allos and Bartholemew, 1955; McAuliffe et al., 1958; Richards and Soper, 1979).

Amendment of the soil with barley straw significantly reduced the accumulation of symbiotically fixed N. This reduction indicates that some N may be necessary for the establishment and functioning of a lentil-Rhizobium symbiotic system. The N requirement of the symbiotic relationship appeared to have been met by soil N alone as the treatment receiving no nitrogen accumulated the greatest amount of fixed N. The soil used in this experiment contained only 12.6 ppm nitrate nitrogen and thus it is concluded that although there is a requirement for some available N to aid in the establishment and functioning of lentil-Rhizobium symbiosis, the N requirement will likely be met by soils containing medium to high levels of available soil N. Enhanced fixation resulting from low levels of starter N has been reported by a number of authors and has been attributed to the alleviation of the "N hunger" stage prior to the onset of significant nodule fixation (Dart and Wildon, 1970; Agboola, 1978; Eaglesham et al., 1983). In addition, Allos and Bartholemew (1959) suggested that total fixation is closely

Table 16. Effect of nitrogen availability on the accumulation of symbiotically fixed nitrogen, as determined by the Classical Difference method (Growth Chamber Experiment A).

Nitrogen rate (ppm)	Accumulation of symbiotically fixed nitrogen (mg pot^{-1})
0	1251 a[1]
0 (1% barley straw)	938 b
30	1105 ab
90	1065 ab
360	-105 c

[1] Means followed by the same lowercase letter in each column are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

related to the amount of growth. Thus, if elevation of the N hunger stage leads to larger, more vigorous plants, as was observed in this experiment, there may be a tendency for plants to fix more nitrogen.

Data indicate that lentils grown in soil ammended with barley straw derived 93 percent of the total plant N from symbiotic fixation (Table 17). The contribution of symbiotically fixed N to total plant N diminished with increasing increments of applied N and made no detectable contribution at the highest rate. With the exception of the 360 ppm N treatment, calculated values for percent nitrogen derived from fixation were exceptionally high. Conditions of the growth chamber were essentially ideal and thus it is unlikely that symbiotically fixed N would make as large a contribution to total crop N under field conditions.

In contrast to the present Saskatchewan Agriculture (1985) recommendations, results from this experiment did not indicate that lentil seed yields may benefit from an induced nitrogen stress. Furthermore, it was found that N stress had detrimental effects on the accumulation of symbiotically fixed N. Although seed yields were not significantly affected by the application of fertilizer N, dry matter yields were found to increase significantly, which indicates that symbiotic fixation did not meet lentil N requirements. Accumulation of symbiotically fixed N diminished with increasing increments of applied N and was completely inhibited by the application of 360 ppm N.

Table 17. Effect of nitrogen availability on percent nitrogen derived from symbiotic nitrogen fixation in lentil shoots (Growth Chamber Experiment A).

Nitrogen rate (ppm)	Percent nitrogen derived from fixation
0	90
0 (1% barley straw)	93
30	82
90	70
360	-

4.1.2 Growth Chamber Experiment B

A growth chamber experiment was conducted in which lentils were subjected to moisture stress applied at different physiological stages. Lentils have an indeterminate flowering habit and it has been suggested that some drought stress is required in the latter part of the flowering process in order to encourage maturation of younger pods and stimulate heavy pod set (Slinkard and Drew, 1982). The objective of this experiment was to study the effects of moisture stress on dry matter, seed and nitrogen yield.

Maturity is difficult to define for lentils due to indeterminacy and complex flowering responses. In this experiment it was observed that rewatering of moisture stressed plants stimulated rapid vegetative growth and a new flush of flowers for all moisture stress treatments. This resurgence of growth effectively extended the bloom stage despite the presence of ripe seed. Thus, for the purpose of this experiment, maturity was defined as the stage at which flowers were no longer present on the plants and were not encouraged to develop even after rewatering. Using this definition, it was observed that water stress lengthened the time required to reach maturity. Unstressed plants had reached maturity 86 days after seeding, whereas stressed plants required 95 days to reach maturity.

Little difference existed between the three moisture stress treatments in terms of length of time required to reach maturity. There were, however, observable differences in the morphology of the plants stressed at different physiological stages. It was observed that lentils allowed to wilt repeatedly from the prebud stage until maturity

developed smaller, more vertically oriented leaves. Reduced leaf size and vertical leaf orientation effectively reduces leaf area and thus are accompanied by a reduction in water loss due to evapotranspiration. Both mechanisms for drought avoidance are commonly exhibited by cowpea (Shackel and Hall, 1979; Turk et al., 1980; Turk and Hall, 1980a 1980b).

Wilting during bloom and early pod fill did not result in a reduction of leaf size as the majority of the vegetative development was complete. However, extensive leaf loss occurred for both treatments shortly after the water was withheld. Thus, at later growth stages lentils responded to moisture stress by reducing leaf area through the abscission of leaves.

Although decreased leaf area reduces the area over which evapotranspiration can occur, it is accompanied by a loss in photosynthetic area. Sprent (1972) postulated that reduced photosynthate production from leaves of water stressed plants may lead to a decrease in nitrogen fixation. This contention was supported by Huang et al. (1975a, 1975b) who found that reduction in photosynthate production by soybeans during periods of water stress led to reductions in nitrogen fixation. Decreased leaf size and (or) leaf drop resulted in decreased photosynthetic area and thus may have led to a reduction in nitrogen fixation in the water stressed plants.

In addition to reducing the area available for photosynthetic activity, extensive leaf loss associated with moisture stresses applied at bloom and pod fill presumably decreased the nitrogen available for translocation out of the leaves to the reproductive structures and thus may have limited seed development. Moisture stress applied at flowering

also resulted in the abortion of floral structures, thereby decreasing pod formation. Where stress was applied during the vegetative stage, fewer flowers wilted and abortion of floral structures did not occur, suggesting that the early stressed lentils developed a drought avoidance mechanism by which flowering was restrained and thus plants did not put a large demand on the limited moisture supply.

Dry matter yields were significantly decreased by the application of moisture stress, regardless of developmental stage at which stress was applied (Table 18). The lowest yield was obtained where lentils were stressed for the longest duration, namely from the prebud growth stage. The reduction in dry matter was due to both reduced leaf and total plant size produced in response to the limited water supply. An attempt was made to collect any senescent leaves and thus reductions in dry matter do not simply reflect loss of leaf dry matter. Lentils which were stressed at bloom and early pod dvelopment had, to a large degree, completed vegetative growth at the time of the stress and thus significant reduction in total dry matter were attributable primarily to reduced seed yields.

Nitrogen accumulation was also observed to be significantly reduced in response to moisture stress (Table 18). No significant differences in the quantity of nitrogen accumulated existed between the three moisture stress treatments. No attempt was made to measure nitrogen fixation and thus conclusions regarding the contribution of fixed N to total nitrogen accumulated by the moisture stressed lentils can not be drawn. However, the literature suggests a number of mechanisms by which the application of a moisture stress may have resulted in a decrease in

Table 18. Effect of water stress applied at different physiological stages on dry matter and nitrogen accumulation in lentil shoots (Growth Chamber Experiment B).

Physiological stage at water stress	Dry matter accumulation (g pot^{-1})	Nitrogen accumulation (mg pot^{-1})
No moisture stress applied	61.8 a[1]	1348 a
Prebud	36.3 c	773 b
Bloom	41.5 b	811 b
Early pod development	40.2 cb	780 b

[1] Means followed by the same letter in each column are not significantly different at P = 0.05. (Duncan's Multiple Range Test).

nitrogen accumulation. Firstly, application of moisture stress may have restricted export of nitrogenous compounds from the roots to the shoots thereby resulting in an apparent decline in N accumulation. Unfortunately, root portions were not recovered and thus the contribution of root N to total plant N could not be determined. Alternatively it was observed that moisture stress limited growth, particularly when applied at the prebud stage. Thus the nitrogen requirement of the stressed lentils was likely limited by the moisture supply and subsequently N uptake from soil and fertilizer sources, as well as symbiotic sources, may have been reduced. Allos and Bartholomew (1959) postulated that total fixation is closely related to the amount of growth. Thus in the smaller, moisture stressed lentils, nitrogen fixation may have been limited. Depression in N accumulation may also reflect inhibition of symbiotic N fixation as a result of decreased photosynthate available to the nodule, as suggested by Sprent (1972) and Huang et al. (1975a, 1975b). Loss of nodule functioning in direct response to moisture stress has been observed in numerous studies and has been cited as the mechanism by which nitrogen accumulation may be limited in moisture stressed plants (Sprent, 1970, 1971, 1972; Pankhurst and Sprent, 1975). Even when moisture stress was applied in the later stages of development, accumulation of N may have been significantly affected by the inhibition of nitrogen fixation. Wally (1983) reported that under controlled conditions lentils continued to fix significant amounts of nitrogen throughout the latter stages of development. Moisture stress applied both at bloom and at early pod development resulted in similar decreases in nitrogen yield, suggesting that rapid accumulation of N in

lentil shoots occurs during the later growth stages and that this rapid accumulation was restricted by the moisture stress. Although the conditions of this experiment did not allow for the distinction between soil, fertilizer and symbiotic N sources, it is possible that application of moisture stress limited uptake, to some degree, from all three sources.

Seed yields were significantly reduced by moisture stress, although no significant differences existed among the three moisture stress treatments (Table 19). Several possible mechanisms by which seed yields were reduced may have occurred. Restricted number of flowers, abortion of floral structures, and reduced seed size may all have contributed to reduced seed yields. Furthermore, the extent to which each mechanism contributed to the reduction in seed yields may have varied amongst treatments depending on the developmental stage at which stress was applied.

Similarly, nitrogen accumulation in the seed was significantly affected by moisture stress (Table 19). Moisture stress applied at pre-bud, bloom and early pod development was equally effective in reducing seed N as no significant differences amongst these treatments existed.

Percent protein in the seed was not significantly affected by moisture stress (Table 19). Thus the reduction in nitrogen accumulated in the seed was a reflection of decreased seed yields.

Harvest index, given by the ratio of seed dry matter to total dry matter, was reduced by moisture stress, although this reduction was statistically significant only when stress was applied at bloom (Table 19). Thus, moisture stress applied at bloom served to limit seed production to a greater extent than vegetative production, presumably

Table 19. Effect of water stress applied at different physiological stages on seed yield, nitrogen accumulation in seed, percent protein of seed and harvest index (Growth Chamber Experiment B).

Physiological stage at water stress	Seed yield (g pot^{-1})	N accumulation in seed (mg pot^{-1})	Percent protein	Harvest index (%)
No moisture stress applied	27.4 a[1]	1046 a	23.8 a	44.4 a
Prebud	15.2 b	559 b	22.9 a	41.8 a
Bloom	14.0 b	507 b	22.8 a	33.8 b
Early pod development	15.5 b	573 b	23.0 a	38.5 ab

[1] Means followed by the same letter in each column are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

because vegetative development was largely complete at the time of stress. Although not significant, the data suggest that moisture stress applied at early pod development similarly limited seed production to a greater extent than vegetative production. Alternatively, moisture stress applied early in development at prebud, resulted in concomitant decreases in both vegetative and reproductive growth.

Thus data indicate that under controlled conditions, moisture stress leads to a reduction in dry matter, nitrogen and seed yield of lentils. This contrasts the contention of Slinkard and Drew (1982) who have suggested that some drought stress may benefit lentil seed yields. However, the conditions of the growth chamber did not facilitate the simulation of moisture stress as would develop under field conditions. In a growth chamber moisture stress develops much faster and generally to a greater extent than in the field. Furthermore, restricted soil volume limits rooting depth and drought can not be avoided by the extension of the root system into lower soil profiles. Thus data obtained in the growth chamber may not accurately reflect the response of lentils to moisture stress under field conditions.

4.1.3 Growth Chamber Experiment C

Results obtained from growth chamber experiment A indicated that although high levels of applied fertilizer N led to increased lentil dry matter yields, accumulation of symbiotically fixed N diminished with increasing increments of applied fertilizer N. Both lentil dry matter yield and accumulation of symbiotically fixed N were reduced by a nitrogen stress, induced by the addition of barley straw to the soil. The

objective of this experiment was to further investigate the effect of nitrogen addition and availability on dry matter yield, nitrogen accumulation and nitrogen fixation of lentils, determined at six different physiological stages. Nitrogen stress was induced by adding barley straw to the soil. Quantity of symbiotically fixed nitrogen was estimated using the Classical Difference method, the ^{15}N Assisted Difference method and the 'A' value method. Nonnodulating soybeans served as a reference crop.

Dry matter yield of lentil shoots continued to increase at each successive harvest for all treatments (Table 20). Maximum rate of dry matter accumulation was influenced by nitrogen availability. Lentils grown on soil amended with barley straw attained a maximum rate of dry matter accumulation between harvest 5 and harvest 6, which corresponds to the pod development stage of growth. It is likely that at this growth stage, dry matter accumulation was due primarily to the development of reproductive structures and to pod filling. In contrast, where nitrogen was applied at a rate of 30 ppm, the maximum rate of dry matter accumulation occurred between harvest 3 and harvest 4, which corresponds to the onset of flowering. This is in agreement with Walley (1983) who observed that under similar conditions, maximum rate of lentil dry matter accumulation occurred at the initiation of reproductive growth. Due to the indeterminate growth habit of lentils, dry matter accumulation during this stage of growth may have been due to both vegetative and reproductive growth. Where N was applied at a rate of 200 ppm, the maximum rate of dry matter accumulation occurred between harvest 2 and harvest 3, which corresponds to the bud stage, prior to first flower.

Table 20. Effect of nitrogen availability on dry matter accumulated by lentil shoots, lentil roots and nonnodulating soybean shoots, determined at successive harvest dates (Growth Chamber Experiment C).

Crop, portion harvested, and nitrogen rate (ppm)	Dry matter accumulation (g pot^{-1})					
	Harvest[1] 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5	Harvest 6
Lentil shoots 0(1% barley straw)	0.7b[2] D[3]	1.9b D	5.1b CD	9.4b C	16.9b B	45.0b A
30	1.4a D	8.4a D	17.3a C	33.6a B	50.3a A	56.0a A
200	1.1a E	7.4a ED	18.6a DC	30.6a CB	41.8a BA	50.3a A
Lentil roots 0(1% barley straw)	0.9a D	2.7b DC	4.1b CB	2.8b DC	5.6b BA	7.4b A
30	1.6a D	8.0a C	9.0a CB	12.5a BA	13.5a A	11.4a BA
200	1.0a C	2.4b CB	6.9abBA	9.8a A	10.5ab A	9.4b A
Nonnod soy shoots 0(1% barley straw)	1.2b D	2.3b C	2.2c C	3.2c B	3.5c B	5.1c A
30	2.3a E	9.6a D	15.6b C	25.9b B	34.5b A	35.3b A
200	2.2a F	8.7a E	21.5a D	34.4a C	58.9a B	71.9a A

1 Days after seeding and physiological stage of lentils: Harvest 1 = day 23, prebud; Harvest 2 = day 34, bud; Harvest 3 = day 42, first flower; Harvest 4 = day 55, bloom; Harvest 5 = day 70, pod development; Harvest 6 = day 90, ripe seed.

2 Means followed by the same lowercase letter in each column within each crop and portion are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

3 Means followed by the same uppercase letter in each row are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

Maximum rate of dry matter accumulation during this stage largely reflects the accumulation of vegetative growth. Thus availability of nitrogen was observed to influence the pattern of dry matter accumulation. Specifically, as N availability increased, maximum rate of dry matter accumulation occurred during progressively earlier growth stages.

With the exception of harvest 3, lentil shoot dry matter accumulation was greatest where nitrogen had been applied at a rate of 30 ppm. Application of 200 ppm N did not lead to yield increases above the 30 ppm N treatment. Lack of response to additional N may have been due, in part, to the method of fertilizer placement. Nitrogen fertilizer had been added to the soil occupying the bottom half of the pot in an attempt to avoid early urea toxicity damage to the lentil seedlings. Unfortunately, it was observed that at the highest rate of N addition, lentil root proliferation was restricted to the upper half of the pots for the first two harvests in avoidance of the toxic levels of urea. Hence the availability of nutrients may have been restricted during early growth and development by the limited rooting volume. Lentils receiving 200 ppm N appeared chlorotic and stunted during early growth stages as compared to lentils receiving N at a rate of 30 ppm. Visual symptoms of N deficiency persisted until approximately day 42 or harvest 3. Examination of the lentil roots at harvest 3 revealed that roots had begun to penetrate the bottom half or the soil volume. Lentil roots had explored the entire soil volume by maturity. Thus lack of early response to high levels of N as compared to 30 ppm N may have been due to the effects of toxic levels of urea on root growth. At later stages of growth and at maturity, lack of significant difference in dry matter

accumulated by lentils receiving N at rates of 30 and 200 ppm N may reflect either early inhibition of growth due to urea toxicity for lentils receiving the latter treatment, or a significant contribution of symbiotically fixed N to total N for lentils receiving the former treatment.

At each harvest date, shoot dry matter yields where lowest were soil had been amended with barley straw (Table 20). Thus it is suggested that N fixation alone did not meet the nitrogen requirement of lentils and that supplemental nitrogen was an important factor in increasing lentil dry matter yields.

Where barley straw was added, accumulation of root dry matter continued to increase until maturity (Table 20). Maximum rate of root dry matter accumulation occurred between harvest 4 and harvest 5, corresponding to the latter part of flowering. Where N was applied at a rate of 30 ppm, maximum rate of dry matter accumulation occurred between harvest 2 and harvest 3, corresponding to the initiation of flowering. Accumulation of root dry matter was not significantly altered beyond this growth stage. These results are in agreement with Walley (1983) who reported that where N was added at a rate of 30 ppm, lentil root growth essentially ceased at the onset of early flowering. Similarly, maximum root dry matter accumulation occurred between harvest 2 and harvest 3 for lentils receiving N at a rate of 200 ppm and further increases in root dry matter were not significant beyond this harvest. Visual observations indicated that the 200 ppm N treatment inhibited early root proliferation in the zone of application and resulted in relatively small amounts of dry matter accumulated at harvest 1 and

harvest 2. Roots of lentils grown on soil amended with 30 ppm N out-yielded both the barley straw and 200 N treatments at all harvest dates.

Dry matter accumulation of nonnodulating soybean shoots continued to increase until the final harvest where soil was amended with both barley straw and 200 ppm N (Table 20). However, where N was applied at a rate of 30 ppm, maximum yields were achieved at harvest 5, 70 days after seeding. It was observed that these plants had begun to develop characteristic N deficiency symptoms as early as harvest 3, and by harvest 5, all leaves were chlorotic with spreading necrotic areas on the lower leaves. Thus it was concluded that for nonnodulating soybeans receiving N at a rate of 30 ppm, dry matter yield did not increase following harvest 5 due to limited N availability. Nitrogen deficiency symptoms did not develop in nonnodulating soybeans receiving N at a rate of 200 ppm and dry matter yields continued to increase until harvest 7, which suggests that N was not limiting to plant growth. In contrast, nonnodulating soybeans grown on soil amended with barley straw developed observable N deficiency symptoms prior to the first harvest which persisted until the final harvest, suggesting that available soil N was strictly limiting to plant growth. However, small but significant dry matter yield increases were obtained at final harvest. This increase likely reflected slight increases in N availability during the latter part of the experiment due to mineralization of organic N.

Throughout the duration of the experiment, the lowest nonnodulating soybean yields were achieved where soil had been amended with barley straw. No significant differences in dry matter yield of nonnodulating soybeans existed between the 30 and 200 ppm N treatment at harvest 1 and

harvest 2. However, for the remainder of the experiment, nonnodulating soybeans receiving 200 ppm N had greater yields than those receiving 30 ppm N. Examination of the nonnodulating soybean roots at harvest 2 revealed that the roots had begun to explore the bottom half of the pot and thus appeared more tolerant to the high levels of urea than the lentils. Thus increasing increments of available N resulted in concomitant increases in nonnodulating soybean yields.

With the exception of the first two harvests, lentil shoots yielded more than nonnodulating soybean shoots for both the barley straw and the 30 ppm N treatments. However, where N was applied at a rate of 200 ppm, nonnodulating soybeans had consistently higher dry matter yields than the lentils. The dry matter yield increment was statistically significant ($P=0.05$) at both harvest 5 and harvest 6. Higher nonnodulating soybean yields may have been due, in part, to the fact that the nonnodulating soybeans were more tolerant to the high levels of urea and hence were able to explore the entire soil volume at earlier growth stages. Alternatively, higher dry matter yields may reflect different maximum yield potentials.

Accumulation of nitrogen followed many of the same patterns established for the accumulation of dry matter (Table 22). Lentils grown on soil amended with barley straw continued to accumulate N in the shoots throughout the duration of the experiment. Furthermore, the maximum rate of N accumulation was achieved between harvest 5 and harvest 6 which corresponds to the maximum rate of dry matter accumulation. Where N was applied at a rate of 30 ppm, the maximum rate of N accumulation occurred between harvest 4 and harvest 5, which corresponds to pod

Table 22. Effect of nitrogen availability on nitrogen accumulation in lentil shoots, lentil roots and nonnodulating soybean shoots, determined at successive harvest dates (Growth Chamber Experiment C).

