Effect of Nitrogen Addition on Yield and Symbiotic Dinitrogen Fixation of Soybeans (*Glycine max. L. merr.* c.v. Maple Amber), Fababeans (*Vicia faba L. minor* c.v. Aladin), and Lentils (*Lens esculenta*)

by

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A thesis
presented to the University of Manitoba
in partial fulfillment of the
requirements for the degree of
Master of Science
in
Department of Soil Science

Winnipeg, Manitoba

ISBN 0-315-37308-3

EFFECT OF NITROGEN ADDITION ON YIELD AND SYMBIOTIC DINITROGEN FIXATION OF SOYBEANS (GLYCINE MAX. L. MERR. c.v. MAPLE AMBER), FABABEANS (VICIA FABA L. MINOR c.v. ALADIN), AND LENTILS (LENS ESCULENTA)

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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

This thesis is dedicated to my wife and parents.

ACKNOWLEDGEMENTS

The author wishes to express his appreciation to:

Dr. R.J. Soper, Professor of Soil Science, University of Manitoba, under whose guidance this work was undertaken, for valuable advice and criticism of this manuscript;

Dr. G. M. Cho, Professor of Soil Science, University of Manitoba, who was extremely generous with advice and equipment regarding ^{15}N methodology and for serving on the committee;

Dr. K. W. Clark, Professor of Plant Science, University of Manitoba, for advice on the acetylene reduction assay and for serving on the committee;

Val Huzel, Greg Morden and Jim Rogers for their technical advice;

Brian Wiebe for assisting with the field work;

Naida Stilwell for helping in the final preparation of this thesis; and

Mrs. H. Thould and Mrs. H. Nemeth for all their help.

Financial assistance provided by the Natural Science and Engineering Research Council of Canada.

ABSTRACT

In Manitoba, soybeans, fababeans and lentils are grown to alleviate local crude protein shortages, diversify agriculture and as a marketable commodities.

Field, lysimeter and growth chamber experiments were undertaken in order to determine: 1) the nitrogen nutritional requirements of Maple Amber soybeans; 2) the amount of nitrogen fixed by Maple Amber; 3) the effect of nitrogen addition on dinitrogen fixation; and 4) the physiological stages of growth during which fixation occurs in Maple Amber. The addition of 0-200 kg N ha^{-1}, 0-100 kg N ha^{-1} and 0-1800 mg N pot^{-1} for field, lysimeter and growth chamber experiments, respectively did not result in significant yield increases. By the classical difference method, Maple Amber soybeans were found to fix 79 kg N ha^{-1}, 71 kg N ha^{-1} and 1216 mg N pot^{-1} for lysimeter, field and growth chamber experiments, respectively when grown on soils which had not received additional nitrogen. Nitrogen addition decreased dinitrogen fixation, the decrease appeared to be proportional to the amount of fertilizer nitrogen utilized. The maximum fixation of dinitrogen occurred from early flowering to mid-pod formation (reproductive development), which corresponded to the period of maximum dry matter and nitrogen accumulation.

The nitrogen nutritional requirements of Aladin fababean were studied in lysimeter and growth chamber experiments. Aladin fababeans did not respond to nitrogen additions of 0-100 kg N ha^{-1} and 0-1800 mg N pot^{-1} in lysimeter and growth chamber experiments, respectively and fixed (by the classical difference method) 250 kg N ha^{-1} and 1645 mg N

pot^{-1}, respectively. As with Maple Amber soybeans, fixation of dinitrogen decreased with addition of fertilizer nitrogen, and the decrease appeared to be proportional to the amount of fertilizer utilized.

The nitrogen nutritional requirements of lentils were studied in growth chamber experiments. Unlike soybeans and fababeans, lentils did respond to additional fertilizer nitrogen and hence, did not appear to fix enough nitrogen for their nutritional requirements. Lentils were also the least fixers of dinitrogen, 200 mg N pot^{-1} compared to 1645 and 1216 mg N pot^{-1} for fababeans and soybeans, respectively. Lentils also appeared to be more susceptible to the toxic effects of high rates of nitrogen addition as urea than soybeans or fababeans.

Various methods of assessing dinitrogen fixation were used: ^{15}N assisted difference method, difference method, "A" value method, acetylene reduction assay and nodule counts. The ^{15}N assisted difference method and "A" value method in most cases gave similar estimates of the amount of dinitrogen fixed by the legumes. However, discrepancies between the two methods occurred when the control and the legume had different fertilizer nitrogen utilization. In such cases, the "A" value method was thought to give a better estimate of fixation. Acetylene reduction assay and nodule counts were suitable as qualitative estimates of fixation. Utilization of only the aerial plant portion for measurement of symbiotic nitrogen fixation by legumes underestimated fixation. Results also showed that fertilizer nitrogen was not uniformly distributed in the plant parts but tended to accumulate preferentially in the roots.

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CHAPTER I

INTRODUCTION

Soybeans, fababeans and lentils as members of the family Leguminosae can obtain part or all of their nitrogen nutritional requirements from the fixation of atmospheric dinitrogen when in association with Rhizobium of the appropriate species. Increased cost of nitrogen fertilizers and the growing world need for high protein feed, both for animal and human consumption, has helped to promote cultivation of these pulse crops.

Soybeans were introduced to Canada in the 1970's; however, until recently, most of the hectarage had been confined to Southern Ontario. The advent of early maturing, long day-length adapted cultivars has expanded production to cool climatic areas such as Manitoba. Maple Presto was the first cultivar to be licensed in Manitoba. However, low yields and the need for additional nitrogen for maximum production (Regitnig, 1983) has led to a decline in production of Maple Presto in favour of the higher yielding Maple Amber (licensed in 1982).

Lentils are an old world crop which have gained interest in Manitoba. Currently, they are grown for human consumption, with the straw being used as a forage. Though probably one of the first cultivated crops, very little is known about their nitrogen nutritional requirements.

Fababeans were introduced in Manitoba to alleviate local crude protein shortages. Though generally considered capable of fixing enough nitrogen for their own nutritional requirement, instances of yield responses to nitrogen addition have been reported on the Canadian

Prairies (Rogalsky, 1972; Sadler, 1975). However, Richards and Soper (1979) found that fababeans did not respond to additional nitrogen.

Research was initiated in 1982 in the Department of Soil Science at the University of Manitoba to determine if the nitrogen nutritional requirements of Aladin fababeans, Maple Amber soybeans, and lentils can be met by fixation of atmospheric dinitrogen. Also of interest was the determination of the amount of nitrogen fixed by these three species and the timing of fixation by Maple Amber soybeans. Three methods of measuring dinitrogen fixation (the acetylene reduction assay, "A" value method and ^{15}N assisted difference method) were also studied. The effect of inclusion of roots and addition of straw residues on measurement of fixation was also examined.

CHAPTER II

LITERATURE REVIEW

2.1 Factors affecting Yield, Protein Content, and Dinitrogen Fixation in Soybeans, Lentils and Fababeans

Soybeans, lentils, and fababeans, members of the family Leguminosae, can obtain a portion or all of their nitrogen nutritional requirements from the fixation of atmospheric dinitrogen when in association with the appropriate rhizobium species. The amount of nitrogen fixed varies with respect to legume species, cultivars within a species, rhizobium strain, supply and form of available nitrogen, availability of other nutrients, and environmental conditions.