Crop, portion harvested, and nitrogen rate (ppm)	Nitrogen accumulation (mg pot^{-1})					
	Harvest[1] 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5	Harvest 6
Lentil shoots 0(1% barley straw)	13b[2] C[3]	38b C	106b C	107b C	322b B	874b A
30	58a C	156a C	308a C	665a B	1114a A	1138a A
200	47a C	158a C	365a CB	543a B	962a A	1086a A
Lentil roots 0(1% barley straw)	20a D	66b DCB	93b CB	53b DC	106b B	154b A
30	35a E	169a D	187a DC	276a A	244a A	215a BA
200	27a B	72b B	185a A	212a A	201a A	192a A
Nonnod soy shoots 0(1% barley straw)	22b DC	20c DC	18c D	28c C	44c B	60c A
30	96a B	225b A	230b A	204b A	235b A	234b A
200	91a D	275a C	489a B	546a B	762a A	791a A

1 Refer to Table 20 for corresponding days after seeding and physiological stage of lentils.

2 Means followed by the same lowercase letter in each column within a crop and portion are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

3 Means followed by the same uppercase letter in each row are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

development, although maximum dry matter accumulation had occurred between harvest 3 and 4. Thus it is suggested that the nitrogen demand of lentils is greater for reproductive, rather than vegetative, growth. Following harvest 5, no significant increases in N yield were obtained. This may indicate that N accumulation during later growth stages was limited by the depletion of soil and fertilizer N sources and (or) the cessation of N fixing activity. Alternatively, the data may suggest that by harvest 5, lentils had attained maximum yields and thus N uptake was limited by yield potential. Similarly, the maximum rate of N accumulation for lentils receiving N at a rate of 200 ppm occurred between harvest 4 and harvest 5 and no significant increases in N accumulated were obtained after harvest 5.

Lentils grown on soil amended with barley straw accumulated significantly less nitrogen in the shoots relative to lentils receiving N at rates of 30 and 200 ppm. Thus nitrogen fixation alone was not sufficient to realize maximum N yield. The N yields of lentils grown on soil amended with 30 and 200 ppm N did not differ significantly at any harvest. Thus, the addition of 200 ppm N did not serve to increase N yield above that obtained with the addition of 30 ppm N. These results may indicate that at low levels of applied N, symbiotically fixed nitrogen makes a significant contribution to total plant N and thus lentils are capable of attaining both maximum dry matter and maximum nitrogen yields.

Maximum nitrogen yield of lentil roots grown in barley straw amended soil was realized at final harvest (Table 22). In contrast, lentil roots attained maximum N yields at harvest 4 with the application

of nitrogen at rates of 30 and 200 ppm. Nitrogen accumulation in lentil roots essentially ceased at bloom and at first flower for lentils receiving 30 and 200 ppm N, respectively.

Nonnodulating soybean shoots continued to accumulate nitrogen throughout the duration of the experiment when grown in soil amended with barley straw (Table 22). Continued accumulation of N suggests the small amounts of N became plant available throughout the duration of the experiment through the process of mineralization of organic N. In contrast, where N was applied at a rate of 30 ppm N, N yields did not increase after harvest 2, despite significant increases in dry matter yield. This suggests that nonnodulating soybeans had depleted available soil and fertilizer N sources by harvest 2. Maximum N yields were obtained at the final harvest for nonnodulating soybeans receiving N at a rate of 200 ppm. Thus data suggest that at a rate of 200 ppm N, nitrogen was not limiting to plant growth and that nonnodulating soybeans may have continued to accumulate N until final harvest.

Lentils grown in soil amended with barley straw and 30 ppm N had significantly higher N yields ($P = 0.05$) than nonnodulating soybeans at all but the first two harvests. Thus data indicate that following harvest 2, symbiotically fixed N contributed significantly to the total N yield of lentil shoots. In contrast, where N was applied at a rate of 200 ppm, lentil N yields were significantly greater than nonnodulating soybean N yields ($P = 0.05$) only at the final two harvests.

The percent nitrogen derived from fertilizer in both lentils and nonnodulating soybeans increased significantly from harvest 1 to harvest 2 (Table 23). This increase may reflect early incorporation of seed N and (or) limited use of fertilizer N during early growth stages.

Table 23. Effect of nitrogen application on percent nitrogen derived from fertilizer (Ndff) in lentil shoots, lentil roots and non-nodulating soybean shoots, determined at successive harvest dates (Growth Chamber Experiment C).

Crop, portion harvested, and nitrogen rate (ppm)	Percent nitrogen derived from fertilizer					
	Harvest[1] 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5	Harvest 6
Lentil shoots						
30	17 b[2] B[3]	29 b A	21 b B	10 b C	6 b D	6 b D
200	42 a C	71 a A	61 a B	61 a B	53 a B	58 a B
Lentil roots						
30	13 b B	21 b A	18 b BA	11 b B	12 b B	15 b B
200	34 a B	55 a A	50 a A	53 a A	37 a B	58 a A
Nonnod soy shoots 30	20 b B	34 b A	32 b A	30 b A	21 b B	35 b A
200	46 a B	73 a A	68 a A	73 a A	66 a A	73 a A

1 Refer to Table 20 for corresponding days after seeding and physiological stage of lentils.

2 Means followed by the same lowercase letter in each column within each crop and portion are not significantly different at P = 0.05.

3 Means followed by the same uppercase letter in each row are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

Following harvest 2, the percent Ndff declined in the shoots of lentils receiving N at a rate of 30 ppm. The significant decline in percent Ndff evidences the dilution of ^{15}N labelled fertilizer derived N with symbiotically fixed N and (or) mineralized soil N. Further decreases in percent Ndff were not obtained after harvest 5 which suggests that little or no symbiotic fixation occurred between pod development and maturity. The percent Ndff in lentils receiving 200 ppm N declined significantly from harvest 2 to harvest 3, which indicates that the incorporation of symbiotically fixed N and (or) mineralized soil N served to dilute ^{15}N labelled fertilizer derived N. However, no further significant decreases in percent Ndff were obtained following harvest 3 which suggests that very little, if any, nitrogen was contributed by symbiotic nitrogen fixation.

At the high rate of fertilizer N application, lentils derived a greater proportion of the total N accumulated from fertilizer N rather than from soil and symbiotically fixed N, as evidenced by the higher percent Ndff values for the 200 ppm N treatment. Increases in percent Ndff reflect both enhanced availability and uptake of fertilizer at the higher rate of N application. In addition, it is likely that increases also reflect suppression of symbiotic fixation.

The percent Ndff in lentil roots also increased from harvest 1 to harvest 2 (Table 23). Again, this increase may have been due to early

incorporation of seed N and (or) limited use of fertilizer N during early growth stages. No significant changes in the percent Ndff of lentil roots occurred after harvest 3 for lentils receiving N at a rate of 30 ppm. The observation that percent Ndff did not change in the roots after harvest 3, yet decreased significantly in the shoots suggests that symbiotically fixed nitrogen was not incorporated in the roots to the same extent as it was in the shoots. Rather it is suggested that fixed N was preferentially transported out of the roots and incorporated in the shoots. Similarly, the percent Ndff of lentil roots grown in soil amended with 200 ppm N did not decrease significantly after harvest 2 with the exception of harvest 5. It is possible that the significant decrease in percent Ndff at harvest 5 was a reflection of experimental error rather than of a real decrease.

Comparison of the percent Ndff in lentil roots between the two nitrogen fertilizer treatments indicated that at all harvests, percent Ndff was greater at the higher rate of N application. Thus, the data suggest that at the lower rate of applied fertilizer N, symbiotically fixed N served to dilute ^{15}N labelled fertilizer N accumulated in lentil roots.

The percent Ndff in nonnodulating soybean shoots increased from harvest 1 to harvest 2 for both N fertilizer treatments (Table 23). The increase in percent Ndff was likely due to early dependence on non-labelled seed nitrogen. Following harvest 2, percent Ndff in nonnodulating soybean shoots did not change significantly for either treat-

ment with the exception of harvest 5 for the 30 ppm N treatment. Percent Ndff in lentil roots also declined significantly at harvest 5. There is no known reason for the significant decreases in percent Ndff at this harvest and thus it was concluded that the decreases were due to experimental error.

Comparison of the percent Ndff values for nonnodulating soybeans receiving 30 and 200 ppm N indicated that at the higher N rate, uptake of fertilizer N made a greater contribution to total plant N than soil N. This is consistent with the observation that, as early as harvest 2, nonnodulating soybeans receiving N at a rate of 30 ppm, had developed visual signs of N deficiency. In contrast, plants receiving N at a rate of 200 ppm were not N deficient and they continued to feed from the fertilizer and soil N sources.

Where N was applied at a rate of 30 ppm, the percent Ndff in nonnodulating soybean shoots did not differ significantly from the percent Ndff in lentil shoots ($P = 0.20$) for the first two harvests. Thus data indicate that during the early growth stages, lentils and nonnodulating soybeans shared similar N sources and symbiotically fixed N did not contribute significantly to total N in lentil shoots. However, percent Ndff in nonnodulating soybeans was determined to be significantly greater ($P = 0.05$) than in lentils for the remaining harvests, which evidences the dilution of ^{15}N labelled fertilizer derived N by symbiotically fixed N in the lentils. Where N was applied at a rate of 200 ppm, percent Ndff in nonnodulating soybeans was significantly greater than the corresponding lentil values only for the final three harvests and only at the twenty percent level of probability. Thus data

suggest that symbiotically fixed nitrogen contributed little to the N status of lentils receiving N at a rate of 200 ppm and subsequently little or no dilution of fertilizer derived N occurred.

Accumulation of fertilizer N in the shoots of lentils receiving N at a rate of 30 ppm increased significantly from harvest 1 to harvest 2 (Table 24). Further increases in fertilizer N accumulation were not statistically significant which suggests that lentils had depleted this N source by harvest 2. In contrast, lentils grown in soil amended with 200 ppm N continued to accumulate fertilizer N throughout the duration of the experiment. Although total N accumulation in lentils receiving 30 and 200 ppm N was similar, lentils receiving the higher rate of applied N accumulated significantly more N from the fertilizer source at each harvest. Accumulation of fertilizer N in the roots of lentils receiving 30 ppm N did not change significantly following harvest 2. However, where N was applied at a rate of 200 ppm, accumulation of fertilizer N in the roots increased until harvest 3. Following harvest 3, no further significant increases in fertilizer N accumulation were obtained.

Accumulation of fertilizer N in nonnodulating soybeans receiving N at a rate of 30 ppm did not increase past the second harvest which indicates that the available fertilizer N source had been depleted (Table 24). In contrast, fertilizer N accumulated in the shoots of nonnodulating soybeans receiving N at a rate of 200 ppm contained to increase

Table 24. Effect of nitrogen application on fertilizer nitrogen accumulation in lentil shoots, lentil roots and non-nodulating soybean shoots, determined at successive harvest dates (Growth Chamber Experiment C).

Crop, portion harvested, and nitrogen rate (ppm)	Fertilizer nitrogen accumulation (mg pot^{-1})					
	Harvest[1] 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5	Harvest 6
Lentil shoots						
30	10 b[2] B[3]	45 b A	65 b A	70 b A	67 b A	68 b A
200	20 a D	111 a D	222 a DC	326 a CB	486 a BA	631 a A
Lentil roots						
30	4 b B	36 a A	33 b A	30 b A	28 b A	32 a A
200	9 a C	40 a CB	94 a BA	110 a A	72 a BA	111 a A
Nonnod soy shoots						
30	20 b D	77 b BA	73 b BA	61 B CB	49 b C	81 b A
200	41 a F	200 a E	332 a D	396 a C	502 a B	577 a A

1 Refer to Table 20 for corresponding days after seeding and physiological stage of lentils.

2 Means followed by the same lowercase letter in each column within each crop and portion are not significantly different at P = 0.05 (t-test).

3 Means followed by the same uppercase letter in each row are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

throughout the duration of the experiment which indicates that this N source was not limiting to growth.

Percent utilization of fertilizer N, applied at a rate of 30 ppm, by lentil shoots did not change significantly following the second harvest although slight increases were obtained (Table 25). Lentils utilized a maximum of 38.8 percent of the fertilizer N applied at a rate of 30 ppm N. Percent utilization of fertilizer nitrogen was increased to a maximum of 52.6 percent at the 200 ppm N rate of fertilizer application. These results are consistent with data obtained during the 1983 field experiment in which percent utilization of fertilizer N increased from 19.4% when 30 kg N ha^{-1} was applied to 30.6% when 100 kg. N ha^{-1} was applied. Some authors have reported both increases in percent utilization of fertilizer N with increased fertilizer N supply (Allos and Bartholomew, 1959; Diebert et al., 1979; Vasilas and Ham, 1984) as well as decreases (Regitnig, 1979).

The data indicate that although percent utilization of fertilizer N increased with increased rate of fertilizer addition, this increase was not achieved until final harvest. Furthermore, percent utilization of fertilizer N was actually higher where N was added at a low rate for the first four harvests. This may have been due, in part, to the fact that

Table 25. Effect of nitrogen application on percent utilization of fertilizer nitrogen by lentil and nonnodulating soybean shoots, determined at successive harvest dates (Growth Chamber Experiment C).

Crop and nitrogen rate (ppm)	Percent utilization of fertilizer nitrogen					
	Harvest[1] 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5	Harvest 6
Lentil shoots						
30	5.6a[2] B[3]	25.0a A	35.9a A	38.8a A	37.1a A	37.4b A
200	1.6b E	9.3b D	18.5b DC	27.2b CB	40.5a BA	52.6a A
Nonnod soy shoots						
30	10.8a C	42.5a BA	40.4aBA	33.9a B	40.5a BA	44.8a A
200	3.4b F	16.6b E	27.7b D	33.0a C	41.9a B	48.0a A

1 Refer to Table 20 for corresponding days after seeding and physiological stage of lentils.

2 Means followed by the same lowercase letter in each column within a crop and portion are not significantly different at P = 0.05 (t-Test).

3 Means followed by the same uppercase letter in each row are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

Table 25A. Level of significance associated with differences between percent utilization of fertilizer nitrogen by lentils and nonnodulating soybeans (Growth Chamber Experiment C).

Nitrogen rate (ppm)	Level of significance (%)					
	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5	Harvest 6
30	NS[1]	NS	NS	NS	NS	NS
200	5	5	15	NS	NS	NS

1 Not significant at P > 0.20.

fertilizer N had been added to the soil in the bottom half of the pots, and at a rate of 200 ppm N, the level of N was inhibitory to root growth during early developmental stages. As a result, it was observed that a greater proportion of the fertilizer N was utilized by the lentils receiving 200 ppm N at the later stages of growth as compared to lentils receiving 30 ppm N. Differences may also be due, in part, to the capacity of lentils to use fertilizer N.

Nonnodulating soybeans utilized a maximum of 44.8 percent of fertilizer N applied at a rate of 30 ppm (Table 25). This value of maximum recovery, achieved at final harvest, did not differ significantly from values obtained at the previous four harvests with the exception of harvest 4. Thus, by harvest 2, nonnodulating soybeans had already utilized all of the available fertilizer N and were unable to recover any more. In contrast, percent utilization of fertilizer N by nonnodulating soybeans receiving N at a rate of 200 ppm continued to increase at each successive harvest. Continued increases in the percent utilization of fertilizer N suggests that fertilizer N uptake was not limited where applications of 200 ppm N had been made.

Nonnodulating soybeans grown in soil amended with 30 ppm N utilized significantly more fertilizer N than those grown in soil amended with 200 ppm N for the first three harvests. However, for the final three

harvests, percent utilization of fertilizer N by nonnodulating soybean was not significantly affected by rate of fertilizer application.

Comparison of the percent utilization of fertilizer nitrogen by lentils and nonnodulating soybeans indicate that the two crops were equally adept in extracting fertilizer N when applied at a rate of 30 ppm (Table 25A). Thus, it is suggested that at the 30 ppm N rate of fertilizer application, the use of N balance techniques to determine quantity of N symbiotically fixed may be justified by the fact that utilization of fertilizer N by the two crops was not significantly different. However, when nitrogen was applied at a rate of 200 ppm, nonnodulating soybean utilized significantly more N than the lentils at the first three harvests. This may reflect better tolerance to high rates of fertilizer N by the nonnodulating soybeans and (or) a greater total N requirement at the earlier growth stages. Percent utilization of fertilizer N applied at a rate of 200 ppm was not significantly different for lentils and nonnodulating soybeans at the final three harvests. Thus in later stages of growth, both crops were equally adept in extracting fertilizer N.

'A' values for lentils receiving N at both 30 and 200 ppm decreased from harvest 1 to harvest 2 (Table 26). High estimates of the soil N pool at harvest 1 likely resulted from the fact that the fertilizer N standard was relatively unavailable at this harvest. Thus lentils did not obtain a representative portion of N from the fertilizer because the fertilizer had been placed in the bottom half of the pots, beyond the depth of the roots during early development.

Table 26. Effect of nitrogen application on lentil and nonnodulating soybean 'A' values, determined at successive harvest dates (Growth Chamber Experiment C).

Crop and nitrogen rate (ppm)	'A' - Values (ppm)					
	Harvest[1] 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5	Harvest 6
Lentils						
30	857b[2] C[3]	446a C	676a C	1529a CB	2871a A	2877a AB
200	1656a A	508a C	781a BC	770b BC	1120b B	927b B
Nonnod soybean						
30	772b A	349a B	387a B	424a B	677a A	338a B
200	1451a A	448a B	563a B	456a B	621a B	450a B

1 Refer to Table 20 for corresponding days after seeding and physiological stage of lentils.

2 Means followed by the same lowercase letter in each column within a crop are not significantly different at P = 0.05 (t-Test).

3 Means followed by the same uppercase letter in each row are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

Table 26A. Level of significance associated with differences between lentil and nonnodulating soybean 'A' values (Growth Chamber Experiment C).

Nitrogen rate (ppm)	Level of significance (%)					
	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5	Harvest 6
30	NS[1]	NS	5	5	5	5
200	NS	NS	NS	15	20	20

1 Not significant at P > 0.20.

Decreased 'A'-values at harvest 2 reflect increased fertilizer N uptake due to root proliferation into the zone of fertilizer N application. Increases in lentil 'A'-values from harvest 3 to harvest 5 where N was added at a rate of 30 ppm and from harvest 2 to harvest 5 where N was added at a rate of 200 ppm, resulted from the uptake of symbiotically fixed N and (or) mineralized soil N.

At harvest 1, the 'A'-value for lentils receiving N at a rate of 200 ppm was significantly higher than for lentils receiving N at a rate of 30 ppm. Again, this evidences the fact that N applied at a rate of 200 ppm was toxic and inhibited N uptake from the fertilizer source. Thus, the soil N pool, which was estimated relative to the fertilizer N standard, was likely overestimated at the high rate of applied N. At harvest 2 and harvest 3, lentil 'A'-values were unaffected by the rate of N applied, suggesting that the lentils obtained N from similar sources in the same proportion. However, at the final 3 harvests, 'A'-values for lentils grown in soil amended with 30 ppm N were significantly greater than for lentils receiving 200 ppm N. This suggests that symbiotic N fixation made a significantly greater contribution to the N yield of lentils receiving 30 ppm N.

Similar to lentil 'A'-values, nonnodulating soybean 'A'-values were significantly higher at the first harvest than at harvest 2 (Table 26). Again, this reflects both the method of placement and application of toxic levels of N. Nonnodulating soybean 'A'-values did not change following harvest 2 with the exception of harvest 5 for the 30 ppm N treatment. The relatively high 'A'-value at this harvest was likely due

to experimental error.

With the exception of harvest 1, nonnodulating soybean 'A'-values were not significantly affected by the rate of N application. This is in agreement with a number of studies in which it was reported that the 'A' values were independent of the rate of fetilizer application (Legg and Allison, 1959; Hunter and Carter, 1965; Legg and Stanford, 1967; Smith and Legg, 1971).

Symbiotically fixed nitrogen was calculated using the 'A'-value method, the ^{15}N Assisted Difference method and the Classical Difference method. Calculation of symbiotically fixed nitrogen using the 'A'-value method indicated that, when nitrogen had been added at a rate of 30 ppm, symbiotic fixation continued to contribute significantly to the total N yield of lentils until harvest 5 (Table 27). Further increases in the quantity of symbiotically fixed N beyond this harvest were not significant. A maximum accumulation of 944 mg of symbiotically fixed N was attained. Where N was applied at a rate of 200 ppm, accumulation of symbiotically fixed N was greatly reduced although the data suggest that the fixation system was still operative and contributing to the total N yield. Examination of the roots indicated that some nodules had established despite high levels of applied fertilizer N, although nodule numbers were greatly reduced and tended to be relatively small.

Accumulation of symbiotically fixed nitrogen was also determined using the ^{15}N Assisted Difference Method and the Classical Difference Method (Tables 28 and 29). Apparent differences in the accumulation of symbiotically fixed N as estimated using these two methods reflects differences in the percent utilization of fertilizer N by

Table 27. Effect of nitrogen application on the accumulation of symbiotically fixed nitrogen in lentils as calculated using the 'A'-value method (Growth Chamber Experiment C).

Nitrogen rate (ppm)	Symbiotically fixed nitrogen (mg pot^{-1})					
	Harvest[1] 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5	Harvest 6
30	8 a[2] C[3]	35 a C	105 a C	430 a B	814 a A	944 a A
200	4 a B	12 b B	39 b B	93 b AB	225 b A	256 b A

1 Refer to Table 20 for corresponding days after seeding and physiological stage of lentils.

2 Means followed by the same lowercase letter in each column are not significantly different at P = 0.05 (t-Test).

3 Means followed by the same uppercase letter in each row are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

Table 28. Effect of nitrogen availability, on the accumulation of symbiotically fixed nitrogen in lentils as calculated using the ^{15}N Assisted Difference method (Growth Chamber Experiment C).