2.1.1 Effect of Nitrogen Addition on the Yield, Protein Content, and Symbiotic Nitrogen Fixation of Soybeans, Lentils, and Fababeans.

The response of soybeans to the addition of nitrogen has been variable. Some researchers have noted increased seed yields and protein content upon the addition of nitrogen (Regitnig, 1983; Dean and Clark, 1980; Behron et al., 1979; Diebert et al., 1979; Ham et al., 1978; Sorrenson and Penas, 1978; Bhangoo and Albritton, 1976; Johnson and Hume, 1972). These workers concluded that nitrogen fixation was not adequate to meet the nitrogen nutritional requirements of soybeans. Other researchers have found no increase in seed yield or protein content of soybeans when fertilizer nitrogen was added (Jones et al., 1981; Rennie et al., 1982; Criswell et al., 1976; Pal and Saxena, 1976; Welch et al., 1973) and concluded that nitrogen fixation was adequate to meet the nitrogen nutritional requirements of soybeans. Welch et al., (1973) working in Southern Illinois found that in only 3 of 133 instances did soybeans respond to fertilizer nitrogen. The soils in this experiment contained less available nitrogen than the surrounding area; however, no amounts were given. Shibles et al. (1975), in a general review of soybeans stated that seed yield responses have been inconsistent; the reports of substantial increases in yield with nitrogen addition were rare, while reports of no increase in yield with nitrogen addition have been frequent.

The lack of response of soybeans to nitrogen addition cannot be interpreted to mean that soybeans can fixed enough nitrogen for their nutritional requirements. The omission of reporting the nitrogen status of the soil in the literature precludes an estimate of the soil contribution which may have been high in many cases. Another reason for the variable response of soybeans to added nitrogen may have been due to soybean - cultivar/rhizobium strain interaction: this topic will be discussed later.

Variation in the physiological stage at which nitrogen intake (via fixation or soil nitrogen) cannot meet the nitrogen nutritional requirements of soybeans has been noted by some researchers.

Before nodule initiation, legumes must rely on nitrogen from soil, seed and fertilizer sources. Hatfield et al. (1974), in a gravel solution culture experiment, demonstrated the importance of soil nitrogen in early soybean development. They found that inoculated Culter soybeans supplied with nitrogen for either four and six weeks after emergence had higher dry matter yields at 6 weeks after emergence than those plants which had received no nitrogen.

Nitrogen stress during reproductive development has been found to reduce seed yields (Streeter, 1981; Dumphy et al., 1979; Brevedan et al., 1978; Egli et al., 1978; Sinclair and De Witt, 1976; Schibles et al., 1975; Thibodeau and Jaworski, 1975). When nitrogen stress occurred during early podfill, the reduced yields were due mainly to a reduction in seed size (Streeter, 1981; Egli et al., 1978). However, yield reductions due to a nitrogen stress occurring at flowering were found to be mainly due to reduction in seed number (Streeter, 1981; Brevedan et al., 1978; Schibles et al., 1975; Thibodeau and Jaworski, 1975).

The stage of growth at which fixation of nitrogen occurred in soybeans was variable and depended on the cultivar. Israel (1981) found in field experiments, that when Ransom and Davis cultivars were inoculated with USDA 31 and 110 Rhizobium both cultivars achieved the same seed yield, dry matter yield, and nitrogen content, though fixation of nitrogen occurred at different stages of growth. The Davis cultivar fixed nitrogen during vegetative development while the Ransom cultivar fixed most of its nitrogen during reproductive growth. Weber et al. (1971), working with the Lee cultivar, suggested that fixation occurred from 3 to 4 days after seeding to near maturity (12 to 13 weeks after seeding); however, 80 percent of the nitrogen was fixed between flowering and green bean stage of development. Regitnig (1983), working with Maple Presto soybeans and Thibodeau and Jaworski (1975), working with soybeans found that fixation was greatest at mid-pod formation.

The form of combined nitrogen has been found to influence the response of soybeans to nitrogen addition. Bezdicek et al., (1974) found that urea increased the dry matter production and grain yield of soybeans to a greater extent than NH_4NO_3. Ham et al., (1975) found that urea, ammonium nitrate, sulfur coated urea and urea formaldehyde all increased the seed yield, percent protein and total protein content, however, urea gave consistently higher results. Rabie (1981) concluded that the urea was the most preferable form of nitrogen for legume nutrition.

There has been general agreement in the literature that fababeans can fix enough nitrogen for their nutritional requirements. Dekhuijzen et al. (1981); Dean and Clark (1980), Richards and Soper (1979), Richards and Soper (1982) and McEwen (1970) found that nitrogen addition did not significantly increase the seed yield or protein content of fababeans.

The yield response of lentils to nitrogen addition has been variable. Mahajan et al. (1972), found a significant increase in the seed yield of lentils upon the addition of 20 and 30 kg N ha^{-1}. Ojha et al. (1977), and Chowdhury et al. (1974), however, determined that nitrogen addition did not increase the seed yield of lentils.

Summerfield and Mauchbauer (1982) noted that advisors in the USA considered it worthwhile and reasonable insurance to apply small amounts of nitrogen to lentils. Lentils grown in Saskatchewan on fields testing less than 30 kg NO_3-N to 60 cm were thought to benefit from an application of 30 kg N ha^{-1} (Slinkard and Drew, 1981).

The omission of reporting the nitrogen status of soils on which lentils were grown hinders the evaluation of whether lentils needed additional nitrogen for maximum yields. The lack of response of lentils to the addition of nitrogen in the work of Ojha et al. (1977), and Chowdhury et al. (1974), may have been because the lentils were receiving enough nitrogen from the soil.

2.1.2 Effect of Nitrogen Addition on Nitrogen Fixation and Nodulation of Legumes

The addition of nitrogen, though it may increase the yield of legumes has been found to affect nitrogen fixation and nodulation.

It has been well established that nitrogen addition suppresses fixation (Regitnig, 1983; Rennie et al., 1982; Rabie, 1981; Dean and Clark, 1980; Manhart and Wong, 1980; Wong, 1980; Diebert et al., 1979; Richards and Soper, 1979; Dean and Clark, 1977; Bhangoo and Albritton, 1976; Ham et al., 1975: Bezdicek et al., 1974) and nodule development (Rabie, 1981; Streeter, 1981; Ham et al., 1975; Aba Shakra et al., 1972; Johnson and Hume, 1972; Benjamin et al., 1971; Harper and Cooper, 1971).

Though most authors agree that nitrogen addition usually suppresses nitrogen fixation and nodule development some promotive effects have been noted. Pankhurst and Jones (1979) found in a solution culture experiment that application of 1.0 mg N day^{-1} $plant^{-1}$ as NH_4NO_3 increased the amount of nitrogen fixed by lotus plants by up to 500 percent. The increase in fixation was coupled with a doubling in nodule fresh weight. Allos and Bartholomew (1955) stated that instances of increases in fixation and nodular development upon addition of nitrogen were probably due to increased plant growth (i.e., the larger plant allocated more photosynthate for the nodules thus increasing nodule development and nitrogen fixation).

Various concepts have been promoted to explain the decrease in fixation and nodule development upon nitrogen addition.