Nitrogen rate (ppm)	Symbiotically fixed nitrogen (mg pot^{-1})					
	Harvest[1] 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5	Harvest 6
0(1%) barley straw	-10 a[2] C[3]	18 a C	86 a C	79 b C	278 b C	814 a A
30	-28 a C	13 a C	87 a C	452 a B	862 a A	916 a A
200	-23 a A	-29 a A	-13 a A	67 b A	217 b A	241 b A

1 Refer to Table 20 for corresponding days after seeding and physiological stage of lentils.

2 Means followed by the same lowercase letter in each column are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

3 Means followed by the same uppercase letter in each row are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

Table 29. Effect of nitrogen availability on the accumulation of symbiotically fixed nitrogen in lentils as calculated using the Classical Difference method (Growth Chamber Experiment C).

Nitrogen rate (ppm)	Symbiotically fixed nitrogen (mg pot^{-1})					
	Harvest[1] 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5	Harvest 6
0(1%) barley straw	-10 a2 C3	18 a C	86 a C	79 b C	278 b C	814 a A
30	-38 a C	1 a C	79 a C	460 a B	878 a A	904 a A
200	-44 a B	-118 a B	-124 b B	-3 b AB	201 b A	295 b A

1 Refer to Table 20 for corresponding days after seeding and physiological stage of lentils.

2 Means followed by the same lowercase letter in each column are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

3 Means followed by the same uppercase letter in each row are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

lentils and nonnodulating soybeans. Using the N balance techniques to calculate symbiotic fixation, it was found that the addition of barley straw to the soil delayed accumulation of fixed N. Thus it is suggested that low levels of fertilizer N may stimulate early growth and development of lentil seedlings, thereby encouraging the development of an active N fixing relationship. Nitrogen applied at a rate of 200 ppm also inhibited N fixation and data suggest that at the high rate of N fertilization, little or no fixation occurred. Negative values for fixation where N had been applied at a rate of 200 ppm may have been due to what appeared to be a greater tolerance to high levels of fertilizer N by the nonnodulating soybeans during early growth stages.

Comparison of the three methods used to determine the quantity of N fixed indicated that where lentils and nonnodulating soybeans utilized similar quantities of fertilizer N, estimates of N fixation were quite consistent.

The effect of varying levels of N availability is illustrated in Figure 1. Estimates of N fixation for lentils receiving 30 and 200 ppm N were calculated using the 'A'-value method. The Classical Difference method was used to calculate the quantity of N fixed where barley straw was added. Lentils accumulated a maximum of 944 mg N pot^{-1} where N was applied at a rate of 30 ppm. At this rate of N application, notable accumulation of symbiotically fixed N began after harvest 2, which corresponds to the bud stage of development. This is in agreement with Walley (1983) who reported that under similarly controlled conditions and N fertilizer application, lentils began fixing significant amounts of N during early flowering. Addition of barley straw delayed fixation

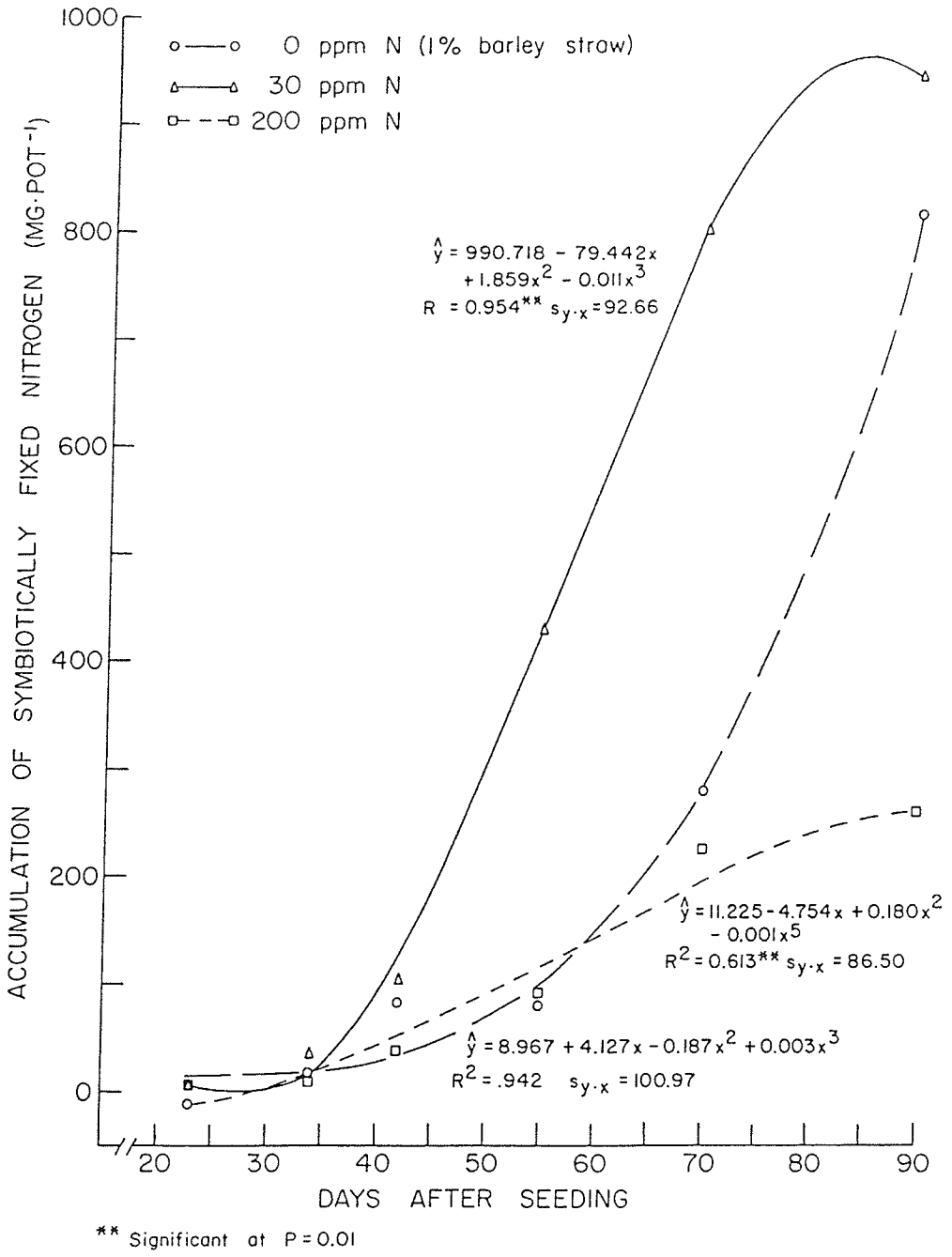

Figure 1. Effect of nitrogen availability on the accumulation of symbiotically fixed nitrogen by lentils.

and resulted in less total accumulation of fixed N as compared to the 30 ppm N treatment. With the addition of barley straw, notable accumulation of symbiotically fixed N began after harvest 4 which corresponds to the bloom stage of development. Although accumulation of symbiotically fixed nitrogen was rapid following harvest 4, total accumulation appeared to have been limited by the maturation of the lentils. Thus lentils grown in soil amended with barley straw accumulated less symbiotically fixed N than lentils receiving 30 ppm N. Although application of 200 ppm N limited the accumulation of fixed N, symbiotic fixation was not completely inhibited. At final harvest, lentils grown on soil amended with 200 ppm N, had accumulated 256 mg N pot^{-1}. Fixation appeared to begin following harvest 2 which corresponds to the initiation of first flower. Lentils receiving both 30 and 200 ppm N began fixing N at the same developmental stage. However, rate of accumulation of fixed N was much less at the higher rate of N fertilizer application and thus total accumulation was limited. Thus it can be concluded that symbiotic N fixation may benefit from the addition of low levels of applied N where available soil N is strictly limiting. Positive effects of applied nitrogen on symbiosis have been reported by several researchers (Pate and Dart, 1961; Dart and Wildon, 1970; Agboola, 1978; Eaglesham et al., 1983). It has been postulated that the beneficial effects of low levels of applied N on symbiosis are explained in terms of an alleviation of the "N hunger" stage of growth during early plant development subsequent to cotyledon consumption and prior to the onset of significant nodule fixation (Pate and Dart, 1961). Although low levels of applied N may benefit N fixation where N is strictly limiting,

high levels of applied N, such as the 200 ppm N treatment, may limit N fixation.

Seed yield, determined at final harvest, was lowest where soil had been amended with barley straw (Table 30). This contrasts the present Saskatchewan Agriculture recommendations which suggest that lentil yields may be enhanced by nitrogen stress (Saskatchewan Agriculture, 1985). Lentil seed yields did not differ significantly where N was added at 30 and 200 ppm. Percent protein of the seed was unaffected by N availability (Table 30).

Harvest index of lentils, which is given by the ratio of seed to total dry matter yield increased with increasing N availability (Table 30). This suggests that increasing increments of available N served to increase reproductive growth to a greater degree than vegetative growth.

Growth Chamber experiment C indicated that lentils may benefit from the application of low levels of fertilizer N. The benefit is seen in both improved N fixation as well as maintenance of maximum seed yields. Application of high levels of fertilizer N limited N fixation in lentils. Furthermore, the increase in N supply did not result in any yield benefits. However, it is important to note that lack of response to high levels of applied fertilizer N may have been due, in part, to toxic effects of urea on plant growth. Nitrogen stress, simulated by the addition of barley straw to the soil limited both quantity of nitrogen fixed as well as yield. It is obviously desirable to retain as much fixation as possible in addition to attempting to maximize yields. Thus it is suggested that lentils will benefit from the application of low levels of fertilizer N if soil N is limiting to growth.

Table 30. Effect of nitrogen application on seed yield, percent protein of seed and harvest index of lentils (Growth Chamber Experiment C).

Nitrogen rate (ppm)	Seed yield (g pot^{-1})	Percent Protein	Harvest index (%)
0(1%) barley straw	16.8 a[1]	23 a	35.6 b
30	22.8 a	23 a	39.3 ab
200	22.1 a	23 a	43.8 a

[1] Means followed by the same letter in each column are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

4.2 FIELD EXPERIMENTS

4.2.1 Field Experiments 1983

An experiment with lentils and nonnodulating soybeans was conducted under field conditions during the summer of 1983. The objective of this experiment was to determine the effects of varying rates of nitrogen fertilizer on dry matter yield, nitrogen yield and nitrogen fixation in lentils. In addition, where nitrogen fertilizer was applied at a rate of 30 kg ha^{-1}, a time-course study was conducted through a series of six harvests. The objective of the time-course study was to examine the accumulation of dry matter, nitrogen and symbiotically fixed nitrogen in lentils over time. Accumulation of symbiotically fixed nitrogen was determined using the 'A'-value method, the ^{15}N Assisted Difference method and the Classical Difference method.

Quantification of nitrogen fixation by a legume necessitates the use of a nonfixing reference plant so that the contribution of soil and (or) fertilizer nitrogen to the nitrogen yield of the fixing plant may be determined. The success of any method used to determine symbiotically fixed N depends largely on the choice of the nonfixing reference crop. When employing nitrogen balance techniques, such as the ^{15}N Assisted Difference method and the Classical Difference method, suitability of a reference crop depends on its utilization of fertilizer and soil N in the same quantities as the legume crop. Thus, in a time-course study, both the test crop and the reference crop must have similar seasonal patterns of fertilizer and soil N uptake.

Application of the 'A'-value method, which is based on differential

isotopic dilution by the test legume and the non-fixing reference crop, is also not without limitations related to the choice of the reference crop. Witty (1983) found that when using the 'A'-value method, field estimates of fixation using peas, french beans, field beans and clover, depended on the nonfixing control used. He contended that this dependency was related to differences in the N uptake patterns of the legumes and the control combinations. He based his contention on the fact that the enrichment of mineral N in the soil falls with time after the application of ^{15}N. Thus, if a legume and a nonlegume are to see the same soil N pool, the nitrogen uptake patterns of the two crops over the growing season must be the same. Even if at maturity it is determined that the test legume and the reference crop took up identical quantities of soil N, the 'A'-value method may be in error (Witty 1983). For example, if the control crop takes up nitrogen earlier than the legume and hence at a higher enrichment of ^{15}N, the apparent soil N pool ('A'-value), which is calculated from the enrichment of the standard, is disproportionately small relative to that of the legume and thus nitrogen fixation is overestimated.

Dissimilarity of maturation date is often seen to preclude similarity in nitrogen uptake patterns and thus Rennie (1982) suggested that a maturation date similar to that of the legume is a necessary prerequisite for a nonfixing control crop.

In addition to sharing identical soil N uptake patterns, either ^{15}N labelled fertilizer must be evenly distributed with depth, or the two crops must have the same rooting patterns (Diebert et al., 1979; Rennie, 1982; Witty, 1983). Similarity of rooting patterns ensures that

the legume and the reference crop feed from the same soil N pool.

Finally, the 'A'-value procedure is based on the concept that a plant, when confronted with two sources of a nutrient, will absorb nutrient from each source in proportion to the amounts available (Fried and Dean, 1952). Furthermore, by definition, the legume and the non-fixing control crop must assimilate fertilizer and soil N in the same proportion (Fried and Broeshart, 1975; Fried and Middleboe, 1977).

Stringent prerequisites for a non-fixing control plant such as those outlined above ensure that the reference crop provides an accurate estimate of the contribution of soil and fertilizer N to total nitrogen in the fixing legume. Walley (1983) demonstrated that under controlled growth bench conditions, nonnodulating soybeans served as an appropriate reference plant for lentils as it met the prescribed prerequisites. Thus nonnodulating soybeans were chosen to serve as the reference crop in the 1983 field experiments.

^{15}N Time Course Study

Lentils and nonnodulating soybeans did not share similar maturation dates. At final harvest, 86 days after seeding, lentils had set ripe seed and were mature, whereas nonnodulating soybeans had not yet fully matured. Degree of leaf senescence was notably affected by the difference in maturation date, and thus was reflected in total dry matter and nitrogen accumulation patterns of the two crops (Table 31, Figures 2-3).

The maximum amount of dry matter accumulated by the lentil crop was 5245 kg ha^{-1}, attained during pod development, 75 days after seeding. An apparent loss of 1172 kg ha^{-1} of dry matter by the lentils, which

Table 31. Dry matter and nitrogen accumulation in lentils and nonnodulating soybeans determined throughout the growing season (Field Experiments 1983).

Days after seeding	Developmental stage of lentils	Dry matter accumulation (kg ha^{-1})		Nitrogen accumulation (kg ha^{-1})	
		Lentils	Nonnod soybeans	Lentils	Nonnod soybeans
24	prebud	51 d[1]	87 d	2.1 d	5.7 d
33	prebud	258 d	218 d	12 d	10 cd
47	first flower	1717 c	1024 c	58 c	28 cb
61	early pod development	4010 b	1982 b	112 b	38 b
75	pod development	5245 a	2800 a	129 a	62 a
86	ripe seed	4073 b	3265 a	112 b	74 a

[1] Means followed by the same letter in each column not significantly different at P = 0.05 (Duncan's Multiple Range Test).

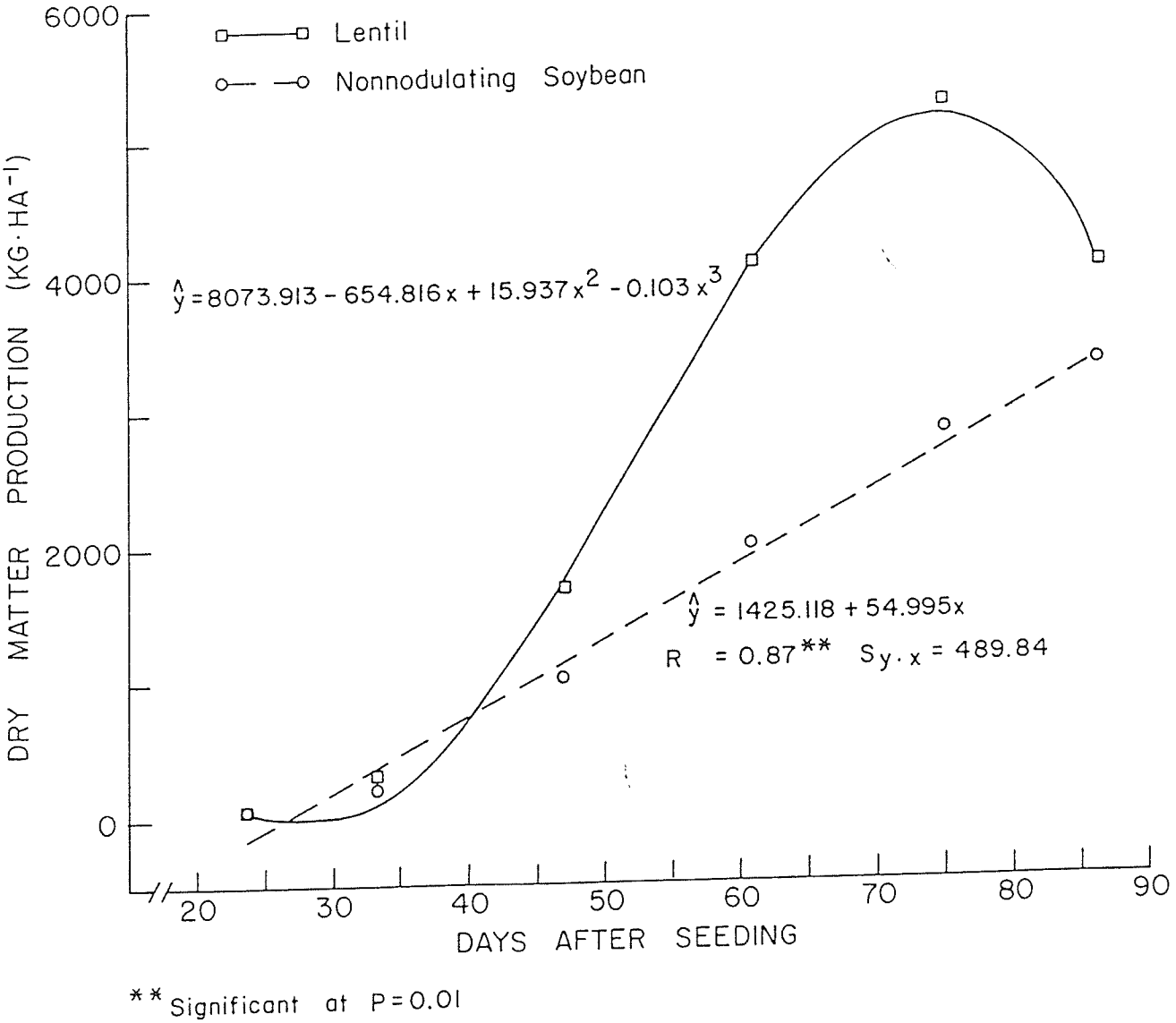

Figure 2. Dry matter accumulated by lentils and nonnodulating soybeans over the growing season (Field Experiments 1983).

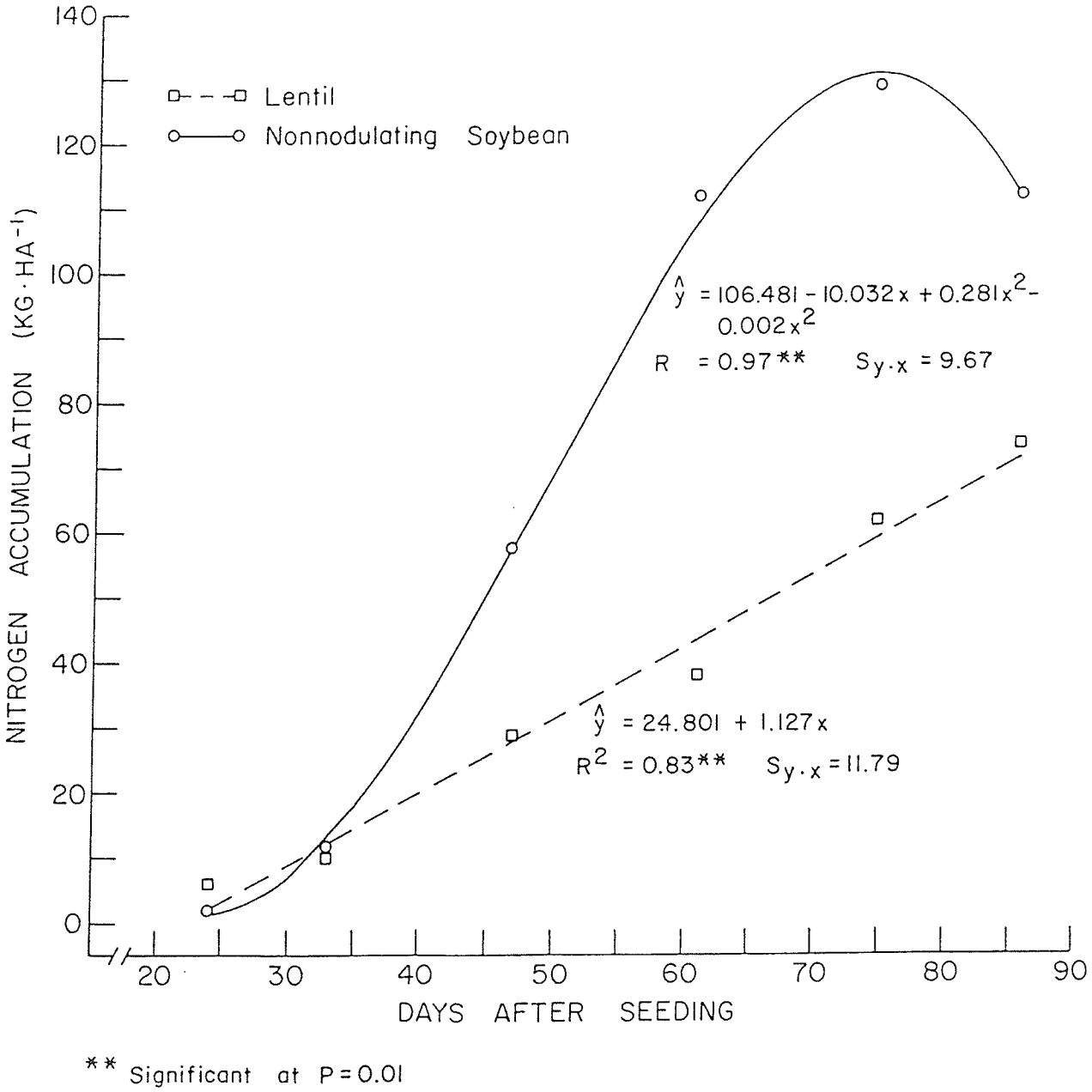

Figure 3. Nitrogen accumulated by lentils and nonnodulating soybeans over the growing season (Field Experiments 1983).

occurred between pod development and maturity, was due to extensive leaf senescence as well as some seed shattering. Nonnodulating soybeans did not share the same dry matter accumulation pattern (Figure 2). Although the nonnodulating soybean yield increase from day 75 to day 86 was not significant, the data suggest that the dry matter yield was still increasing at final harvest. Extensive leaf senescence, associated with maturity was not noted in the field.

The maximum rate of dry matter accumulation by the lentils occurred between day 47 and day 61. This corresponds to the early pod fill stage of development.

Both lentils and nonnodulating soybeans had nitrogen accumulation patterns similar to that of the respective dry matter accumulation patterns. The lentil crop attained a maximum nitrogen content of 129 kg ha^{-1} at the pod development stage, 75 days after seeding. Loss of 17 kg N ha^{-1} following this harvest reflects leaf senescence and seed shattering which occurred as maturity was approached. A similar decrease in the N content of nonnodulating soybeans was not observed. Although the increase in N content from day 75 to day 86 was not significant, the data suggest that N content of the above ground portion of nonnodulating soybeans may have still been increasing at final harvest.

Percent nitrogen derived from fertilizer was similar for both lentils and nonnodulating soybeans at each successive harvest date (Table 32). The decline in Ndff over the growing season evidences the dilution of ^{15}N labelled fertilizer by soil N and soil and (or) atmospheric N in the nonnodulating soybeans and lentils, respectively. The 'A'-value method is based on the concept that a plant absorbs

Table 32. Percent nitrogen derived from fertilizer (Ndff), fertilizer nitrogen accumulation and percent utilization of fertilizer nitrogen in lentils and nonnodulating soybeans determined throughout the growing season (Field Experiments 1983).

Days after seeding	Developmental stage of lentils	Percent nitrogen derived from fertilizer (Ndff)		Fertilizer nitrogen accumulation (kg ha^{-1})		Percent utilization of fertilizer nitrogen	
		Lentils	Nonnod soy	Lentils	Nonnod soy	Lentils	Nonnod soy
24	prebud	18.42 a[1]	22.95 a	0.40 e	1.28 b	1.40 e	4.25 b
33	prebud	17.82 a	16.80 b	2.25 d	1.70 b	7.38 d	5.65 b
47	first flower	12.10 b	12.98 bc	6.85 cb	3.68 a	22.92 cb	12.25 a
61	early pod development	7.85 bc	10.98 c	8.73 a	4.20 a	29.08 a	13.98 a
75	pod development	5.85 c	5.28 d	7.53 ab	3.25 a	24.88 ab	10.83 a
86	ripe seed	5.18 c	4.93 d	5.80 c	3.63 a	19.40 c	12.10 a

[1] Means followed by the same letter in each column are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

nutrients from soil and from fertilizer in proportion to the respective quantities available (Fried and Dean, 1952) and that both the test crop and the reference crop assimilate fertilizer and soil N in the same proportion (Fried and Broeshart, 1975). Based on this assumption, similarities in Ndff values for lentils and nonnodulating soybeans means that the two crops derived N from the same sources, namely soil and fertilizer, and little or no fixation occurred.

With the exception of the first harvest taken at 24 days, uptake of fertilizer N and the resulting fertilizer N accumulation was greatest in the lentils (Table 32). Thus estimation of nitrogen fixed using nitrogen balance methods including the ^{15}N Assisted Difference method and the Classical Difference method is discredited by the fact that the two crops did not obtain the same quantity of nitrogen from the fertilizer source. Only when percent utilization of the fixing system is identical to that of the nonfixing system can N balance methods accurately quantify nitrogen fixation. At all but the first and second harvest, percent utilization of fertilizer N by lentils was significantly greater than the soybeans at the five percent level of probability.

Figure 4 demonstrates the difference in fertilizer N uptake patterns between the two crops. Declining soil enrichment following the application of ^{15}N labelled fertilizer coupled with different N uptake patterns can contribute to error incurred when using dilution techniques (Witty, 1983). Thus it is likely that the different N uptake patterns exhibited by the two crops contributed, to some degree, to error in the calculation of fixed N using the 'A'-value method in this experiment.

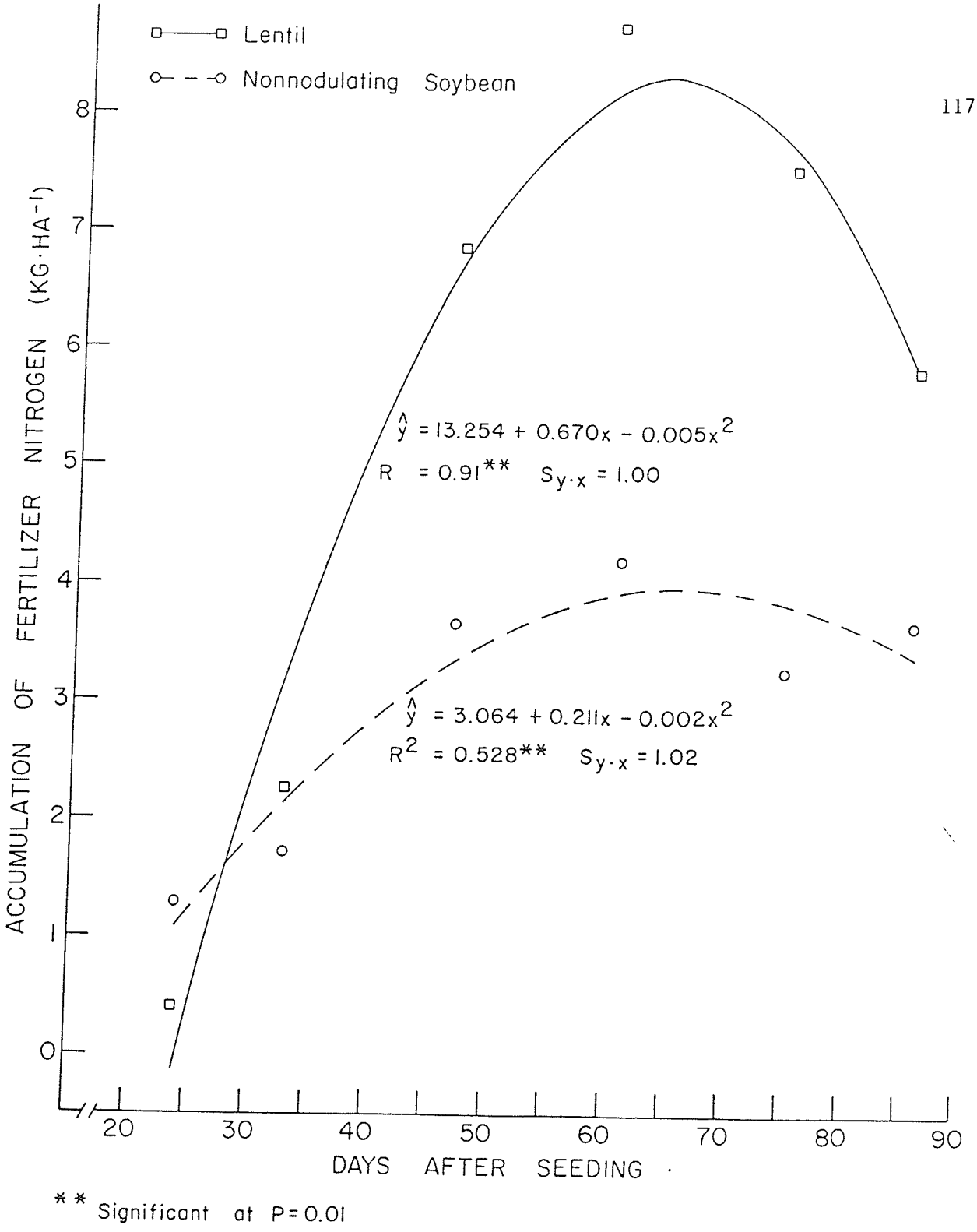

Figure 4. Fertilizer nitrogen accumulation in lentils and nonnodulating soybeans throughout the growing season (Field Experiments 1983).

'A'-values for lentils and nonnodulating soybeans are reported in Table 33. Decreases in the Ndff for both crops resulted in concomitant increases in 'A'-values over the growing season. At each of the harvest dates, 'A' values for lentils and nonnodulating soybeans were of similar magnitude, indicating that insigificant fixation had occurred.

Accumulation of symbiotically fixed nitrogen, as calculated using the 'A'-value method, the ^{15}N Assisted Difference method and the Classical Difference method, is reported in Table 34.

Determination of symbiotically fixed nitrogen using the 'A'-value method indicated that little or no fixation occurred during the growing season. High levels of available soil N (50.9 kg N ha^{-1}) may have inhibited fixation. Large negative values for N fixation were due to variation in percent nitrogen derived from fertilizer, from which 'A'-values were calculated. Calculation of nitrogen fixed using the ^{15}N Assisted Difference method and the Classical Difference method indicated that significant amounts of fixed nitrogen were accumulated by the lentils. As evidenced by the relatively low utilization of fertilizer N by nonnodulating soybeans, it is likely that the contribution of soil N to total N in the lentils, as measured by the nonnodulating soybeans, was underestimated. Underestimation of soil N would result in an overestimation of N fixed. However, as soil N accumulated in the lentils cannot be directly measured, both N balance techniques were invalidated by the differences in percent utilization of fertilizer N by the two crops.

The ^{15}N time-course clearly demonstrated the importance of identifying and using a suitable reference crop. Under the conditions

Table 33. 'A' values for lentils and nonnodulating soybeans determined throughout the growing season (Field Experiments 1983).

Days after seeding	Developmental stage of lentils	'A' Value (kg ha^{-1})	
		Lentils	Nonnod soy
24	prebud	145.96 d[1] A[2]	105.15 b A
33	prebud	142.11 d A	152.73 b A
47	first flower	229.29 c A	201.36 b A
61	early pod development	355.29 b A	246.13 b A
75	pod development	486.25 a A	574.41 a A
86	ripe seed	555.30 a A	671.92 a A

1 Means followed by the same lowercase letter in each column are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

2 Means followed by the same uppercase letter in each row are not significantly different at P = 0.20 (t-test).

Table 34. Accumulation of symbiotically fixed nitrogen in lentils as calculated using the 'A'-value method, the ^{15}N Assisted Difference method and the Classical Difference method (Field Experiments 1983).

Days after seeding	Developmental stage of lentils	Symbiotically fixed nitrogen (kg ha^{-1})		
		'A' Value method	^{15}N Assisted Diff. method	Classical Diff. method
24	prebud	0.2 b1	-2.8 c	-3.7 c
33	prebud	-1.5 b	1.3 c	1.8 c
47	first flower	4.6 b	25 b	29 b
61	early pod development	31 a	69 a	73 a
75	pod development	-22 c	63 a	67 a
86	ripe seed	-23 c	36 a	38 b

1 Means followed by the same letter in each column not significantly different at P = 0.05 (Duncan's Multiple Range Test).

of this study, nonnodulating soybeans did not meet all of the prerequisites of an appropriate reference plant for lentils, and thus estimation of quantity of N fixed was suspect, particularly when N balance techniques were employed.

Application of ^{15}N labelled fertilizer allowed for the determination of the percentage of nitrogen derived from fertilizer in both lentils and nonnodulating soybeans. The data indicated the lentils and nonnodulating soybeans derived the same percentage of total plant N from the fertilizer N source at each harvest date. These results strongly suggest that the two crops fed from similar N sources and thus, it is likely that lentils did not derive N from symbiotic fixation. High levels of available soil N coupled with unfavourable droughty conditions likely resulted in the inhibition of nitrogen fixation.

Nonnodulating soybeans did not meet all of the prerequisites of an appropriate reference crop for lentils and thus did not provide suitable estimates of the contribution of soil and fertilizer N to total N yield of lentils when using N balance techniques. However, droughty conditions may have influenced seasonal growth and nitrogen uptake patterns of lentils and nonnodulating soybeans. Thus the possibility exists that, under favorable environmental conditions, nonnodulating soybeans may be a suitable reference crop for lentils.

Nitrogen Fertilizer Rate Study

Nitrogen was applied at rates of 0, 30, 100 and 200 $kg.ha^{-1}$ to lentils and at rates of 0, 30 and 100 $kg\ ha^{-1}$ to nonnodulating soybeans. For nonfixing plants, the 'A'-value has been demonstrated to be

independent of rate of application (Legg and Allison, 1959; Legg and Stanford, 1967; Fried and Broeshart, 1975) and thus, in light of the high cost of ^{15}N labelled fertilizer, it was deemed unnecessary to apply nitrogen at a rate of 200 kg ha^{-1} to the nonnodulating soybeans. However, nitrogen was added at both 30 and 100 kg N ha^{-1} to the nonnodulating soybeans in order to check that the 'A'-value was indeed rate independent under the conditions of this experiment.

Despite relatively high levels of available soil N (50.9 kg N ha^{-1}), supplemental nitrogen was found to be an important factor in attaining high dry matter yields (Table 35). The addition of 30 and 100 kg N ha^{-1} significantly increased the dry matter yield of lentils. Application of 100 kg N ha^{-1} resulted in the highest dry matter yield of 4544 kg ha^{-1}, although this yield was not significantly greater than that attained when 30 kg N ha^{-1} was added. Positive yield responses indicated that soil and symbiotically fixed sources of N were not adequate to meet plant growth demands. Application of 200 kg N ha^{-1} did not increase yields above that of the check treatment. It is possible that lack of response to high levels of fertilizer N was related to the droughty conditions which persisted throughout the growing season. Viets (1962) cited several studies in which application of fertilizer N resulted in an increased depletion rate or an increased total use of soil water. Leubs and Laag (1969) suggested that at high levels of fertility, increased total use of soil water may lead to an early exhaustion of soil water during rainless periods. Early exhaustion of the soil water supply can result on severe water stress during critical growth stages thereby limiting yield. Application of

Table 35. Effect of nitrogen application on various yield components of lentils (Field Experiments 1983).

Nitrogen Applied (kg ha^{-1})	Total Dry Matter Yield (kg ha^{-1})	Seed Yield (kg ha^{-1})	Harvest Index (%)	Total Nitrogen Accumulation (kg ha^{-1})	Seed Nitrogen Accumulation (kg ha^{-1})
0	3468 b[1]	1984 a	57.3 a	98 b	82 a
30	4073 a	2208 a	54.2 a	112 a	91 a
100	4544 a	2459 a	54.1 a	125 a	102 a
200	3464 b	2034 a	58.7 a	100 b	86 a

[1] Means followed by the same letter in each column are not significantly different at P = 0.10 (Duncan's Multiple Range Test).

200 kg N.ha^{-1} may have encouraged early luxuriant use of soil water, exhausting the limited supply prior to rapid vegetative growth, thus limiting final dry matter yields. Nitrogen fertilizer application did not significantly affect seed yields although the data suggest that application of 30 and 100 kg N ha^{-1} resulted in positive yield responses (Table 35). Lentil seed yields were very high at all levels of fertilizer application. The lentil plant has an indeterminate flowering habit and Slinkard and Drew (1982) have suggested that some drought stress is needed, particularly during the latter part of the flowering process, in order to facilitate maturation of younger pods and maximize seed yields. Thus the relatively high seed yields attained during the 1983 field season may have been due, in part, to drought stress.

Harvest index, which is given by the ratio of seed yield to total dry matter yield, was not significantly affected by the rate of nitrogen applied (Table 35). However, slight decreases in harvest index when rates of 30 and 100 kg N ha^{-1} were applied may indicate that supplemental nitrogen had a greater stimulus on vegetative, rather than reproductive growth. The highest harvest index was obtained when nitrogen was added at a rate of 200 kg N ha^{-1}. If, as Luebs and Laag (1969) suggest may occur, high levels of fertility led to an early exhaustion of soil water, lentils receiving 200 kg N ha^{-1} may have been subjected to a moisture stress earlier in the season than the other treatments. This early water stress may have resulted in the favored production of reproductive rather than vegetative growth. This possibility is supported by Slinkard and Drew (1982) who have suggested that, due to an

indeterminate growth habit, lentils may need some moisture stress to encourage seed set.

Total plant nitrogen accumulation paralled dry matter accumulation (Table 35). Maximum N contents were attained when nitrogen was applied at rates of 30 and 100 kg ha^{-1}. Application of 200 kg N ha^{-1} did not significantly increase crop N content over the check. Nitrogen accumulated in the seed reflected seed yields and was not significantly affected by supplemental nitrogen (Table 35). However, data suggests that the application of 100 kg N ha^{-1} resulted in the highest seed N content.

Dry matter and nitrogen yields of soybeans are reported in Table 36. Application of supplemental nitrogen led to significant dry matter yield reduction. It is likely that supplemental nitrogen encouraged luxurious water consumption, depleting the limited soil water supply prior to stages of rapid growth and dry matter accumulation. It has been demonstrated that application of excess N fertilizer may result in increasing increments of total soil water use (Viets, 1962) and thus, where water is limited, higher rates of nitrogen have the greatest depressive effects on yield.

Very low seed yields for all nonnodulating soybean treatments evidence the fact that nonnodulating soybeans were harvested prior to maturity (Table 36). As with dry matter yields, seed yields decreased

Table 36. Effect of nitrogen application on various yield components of nonnodulating soybeans (Field Experiments 1983).

Nitrogen Applied (kg ha^{-1})	Total Dry Matter Yield (kg ha^{-1})	Seed Yield (kg ha^{-1})	Total Nitrogen Accumulation (kg ha^{-1})	Seed Nitrogen Accumulation (kg ha^{-1})
0	3720 a[1]	537 a	91 a	34 a
30	3265 ab	434 a	74 ab	25 a
100	2798 b	235 b	66 b	15 b

[1] Means followed by the same letter in each column are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

significantly with the addition of 100 kg N ha^{-1}. The addition of 30 kg N ha^{-1} also led to a reduction in seed yield although this reduction was not significant.

Total nitrogen accumulation and seed nitrogen accumulation reflected dry matter yields and seed yields, respectively (Table 36). Fertilizer nitrogen applied at a rate of 100 kg. $N.ha^{-1}$ served to decrease both total N and seed N accumulation.

Percent nitrogen derived from fertilizer in both lentils and nonnodulating soybeans increased with increasing increments of applied N (Table 37). When comparing a nodulating legume to a nonnodulating control, it is generally expected that the fixing system has lower Ndff values, indicating the dilution of ^{15}N labelled fertilizer N by atmospheric N. Where nitrogen was added at a rate of 30 kg $N.ha^{-1}$, lentils and nonnodulating soybeans derived similar amounts of nitrogen from the fertilizer source. However, where nitrogen was added at a rate of 100 kg $N.ha^{-1}$, nonnodulating soybeans had a lower Ndff value than the lentils. This may suggest that the two crops were not feeding from the same soil N pool and thus the nonnodulating soybeans were not valid controls at the 100 kg $N.ha^{-1}$ rate of fertilizer application.

Lentils accumulated more fertilizer nitrogen than the reference crop (Table 37). Application of 100 kg N ha^{-1} resulted in the accumulation of 27.7 kg ha^{-1} of fertilizer nitrogen in the lentils whereas nonnodulating soybeans accumulated only 9.8 kg ha^{-1}. Again, differences in total fertilizer N accumulation may reflect different N

Table 37. Effect of nitrogen application on various nitrogen uptake components by lentils and nonnodulating soybeans (Field Experiments 1983).