The amount of photosynthate allocated to nodules has been found to change upon the addition of nitrogen. Thibodeau and Jaworski (1975), working with soybeans and Dekhuijzen et al. (1981), working with fababeans found a close and competitive relationship existed between NO_3 reduction and nitrogen fixation. This was further corroborated by work done by Streeter (1981) and Latimore et al. (1977). These authors, using $^{14}CO_2$, found that the CO_2 assimilated by soybean plants and subsequent transport as photosynthate to the nodules was reduced when plant roots were supplied with nitrate, and resulted in retarded nodule development and hence decreased fixation. Streeter (1981) also noted that the presence of NO_3-N reduced the concentration of reducing sugars in the sap. Wong (1980) found that lentils grown in a solution containing the reducing sugars glucose, sucrose and fructose in addition to nitrate fixed similar amounts of nitrogen as plants grown in a NO_3 free media. Wong (1980) stated that the added sugars alleviated the inhibitory effects of nitrogen on symbiotic nitrogen fixation through increased carbohydrate supply such that lentils supported both fixation and nitrate reduction activity and by inhibited nitrate accumulation and lowered nitrate reductase activity in the leaves.

Rabie (1981), in a review of the literature, stated that nitrite production via the reduction of nitrate by Rhizobium bacteroid nitrate reductase has been postulated to inhibit nitrogen fixation. He indicated that nitrite production could inhibit nitrogenase activity directly or through the binding of an NO compound with leghaemoglobin, thus interfering with oxygen-leghaemoglobin binding and decreasing nitrogen fixation due to increased partial pressure of oxygen around the nitrogenase enzyme. Manhart and Wong (1980) and Gibson and Pagan (1977), however, concluded that it was unlikely that nitrite produced from nitrate by bacteroid nitrate reductase played a significant role in the inhibitory effect of nitrate on the nitrogenase activity of nodules.

Rabie also indicated that nitrate appeared to mitigate the action of indoleacetic acid (IAA) in the development of the legume rhizobium symbiosis. The addition of nitrate did not affect the conversion of tryptophan to IAA, but the lower IAA concentration upon addition of nitrate was due to nitrate catalyzed destruction of IAA (Tanner and Anderson, 1963). The conversion of tryptophan to IAA was decreased, however, with the addition of ammonium. Valera and Alexander (1965) showed that alfalfa plants provided with both IAA and nitrate nodulate similarly to plants grown in a nitrate free media while those grown in medium that contained only nitrate nodulated poorly.

All of the mechanisms proposed, except the action of Rhizobium bacteroid nitrate reductase, for the decrease in nitrogen fixation and nodulation upon nitrogen addition have been proven experimentally and since no one mechanism seemed to be more valid than any other, it can only be assumed at the present time that all mechanisms act concurrently.

Gates and Muller (1979) working on the effect of N, P and S on nodulation of soybeans in solution culture showed that any imbalance in these three nutrients inhibited nodular development. The greatest development of nodular material occurred at the highest level of addition of all three nutrients.

The inhibitory effect of combined nitrogen on fixation and nodulation has also appeared to be influenced by the form of the nitrogen applied.

Mahon (1977) in a greenhouse experiment found that a 10 mM concentration of NO_3-N decreased the nitrogenase activity of peas by 95 percent while an equal concentration of NH_4 decreased activity only 16 percent. Soybeans grown in a nutrient solution containing 18 mM of urea-N produced nodules capable of fixing nitrogen while a 2 mM solution of NO_3-N inhibited nodulation (Vigue et al, 1977). Diatloff (1968) reported that nitrate forms of nitrogen had a greater inhibitory effect on nodulation than ammonium forms. Rabie (1981) stated that nitrate inhibited nodulation to a greater extent than ammonium and that ammonium inhibited nodulation to a greater extent than urea.

2.1.3 Cultivar - Rhizobium Strain Effect on Yield and Nitrogen Fixation

Researchers have noted that the yield of soybean cultivars depended on the strain of *Rhizobium japonicum* used in inoculation.

Nangju (1980) evaluating the response of cultivars Bossier, Juniper, and TGm 294-4-2371 (originating from America) and Malayara, Orba, and TGm 686 (originating from South East Asia) to native and applied Rhizobium in Nigeria, found that the South East Asian cultivars nodulated adequately with native rhizobium and that inoculation with Nitragen S culture did not significantly improve yield. In contrast, the American cultivars nodulated poorly with native rhizobium but inoculation with Nitragen Corporation S Culture significantly increased the seed yields. The increased seed yields of the American cultivars when inoculated with Nitragen S culture was thought to be due to increased fixation through a better symbiotic relationship between the Rhizobium and cultivars.

Israel (1981) found that Ransom and Davis cultivars inoculated with USDA 110 strain of Rhizobium japonicum had significantly higher yields than when inoculated with USDA 31. The increased yield of plants inoculated with USDA 110 over plants inoculated with USDA 31 was attributed to greater nitrogen fixation by a better symbiotic relationship between the cultivar and rhizobium.

The influence of method of inoculation (slurry vs. granular) and supplier of inoculum on the yields of Amsoy 71 and Beeson cultivars of soybeans was studied by Nelson et al. (1978). They found that the yields of Amsoy 71 were not effected by method of inoculation or from which supplier the Rhizobium had come. The yield of the Beeson cultivar, however, was significantly lower when inoculated with slurry inoculum from Agriculture Laboratories than inoculants from Nitragen and Kalo laboratories. Incompatibility of the Agriculture Labs seed inoculum with the Beeson cultivar was cited as reason for the reduced yield. Thus, the reports that soybeans could not fix enough nitrogen for their own needs may have been due in part to the incompatibility of the cultivar and the inoculant.

2.1.4 Environmental Influences on Yield and Nitrogen Fixation of Legumes

2.1.4.1 Effect of Carbon Addition and CO_2 Enrichment on Yield and Nitrogen Fixation in Legumes

Carbon dioxide enrichment has been found to increase the seed yield and nitrogen fixation of legumes.

Sionet *et al*. (1982), working with soybeans, and Day *et al*. (1979), working with fababeans, in growth chamber experiments on CO_2 enrichment found that increased dry matter production, seed yield and nitrogenase activity were coupled to an increased production of photosynthate.

The responses of soybeans to the addition of straw residues have been variable. Shivashankar *et al*. (1976), in a growth chamber experiment found that straw incorporation increased the dry matter production, seed yield and nitrogenase activity of soybeans in a similar manner to CO_2 enrichment. The increased nitrogenase activity with the incorporation of straw residues was thought to be due to immobilization of soil nitrogen and increased photosynthate supply to the nodules from an increased photosynthate production. The increased photosynthetic production was attributed to increased CO_2 supply from the soil to the upper plant portions which resulted in increased dry matter production and seed yield. They proposed that straw addition may replace CO_2 enrichment as a method for increasing the seed yield of soybeans. In contrast, Criswell *et al*. (1976), and Weber (1966) in field experiments found no effect of addition of corn cobs on the seed yield of soybeans. The amount of nitrogen fixed, however, increased with cob addition due to immobilization of soil nitrogen. Wagner and Zapata (1982), in a field experiment, found that the addition of sugar decreased dry matter production of the nodulated soybeans slightly and the reference crop dramatically. The addition of sugar, however, increased the fraction and the amount of nitrogen in the soybeans derived from fixation by immobilizing soil nitrogen.

2.1.4.2 Effect of Water Stress on Yield and Nitrogen Fixation

Water stress has been found to decrease yield and nitrogen fixation of legumes.