Nitrogen applied ($kg.ha^{-1}$)	Nitrogen derived from fertilizer (Ndff) (%)		Fertilizer nitrogen accumulation ($kg\ ha^{-1}$)		Utilization of fertilizer nitrogen (%)	
	Lentils	Nonnod soy	Lentils	Nonnod soy	Lentils	Nonnod soy
0	-	-	-	-	-	-
30	5.2 c[1]	4.9 b[2]	5.8 c	3.6 a	19.4 b	12.1 a
100	24.7 b	14.9 a	27.7 b	9.8 a	30.6 a	9.8 a
200	45.4 a	-	45.3 a	-	27.2 a	-

1 Means followed by the same letter in each column are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

2 Means followed by the same letter in each column are not significantly different at P = 0.05 (t-test).

demands. The large discrepancy in fertilizer N accumulation may also indicate that the N uptake patterns of the two crops were dissimilar. Different maturation dates further indicate that N uptake patterns were not the same as it is unlikely that periods of high N demands exerted by the two crops coincided with one another. Although the quantity of fertilizer nitrogen accumulated by the lentils and nonnodulating soybeans was not significantly different ($P = 0.05$) for the 30 kg N ha^{-1} treatment, the time-course study had demonstrated differences in N uptake patterns.

Percent utilization of fertilizer N by lentils increased from 19.4% when 30 kg N ha^{-1} was applied to 30.6% when 100 kg N ha^{-1} was applied (Table 37). No further increases resulted from the application of 200 kg N ha^{-1}. Although percent utilization of N fertilizer has been demonstrated to increase with increasing rates of applied N (Allos and Bartholemew, 1959; Diebert et al., 1979; Vasilas and Ham, 1984), it is more usual to observe decreases in percent utilization of fertilizer N with increasing N rates, as was reported by Regitnig (1979).

Percent utilization of fertilizer nitrogen by the nonnodulating soybeans was not significantly affected by an increase in the rates of applied N (Table 37).

At both 30 and 100 kg N ha^{-1}, percent utilization of fertilizer N was greater for lentils than for nonnodulating soybeans. Significant differences ($P = 0.05$) in the percent utilization of fertilizer N invalidates the use of N balance methods for determining the quantity of symbiotically fixed nitrogen.

'A'-values, calculated for both lentils and nonnodulating soybeans, are reported in Table 38. Lentil 'A'-values decreased in response to added N. Both soil and symbiotically fixed N dilute ^{15}N labelled fertilizer N. Thus, if 'A'-(soil) values are indeed independent of fertilizer N rates, as with the nonnodulating soybeans, the decline in lentil 'A'-values as fertilizer N rates increase indicates a decrease in symbiotic nitrogen fixation. Decreased N fixation in response to additions of fertilizer N has been demonstrated in a number of crops (Allos and Bartholemew, 1955; McAuliffe et al., 1958; Munns, 1968; Richards and Soper, 1979). Nonnodulating soybean 'A'-values were not affected by the rate of fertilizer N applied.

Lentil and nonnodulating soybean 'A'-values were not significantly different ($P = 0.20$) where N was applied at a rate of 30 kg ha^{-1}. Lack of significant difference suggests that even at this relatively low level of fertilizer N application, lentils did not symbiotically fix nitrogen. At nitrogen fertilizer rates of 100 and 200 kg ha^{-1}, lentil 'A'-values were significantly lower ($P = 0.10$) than nonnodulating soybean 'A'-values. Lower 'A'-values resulted from the fact that lentils derived a greater percentage of total crop N from the fertilizer than did the nonnodulating soybeans.

Symbiotically fixed nitrogen was determined by the 'A'-value method, the ^{15}N Assisted Difference method and the Classical Difference method (Table 39). The ^{15}N Assisted Difference method

Table 38. Effect of nitrogen application on 'A'-values of lentils and nonnodulating soybeans (Field Experiments 1983).

Nitrogen Applied (kg ha^{-1})	'A'-Value (kg ha^{-1})	
	Lentil	Nonnodulating soybean
30	555.3 a[1]	584.2 a[2]
100	316.8 b	608.4 a
200	251.6 b	

1 Means followed by the same letter in each column not significantly different at P = 0.05 (Duncan's Multiple Range Test).

2 t-test.

Table 39. Effect of nitrogen application on the accumulation of symbiotically fixed nitrogen as calculated using the 'A'-value method, the ^{15}N Assisted Difference method and the Classical Difference method (Field Experiments 1983).

Nitrogen applied (kg ha^{-1})	Symbiotically fixed nitrogen (kg ha^{-1})		
	'A'-value method	^{15}N Assisted Difference method	Classical Difference method
0	-	7.2 b	7.2 b
30	-11 a[1]	36 a	38 a
100	-91 b	41 a	64 b
200	-102	-	-

[1] Means followed by the same letter in each column not significantly different at P = 0.05 (Duncan's Multiple Range Test).

and the Classical Difference method differed in estimating quantity of N fixed. Differences in the two methods are attributable to the higher recovery of fertilizer N by the lentils. Use of these two N balance techniques was discredited by the fact that lentils and nonnodulating soybeans did not have the same percent utilization of fertilizer N.

'A'-values for the nonnodulating soybeans receiving 30 kg N ha^{-1} and 100 kg N ha^{-1} were not significantly different. Thus calculation of symbiotically fixed nitrogen using the 'A'-value method was not significantly affected by the rate of fertilizer applied to the reference crop. Estimates of quantity of N fixed reported in Table 39 were calculated using nonnodulating soybeans treated with 100 kg N ha^{-1} as the control. Calculation of symbiotically fixed nitrogen using the 'A'-value method led to large negative values of fixed N, particularly for lentils receiving 100 kg N ha^{-1} and 200 kg N ha^{-1}. Errors incurred using this method were due to variation related to the parameters from which the 'A'-values were calculated, most notably the percentage of N derived from fertilizer.

Direct evidence regarding the quantity of N symbiotically fixed by lentils was not obtained. However, in light of the lentil yield increases associated with added fertilizer N, it can be concluded that, under the condition of this experiment, N fixation did not adequately meet the nitrogen demands of lentils.

Calculation of the quantity of N symbiotically fixed using both the ^{15}N Assisted Difference method and the Classical Difference method was discredited by the fact that the two crops did not utilize the same percentage of applied fertilizer N. Use of the 'A'-value method for

determining N fixed was also suspect as the lentils were more adept at utilizing applied fertilizer N than were the nonnodulating soybeans, particularly at high rates of fertilizer N application. However, where N was applied at a rate of 30 kg ha^{-1}, lentils and nonnodulating soybeans shared similar Ndff values which may suggest that the lentils did not symbiotically fix nitrogen at this rate of fertilizer N application. It is unlikely that symbiotic fixation was operative at the higher levels of applied N. Droughty conditions coupled with relatively high levels of available soil N likely resulted in the inhibition of symbiotic fixation.

4.2.2 Field Experiments 1984

The effect of nitrogen application on nitrogen fixation, nitrogen accumulation and yield of lentils was studied during the 1984 growing season under field conditions. Two experimental locations, designated Haywood and Morden were used in this study. Studies conducted by Chowdhury et al. (1974) and Sekhon et al. (1978) indicated that application of fertilizer N did not benefit lentil yield. However, nitrogen rates used in these studies did not exceed 25 kg N ha^{-1}. McEwen (1970) suggested that application of small amounts of fertilizer N may have little or no effect on the yield of legumes if the application of fertilizer N does not exceed the amounts usually fixed from the atmosphere. That is, small dressings of fertilizer nitrogen may lessen fixation of atmospheric nitrogen by rhizobia so that there is no net gain in nitrogen available for crop use. Results from the previous year's study indicated that lentils may benefit from the addition of

supplemental nitrogen. The experiments described here further tested the effects of fertilizer nitrogen, applied at varying rates, on lentil production.

In addition, an experiment in which lentils and nonnodulating soybeans were grown in lysimeters was conducted at the Haywood site. Both crops received ^{15}N labelled fertilizer applied at a rate of 30 kg N ha^{-1}. Lysimeters were utilized in order to facilitate the excavation and examination of lentil and nonnodulating soybean roots. The objective of the lysimeter study was to determine the extent to which consideration of root N may influence the determination of symbiotically fixed nitrogen.

The application of ^{15}N labelled fertilizer facilitated the determination of symbiotically fixed N. Accumulation of symbiotically fixed nitrogen was calculated using the 'A'-value method, the ^{15}N Assisted Difference method and the Classical Difference method. Nonnodulating soybeans served as a reference crop. In addition, nitrogenase activity was evaluated throughout the growing season using the acetylene reduction technique.

On June 2nd, a severe windstorm swept the Haywood site, causing considerable damage to the newly emerged lentils. The lentils, which had reached a height of approximately five centimeters were completely stripped of leaves and, in some cases, shorn off at ground level. The nonnodulating soybeans had germinated at this time but had not yet emerged and thus were undamaged by the storm. Although lentils recovered from the effects of the storm, damage to the crop was extensive and regrowth was slow.

Very little crop cover existed at the time of the windstorm and evidence of soil drifting was noted. The effects of soil drifting were particularly notable in the area of the lysimeter experiment as the lip of the exposed lysimeters served to collect drifting soil. Although it is not known to what degree the soil drifting may have influenced experimental results, it is likely that some cross-contamination of treatment plots, treatment subplots and lysimeters occurred. In addition to encouraging variability amongst treatments, cross-contamination of ^{15}N labelled treatment subplots and lysimeters may have diluted the ^{15}N labelled soil pool and thus led to an underestimation of the contribution of fertilizer N to total crop N and an overestimation of crop 'A'-values.

On July 7th, a hailstorm occurred at the Haywood site damaging both lentils and nonnodulating soybeans. The lentils, having recovered from the previous storm, were only 5-7.5 cm in height and had initiated branching. Many of the lentil plants were knocked to the ground and some leaf loss was noted. The nonnodulating soybeans had reached the 2-3 trifoliate leaf stage at the time of the storm and many of these leaves were either dislodged or badly shredded by the hail. Both lentils and nonnodulating soybeans recovered from this second storm and although some dry matter was lost, very few plants were damaged beyond recovery. Lentils at Haywood required 91 days to mature whereas the crop at Morden matured in 74 days. It is thought that the damage caused by the two storms at Haywood prolonged the duration of the growing season as vegetative regrowth following each storm lengthened the vegetative stage.

During the latter part of the growing season, grasshoppers infested the Haywood site causing considerable damage to both lentils and non-nodulating soybeans. While plants were not completely stripped, some areas of the experimental site were visibly damaged. It is likely that the considerable variation in dry matter yields obtained at this location was due, in part, to the damage caused by the grasshoppers.

Finally, the Haywood site tended to be somewhat droughty throughout much of the growing season and is likely that a moisture deficit limited growth. Although growing season precipitation was below the long-term average at Morden, available soil moisture appeared to be sufficient to support both lentil and nonnodulating soybean growth.

^{15}N Fertilizer Rate - Time Course Study

Dry matter yields were determined at bloom and at ripe seed stages of lentils at both experimental locations. Additional harvests at Haywood for treatments receiving nitrogen at rates of 0 and 30 kg N ha^{-1} and at Morden for the check treatment were conducted for the purpose of determining nitrogen fixation. Dry matter yields for lentils and nonnodulating soybeans are reported in Table 40 and 41, respectively.

Lentils and nonnodulating soybeans did not share similar maturation dates. At both sites, lentils reached maturity prior to nonnodulating soybeans. Leaf senescence, associated with approaching maturity, was extensive by lentils, whereas loss of dry matter appeared to be much less by the nonnodulating soybeans. Difference in maturation date and thus degree of leaf senescence was reflected in the dry matter accumula-

Table 40. Effect of nitrogen application on dry matter yield of lentils determined throughout the growing season (Field Experiments 1984).

Location and nitrogen rate (kg N ha^{-1})	Dry Matter Yield (kg ha^{-1})				
	Harvest[1] 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5
Haywood 0	338 a[2] C[3]	690 a B	924 a AB	1126 a A	1050 a A
30	336 a C	776 a B	875 a AB	1000 a AB	1120 a A
90			1057 a A		1389 a A
180			1397 a A		1180 a A
Morden 0	1861 C	2453 a C	3656 B	5072 A	3923 a B
30	-	3041 a B	-	-	4348 a A
90	-	3114 a B	-	-	4662 a A
180	-	3172 a B	-	-	4936 a A

1 Days after seeding and physiological stage of lentils:
Haywood - Harvest 1 = day 49, bud; Harvest 2 = day 58, early bloom; Harvest 3 = day 66, bloom; Harvest 4 = day 74, early pod; Harvest 5 = day 91, ripe seed.
Morden - Harvest 1 = day 48, bud; Harvest 2 = day 57, bloom; Harvest 3 = day 65, early pod; Harvest 4 = day 73, pod development; Harvest 5 = day 79, ripe seed.

2 Means followed by the same lowercase letter in each column within a site are not significantly different at P = 0.05 (Duncan's Multiple Range Test, t-test).

3 Means followed by the same uppercase letter in each row are not significantly different at P = 0.05 (Duncan's Multiple Range Test, t-test).

Table 41. Effect of nitrogen application on dry matter yield of nonnodulating soybeans determined throughout the growing season (Field Experiments 1984).

Location and nitrogen rate (kg N ha^{-1})	Dry Matter Yield (kg ha^{-1})				
	Harvest[1] 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5
Haywood 0	529 a[2] D[3]	710 a C	712 a C	947 a B	1819 a A
30	503 a D	890 a C	1037 a BC	1268 a B	1992 a A
90					2088 a
Morden 0	1060 E	1580 a D	2078 C	3014 B	3812 a A
30	-	1807 a B	-	-	2522 a A
90	-	-	-	-	3827 a

1 Refer to Table 40 for corresponding days after seeding.

2 Means followed by the same lowercase letter in each column within a site are not significantly different at P = 0.05 (Duncan's Multiple Range Test, t-test).

3 Means followed by the same uppercase letter in each row are not significantly different at P = 0.05 (Duncan's Multiple Range Test, t-test).

tion patterns of the two crops. Rennie (1982) suggested that a nonfixing reference crop should have a maturation date similar to that of the legume, as dissimilarity of maturation date often precludes similarity in N uptake patterns, a necessary prerequisite for the control crop.

In general, lentil dry matter yields obtained at Haywood were extremely low despite relatively high levels of available soil NO_3-N. Low yields were attributed to a combination of the early damage to the crop by the June 2nd windstorm, the July 7th hail storm, the later grasshopper infestation and the droughty conditions which persisted throughout the growing season. In contrast, lentil dry matter yields obtained at Morden were relatively high and were consistent with yields obtained during the 1983 field season.

Supplemental nitrogen did not lead to significant dry matter yield increases of lentils at either experimental site. High levels of available soil NO_3^--N at the Haywood site were sufficient to meet the N demands of the low yielding lentils. Thus it was apparent that factors other than N availability limited lentil growth at this site. Although positive yield responses to nitrogen addition at Morden were not statistically significant, nonsignificant yield increases were obtained both at bloom and at ripe seed, suggesting that soil and symbiotically fixed sources of N may not have been sufficient for optimal growth.

At Haywood, the maximum rate of dry matter accumulation occurred between the bud and early bloom stage for lentils receiving nitrogen at rates of 0 and 30 kg ha^{-1}. This is consistent with a study conducted by Walley (1983) in which it was reported that under controlled growth bench conditions, maximum rate of dry matter accumulation occurred as

lentils made the transition from vegetative to reproductive growth. In contrast, the maximum rate of dry matter accumulation for lentils at Morden receiving no fertilizer N occurred later in the growing season, corresponding to the period between early pod and pod development stages of growth. This is consistent with results obtained during the 1983 field season in which maximum dry matter accumulation occurred during the early pod fill stage of development. Moisture did not appear as limiting at the Morden site as at the Haywood site and thus, due to the indeterminate flowering habit of lentils, some vegetative as well as reproductive growth may have been stimulated even in the latter growth stages. In particular, the flowering stage was not limited resulting in the production of a large number of pods and thus rapid dry matter accumulation was likely due to the rapid filling of immature pods.

Nonnodulating soybeans did not respond significantly to the application of fertilizer nitrogen at either experimental location. At Haywood, yields were relatively low and thus high levels of soil NO_3^--N may have met crop N demands. However, it should be noted that at all but the first harvest at Haywood, additions of fertilizer N appeared to increase yields, although not significantly. Lack of significance may reflect variability caused by adverse environmental conditions rather than the absence of real yield responses.

Yields obtained at Haywood for both lentils and nonnodulating soybeans were similar throughout the growing season with the exception of the final harvest. Data indicate that nonnodulating soybeans outyielded lentils at final harvest, reflecting differences in dry matter accumulation patterns and leaf senescence. In contrast, lentil dry matter

yields at Morden were consistently higher than nonnodulating soybean yields. Increased lentil yields may be indicative of improved nitrogen nutrition due to N fixation.

The accumulation of nitrogen in the aerial portions of lentils is reported in Table 42. Many of the trends reported for dry matter yields are reflected in nitrogen accumulation patterns. Generally, nitrogen yields were much lower at Haywood than at Morden. Nitrogen yields obtained at Morden were in keeping with those reported for the 1983 growing season. Although trends at Haywood suggested a positive response to supplemental nitrogen at bloom and ripe seed, significant responses were not obtained. Lack of significant response was not surprising in light of the fact that both dry matter and nitrogen yields were very low at this site. Subsequently, lentil N requirements were very low and thus soil and symbiotically fixed N were sufficient to meet any N demands.

In contrast, significant nitrogen yield responses to applied N were obtained at the Morden site where higher yielding lentils placed a greater demand on N supply. Application of 90 and 180 kg N ha^{-1} significantly increased the accumulation of nitrogen in lentils harvested at bloom. Application of 30 kg N ha^{-1} did not result in a significant N yield increase at this harvest. This is in keeping with results obtained by McEwen (1970) who reported that small dressings of fertilizer N may have no measurable effect on the yield of legumes. McEwen suggested that small dressings of fertilizer nitrogen may lessen fixation of atmospheric N so that there is no net gain in nitrogen available to the plant. Similar results were reported by Richards and

Table 42. Effect of nitrogen application on nitrogen accumulation in lentils determined throughout the growing season (Field Experiments 1984).

Location and nitrogen rate (kg N ha^{-1})	Nitrogen Accumulation (kg ha^{-1})				
	Harvest[1] 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5
Haywood 0	8.2 a[2] C[3]	16.2 a B	21.2 a AB	25.5 A	23.5 a A
30	8.1 a C	19.3 a B	22.2 a B	21.9 a B	28.7 a A
90			36.0 a A		31.5 a A
180			28.1 a A		29.2 a A
Morden 0	59.5 D	68.7 b CD	83.3 CB	102.6 AB	107.4 b A
30	-	74.7 b B	-	-	120.0 ab A
90	-	90.8 a B	-	-	120.2 ab A
180		101.0 a B			144.2 a A

1 Refer to Table 40 for corresponding days after seeding and physiological stage of lentils.

2 Means followed by the same lowercase letter in each column within a site are not significantly different at P = 0.05 (Duncan's Multiple Range Test, t-test).

3 Means followed by the same uppercase letter in each row are not significantly different at P = 0.05 (Duncan's Multiple Range Test, t-test).

Soper (1979) who found that addition of N fertilizer to fababeans resulted in a reduction in symbiotic fixation with no corresponding increase in N uptake. Thus N yield responses to the application of 90 and 180 kg N ha^{-1}, suggest that these amounts exceeded the amount of nitrogen symbiotically fixed by the check treatment. Furthermore, it can be concluded that N yield of the check treatment was limited by the dependence on soil and symbiotically fixed sources of N. At final harvest, only the application of 180 kg N ha^{-1} resulted in a significant increase in N yield. Yield increases resulting from the application of 30 and 90 kg N ha^{-1} were not statistically significant. However, data suggest that even small dressings of fertilizer N may lead to increased N yield in lentils.

Nitrogen accumulation by lentils at Haywood was most rapid between bud and early bloom, corresponding to the maximum rate of dry matter accumulation. Similarly, maximum N accumulation rate at Morden corresponded with the maximum dry matter accumulation rate and occurred between early pod and pod development.

Nitrogen yield of nonnodulating soybeans was not significantly affected by the application of fertilizer N at the Haywood site (Table 43). Limited dry matter production and relatively low N yields limited the crop N demands and thus high levels of soil NO_3^--N appeared to meet nonnodulating soybean N requirements. Application of 90 kg N ha^{-1} led to a significant increase in final N yields at Morden. Application of 30 kg N ha^{-1} did not significantly affect N yield.

Comparison of nitrogen yields at Haywood for lentils and nonnodulating soybeans revealed that N uptake by the two crops was not

Table 43. Effect of nitrogen application on nitrogen accumulation in nonnodulating soybeans determined throughout the growing season (Field Experiments 1984).

Location and nitrogen rate (kg N ha^{-1})		Nitrogen Accumulation (kg ha^{-1})				
		Harvest[1] 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5
Haywood	0	12.0 a[2] C[3]	18.7 a B	14.1 BC	15.9 BC	30.1 a A
	30	11.6 a C	17.5 a B	20.5 B	20.5 B	27.3 a A
	90					35.0 a
Morden	0	28.1 C	30.8 a C	37.6 C	50.4 B	67.3 b A
	30	-	37.6 a A	-	-	56.1 b A
	90	-	-	-	-	81.5 a

1 Refer to Table 40 for corresponding days after seeding.

2 Means followed by the same lowercase letter in each column within a site are not significantly different at P = 0.05 (Duncan's Multiple Range Test, t-test).