Carlson *et al*. (1982), found that soybean yields decreased 20 to 50 percent upon moisture stress and the yield reduction was a function of the cultivar. This indicated that soybean cultivars varied in their ability to withstand drought and that, in areas where drought was prevalent, selection should have been made for cultivars which gave the highest yields under such conditions.

Alessi and Power (1982) found that row spacing had an influence on the seed yield of soybeans grown under drought conditions. They found that the total water use over the season was greatest and the soybean yield least from the smaller (15 cm) than the larger 100 cm row spacings. They suggested that under extreme drought conditions early season uptake of water left less water available during podfill, and thus reduced yields for soybeans grown on 15 cm row spacing.

Water stress has been found to decrease nitrogen fixation in legumes (Tu and Hietkamp, 1977; Sprent, 1972; Sprent, 1977; Kuo and

Boersma, 1971). Sprent (1971) found that when the fresh weight of detached nodules was reduced to eighty percent of its maximum fully turgid value, nitrogenase activity, as measured by the acetylene reduction assay, stopped. She stated that such nodules would be shed by the plant. In a field experiment with fababeans, Sprent (1971) found that maximum fixation occurred at field capacity; soil moisture levels above and below this level reduced fixation.

Weber (1966), working with nodulating and non-nodulating soybean isolines determined that moisture stress decreased seed yields of both isolines. The amount of nitrogen needed to equalize the yield of the non-nodulating isoline with that of the nodulating was significantly less under stressed than non-stressed conditions and indicated by the classical difference method that fixation was reduced.

2.1.4.3 Effect of Temperature on Yield and Nitrogen Fixation of Legumes

A number of workers have studied the effect of temperature on yield and nitrogen fixation of legumes.

As temperature was increased from 15 to 30C an increase in the growth rate and nitrogen content of the soybean plants occurred (Trang and Giddens, 1980). Duke et al. (1979), found that soybean plants grown at a root temperature of 13C did not nodulate but plants which were switched from 13C to 20C root temperature nodulated and fixed nitrogen. A decline in acetylene reduction activity occurred upon switching plants from the 20C to the 13C root temperature bath. An Arrhenius plot of acetylene reduction during the decline in root temperature for the period immediately after switching the plants from

20C to 13C bath until the root temperature reached 13C showed a steady decrease in nitrogenase activity between 20C and 15C with an activation energy of 13.7 Kcal mol^{-1}. A sharp inflection occurred in the plot at 15C corresponding to an increase in the activation energy to 52 Kcal mol^{-1} for temperatures below the inflection. The Arrhenius plot indicated that at temperatures below 15C more energy was needed for each mole of acetylene reduced than for temperatures above 15C.

Schwerter and Harper (1980) found that exposure of shoots and roots of intact soybean plants to a low temperature, 18C as compared to 27C, decreased nodule activity. Exposure of only the shoot portion to the lower temperature gave similar results indicating that decreased nitrogen fixation with lower temperatures may have been due in part to decreased photosynthate supply to the nodules from the shoots.

Kuo and Boersma (1971) found that increasing the root temperature from 15.6C to 27C increased nitrogen fixation and seed yield of soybeans but temperatures above 27C decreased both parameters.

2.2 Methods of Measuring the Amount of Dinitrogen fixed by Legumes

Various methods have been utilized by researchers for determining qualitatively and quantitatively the amount of dinitrogen fixed by legumes. These methods have been summarised in reviews, (Hauck and Bremmer 1976; LaRue and Patterson, 1981; Hardy and Holstein, 1977; Ham, 1978; Hardy et al., 1972) thus, only the methods pertinent to this thesis will be discussed. The methods utilized in this thesis were, the classical difference method, the ^{15}N assisted difference method, the "A" value method and the acetylene reduction assay.

The difference method, as used by Weber (1966), involved the determination of the total amount of nitrogen in the legume and the non-fixing reference crop. The difference in total nitrogen between the two crops estimated the amount of nitrogen fixed by the legume. Three approaches to the difference method have been used: 1) comparison of a legume with an uninoculated legume of the same species, 2) comparison of a legume with a non-nodulating legume, and 3) comparison of a legume with a non-legume. The major assumptions used in the difference method were that the legume and the control crop will take up the same amount of soil nitrogen and that combined nitrogen is used preferably over symbiotically fixed nitrogen.

The first assumption, that the nitrogen uptake of the legume and the control crop is identical, has been found to be not entirely valid. LaRue and Patterson (1981) noted that the estimate of fixation depended on the arbitrary choice of the non-fixing control; the estimated amount of nitrogen fixed by ladino clover when orchard grass and tall fescue were used as controls was 165 and 189 kg N ha^{-1}, respectively. Herridge (1982) stated that use of the difference method in soils which were low in available nitrogen may overestimate fixation since the volume of soil explored by the legume and the reference crop may have been quite different due to the restricted root growth of the non-fixing nitrogen deficient plant.

Allos and Bartholomew (1959) indicated that the second assumption that combined nitrogen is used preferably over symbiotically fixed nitrogen, was generally considered true for most legumes.

The ^{15}N assisted difference method, a modification of the difference method in which ^{15}N labelled nitrogen fertilizer was added to both crops was used by Richards and Soper (1979). After harvest both the nodulated fixing crop and the non-nodulated non-fixing crop were analyzed to determine the quantity of labelled nitrogen present. The fertilizer nitrogen contribution was determined by calculating the dilution of fertilizer ^{15}N in the plant. Subtraction of the contribution of fertilizer nitrogen from the total nitrogen in the non-fixing crop gave the amount of soil nitrogen taken up by the plant. Subtraction of the contribution of fertilizer nitrogen from the total nitrogen in the fixing crop gave the amount of soil and fixed nitrogen in the fixing plant. Further subtraction of soil nitrogen in the non-fixing control from the nitrogen in the fixing crop resulted in an estimate the amount of nitrogen fixed by the legume crop. The calculation was represented by the equation:

$$S = P - B - F - {}^{15}N \qquad (2.2.1)$$

where

- S = quantity of nitrogen symbiotically fixed.
- P = total nitrogen in the aerial portions of the fixing plant.
- B = contribution of soil nitrogen as measured by the non-fixing crop.
- F = contribution of seed nitrogen.
- ^{15}N = contribution of fertilizer nitrogen as measured by tracer ^{15}N. (This factor is omitted in treatments where no fertilizer nitrogen was added).

The use of ^{15}N fertilizer in the modified difference method allowed for the differential fertilization of the legume (fixing crop) and the control crop (non-fixing). High rates of nitrogen were added to the non-fixing control such that the problem in the classical difference method (the difference in soil volume explored by the control crop and the fixing crop due to the control being nitrogen deficient) was overcome. The addition of fertilizer to the legume was not necessary if only the amount of nitrogen fixed was to be determined.

To determine if fixation occurred and how much nitrogen was fixed, the classical and ^{15}N assisted difference methods relied on yield dependant criteria (i.e., the total nitrogen content of the fixing and non-fixing crops). Variation in growth of the non-fixing crop (i.e., total nitrogen accumulation) due to conditions not related to nitrogen (i.e., temperature) may have caused erroneous results in the determination of fixation.

McAuliffe et al. (1958), proposed the use of the following isotope dilution formula for determining the amount of nitrogen fixed by a legume:

$$TNF = (1 - a/b) * TNL \qquad (2.2.2)$$

where

TNF = total nitrogen fixed by the legume.
a = percent atom excess ^{15}N in the legume.
b = percent atom excess ^{15}N in the control.
TNL = total nitrogen in the legume.

The formula described above does not utilize the total amount of nitrogen accumulated in the non-fixing control thus the problem of variation in total nitrogen accumulation in the control due to factors other than nitrogen was overcome. In order to utilize this method, the amount of fertilizer nitrogen and the percent atom ^{15}N excess in the fertilizer applied to both the legume and the control had to be the same. The major problem was that the addition of nitrogen at high rates (to ensure a healthy non-fixing control plant) decreased the amount of nitrogen fixed by the legume.

The modified "A" value concept of Fried and Broeshart (1975) overcame the problem with the method of McAuliffe which allowed high fertilization of the barley and thus ensured a healthy control crop. Any difference in the volume of soil explored between the control crop and the fixing crop was not due to the control crop being nitrogen deficient. Low fertilization of the legume such that the added nitrogen did not suppress fixation was also possible.

The "A" value method, proposed by Fried and Dean (1952), was based on the concept that the availability of a nutrient in soil could be determined in terms of a fertilizer standard. The main assumption was that if a plant was confronted with two or more sources of a nutrient, the plant would take up from each source in proportion to their respective availabilities. The following equation was proposed for determining the amount of available nutrient in the soil in fertilizer equivalent units:

$$A = B\,(1 - y)/y \qquad (2.2.3)$$

where

A = the available nutrient in the soil in terms of fertilizer.
B = the quantity of nutrient added to the soil as fertilizer.
y = the fraction of nutrient in the plant derived from the fertilizer.

Determination of y by the use of radioactive or stable isotopes allowed for the calculation of "A" assuming that all the fertilizer was available to the plant. The "A" value according to Fried and Dean was independent of the amount of fertilizer added.

The modification proposed by Fried and Broeshart (1975) involved the determination of the "A" value of the fixing crop and the "A" value of the non-fixing crop. The "A" value of the non-fixing crop represented the plant available soil nitrogen while the "A" value of the legume (fixing crop) represented the availability of soil and fixed nitrogen. Subtraction of the "A" value of the control crop from the "A" value of the legume gave fixed nitrogen in terms of a fertilizer standard. Multiplication of the difference in "A" values by the percent utilization of fertilizer N by the legume gave the amount of nitrogen fixed by the legume. This was represented by the equation:

$$\text{Amount Fixed} = (A_{leg} - A_{cont}) * UtFert_{leg} \qquad (2.2.4)$$

where:

$UtFert_{leg}$ = the percent utilization of fertilizer N by the legume.
A_{leg} = the "A" value of the legume.
A_{cont} = the "A" value of the control.

They indicated that the modified "A" value method allowed for differential fertilization of the legume and the control (high amounts of nitrogen fertilizer applied to the control species and low amounts of nitrogen applied to the legume) and did not rely on yield dependent criteria of the control (i.e., the total nitrogen accumulated in the control). Thus, the major problems associated with the difference, ^{15}N assisted difference and isotopic dilution (as proposed by McAuliffe et al., (1958)) methods may have been overcome if the assumptions that the "A" value of the control does not change with the addition of nitrogen was true and the amount of soil nitrogen taken up by the control was independent of differential fertilization. If, however, the "A" value of the control increased with increasing nitrogen additions (due to a priming effect) the amount of nitrogen fixed by the legume would be underestimated by this method since the priming effect would not be accounted for in the "A" value of the legume.

The modified "A" value technique of Fried and Broeshart (1975) was mathematically identical to the formula of McAuliffe et al., (1958), if the amount of nitrogen fertilizer and the percent ^{15}N excess in the fertilizer added to the control crop and the fixing crop were identical.

Incorporation of ^{15}N fertilizer into the soil organic fraction by the addition of ^{15}N fertilizer with carbon substrates was used by Talbott et al., (1982). Incorporation of ^{15}N fertilizer into the soil organic fraction meant that a portion of the soil organic pool was labelled. Dilution of the nitrogen taken up from the soil organic pool by fixed nitrogen, when compared to the non-fixing control, resulted in an estimate of nitrogen fixation. Talbott et al. (1982), stated that this method was preferable to that proposed by Fried and Broeshart in that the uptake of combined nitrogen by the plant was solely from the mineralization of organic nitrogen and resulted in only one input of nitrogen to the plant other than fixation. Further stated was that, in the method proposed by Fried and Broeshart, the nitrogen inputs to the legume consisted of soil nitrogen, fixed nitrogen and fertilizer nitrogen and that the soil nitrogen contribution could not be easily separated from symbiotically fixed nitrogen. That the nitrogen taken up by the plant arised solely from the mineralization of organic nitrogen may not be true if appreciable amounts of inorganic nitrogen occurred in the lower portions of the soil profile. They also stated that this approach was preferable because the incorporation of carbon substrate resulted in immobilization of soil nitrogen and promotion of fixation. However, the immobilization of soil nitrogen may have led to a nitrogen deficient condition in the control crop. Under this condition the soil volume explored by the control crop may have been less than that óf the fixing crop. If appreciable amounts of soil nitrogen were in the volume of soil explored by the fixing crop that was not explored by the roots of the control, nitrogen fixation would have been overestimated.

The formula used by Talbott <u>et</u> <u>al</u>. (1982), for determining the amount of nitrogen fixed was identical to that of McAuliffe (1958) since the amount of fertilizer nitrogen and the percent ^{15}N excess in the fertilizer added to the control and fixing crop were the same. If one of these factors had been different (i.e., the amount of fertilizer applied to both crops) then the "A" value formula would have been used.

The acetylene reduction assay has been used by many researchers for the determination of nitrogen fixation in legumes. The nitrogenase enzyme, responsible for the conversion of atmospheric dinitrogen to ammonia, has been found to reduce acetylene to ethylene. The use of a conversion factor allowed an estimation of the amount of nitrogen fixed. The equation used in the quantification of nitrogen fixation, as given by Hardy and Holsten (1977), was as follows:

$$g\ N_2\ [C_2H_2]\ \text{fixed}\ hr^{-1}\ \text{sample}^{-1} = \frac{ebi}{s}*c*r*v*\frac{1}{t}*\frac{1}{f}*28 \quad (2.2.5)$$

where:

e, b, i and s are the peak height, or area, for C_2H_2 in analyzed sample of 50 ml from, respectively,

(i) experimental sample incubated with C_2H_2,
(ii) experimental sample preincubated in 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), and
(iv) C_2H_4 standard;

c = concentration of ethylene in standard expressed as moles/litre at S.T.P.
r = ration 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 litres at S.T.P.
t = time of incubation in hours.
f = the conversion factor for moles of C_2H_2 reduced to moles N_2 fixed.
28 = molecular weight of N_2.

The theoretical value given for conversion factor given was three. LaRue and Patterson (1981) in a review of the literature indicated that a more appropriate factor for in vitro measurement of nitrogenase activity would be four. Hudd et al. (1980), using ^{15}N labelled N_2 gas to determine the conversion factor for fababeans found that a factor of 5.75 was applicable. Zablotowicz et al. (1980), stated in their review of the literature, that ATP dependent H_2 evolution by nitrogenase enzyme activity may consume 20 to 40 percent of the energy supplied to the nitrogenase enzyme. The hydrogenase activity of nitrogenase enzyme probably accounted for the deviation of the conversion factor from its theoretical value of three.

Hardy et al. (1973), comprehensively reviewed the advantages and limitations of the acetylene reduction assay and, thus, only the major advantages and disadvantages will be discussed.