3 Means followed by the same uppercase letter in each row are not significantly different at P = 0.05 (Duncan's Multiple Range Test, t-test).

significantly different (P = 0.05) at corresponding levels of N fertilizer application. This suggests that lentils at the Haywood site fixed little or no atmospheric nitrogen. In contrast, at Morden, lentil N yields were significantly higher (P = 0.05) than nonnodulating soybean yields which received N at similar rates. The difference in nitrogen yields suggests that symbiotically fixed nitrogen contributed significantly to the total N yield of lentils at Morden.

At Haywood, the maximum rate of nitrogen accumulation for nonnodulating soybeans occurred between day 74 and day 91, as opposed to N accumulation by lentils which reached a maximum between day 49 and day 58. Similarly, maximum rate of N accumulation by nonnodulating soybeans at Morden occurred later in the season than by lentils. Nonnodulating soybeans accumulated N at a maximum rate between day 73 and day 79, whereas lentils reached a maximum rate of N accumulation between day 65 and day 73. Differences in N accumulation patterns may have been due, in part, to the fact that the lentils matured more rapidly than the nonnodulating soybeans. Diebert et al. (1979) reported that soybeans tend to utilize proportionately more N during latter growth stages than other crops. Witty (1983) has reported that the enrichment of mineral N in the soil falls with time after the application of ^{15}N and thus if a legume and a nonlegume are to see the same soil N pool, the nitrogen uptake patterns of the two crops over the growing season must be the same. In calculating 'A'-values, the magnitude of error incurred due to differences in N uptake patterns is dependent on the rate of decline in soil enrichment (Witty 1983). Rate of decline was not evaluated for this experiment and thus the magnitude of error could not be determined.

However, it is possible that because the nonnodulating soybeans took up N later than the lentils, enrichment of the soil N pool was lower.

Lentil seed yields were determined at final harvest and are reported in Table 44. Yields at Haywood were very low, reflecting the adverse growing conditions whereas those obtained at Morden were extremely good. No significant seed yield responses were obtained at either site although data obtained at Morden suggest that yields increased in response to increasing increments of supplemental N.

Accumulation of nitrogen in the seed was also not significantly affected by the rate of nitrogen application (Table 44). However, non-significant responses to applied N at Morden suggest that supplemental N may be necessary for maximum seed N content.

Percent protein in the seed was not significantly affected by the application of fertilizer N at either experimental location (Table 44). Values for percent protein obtained in this experiment were in keeping with the average of 25 percent reported by Summerfield (1981).

Harvest index, given by the ratio of seed yield to total dry matter yield, was unaffected by the rate of nitrogen addition (Table 44). An exception exists at Haywood where the harvest index declined significantly with the addition of 180 kg N ha^{-1}. The decline in harvest index indicates that the high rate of fertilizer N favoured vegetative rather than reproductive growth. Harvest index at Morden was unaffected by rate of fertilizer N addition. Thus increases in dry matter production due to increased fertilizer addition was coupled with increased seed yields. Harvest index at Morden was higher than at Haywood at all levels of N application, indicating that lentils at Morden yielded

Table 44. Effect of nitrogen application on seed yield, seed nitrogen accumulation, percent protein and harvest index of lentils (Field Experiments 1984).

Location and nitrogen rate (kg N ha^{-1})	Seed yield (kg ha^{-1})	Seed nitrogen accumulation (kg ha^{-1})	Percent protein	Harvest index (%)
Haywood 0	345 a[1]	12.4 a	22.3 a	31 a
30	381 a	16.1 a	26.3 a	33 a
90	447 a	19.0 a	26.5 a	30 a
180	293 a	13.9 a	29.6 a	24 b
Morden 0	1846 a	76.4 a	25.8 a	47 a
30	2020 a	84.0 a	25.9 a	46 a
90	2193 a	91.2 a	25.9 a	47 a
180	2285 a	102.0 a	27.8 a	46 a

[1] Means followed by the same letter in each column within a site are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

proportionately higher than at Haywood.

Determination of fertilizer derived nitrogen was facilitated by the use of ^{15}N labelled fertilizer. Percent nitrogen derived from fertilizer (Ndff) in both lentils and nonnodulating soybeans is reported in Table 45. The decline in Ndff over the growing season at all levels of fertilizer N evaluated, evidences the dilution of ^{15}N labelled fertilizer by soil N, and soil and (or) atmospheric N in the nonnodulating soybeans and lentils, respectively.

Where nitrogen fertilizer was applied at a rate of 30 kg N ha^{-1}, a greater proportion of nitrogen in the nonnodulating soybeans was derived from fertilizer N than in the lentils as evidenced by the higher Ndff values. This suggests that at this rate of fertilizer application, lentils derived some of the total crop N requirements from atmospheric N which served to dilute ^{15}N labelled fertilizer derived N. However, the addition of 90 kg N ha^{-1} resulted in similar Ndff values for the two crops at both Haywood and Morden. Thus it is suggested that at higher levels of fertilizer application, lentils and nonnodulating soybeans derived nitrogen in similar proportion from the same sources, namely soil and fertilizer N. It follows that symbiotically fixed nitrogen did not contribute to the total N yield of lentils.

Application of increasing increments of supplemental nitrogen resulted in signficant increases in Ndff values for both lentils and nonnodulating soybeans. Thus, increases in fertilizer N availability resulted in concomittant increases in the contribution of fertilizer N to total crop N.

Application of supplemental N resulted in significant increases in

Table 45. Effect of nitrogen application on percent nitrogen derived from fertilizer in lentils and nonnodulating soybeans determined throughout the growing season (Field Experiment 1984).

Location and nitrogen rate (kg N ha^{-1})	Percent nitrogen derived from fertilizer				
	Harvest[1] 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5
Haywood lentils 30	13.2 A[2]	10.2 AB	11.0 b[3] AB	7.6 B	7.3 c B
90	-	-	40.3 a A	-	29.0 b B
180	-	-	-	-	55.1 a
Nonnod soybeans 30	17.4 AB	18.2 A	15.4 ABC	12.0 C	12.8 b BC
90	-	-	-	-	25.0 a
Morden lentils 30	-	10.5 A	-	-	7.7 b B
9	-	-	-	-	27.6 a
Nonnod Soybeans 30	-	18.3 A	-	-	13.1 b B
90	-	-	-	-	26.1 a

1 Refer to Table 40 for corresponding days after seeding and physiological stage of lentils.

2 Means followed by the same uppercase letter in each row are not significantly different at P = 0.05 (Duncan's Multiple Range Test, t-test).

3 Means followed by the same lowercase letter in each column within a site and crop are not significantly different at P = 0.05 (Duncan's Multiple Range Test, t-test).

fertilizer nitrogen accumulation in both crops (Table 46). Fertilizer nitrogen accumulation tended to be very low at Haywood, reflecting poor dry matter and nitrogen yields. Accumulation of fertilizer nitrogen by lentils and nonnodulating soybeans was not significantly different ($P = 0.05$) throughout the growing season at levels of fertilizer N evaluated. Lack of significant differences may be taken to indicate that lentils and nonnodulating soybeans shared identical fertilizer N uptake patterns. However, it is more likely that the lack of statistical significance occurred as a result of very low levels of fertilizer N accumulation accompanied by a relatively high degree of variation amongst replicates.

Application of 30 kg N ha^{-1} at Morden resulted in the accumulation of similar amounts of fertilizer N in the two crops, as determined at final harvest. However, a significant difference ($P = 0.05$) in the values obtained at harvest 2 indicate that fertilizer nitrogen uptake patterns of the two crops were not the same. The data suggest that nonnodulating soybeans accumulated fertilizer N earlier in the growing season than lentils. The fact that fertilizer N accumulation in nonnodulating soybeans did not increase significantly following harvest 2 is particularly surprising in light of the fact that total nitrogen accumulation by this crop was greatest between harvest 4 and harvest 5 (Table 43). Such a discrepancy may indicate that, following the second harvest, nonnodulating soybeans fed from a soil N pool which excluded ^{15}N labelled fertilizer such as that which may have existed lower in the soil profile. Alternatively, nonnodulating soybeans may have taken up all of the fertilizer N they could early in the season and were

Table 46. Effect of nitrogen application on fertilizer nitrogen accumulation in lentils and nonnodulating soybeans determined throughout the growing season (Field Experiments 1984).

Location and nitrogen rate (kg N ha^{-1})	Fertilizer nitrogen accumulation (kg ha^{-1})				
	Harvest[1] 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5
Haywood lentils 30	1.1 B[2]	1.9 AB	2.3 b[3] A	1.7 AB	2.0 c AB
90	-	-	11.5 a A	-	9.0 b A
180	-	-	-	-	16.1 a
Nonnod soybeans 30	2.0 B	3.2 A	3.1 A	2.4 AB	3.5 b A
90	-	-	-	-	8.9 a
Morden lentils 30	-	2.7 B	-	-	9.2 b A
90	-	-	-	-	33.0 a
Nonnod Soybeans 30	-	7.0 A	-	-	7.4 b A
90	-	-	-	-	21.7 a

1 Refer to Table 40 for corresponding days after seeding and physiological stage of lentils.

2 Means followed by the same uppercase letter in each row are not significantly different at P = 0.05 (Duncan's Multiple Range Test, t-test).

3 Means followed by the same lowercase letter in each column within a site are not significantly different at P = 0.05 (Duncan's Multiple Range Test, t-test).

unable to utilize fertilizer N beyond harvest 2.

Lentils accumulated significant amounts of fertilizer nitrogen between harvest 2 and harvest 5 indicating that lentils fed from a soil N pool which included ^{15}N labelled fertilizer. This may suggest that lentils and nonnodulating soybeans did not feed from the same soil N pool. This possibility is further evidenced by the fact that lentils accumulated significantly more ($P = 0.05$) fertilizer N applied at a rate of 90 kg ha^{-1} than nonnodulating soybeans.

Only when percent utilization of fertilizer N by the fixing system is identical to that of the nonfixing system can N balance methods accurately quantify nitrogen fixation. At Haywood no significant differences ($P = 0.05$) existed between the percent utilization of fertilizer N by lentils and nonnodulating soybeans (Table 47). However, as indicated earlier, lack of significant differences may have been an artifact of low levels of fertilizer recovery coupled with high variability between replicates rather than an indication that lentils and nonnodulating soybeans utilized similar amounts of fertilizer N. At Morden, percent utilization of fertilizer N by lentils and nonnodulating soybeans was significantly different at harvest 2 where N had been applied at a rate of 30 kg ha^{-1} and at final harvest where N had been applied at a rate of 90 kg N ha^{-1}. No significant differences existed between the percent utilization of fertilizer N applied at a rate of 30 kg N ha^{-1} as determined at final harvest. Data suggest that, at Morden, lentils utilized a greater percent of applied fertilizer N later in the growing season than nonnodulating soybeans, invalidating the use of N balance sheets for the determination of symbiotically fixed N.

Table 47. Effect of nitrogen application on percent utilization of fertilizer nitrogen by lentils and nonnodulating soybeans, determined throughout the growing season (Field Experiments 1984).

Location, crop and nitrogen rate (kg N ha^{-1})	Percent utilization of fertilizer nitrogen				
	Harvest[1] 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5
Haywood lentils 30	3.8 B[2]	6.4 AB	7.7 a[3] A	5.8 AB	6.8 b AB
90	-	-	12.8 b A	-	10.0 a A
180	-	-	-	-	8.9 ab
Nonnod soybeans 30	6.7 B	10.6 A	10.4 A	8.2 AB	11.6 a A
90	-	-	-	-	9.9 a
Morden lentils 30	-	8.9 B	-	-	30.7 a A
9	-	-	-	-	36.6 a
Nonnod Soybeans 30	-	23.3 A	-	-	24.7 a A
90	-	-	-	-	24.1 a

1 Refer to Table 40 for corresponding days after seeding and physiological stage of lentils.

2 Means followed by the same uppercase letter in each row are not significantly different at P = 0.05 (Duncan's Multiple Range Test, t-test).

3 Means followed by the same lowercase letter in each column within a site are not significantly different at P = 0.05 (Duncan's Multiple Range Test, t-test).

'A'-values for both lentils and nonnodulating soybeans increased throughout the growing season (Table 48). Increases reflect decreases in percent Ndff due to accumulation of soil, and soil and (or) symbiotically fixed nitrogen by nonnodulating soybeans and lentils, respectively.

At Haywood, increasing increments of applied fertilizer N did not significantly affect lentil 'A'-values. However, lack of significance was likely due to the extreme variability caused by adverse environmental conditions. The data suggest that increasing increments of applied N resulted in decreased lentil 'A'-values. The application of both 90 kg N ha^{-1} and 180 kg N ha^{-1} resulted in similar reductions. Thus it is suggested that the higher rates of applied fertilizer N may have inhibited fixation. Similarly, at Morden, lentil 'A'-values decreased with the addition of 90 kg N ha^{-1} which again suggests that this rate of N application may have inhibited fixation.

Nonnodulating soybean 'A'-values were not significantly affected by the application of fertilizer N. This is in keeping with a number of studies in which it was demonstrated that 'A'-values of nonfixing crops were independent of the rate of fertilizer application (Legg and Allison, 1959; Legg and Stanford, 1967; Fried and Broeshart, 1975).

Lentil 'A'-values reflect both soil and symbiotically fixed nitrogen whereas nonnodulating soybean 'A'-values reflect only soil nitrogen. At Haywood, where N was applied at a rate of 30 kg N ha^{-1}, lentil 'A'-values were higher than those calculated for nonnodulating soybeans, indicating that fixed nitrogen may have contributed to the total N yield of lentils. However, differences in 'A'-values were

Table 48. Effect of nitrogen application on 'A'-values for lentils and nonnodulating soybeans, determined throughout the growing season (Field Experiments 1984).

Location, crop and nitrogen rate (kg N ha^{-1})	'A'-value (kg ha^{-1})				
	Harvest[1] 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5
Haywood lentils 30	203.0 A[2]	276.4 A	255.9 a[3] A	426.6 A	469.0 a A
90	-	-	135.8 a A	-	241.8 a A
180	-	-	-	-	222.5 a
Nonnod soybeans 30	156.6 BC	137.5 C	168.2 ABC	232.8 A	206.8 a AB
90	-	-	-	-	285.1 a
Morden lentils 30	-	267.7 B	-	-	364.4 a A
9	-	-	-	-	267.7 b
Nonnod Soybeans 30	-	137.0 B	-	-	207.0 a A
90	-	-	-	-	260.0 a

1 Refer to Table 40 for corresponding days after seeding and physiological stage of lentils.

2 Means followed by the same uppercase letter in each row are not significantly different at P = 0.05 (Duncan's Multiple Range Test, t-test).

3 Means followed by the same lowercase letter in each column for each crop within a site are not significantly different at P = 0.05 (Duncan's Multiple Range Test, t-test).

statistically significant only at harvest 1 and harvest 2, and only at the 10% level of probability, as indicated in Table 48A. Similarly, 'A'-values calculated for lentils and nonnodulating soybeans were not significantly different at rates of 90 and 180 kg N ha^{-1}. Statistical analysis which indicated that significant differences between 'A'-values calculated for lentils and nonnodulating soybeans did not exist suggests that no measureable fixation occurred. However, lack of statistical significance may have been an artifact of the variability associated with the calculation of 'A'-values, namely the determination of Ndff and rate of ^{15}N labelled fertilizer added rather than a real indication that fixation did not take place. Extreme variability encountered at the Haywood site was likely due, in part, to the June 2nd windstorm which may have cross-contaminated ^{15}N labelled treatment subplots.

Lentil 'A'-values at Morden, where N had been applied at a rate of 30 kg N ha^{-1}, were significantly greater than nonnodulating soybean 'A'-values ($P = 0.20$). However, where N was added at a rate of 90 kg N ha^{-1}, 'A'-values were not significantly different. Thus evidence suggests that high rates of fertilizer N inhibited fixation at this site.

Symbiotically fixed N was calculated using the 'A'-value method, the ^{15}N Assisted Difference method and the Classical Difference method (Tables 49-51). The ^{15}N Assisted Difference method and the Classical Difference method differed only slightly in the calculation of symbiotically fixed N. Differences in the two methods reflect different amounts of fertilizer recovery by lentils and nonnodulating soybeans. Use of these two N balance techniques is descredited by the fact that

Table 48A. Level of significance associated with differences between lentils and nonnodulating soybean 'A'-values determined by the t-test procedure (Field Experiments 1984).

Location, and nitrogen rate (kg N ha^{-1})	Level of significance (%)				
	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5
Haywood					
30	10	10	N.S.[1]	N.S.	N.S.
90	-	-	N.S.	-	N.S.
180	-	-	-	-	N.S.
Morden					
30	-	20	-	-	20
90	-	-	-	-	N.S.

1 'A'-value means for lentils and nonnodulating soybeans are not significantly different at $P \leq 0.20$.

Table 49. Effect of nitrogen application on the accumulation of symbiotically fixed nitrogen as calculated using the 'A'-value method, determined throughout the growing season (Field Experiments 1984).

Location, and nitrogen rate (kg N ha^{-1})	Symbiotically fixed nitrogen (kg ha^{-1})				
	Harvest[1] 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5
Haywood					
30	1.1 B[2]	8.6 AB	6.9 a AB	6.6 AB	12.7 a[3] A
90	-	-	-4.8 a A	-	-6.0 a A
180	-	-	-	-	-6.9 a
Morden					
30	-	4.0 B	-	-	47.6 a A
90	-	-	-	-	-8.0 b B

1 Refer to Table 40 for corresponding days after seeding and physiological stage of lentils.

2 Means followed by the same uppercase letter in each row are not significantly different at P = 0.05 (Duncan's Multiple Range Test, t-test).

3 Means followed by the same lowercase letter in each column for each crop within a site are not significantly different at P = 0.05 (Duncan's Multiple Range Test, t-test).

Table 50. Effect of nitrogen application on the accumulation of symbiotically fixed nitrogen as calculated using the ^{15}N Assisted Difference method throughout the growing season (Field Experiments 1984).

Location, and nitrogen rate (kg N ha^{-1})	Symbiotically fixed nitrogen (kg ha^{-1})				
	Harvest[1] 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5
Haywood					
0	-3.8 a[2] B[3]	-2.5 a B	7.1 a A	9.6 a A	-6.6 a B
30	-2.6 a A	3.1 a A	2.5 b A	2.1 b A	2.8 a A
90	-	-	-	-	-3.6 a
Morden					
0	31.4 B	37.9 a B	45.7 A	52.2 A	40.1 ab AB
30	-	41.4aa B	-	-	62.2 a A
90	-	-	-	-	27.5 b

1 Refer to Table 40 for corresponding days after seeding and physiological stage of lentils.

2 Means followed by the same uppercase letter in each row are not significantly different at P = 0.05 (Duncan's Multiple Range Test, t-test).

3 Means followed by the same lowercase letter in each column within a site and crop are not significantly different at P = 0.05 (Duncan's Multiple Range Test, t-test).

Table 51. Effect of nitrogen application on the accumulation of symbiotically fixed nitrogen, as calculated using the Classical Difference method, determined throughout the growing season (Field Experiments 1984).

Location, and nitrogen rate (kg N ha^{-1})	Symbiotically fixed nitrogen (kg ha^{-1})				
	Harvest[1] 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5
Haywood					
0	-3.8 a[2] B[3]	-2.5 a A	7.1 a A	9.6 a A	-6.6 a B
30	-3.5 a A	1.8 a A	1.7 b A	1.4 b A	1.4 a A
90	-	-	-	-	-3.4 a
Morden					
0	31.4 B	37.9 a B	45.7 A	52.2 A	40.1 a AB
30	-	37.1 a B	-	-	63.9 a A
90	-	-	-	-	38.8 a

1 Refer to Table 40 for corresponding days after seeding and physiological stage of lentils.

2 Means followed by the same uppercase letter in each row are not significantly different at P = 0.05 (Duncan's Multiple Range Test, t-test).

3 Means followed by the same lowercase letter in each column within a site and crop are not significantly different at P = 0.05 (Duncan's Multiple Range Test, t-test).

lentils and nonnodulating soybeans did not share the same percent utilization of fertilizer N.

Calculation of symbiotically fixed nitrogen using the 'A'-value method was statistically valid for harvest 1 and harvest 2 at Haywood and where N was applied at a rate of 30 kg N ha^{-1} at Morden. Values other than these were not statistically valid, as significant differences did not exist between lentil and nonnodulating soybean 'A' values, and thus it must be concluded that no measureable amounts of N fixation occurred. Calculation of symbiotically fixed N at Haywood, where applicable, indicated that very little nitrogen was symbiotically fixed. However, at Haywood total crop N accumulation was very low and thus the 8.6 kg of symbiotically fixed N accumulated at harvest 2 accounted for 44% of the total lentil N yield. At Morden, only 4 kg of symbiotically fixed N had been accumulated by the bloom stage. However, significant amounts of N were fixed following the initiation of flowerings resulting in a total accumulation of 47.6 kg of symbiotically fixed N. Thus, at final harvest, symbiotically fixed N accounted for 40% of the total N yield of lentils receiving fertilizer N at a rate of 30 kg N ha^{-1}. Application of 90 kg N ha^{-1} completely inhibited N fixation at Morden, as evidenced by the lack of statistically significant differences between lentil and nonnodulating soybean 'A'-values.

A further evaluation of nitrogen fixation was accomplished through the use of the acetylene reduction technique. Micromoles of ethylene produced at each harvest are reported in Table 52. No attempt was made to quantify N fixation using this technique as such determination can be erroneous and misleading. Rennie et al. (1978) suggested that because

Table 52. Effect of nitrogen application on symbiotic nitrogen fixation (C_2H_2 reduction) at different developmental stages of lentils (Field Experiments 1984).

Location and nitrogen applied (kg N ha^{-1})	μ mol $plant^{-1}$ hr^{-1} of ethylene produced at different developmental stages			
Haywood	Bud[1]	Early bloom	Early pod	Ripe seed
0	1.31a[3]	0.33a	0.04a	0.02a
30	0.69b	0.13b	0.04a	0.03a
90	0.30c	0.15b	0.02a	0.02a
180	0.09c	0.11b	0.02a	0.01a
Morden	Bud[2]	Bloom	Pod development	Ripe seed
0	1.22a	1.59a	0.04a	0.03a
30	0.98a	1.06a	0.02a	0.03a
90	0.06b	0.06b	0.05a	0.02a
180	0.03b	0.05b	0.04a	0.03a

1 Days after seeding at Haywood: Bud = 49, Early bloom = 58, Early pod = 74, Ripe seed = 91.

2 Days after seeding at Morden: Bud = 48, Bloom = 57, Pod development = 73, Ripe seed = 79.

3 Means followed by the same lower case letter in each column are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

C_2H_2 reduction is a short-term kinetic measurement, the existence of diurnal and seasonal variations in nitrogenase activity makes extrapolation to total dinitrogen fixed over the growing season questionable. Sprent (1972) reported that factors such as light intensity, surface soil moisture and soil temperature may affect nitrogenase activity and thus acetylene reduction. In addition, Bremner (1965) confirmed that it is not valid to convert C_2H_2 reduction values to N_2 fixed using a theoretical conversion factor of 3. Thus the acetylene reduction technique was employed only as a qualitative evaluation of N fixing activity. An examination of the micromoles of ethylene produced at each harvest indicated that nitrogenase activity was very low at both sites. Regitnig (1983) reported that ethylene production of root nodules of soybeans ranged from 0.04 to 9.57 micromoles under similar conditions. Ethylene production at Haywood indicated that active fixation only occurred during bud and early bloom, and that the occurrence of N fixation was inhibited with increasing increments of applied fertilizer N, as was suggested by the determination of symbiotically fixed N using the 'A'-value method. Ethylene production at Morden also indicated that active fixation occurred during bud and bloom and that the occurrence of N fixation was inhibited with increasing increments of applied fertilizer N. Although the 'A'-value method indicated that N fixation was inhibited at high levels of fertilizer N, it also indicated that lentils accumulated significant amounts of symbiotically fixed N between bloom and ripe seed where low levels of fertilizer N were applied. This appears to be in direct contrast to the evidence supplied by the acetylene reduction technique. However, it is important to note that

harvest dates for both 'A'-value measurements and acetylene reduction measurements were relatively far apart. Furthermore, it is possible that the acetylene reduction technique may have underestimated N fixation activity at pod development due to environmental factors affecting nitrogenase activity at the time of measurement. Alternatively, values obtained using the C_2H_2 technique may indicate that calculation of symbiotically fixed N using the 'A'-value technique was in error.

Little can be concluded regarding the effect of fertilizer N on nitrogen fixation due to the fact that nonnodulating soybeans served as an inappropriate control for lentils. The two crops did not share similar maturation dates nor did they exhibit similar nitrogen and dry matter accumulation patterns. Furthermore, evidence of different fertilizer uptake patterns suggests that the two crops did not feed from the same soil N pool.

Despite difficulties encountered in determining N fixation, positive lentil dry matter, nitrogen and seed yield responses at Morden indicate that nitrogen fixation did not meet lentil N requirements and thus lentils may benefit from the addition of fertilizer N.

Lysimeter Study

Dry matter yields of the shoots and roots of both lentils and nonnodulating soybeans were determined and are reported in Table 53. The extremely low yields for both crops are consistent with yields obtained in the ^{15}N Fertilizer Rate - Time Course study conducted at this site. Lentil shoots attained a maximum dry matter yield of 1460 $kg.ha^{-1}$ at bloom. Significant lentil dry matter was lost from the

Table 53. Dry matter and nitrogen accumulation in the shoots and roots of lentils and nonnodulating soybeans, determined throughout the growing season (Field Experiments 1984).

Days after seeding	Developmental stage of lentils	Dry matter accumulation (kg ha^{-1})						Nitrogen Accumulation (kg.ha^{-1})					
		Lentils			Nonnod soybeans			Lentils			Nonnod soybeans		
		Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total
49	Bud	310 c[1]	1536 a	1846 b	226 d	626 c	852 c	10 c	27 a	37 c	7 c	13 b	20 c
58	Early bloom	1004 b	1655 a	2659 ab	591 cd	1910 ab	2500 b	25 ab	30 a	55 a	14 bc	25 a	39 b
66	Bloom	1460 a	1912 a	3372 a	850 bc	2238 a	3188 ab	28 a	28 a	56 a	16 b	32 a	48 ab
74	Early pod	932 b	2222 a	3154 a	1447 a	1973 ab	3420 a	21 b	26 a	47 ab	26 a	30 a	56 a
91	Ripe Seed	1078 b	1659 a	2737 ab	1315 a	1398 b	2713 ab	22 b	22 a	44 bc	20 ab	21 ab	41 b

[1] Means followed by the same letter in each column are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

shoot through leaf senescence and (or) grasshopper damage. Nonnodulating soybean shoots attained a maximum dry matter yield of 1447 kg, 74 days after seeding. Significant increases in nonnodulating soybean shoot yields after day 66 indicate that lentils and nonnodulating soybeans did not share similar growth patterns.

Although not statistically significant, dry matter yield of lentil roots increased until early pod development. Between early pod and ripe seed root yields decreased by 563 kg ha^{-1}. The decrease in yield at maturity likely represents a loss of integrity and sloughing off of older roots. Nonnodulating soybean roots attained a maximum dry matter yield of 2288 kg ha^{-1} 66 days after seeding, decreasing significantly to 1398 kg ha^{-1} at final harvest. Similar to the lentils, the apparent loss of root dry matter was likely due to sloughing off of older roots.

Dry matter yield of roots was consistantly greater than the corresponding shoot dry matter yields of both lentils and nonnodulating soybeans. Contribution of shoot dry matter to total dry matter of the two crops is reported in Table 54. At bloom, 66 days after seeding, lentil shoots contributed a maximum of 43.3 percent to the total dry matter yield. In contrast, nonnodulating soybean shoots accounted for a greater proportion of total dry matter at each successive harvest and at final harvest accounted for 48.5 percent of the total dry matter.

With the exception of the first harvest, total dry matter yield of lentils and nonnodulating soybeans was very similar although the distribution of dry matter between shoots and roots was not.

The accumulation of nitrogen in the shoots and roots of the two

Table 54. Contribution of shoots to total dry matter and nitrogen yield of lentils and nonnodulating soybeans (Field Experiments 1984).

Days after seeding	Dry matter yield of shoots (Percent of total yield)		Nitrogen accumulation in shoots (Percent of total N accumulated)	
	Lentils	Nonnod soybean	Lentils	Nonnod soybean
49	16.8	26.5	27.0	35.0
58	37.8	23.6	45.4	35.9
66	43.3	27.1	50.0	34.0
74	29.5	42.3	44.7	53.1
91	39.4	48.5	50.0	48.8

crops is reported in Table 53. Crop N, calculated from Kjeldahl N and dry matter yields, closely reflected dry matter yield trends. The maximum rate of nitrogen accumulation in lentil shoots occurred between bud and early bloom, corresponding to day 49 and day 58, respectively. In contrast, the maximum rate of N accumulation in nonnodulating soybean shoots occurred between day 66 and day 74. Thus, when considering nitrogen accumulated in the aerial portion of lentils and nonnodulating soybeans, data suggest that lentils and nonnodulating soybeans do not share similar N uptake patterns. Witty (1983) contends that a similar N uptake pattern to the fixing legume is a necessary prerequisite for a nonfixing control crop and thus, on the basis of shoot N accumulation, nonnodulating soybeans are rejected as an appropriate reference crop for lentils. However, determination of total N in the two crops indicates that the maximum rate of N accumulation in both lentils and nonnodulating soybeans occurred between day 49 and day 58. Thus determination of total crop N indicated that lentils and nonnodulating soybeans may have shared similar N uptake patterns although the partitioning of nitrogen between shoots and roots was not similar.

The contribution of nitrogen accumulated in the shoots to total N is reported in Table 54. Percent nitrogen in lentil shoots was greater than the percent N in the roots. Thus at final harvest lentil shoots, which accounted for only 39.4 percent of the total dry matter yield, contributed 50 percent of the total crop N. Lentil shoot N made a greater contribution to total N earlier in the growing season than nitrogen in the shoots of nonnodulating soybeans.

Throughout the growing season, nitrogen in the shoots of lentils

accounted for less than half of the total crop N. Generally, under field conditions, only the aerial portion of the crop is harvested for determinations of symbiotically fixed N. Hence conclusions drawn and reported in regards to quantity of nitrogen symbiotically fixed by a legume where only aerial portions are harvested, may be based on a relatively small proportion of the total crop N.

Percent nitrogen derived from fertilizer is reported in Table 55. Ndff values for the roots of both lentils and nonnodulating soybeans were significantly lower than shoot Ndff values. Data suggest that fertilizer nitrogen was preferentially accumulated in the shoots of both crops whereas soil, and soil and (or) symbiotically fixed N was preferentially accumulated in the roots.

Comparison of Ndff values for lentils and nonnodulating soybeans, calculated using shoot N and total plant N, indicated lack of statistical significance at the five percent level of probability. Lack of significant differences suggest that little or no fixation took place.

Fertilizer nitrogen accumulation, reported in Table 56, tended to be very low in both lentils and nonnodulating soybeans, reflecting poor dry matter and nitrogen yields. Accumulation of fertilizer N in both shoot and total plant by lentils and nonnodulating soybeans was not significantly different ($P = 0.05$) at any harvest. Lack of significant differences may indicate that lentils and nonnodulating soybeans shared similar fertilizer N uptake patterns. However, it is more likely that lack of statistical significance occurred as a result of very low levels of fertilizer N accumulation accompanied by a relatively high degree of variation amongst replicates. Data suggest that lentils accumulated

Table 55. Percent nitrogen derived from fertilizer in shoots and roots of lentils and nonnodulating soybeans (Field Experiments 1984).

Days after seeding	Developmental stage of lentils	Percent Nitrogen Derived from Fertilizer					
		Lentils			Nonnod soybeans		
		Shoot	Root	Total	Shoot	Root	Total
49	Bud	19.7 a[1]	5.6 a	9.3 ab	21.3 a	6.8 a	11.8 a
58	Early bloom	17.4 ab	6.1 a	11.2 a	10.8 b	3.5 b	6.3 b
66	Bloom	11.9 c	5.4 a	8.7 ab	9.6 b	3.7 b	5.8 b
74	Early pod	11.8 c	4.6 a	7.8 b	6.7 b	3.0 b	4.7 b
91	Ripe Seed	13.0 cb	5.1 a	9.1 ab	6.2 b	4.3 b	5.5 b

[1] Means followed by the same letter in each column are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

Table 56. Fertilizer nitrogen accumulation in shoots and roots of lentils and nonnodulating soybeans (Field Experiments 1984).

Days after seeding	Developmental stage of lentils	Fertilizer Nitrogen Accumulation (kg N ha^{-1})					
		Lentils			Nonnod soybeans		
		Shoot	Root	Total	Shoot	Root	Total
49	Bud	1.9 c[1]	1.4 ab	3.3 b	1.4 a	0.8 a	2.3 a
58	Early bloom	4.4 a	1.8 a	6.2 a	1.6 a	0.9 a	2.4 a
66	Bloom	3.4 ab	1.5 ab	4.9 ab	1.6 a	1.2 a	2.8 a
74	Early pod	2.5 bc	1.2 b	3.7 b	1.7 a	0.9 a	2.6 a
91	Ripe Seed	2.9 bc	1.1 b	4.0 b	1.2 a	0.9 a	2.1 a

[1] Means followed by the same letter in each column are not significantly different at P = 0.05.

more fertilizer N than nonnodulating soybeans.

Utilization of fertilizer N by both crops was extremely low (Table 57). It is likely that poor yields limited total N demands and thus supplemental nitrogen was not utilized to a great extent. In addition, the June 2nd windstorm may have removed significant amounts of the fertilizer N which had been applied to the surface and incorporated to a depth of 10 cm.

Percent utilization of applied fertilizer N by the nonfixing control crop must be identical to that of the test legume for N balance techniques to accurately quantify symbiotically fixed N. Percent utilization of fertilizer N by lentils and nonnodulating soybeans, as calculated using shoot N and total N, was significantly different ($P = 0.10$) at day 58. The data indicate that percent utilization of fertilizer nitrogen was consistently higher for lentils than nonnodulating soybeans at all other harvests. However, these differences were not statistically significant. However, as indicated earlier, lack of statistically significant differences may have been an artifact of low levels of fertilizer recovery coupled with high variability between replicates rather than an indication that the crops utilized the same percent of the applied fertilizer N.

'A'-values were calculated using shoot and total plant Ndff values for both lentils and nonnodulating soybeans (Table 58). Where contribution of roots was included in the calculations, 'A'-values were higher for both crops. 'A'-values increased in response to the low root Ndff values. Levels of significance associated with differences between lentil and nonnodulating soybean 'A'-values determined by the T-test

Table 57. Percent utilization of fertilizer nitrogen by shoots and roots of lentils and nonnodulating soybeans (Field Experiments 1984).

Days after seeding	Developmental stage of lentils	Percent utilization of fertilizer nitrogen					
		Lentils			Nonnod soybeans		
		Shoot	Root	Total	Shoot	Root	Total
49	Bud	6.3 c[1]	4.7 ab	11.0 b	4.9 a	2.8 a	7.8 a
58	Early bloom	14.6 a	5.9 a	20.5 a	5.3 a	3.0 a	8.1 a
66	Bloom	11.3 ab	4.9 ab	16.2 ab	5.3 a	4.2 a	9.4 a
74	Early pod	8.3 bc	4.1 b	12.4 b	5.8 a	3.0 a	8.8 a
91	Ripe Seed	9.7 bc	3.7 b	13.4 b	4.2 a	3.0 a	7.15 a

[1] Means followed by the same letter in each column are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

Table 58. 'A'-values for lentils and nonnodulating soybeans determined throughout the growing season, calculated using shoot and total plant sample(Field Experiments 1984).

Days after seeding	Developmental stage of lentils	'A'-value (kg ha^{-1})			
		Lentil		Nonnod soybean	
		Shoots	Total	Shoots	Total
49	Bud	135.9 b[1]	294.5 ab	112.3 b	227.3 b
58	Early bloom	148.4 b	243.2 b	322.0 ab	532.0 ab
66	Bloom	233.7 a	326.4 ab	283.3 ab	492.3 ab
74	Early pod	227.9 a	357.9 a	424.4 ab	620.4 a
91	Ripe seed	201.4 ab	303.3 ab	473.0 a	556.5 ab

1 Means followed by the same letter in each column, are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

procedure are reported in Table 58A. 'A'-values calculated from shoot Ndff values for lentils and nonnodulating soybeans were not significantly different even at the twenty percent level of probability for the first three harvests. Thus it must be concluded that no fixation occurred. Lentil 'A'-values determined at early pod and ripe seed were significantly lower than nonnodulating soybean 'A' values. These results reflect the fact that lentils derived a greater proportion of the plant N from the fertilizer and thus it can be concluded that the two crops did not see the same soil N pool.

'A'-values calculated using both total plant and shoot Ndff values further evidence the fact that the two crops did not see the same N pool. At all but the first harvest, lentil 'A'-values were consistently lower than nonnodulating soybean 'A'-values. An assumption basic to the 'A'-value method is that the test crop and the nonfixing reference crop assimilate soil and fertilizer N in identical proportion (Fried and Broeshart, 1975; Fried and Middleboe, 1977). The data indicate that under the conditions of the lysimeter experiment, this assumption was in error, invalidating the use of the 'A'-value method for the quantification of symbiotically fixed N.

In order to illustrate the problems encountered in evaluating fixed N using an inappropriate control crop, symbiotically fixed N was determined using the 'A'-value method, the ^{15}N Assisted Difference method and the Classical Difference method (Table 59). Significant negative values for symbiotically fixed N were calculated when the 'A'-value method was employed. Clearly these estimates are in error, demonstrating the importance of using a reference crop which assimilates soil

Table 58A. Level of significance associated with differences between lentil and nonnodulating soybean 'A'-values determined by the t-test procedure (Field Experiments 1984).

Days after seeding	Level of significance	
	Shoots	Total plant
49	NS[1]	NS
58	NS	15
66	NS	5
74	5	5
91	5	5

1 'A'-value means for lentils and nonnodulating soybeans are not significantly different at $P \leq .20$.

Table 59. Accumulation of symbiotically fixed nitrogen in lentils determined using the 'A'-value method, the ^{15}N Assisted Difference method and the Classical Difference method; calculated using shoot and total plant samples (Field Experiments 1984).

Days after seeding	Developmental stage of lentils	Symbiotically fixed nitrogen (kg ha^{-1})					
		'A'-value method		^{15}N Assisted Difference method		Classical Difference method	
		Shoot	Total Plant	Shoot	Total Plant	Shoot	Total Plant
49	Bud	0.6 a[1]	8.3 a	2.5 a	16.0 a	3.0 a	17.0 a
58	Early bloom	-26.6 cd	-60.7 c	8.2 a	12.2 a	11.0 a	16.0 a
66	Bloom	-7.1 ab	-28.7 b	10.2 a	5.9 a	12.0 a	8.0 a
74	Early pod	-16.8 cb	-33.3 b	-5.8 a	-10.1 a	-5.0 a	-9.0 a
91	Ripe seed	-28.9 d	-38.6 b	0.3 a	1.1 a	2.0 a	3.0 a

1 Means followed by the same letter in each column are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

and fertilizer N in identical proportion to the fixing plant.

Data suggested that differences existed between the percent utilization of fertilizer N by the two crops and thus little confidence can be placed on the determination of symbiotically fixed nitrogen using N balance techniques. Little difference existed between the calculation of symbiotically fixed N using the ^{15}N Assisted Difference method and the Classical Difference method. Differences which did occur reflected different amounts of fertilizer recovery by lentils and non-nodulating soybeans. Higher estimates of symbiotically fixed N at harvest 1 and harvest 2, when total plant N was used in calculations rather than shoot N alone, resulted from the fact that lentils had a much greater amount of N in the root portion of the plant early in the season than the nonnodulating soybeans.

Although the lysimeter experiment failed to determine the quantity of nitrogen symbiotically fixed by lentils, the study served to illustrate the large contributions which rooting systems may make to total crop N. Thus it is recommended that care be taken in evaluating and reporting the quantity of nitrogen symbiotically fixed by a legume, particularly if calculations are based on nitrogen accumulated in the aerial portion of the crop.

5. SUMMARY AND CONCLUSIONS

Field and growth chamber studies were conducted to evaluate the effect of various soil conditions, including nitrogen and moisture availability on the yield and symbiotic nitrogen fixing potential of lentils. In addition, methods of estimating the quantity of nitrogen symbiotically fixed were evaluated.

In both field and growth chamber studies, supplemental N was found to be an important factor in attaining high dry matter and seed yield of lentils. Growth chamber studies revealed that nitrogen stress, simulated by ammending the soil with barley straw, limited dry matter and seed yields whereas increasing increments of supplemental N, up to a maximum of 360 ppm N, continued to promote yield increases. Similarly, in field studies where environmental conditions were not limiting, dry matter yields continued to increase with applications of up to 180 kg N ha^{-1}. Although seed yield responses to applied N under field conditions were not statistically significant, results suggest that increased N supply resulted in concomitant seed yield increases. Application of 100 kg N ha^{-1} increased seed yield by as much as 475 kg N ha^{-1}, resulting in a maximum seed yield of 2,459 kg ha^{-1}.

Although high levels of applied N generally resulted in positive yield responses, low levels of applied N did not significantly affect dry matter or seed yield in either growth chamber or field experiments. It was concluded that low levels of applied N, coupled with available soil N, depressed symbiotic nitrogen fixation so that there was no net gain in the quantity of N available to the lentils. Application of

higher rates of fertilizer N, coupled with soil N, exceeded the quantity of nitrogen normally fixed by lentils and thus yield responses were obtained. Growth chamber studies revealed that nitrogen stress, simulated by the addition of barley straw to the soil, limited lentil yields as compared to yields obtained on unammended soil. It is apparent from these results that lentils are not capable of symbiotically fixing enough atmospheric nitrogen to meet optimum plant growth requirements. Supplemental nitrogen is required to produce maximum seed yield on soils low to medium in available NO_3-N. Amounts as low as 30 kg N ha^{-1} may benefit yields where available soil N is strictly limiting.