One of the major benefits indicated was its sensitivity. Hardy et al. (1973) stated that the sensitivity of the acetylene reduction assay was 10^3 to 10^4 times more sensitive than ^{15}N methods. Another benefit was the cost. The cost of acetylene reduction assay was shown to be many times less than that of ^{15}N analysis. Also, a number of acetylene reduction samples could be run in the time it takes for one Kjeldahl or ^{15}N determination.

One of the major disadvantages, previously discussed, was that the conversion factor of moles ethylene produced to moles of nitrogen fixed varied greatly depending on the crop and cultivar studied. Other major disadvantages were seasonal variation in nitrogenase activity and that only a few plants were used to measure fixation.

As discussed, each method used in measuring nitrogen fixation had its own inherent advantages and disadvantages. Thus, care must be taken in interpreting the results, but meaningful information can be extracted using these methods.

CHAPTER III

ANALYTICAL PROCEDURES

3.1 Soil Analysis

3.1.1. Nitrate Nitrogen

Soil NO_3-N was estimated by the hydrazine reduction method of Kamphake et al. (1967). Bulk density data was used to convert parts per million nitrate nitrogen into kilograms of nitrate nitrogen per hectare for field samples.

3.1.2 Phosphorus

Plant available phosphorus was estimated by a modification of the $NaHCO_3$ method of Olson et al. (1954) used by the Manitoba Soil Testing Laboratory. Phosphorus was extracted from 2.5 grams of soil, to which 0.5 grams of pretreated charcoal has been added, with 50 ml of 0.5 M $NaHCO_3$ which had been standardized to pH 8.5. The sample plus extracting solution was shaken for 30 minutes on an Eberbach reciprocation shaker set on slow speed. The soil extract was filtered through Whatman No. 30 filter paper and the phosphorus level in the filtrate determined by the Murphy and Riley (1962) acid molybdate method.

3.1.3 Potassium

Plant available potassium was determined by the following procedure. An extraction solution of 25 ml of 1.0 M NH_4OAc was added to 2.5 g of soil. The soil plus extracting solution was shaken for one hour, filtered, and the potassium concentration in the filtrate determined by flame photometry.

3.1.4 Sulfur

The plant available sulfate sulfur in the soil was extracted by shaking a 1:20 soil to 0.001 M $CaCl_2$ mixture for thirty minutes. The mixture was then filtered through Whatman No. 42 paper. The amount of sulfate sulfur in the filtrate was determined by the method of Lazrus et al. (1966) on a Technicon Autoanalyzer II with a wavelength set at 460 nm.

3.1.5 Zinc and Copper

Extractable zinc and copper was determined by the method described by Lindsay and Norvell (1969). The 2:1 DTPA extracting solution (0.005 M DTPA, 0.01 M $CaCl_2$ and 0.1 M triethanolamine) to soil mixture was shaken for two hours. After shaking the extract was then filtered and the zinc and copper concentration in the filtrate determined using a Perkin Elmer 560 atomic absorption spectrophotometer.

3.1.6 Organic Matter

The percent organic matter in the soil was determined by the Walkley Black method (Allison, 1965). An automatic titrator was used to back titrate the excess K_2CrO_7 with $FeSO_4$.

3.1.7 Inorganic Carbonate

The inorganic carbonate content was determined by the following procedure. A 1.0 g sample of soil was digested in 40 ml of 0.1 M HCl for 10 minutes. The CO_2 evolved was collected using a Nesbitt tube containing ascarite and magnesium perchlorate. The change in the mass of the ascarite was taken as the mass of the CO_2 evolved.

3.1.8 pH and Conductivity

Soil pH was determined on a water saturated soil paste using a standard glass-calomel combination electrode.

Conductivity of the soil was determined on the same soil paste using a Radiometer conductivity meter with a standard conductivity cell.

3.1.9 Field Capacity

The field capacity of the soil was determined by the following procedure. Soil was added to a plastic cylinder (one end of which had been covered to keep the soil in). Water was then added to the soil until the wetting front had moved between one quarter and halfway down the cylinder. The top of the cylinder was then covered with parafilm and the cylinder and soil incubated for 48 hours. After incubation, a soil sample was taken from the centre of the wetted soil. The soil sample was then weighed and oven dried at $105^{0}C$ for 48 hours reweighed and the percent moisture at field capacity calculated.

3.2 PLANT ANALYSIS

3.2.1 Percent Nitrogen in the Plant Material

The nitrogen in the plant was determined by the modified Kjeldahl Gunning method described by Jackson (1959). The digestion accelerators used were Keldtab[1] and Kelpac.[2] The NH_3 released from the digested

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1 Supplier. ITECATOR Box 70 S-26301 Hoganas Sweden
Telex 72695 042 -42330

2 Supplier. Canadian Lab Supplier Ltd., 80 Jutland Road,
Toronto, Ontario

plant material was trapped in 50 ml of 0.1 N H_2SO_4 The amount of NH_3 in the flask was determined by back titration with standard 0.1 N NaOH.

3.2.2. Atom Percent ^{15}N

The atom percent ^{15}N in plant samples which were enriched with ^{15}N, and samples used to determine the ^{15}N background, was determined with a mass spectrometer.

The solution in the flasks which contained the nitrogen released from the plant material during the Kjeldahl procedure (Section 3.2.1) was acidified by adding one drop of concentrated sulphuric acid. The solution was then concentrated, by the evaporation of the water in the solution, to a volume of approximately 7 ml. The evaporation process was done on either a hot plate or in an oven. The concentrated samples were stored in test tubes until they could be analyzed. The procedure used for fertilizer standards which contained ^{15}N was the same as that used for the plant samples.

The samples were transferred from the test tubes to reaction flask and nitrogen gas was produced by the oxidization of the sample with sodium hypobromite in the absence of air. The vacuum system used to remove the air was similar to that described by Fehr (1969). The use of liquid nitrogen and KOH for reducing the amount of water vapour in the system was discarded and a method suggested by Cho[3] was used. The method entailed placing a strong desiccating agent Drierite[4] (anhydrous calcium sulfate) into collection tubes.

A Micromass 602 mass spectrometer in the single collector scanning mode was used to analyse the N_2 gas for $^{14}N/^{15}N$ ratios based on the ion current intensities of mass twenty eight and mass twenty nine.

The equations used in the calculation of the atom percent ^{15}N in the sample from the peak ratios as given by Bremner (1965) are:

$$\text{Atom \% } ^{15}N = 100/(2R + 1) \qquad (3.2.1)$$

and

$$R = (^{14}N\ ^{14}N) \ /\ (^{15}N/^{14}N) \qquad (3.2.2)$$

where:

R is the ratio of peak heights (ion currents) corresponding to mass 28 ($^{14}N\ ^{14}N$) and mass 29 ($^{14}N\ ^{15}N$).

Bremner (1965) suggested the following formula for defining atom % ^{15}N:

$$\text{Atom \% } ^{15}N = \frac{(^{14}N\ ^{15}N) + 2(^{15}N\ ^{15}N) * 100}{2(^{14}N\ ^{14}N) + 2(^{14}N\ ^{15}N) + 2(^{15}N\ ^{15}N)} \qquad (3.2.3)$$

Calculations involved only mass 28 and 29 ion currents since mass 30 ion currents can be eliminated from the equation based on the equilibrium:

$$Keg = (^{14}N\ ^{15}N)^2/(^{14}N\ ^{14}N)\ (^{15}N\ ^{15}N) = 4 \qquad (3.2.4)$$

The value of 4.0 has been justified theoretically and experimentally. Rearrangement of equation 3.2.4, substitution of it into 3.2.3, and simplification gives:

$$\text{Atom \% } ^{15}N = 100/(2R +1) \qquad (3.2.2)$$

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[3] Dr. C. M. Cho, Professor, Department of Soil Science University of Manitoba, Winnipeg, Manitoba.

[4] Supplier. W.B. Hammond Driete Company, Xenia, Ohio.

3.3 <u>ACETYLENE REDUCTION ASSAY</u>

3.3 ACETYLENE REDUCTION ASSAY

An acetylene reduction assay was performed on soybean nodules to determine the nitrogenase activity in the nodules. Only the methodology used in the laboratory analysis of the ethylene produced is discussed here; the obtaining of the samples in the field is discussed later. A Varian Model 3700 gas chromatograph coupled with a Vista 401 computer console was used to determine the amount of ethylene produced by Soybean nodules. The column was packed with poropak T. Calibrating gas[5] which contained 100 ppm ethylene in helium was used as a standard. One millilitre of gas was removed from the vacutainers and injected into the chromatograph with a gas tight locking syringe.[6] Moles of ethylene produced were then calculated from the standard count. The conditions of the chromatograph were:

> Inlet Temperature - 70C, Detector Temperature - 100C, Detector Type - Flame Ionization, Oven Temperature - 40C, Nitrogen gas flow rate - 30 ml minute $^{-1}$, Hydrogen gas flow rate - 20 ml minute $^{-1}$, Oxygen flow rate - 300 ml minute^{-1}, column size - 1.83 mm in length with a 2 mm internal diameter.

3.4 Statistical analysis

Statistical Analysis was done by Duncans Multiple Range Test, as in the Statistical Analysis System[7] (SAS) package on the University of Manitoba main frame computer.

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5 Manufacturer: Altech Associates, 2051 Waukeyan Road, Deerfield Il. 60015.

6 Manufacturer: Dynatech Corporation, Baton Rouge, Louisiana.

7 SAS Users Guide: Statistics 1982 Edition, SAS Institute .

CHAPTER IV

GROWTH CHAMBER EXPERIMENT 1: JANUARY 1982

4.1 Introduction

Fababeans, soybeans and lentils can derive some of their nitrogen nutritional requirements from symbiotic fixation of atmospheric dinitrogen. Researchers have agreed that fababeans can fix enough nitrogen for their nutritional requirements and hence do not respond to additional nitrogen. Regitnig (1983), however, found that Maple Presto soybeans could not fix enough nitrogen for maximum production. The little work done on lentils suggested that they may need additional nitrogen for maximum production (Summerfield and Mauchbauer, 1982; Slinkard and Drew 1981).

A growth chamber experiment was initiated to: determine the effect of nitrogen addition on the dry matter yield and nitrogen content of fababeans (Vicia faba cv. Aladin), soybeans (Glycine max. L. merr. cv. Maple Amber) and lentils (Lens esculenta. common Chilean), determine the effect of nitrogen addition on nitrogen fixation, and evaluate the ^{15}N assisted difference and "A" value methods for measuring dinitrogen fixation.

4.2 Materials and Methods

4.2.1 Soil

A Gleyed Dark Grey Chernozem of the Poppleton Association (gravelly phase) located near Steinbach, Manitoba was chosen for use in the first growth chamber experiment (Michalyna, 1982) because of its low NO_3-N content. The soil was obtained in the fall from the 0 - 15 cm depth and stored in an unheated shed. Prior to the experiment, the soil was air dried at 30C, mixed and passed through a 2 mm sieve. A subsample was taken for analysis.

At the termination of the experiment, soil from each pot was dried, mixed and subsampled. The subsamples were ground to pass through a 2 mm sieve and NO_3-N analysis performed.

4.2.2 Experimental Design and Procedure

A completely randomized experiment containing sixteen treatments and three replicates was undertaken (Table 1). The crops studied were lentils, soybeans, fababeans and barley (*Hordeum vulgare* cv. *Bonanza*).

Six kilograms of air dried soil was added to pots which had been previously washed with tap water, dried and had a plastic inner liner inserted. Each crop received nitrogen treatments of 0, 600, 1200 and 1800 mg N per pot. The form of the nitrogen used in this experiment was urea (4.549 atom % ^{15}N excess).

Basal applications of 100 ppm P as KH_2PO_4, 200 ppm K as KCl and KH_2PO_4, 5 ppm Cu as $CUSO_45H_2O$, 10 ppm Zn as $ZnSO_47H_2O$ and 7.4 ppm S as $CuSO_45H_2O$ and $ZnSO_47H_2O$ were added to each pot. All nutrients were added to 100 mL. The water plus nutrients were then poured uniformly over the soil surface. The top two-thirds of the soil in the pots was watered to field capacity by mass immediately after addition of the nutrients. The amount of water added was calculated on an oven-dried weight basis.

After the nutrients and water additions, the pots were incubated at room temperature (approximately 23C) for one week; due to lack of availability of growth chamber space.

Table 1. Treatments used in growth chamber experiment 1: January 1982.

Treatment Number	Nitrogen Added (mg N/pot)	Type of Plant
1	0	Fababean
2	600	Fababean
3	1200	Fababean
4	1800	Fababean
5	0	Soybean
6	600	Soybean
7	1200	Soybean
8	1800	Soybean
9	0	Lentils
10	600	Lentils
11	1200	Lentils
12	1800	Lentils
13	0	Barley
14	600	Barley
15	1200	Barley
16	1800	Barley

The imbibition of lentil, soybean and fababean seeds and inoculation with Nitragin Corporation C[1], S and Q cultures,

1 Nitragin Corporation, 3101 W. Custer Avenue
Milwaukee, W.I. 53209

respectively occurred twenty-four hours prior to seeding. Inoculation and imbibition of the seeds was accomplished by placing wet paper towels in the bottom of large pans to which an equal volume of seed and rhizobium of the appropriate species were added, mixed and covered with four layers of wet paper towels.

Four, six, eight and eight seeds of fababeans, soybeans, lentils and barley, respectively were placed in their designated containers at a depth of two centimeters. The top two thirds of the soil in the pots was maintained at field capacity until one week after emergence, at which time, the soil in each pot was watered to field capacity by mass. The pots were maintained at field capacity until harvest. The maintenance of the pots at two-thirds field capacity and field capacity was done by daily watering by mass.

At the change in the water regime, the plants were thinned to two, three, four and four plants per pot for fababeans, soybeans, lentils and barley, respectively. Random rearrangement of the pots once a week helped to ensure equal competition for light among plant species.

The aerial portions of the plants were harvested 65 days after seeding corresponding to early podfill of the legumes. Tap roots of the legumes were removed from the pots with a trowel after the above ground portions had been harvested. The soil was washed off the roots and the nodules counted.

The plant samples were air dried at 30C for 48 hours, weighed and then ground in a Wiley mill to pass through a 2 mm sieve. Nitrogen analysis was performed on all samples and the percent ^{15}N determined on samples which were enriched with ^{15}N. Percent ^{15}N was also determined on some samples which were not enriched with ^{15}N to determine the amount of background ^{15}N. The results are reported on an oven dried basis.