In a growth chamber study, moisture availability was also found to be an important factor in attaining high dry matter and seed yield of lentils. Dry matter yields were significantly decreased by moisture stress; the degree of reduction dependent on the physiological stage at which stress was applied. The lowest dry matter yield was obtained where lentils were stressed for the longest duration, namely from the prebud growth stage. This reduction was due to both reduced leaf and total plant size produced in response to the limited water supply. Lentils which were stressed at bloom and early pod development had, to a large degree, completed vegetative growth at the time of the stress and thus significant reductions in total dry matter were attributed primarily to reduced seed yields. Physiological stage at which stress was applied did not influence the degree to which seed yields were reduced. It was concluded that several possible mechanisms, including restricted number of flowers, abortion of floral structures and reduced seed size, may have contributed to reduced seed yields. Furthermore, the extent to

which each mechanism contributed to reduced seed yields was likely dependent on the developmental stage at which stress was applied. From these results, it is apparent that moisture stress may elicit a complicated response mechanism in lentils, and may result in a reduction in both dry matter and seed yield.

Quantity of nitrogen symbiotically fixed in lentils was estimated using three methods: 1) the 'A'-value method; 2) the ^{15}N Assisted Difference method; and 3) the Classical Difference method. Employing these methods necessitated the use of a non-fixing reference crop. In all studies conducted, nonnodulating soybeans served as the reference crop. Under field conditions, difficulties were encountered in quantifying symbiotically fixed N due to the fact that nonnodulating soybeans did not meet all of the requirements of an ideal reference crop for lentils. In both years of field experimentation, lentils and nonnodulating soybeans did not share similar maturation dates, nor did they exhibit similar nitrogen accumulation patterns. Generally, it was found that lentils utilized a greater percentage of the applied fertilizer N than the nonnodulating soybeans. The differences which existed between the lentils and the nonnodulating soybeans rendered estimation of the quantity of nitrogen symbiotically fixed suspect, particularly where N balance techniques, which are based on the assumption that both the test crop and the reference crop utilize identical quantities of soil and fertilizer N, were employed. In contrast, the 'A' Value method assumes only that soil and fertilizer N is utilized by the two crops in similar proportions, and thus it was concluded that the 'A' Value method was a more reliable method of estimating the quantity of nitrogen symbiotic-

ally fixed under field conditions. Under conditions of the growth chamber, nonnodulating soybeans met all of the requirements of an appropriate control crop and subsequently little difference existed between the three methods used to estimate quantity of N fixed.

Both growth chamber and field studies revealed that throughout the growing season, nitrogen in the lentil roots may account for as much as fifty percent of the total crop N. Generally under field conditions, only the aerial portion of the crop is harvested for the determination of symbiotically fixed N. Hence conclusions drawn and reported in regard to the total contribution of symbiotically fixed N to lentil N yield where only the aerial portions are harvested, may be based on a relatively small proportion of the total crop N.

Despite difficulties encountered in determining the quantity of N symbiotically fixed by lentils under field conditions, dry matter and seed yield responses to applied fertilizer N indicated that symbiotic N fixation alone did not meet lentil N requirements. Yield responses to applied N under growth chamber conditions were obtained at rates as low as 30 ppm which suggested that lentils derived only a small quantity of N from symbiotic fixation. Studies showed that although addition of supplemental N generally increased the total nitrogen in the dry matter, both the percent of the total N derived from fixation as well as the actual quantity of N derived from fixation decreased with increased N fertilizer application. In addition, growth chamber studies demonstrated that N availability in the soil may influence the onset and duration of active fixation. Where N was applied at a rate of 30 ppm N, notable accumulation of symbiotic nitrogen fixation began at the bud

stage of development. Nitrogen stress delayed the onset of active fixation until the bloom stage and subsequently reduced the quantity of N symbiotically fixed. Thus it was concluded that some nitrogen may be required to promote early establishment and functioning of a symbiotic nitrogen fixing system in lentils.

LITERATURE CITED

Agboola, A.A. 1978. Influence of soil organic matter on cowpeas response to N fertilizer. Agron. J. 70: 25-28.

Allos, H.F. and M.V. Bartholomew. 1955. Effect of nitrogen on symbiotic fixation. Soil Sci. Soc. Amer. Proc. 19: 182-184.

Allos, H.F. and W.V. Bartholomew. 1959. Replacement of symbiotic fixation by available nitrogen. Soil Science 87: 61-66.

Bell, F. and P.S. Nutman. 1971. Experiments on nitrogen fixation by nodulated lucerne. Plant Soil Sp. Vol.: 231-264.

Bennett, J.M. and S.L. Albrecht. 1984. Drought and flooding effects on N_2 fixation, water relations, and diffusive resistance of soybean. Agron. J. 76: 735-740.

Bergerson, F.J. 1970. The quantitative relationship between nitrogen fixation and the acetylene reduction assay. Aust. J. Biol. Sci. 23: 1015-1025.

Bergerson, F.J. 1978. Physiology of legume symbiosis. In Interactions Between Non-pathogenic Soil Microorganisms and Plants. (Eds. Y.R. Dommergues and S.V. Krupa) New York: Scientific Pub. Company.

Bhangoo, M.S. and D.J. Albritton. 1976. Nodulating and nonnodulating Lee soybean isolines response to applied nitrogen. Agron. J. 68: 642-645.

Bremner, J.M. 1965. Total nitrogen. In Methods of Soil Analysis. Part II. Chemical and Microbiological Properties. pp. 1149-1178. Monograph No. 9 in the series of Agronomy, Am. Soc. of Agron. Madison, Wisconsin. C.A. Black (Ed.).

Bremner, J.M. 1965. Isotope-ratio analysis of nitrogen in nitrogen-15 investigations. In Methods of Soil Analysis. Part II. Chemical and Microbiological Properties. pp. 1274-1282. Monograph No. 9 in the series of Agronomy, Am. Soc. of Agron. Madison, Wisconsin. C.A. Black (Ed.).

Broadbent, F.E. 1970. Variables affecting "A" values as a measure of soil nitrogen availability. Soil Sci. 110: 19-23.

Chen, P.C. and D.A. Phillips. 1977. Induction of root nodule senescence by combined nitrogen in Pisum sativum (L.). Plant Physiol. 59: 440-442.

Chowdhury, S.L., S. Ram and G. Giri. 1974. Effect of inoculum, nitrogen and phosphorus on root nodulation and yield of lentil varieties. Indian J. Agron. 19: 274-276.

Dart, P.J. and D.C. Wildon. 1970. Nodulation and nitrogen fixation by Vigna sinensis and Vicia atropurpurea: the influence of concentration, form and site of application of combined nitrogen. Aust. J. Agric. Res. 21: 45-56.

Dart, P. 1977. Infection and development of leguminous nodules. In A Treatise on Dinitrogen Fixation. Section III. Biology. R.W.F. Hardy and W.S. Silver (Eds.).

Deibert, E.J., M. Bijeriego and R.A. Olson. 1979. Utilization of ^{15}N fertilizer by nodulating and nonnodulating soybean isolines. Agron. J. 71: 717-723.

Delwiche, C.C. and P.L. Steyne. 1970. Nitrogen isotope fractionation in soils and microbial reactions. Envir. Sci. Tech. 4: 929-935.

Eaglesham, A.R.J., S. Hassouna and R. Seegers. 1983. Fertilizer N effects on N_2 fixation by cowpea and soybean. Agron. J. 75: 61-66.

Ellis, J.H. and Wm. H. Shafer. 1943. Report of Reconnaissance Soil Survey of South-Central Manitoba. Manitoba Department of Agriculture, (Publisher), Winnipeg, Manitoba.

Engin, M. and J.I. Sprent. 1973. Effects of water stress on growth and nitrogen-fixing activity of Trifolium repens. New Phytol. 72: 117-126.

Erhlich, W.A., E.A. Poyser and L.E. Pratt. 1957. Report of Reconnaissance Soil Survey of Carberry Map Sheet Area. Manitoba Department of Agriculture (Publisher). Winnipeg, Manitoba.

Fried, M. and H. Broeshart. 1975. An independent measurement of the amount of nitrogen fixed by a legume crop. Plant and Soil 43: 707-711.

Fried, M. and H. Broeshart. 1981. A further extension of the method for independently measuring the amount of nitrogen fixed by a legume crop. Plant and Soil 62: 331-336.

Fried, M. and L.A. Dean. 1952. A concept concerning the measurement of available soil nutrients. Soil Sci. 73: 263-271.

Fried, M. and V. Middleboe. 1977. Measurement of amount of nitrogen fixed by a legume crop. Plant and Soil 47: 713-715.

Ham, G.E. 1973. Use of ^{15}N in evaluating symbiotic N_2 fixation of field grain soybeans (unpublished report).

Hardy, R.W.F., Burns, R.C., Hebert, R.P., Holsten, R.D., and Jackson, E.K. 1971a. Biological nitrogen fixation: a key to world protein. Plant Soil Spc. Vol.: 561-590.

Hardy, R.W.F., R.C. Burns and R.D. Holsten. 1973. Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. Soil Biol. Biochem. 5: 47-81.

Hardy, R.W.F. and R.D. Holsten. 1977. Methods for measurement of dinitrogen fixation. In A Treatise on Dinitrogen Fixation. Section IV: Agronomy and Ecology. (Eds. R.W.F. Hardy and A.H. Gibbons). pp. 451-486. New York: Wiley Interscience Pub.

Hardy, R.W.F., R.D. Holsten, E.K. Jackson and R.C. Burns. 1968. The acetylene-ethylene assay for N_2 fixation: laboratory and field evaluation. Plant Physiol. 43: 1185-1207.

Hartt, C.E. 1967. Effect of moisture supply on translocation and storage of ^{14}C in sugarcane. Plant Physiol. 42: 338-346.

Hatfield, J.L., D.B. Egli, J.E. Leggett and D.E. Peaslee. 1974. Effect of applied nitrogen on the nodulation and early growth of soybeans (Gleine max (L.) Merr). Agron. J. 66: 112-114.

Hauck, R.D. and J.M. Bremner. 1976. Use of tracers for soil and fertilizer nitrogen research. Advances in Agron. 28: 219-266.

Havelka, V.D., M.G. Boyle and R.W.F. Hardy. 1982. Biological nitrogen fixation. In Nitrogen in Agricultural Soils. (Ed. F.J. Stevenson). pp. 365-413. Madison, Wisconsin: American Society of Agronomy, Inc.

Herridge, D.F. 1982. Assessment of nitrogen fixation. In Nitrogen Fixation in Legumes, J.M. Vincent (Ed.).

Hiler, E.A., C.H.M. van Baval, M.M. Hossain and W.R. Jordan. 1972. Sensitivity of southern peas to plant water deficit at three growth stages. Agron. J. 64: 60-64.

Hill-Cottingham, D.G. and C.P. Lloyd-Jones. 1980. The influence of nitrate supply on nitrogen fixation during growth of the field bean Vicia faba in sand. Physiol. Plant 48: 116-120.

Huang, Chi-Ying, J.S. Boyer and L.N. Vanderhoef. 1975a. Acetylene reduction (nitrogen fixation) and metabolic activities of soybean having various leaf and nodule water potentials. Plant Physiol. 56: 222-227.

Huang, Chi-Ying and J.S. Boyer. 1975b. Limitations of acetylene reduction (nitrogen fixation) by photosynthesis on soybean having low water potentials. Plant Physiol. 56: 228-232.

Hunter, A.S. and D.L. Carter. 1965. Studies of methods for measuring forms of available soil nitrogen. Soil Sci. 100: 112-117.

Jackson, M.L. 1958. Nitrogen determination for soil and plant tissue. In Soil Chemical Analysis. pp. 183-189. Prentice-Hall Inc., Eaglewood Cliffs, New Jersey.

Kamphake, L.J., S.A. Hannah and J.M. Cohen. 1967. Automated analysis for nitrate by hydrazine reduction. Water Res. 1: 205-216.

Kuo, T. and L. Boersma. 1971. Soil water suction and root temperature effects on nitrogen fixation in soybeans. Agron. J. 63: 901-904.

Lawn, R.J. 1982. Response of four grain legumes to water stress in southeastern Queensland. III. Dry matter production, yield and water use efficiency. Aust. J. Agric. Res. 33: 511-521.

Lazrus, A.J., K.C. Hill and J.P. Lodge. 1966. A new colormetric micro-determination of sulphate ion in Automation in Analytical Chemistry, Technicon Symposium 1965. pp. 291-293. Mediad.

Legg, J.O. and Allison, F.E. 1959. Recovery of ^{15}N-tagged nitrogen from ammonium-fixing soils. Soil Sci. Soc. Am. Proc. 23: 131-134.

Legg, J.O. and G. Stanford. 1967. Utilization of soil and fertilizer N by oats in relation to the available N status of soils. Soil Sci. Soc. Amer. Proc. 31: 215-219.

Liu, M.C., and H.H. Hadley. 1971. Relationships of nitrate reductase activity to protein content in related nodulated and nonnodulating soybeans. Crop Sci. 11: 467-471.

Luebs, R.E. and A.E. Laag. 1969. Evapotranspiration and water stress of barley with increased nitrogen. Agron. J. 61: 921-924.

Manitoba Agriculture. 1980. Lentil Production in Manitoba. Manitoba Department of Agriculture, Winnipeg, Manitoba.

McAuliffe, C., D.S. Chamdee, H. Vribe-Arango and W.W. Woodhouse. 1958. Influence of inorganic nitrogen on nitrogen fixation by legumes as revealed by ^{15}N. Agron. J. 50: 334-347.

McEwen, J. 1970. Fertilizer nitrogen and growth regulators for field beans (Vicia faba (L.)). I. The effects of seed bed applications of large dressings of fertilizer nitrogen and the residual effects on following winter wheat. J. Agric. Sci. 74: 61-66.

Munns, D.N. 1968. Nodulation of Medicago sativa in solution culture. III. Effects of nitrate on root-hairs and infection. Plant Soil 29: 33-47.

Murphy, J. and J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chem. Acta. 27: 31-36.

Norihau, A. and H. Nishi. 1983. Rapid and simple method for measuring dinitrogen fixation by soybean root nodules. Soil Biol. Biochem. 15: 619-621.

Ojha, S.N., R.K. Roy and J.N. Jha. 1977. Effect of nitrogen, phosphorus and Rhizobium inoculation on grain yield of lentil under irrigated condition. Indian J. Agron. 15: 250.

Olson, S.R., C.W. Cole, F.S. Watanabe and L.A. Dean. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Dept. Agric. Arc. 939.

Pankhurst, C.E. and J.I. Sprent. 1975. Effects of water stress on the respiratory and nitrogen-fixing activity of soybean root nodules. J. Exp. Bot. 26: 287-304.

Pate, J.S. and P.J. Dart. 1961. Nodulation studies in legumes. IV. The influence of inoculum strain and time of application of ammonium nitrate on symbiotic response. Plant Soil 15: 329-346.

Pate, J.S., B.E.S. Gunning and L.G. Briarty. 1969. Ultra structure and functioning of the transport system of the leguminous root nodule. Planta 85: 11.

Pratt, P.E. 1965. Potassium. In Method of Soil Analysis, Part II. (Ed. C.A. Black). pp. 1022-1030. Madison, Wisc.: American Society of Agronomy.

Regitnig, P.J. 1979. The effects of nitrogen fertilization on symbiotic nitrogen fixation, yield and protein content in soybeans and blackbeans. B.S.A. Thesis, University of Manitoba.

Regitnig, P.J. 1983. The effect of fertilizer nitrogen on yield, protein content and symbiotic nitrogen fixation in soybeans (Glycine max (L.) var. Maple Presto). M.Sc. Thesis, University of Manitoba.

Rennie, R.J. 1982. Quantifying dinitrogen (N_2) fixation in soybeans by ^{15}N isotope dilution: the question of the nonfixing control plant. Can. J. Bot. 60: 856-861.

Rennie, R.J. 1984. Comparison of N balance and ^{15}N isotope dilution to quantify N_2 fixation in field-grown legumes. Agron. J. 76: 785-790.

Rennie, R.J., E.A. Paul and L.E. Johns. 1976. Natural nitrogen - 15 abundance of soil and plant samples. Can. J. Soil Sci. 56: 43-50.

Rennie, R.J. and D.A. Rennie. 1983. Techniques for quantifying N_2 fixation in association with nonlegumes under field and greenhouse conditions. Can. J. Microbiol. 29: 785-800.

Rennie, R.J., D.A. Rennie and M. Fried. 1978. Concepts of ^{15}N usage in dinitrogen fixation studies. In Isotopes in Biological Dinitrogen Fixation. pp. 107-131. International Atomic Energy Agency. (Proceedings of an Advisory Group Meeting, Vienna, Nov. 1977). Vienna, Austria.

Richards, J.E. and R.J. Soper. 1979. Effect of N fertilizer on yield, protein content, and symbiotic N fixation in fababeans. Agron. J. 71: 807-811.

Rizk, S.G. 1966. Atmospheric nitrogen fixation of legumes under Egyption conditions. J. of Microbiol. 1: 33-45.

Saskatchewan Agriculture. 1985. Varieties of grain crops for Saskatchewan 1985. Plant Industry Branch. Saskatchewan Agriculture.

Saxena, M.C. 1981. Agronomy of lentils. In Lentils. (Eds. C. Webb and G. Hawtin). pp. 111-131. Farnham Royal: Commonwealth Agricultural Bureaux.

Semu, E. and D.J. Hume. 1979. Effects of inoculation and fertilizer N levels on N_2 fixation and yields of soybeans in Ontario. Can. J. Plant Sci. 59: 1129-1137.

Shackel, K.A. and A.E. Hall. 1979. Reversible leaflet movements in relation to drought adaptation of cowpeas (Vigna unguiculata (L.) Walp). Aust. J. Plant Physiol. 6: 265-276.

Shaykewich, C.F. 1965. The relationship between soil components and soil physical constants of some Manitoba soils. M.Sc. Thesis, University of Manitoba, Winnipeg, Manitoba.

Slinkard, A.E. and B.N. Drew. 1982. Lentil production in western Canada. University of Saskatchewan. Publication No. 413. Agdex No. 149/10.

Smith, S.J. and J.O. Legg. 1971. Reflection on the "A" value concept of soil nutrient availability. Soil Sci. 112: 373-375.

Smith, R.E. and W. Michalyna. 1973. Soils of the Morden-Winkler area. Manitoba Department of Agriculture (Publisher). Winnipeg, Manitoba.

Sprent, J.I. 1971. Effects of water stress on nitrogen fixation in root nodules. Plant Soil Sp. Vol.: 225-228.

Sprent, J.I. 1971. The effects of water stress on nitrogen-fixing root nodules. I. Effects on the physiology of detached soybean nodules. New Phytol. 70: 9-17.

Sprent, J.I. 1972. The effects of water stress on nitrogen-fixing root nodules. II. Effects on the fine structure of detached soybean nodules. New Phytol. 71: 443-450.

Sprent, J.I. 1972. The effects of water stress on nitrogen-fixing root nodules. IV. Effects on whole plants of Vicia faba and Glycine max. New Phytol. 71: 603-611.

Sprent, J.I. 1976. Nitrogen fixation by legumes subjected to water and light stresses. In Symbiotic Nitrogen Fixation in Plants. Cambridge University Press. P.S. Nutman (Ed.).

Sekhon, H.S., J.N. Kaul and B.S. Dahiya. 1978. Response of lentil to Rhizobium inoculation and N fertilizer. J. Agric. Sci. 90: 325-327.

Summerfield, R.J. 1981. Adaptation to environments. In Lentils. (Eds. C. Webb and G. Hawton. pp. 91-111. Farnham Royal: Commonwealth Agricultural Bureaux.

Summerfield, R.J., P.J. Dart, P.A. Huxley, A.R.J. Eaglesham, F.R. Minchin and J.M. Day. 1977. Nitrogen nutrition of cowpea (Vigna unguiculata). I. Effects of applied nitrogen and symbiotic nitrogen fixation on growth and seed yield. Exp. Agric. 13: 129-142.

Turk, K.J., A.E. Hall and C.W. Asbell. 1980. Drought adaptation of cowpea. I. Influence of drought on seed yield. Agron. J. 72: 413-420.

Turk, K.J. and A.E. Hall. 1980. Drought adaptation of cowpea. II. Influence of drought on plant water status and relations with seed yield. Agron. J. 72: 421-427.

Turk, K.J. and A.E. Hall. 1980. Drought adaptation of cowpea. III. Influence of drought on plant growth and relations with seed yield. Agron. J. 72: 428-433.

Turk, K.J. and A.E. Hall. 1980. Drought adaptation of cowpea. IV. Influence of drought on water use, and relations with growth land seed yield. Agron. J. 72: 434-439.

Vasilas, B.L. and G.E. Ham. 1984. Nitrogen fixation in soybeans: An evaluation of measurement techniques. Agron. J. 76: 759-764.

Viets, F.G. 1962. Fertilizers and the efficient use of water. Advance. Agron. 14: 223-264.

Weber, C.R. 1966. Nodulating and nonnodulating soybean isolines: I. Agronomic and chemical attributes. Agron. J. 58: 43-46.

Williams, W.A., M.B. Jones and C.C. Delwiche. 1977. Clover N-fixation measurement by total-N difference and ^{15}N "A"-values in lysimeters. Agron. J. 69: 1023-1024.

Walley, F.L. 1983. symbiotic nitrogen fixation in lentils during an 86-day growing period. B.Sc. Thesis, Univeristy of Manitoba, Winnipeg, Manitoba.

Witty, J.F. 1983. Estimating N_2-fixation in the field using ^{15}N-labelled fertilizer: some problems and solutions. Soil Biol. Biochem. 15: 631-640.

Wong, P.P. 1980. Nitrate and carbohydrate effects on nodulation and nitrogen fixation (acetylene reduction) activity of lentil (Lens esculenta) Moench. Plant Physiol. 66: 78-81.