The conditions of the growth chamber experiment were: Temperature 20C day/17C night, Day length 14 hours (8 a.m. to 10 p.m.), Humidity 75%, Light intensity 700 uE m^{-2} s^{-1}.

4.3 Results and Discussion

The physical and chemical characteristics of the soil used in this growth chamber experiment are outlined in Table 2.

Table 2. Soil characteristics growth chamber experiment 1: January 1982.

Soil Association - Poppleton (gravely phase)
Soil type - Gleyed Dark Grey Chernozem
Texture - Loamy Fine Sand
NO_3-N (ppm) - 25.0
Available K (ppm) - 21.1
Available P (ppm) - 35.0
SO_4-S (ppm) - 6.0
Percent Organic Matter - 2.5
pH - 8.0
Calcium Carbonate Content - Low
Field Capacity = 22%
Conductivity - dSm^{-1} = 0.2

4.3.1 Effect of Nitrogen Addition on the Dry Matter Yield, Percent Nitrogen, and Total Nitrogen Accumulation in the Aerial Portions of Barley, Fababeans, Soybeans, and Lentils

Nitrogen addition significantly increased the dry matter yield, percent nitrogen and nitrogen accumulation in the aerial plant portions of barley (Table 3).

Table 3. The effect of rate of nitrogen addition on the dry matter yield, percent nitrogen and nitrogen accumulation in the above ground portion of barley plants.

Nitrogen Added (mg N/pot)	Dry Matter Yield (g/pot)	Percent N	Total N (mg N/pot)
0	21.9[1]A[2]	.80 A	175 A
600	28.6 B	1.72 B	494 B
1200	30.0 B	3.14 C	941 C
1800	31.7 C	3.38 C	1066 D

1 The results are expressed on an oven-dried basis.
2 Duncan's Multiple Range Test: Means followed by the same letter are not significantly different at P = 0.05.

Nitrogen addition, however, did not significantly affect the dry matter yield, percent nitrogen and total amount of nitrogen accumulated in the above ground portion of fababean and soybean plants (Tables 4 and 5). The lack of response of fababeans and soybeans to the addition of nitrogen indicated that uptake of soil nitrogen and symbiotic nitrogen fixation satisfied their nitrogen nutritional requirements. Other workers have also found that even on soils low in available nitrogen, fababeans did not need supplementary nitrogen. (Dekhuyzen *et* al., 1981; Dean and Clark, 1980b; Richards and Soper, 1979a; Richards and Soper, 1979b; McEwen, 1970).

Table 4. The effect of rate of nitrogen addition on the dry matter yield, percent nitrogen and nitrogen accumulation in the above ground portion of fababean plants.

Amount of Nitrogen Added (mg N/pot)	Dry Matter Yield (g/pot)	Percent Nitrogen	Total Nitrogen (mg N/pot)
0	34.4[1]A[2]	4.47 A	1535 A
600	32.4 A	4.18 A	1354 A
1200	32.3 A	4.09 A	1316 A
1800	34.0 A	3.58 A	1256 A

1 The results are expressed on an oven-dried basis.
2 Duncan's Multiple Range Test: Means followed by the same letter are not significantly different at $P = 0.05$.

Table 5. The effect of rate of nitrogen addition on the dry matter yield, percent nitrogen and nitrogen accumulation in the above ground portion of soybean plants.

Amount of Nitrogen Added (mg N/pot)	Dry Matter Yield (g/pot)	Percent Nitrogen	Total Nitrogen (mg N/pot)
0	24.5[1]A[2]	3.78 A	921 A
600	25.8 A	3.70 A	949 A
1200	26.0 A	4.23 A	985 A
1800	23.8 A	4.23 A	1003 A

1 The results are expressed on an oven-dried basis.
2 Duncan's Multiple Range Test: Means followed by the same letter are not significantly different at $P = 0.05$.

In contrast, Regitnig (1983) in a growth chamber experiment under similar conditions found increased dry matter yield, percent protein, and total amount of nitrogen accumulated in the above ground portion of Maple Presto soybeans. Thus, Maple Amber soybeans appear better able to satisfy their nitrogen nutritional requirements through the fixation of atmospheric dinitrogen than Maple Presto.

Nitrogen addition of 600 and 1200 mg N pot^{-1} increased the dry matter yield of lentils, however, the increase was not significant. The 1800 mg N pot^{-1} treatment significantly decreased the dry matter yield of lentils when compared to the dry matter yields associated with the 600 and 1200 mg N pot^{-1} treatments (Table 6).

The addition of nitrogen significantly increased the percent nitrogen in the above ground portion of lentils but there was no significant difference among nitrogen amendments (Table 6).

Table 6. The effect of rate of nitrogen addition on the dry matter yield, percent nitrogen, and total nitrogen accumulation in the above ground portion of lentil plants.

Amount of Nitrogen Added (mg N/pot)	Dry Matter Yield (g/pot)	Percent Nitrogen	Total Nitrogen (mg N/pot)
0	17.1[1]A[2]B	1.87 A	320 A
600	20.0 A	2.85 B	552 C
1200	20.5 A	2.65 B	543 C
1800	13.5 B	3.41 B	455 B

1 The results are expressed on an oven-dried basis.
2 Duncan's Multiple Range Test: Means followed by the same letter are not significantly different at P = 0.05.

Nitrogen addition also significantly increased the total nitrogen content of lentils (Table 6). However, the 1800 mg N pot^{-1} when compared to the 600 and 1200 mg N pot^{-1} additions significantly decreased the total nitrogen accumulated in the above ground portion of lentils, though plants which had received the 1800 mg N pot^{-1} treatment still had greater nitrogen accumulation in the aerial portion than those which had not received any nitrogen. There was no significant difference in total nitrogen accumulation of lentils which had recieved the 600 and 1200 mg N pot^{-1} treatments (Table 6).

The significant decline in the dry matter yield and total nitrogen accumulated in the above ground portion of lentils with the 1800 mg N pot^{-1} treatment when compared to plants which had recieved the 600 mg N pot^{-1} and 1200 mg N pot^{-1} treatments indicated that the high rate of urea addition was toxic to plant growth. Lentils appeared to be more susceptible to the toxic effects associated with high rates of urea addition than soybeans, fababeans and barley since the yield and nitrogen content of these crops were not adversely affected by the addition of 1800 mg N pot^{-1} (Tables 3, 4, and 5).

The lack of increase in dry matter yield, percent nitrogen, and total nitrogen accumulated in the above ground portion of the lentils receiving 1200 mg N pot^{-1} over those plants receiving 600 mg N pot^{-1} suggested that either the nitrogen nutritional requirement of lentils was satisfied with the addition of 600 mg N pot^{-1} or that the 1200 mg N pot^{-1} addition was toxic to plant growth.

The increase in the total nitrogen content of the lentils upon addition of nitrogen suggested that lentils did not receive enough soil and fixed nitrogen for maximum production. Although this was growth chamber data, it appeared to substantiate the assessment by Summerfield and Mauchbauer (1982) and Slinkard and Drew (1981) that lentils grown on soils low in nitrogen need supplemental nitrogen for maximum yields and protein content.

4.3.2 Accumulation and Percent Utilization of Fertilizer Nitrogen in the Aerial Portion of Fababeans, Soybeans, Lentils, and Barley as Affected by Rate of Nitrogen Addition

Accumulation of fertilizer nitrogen by fababeans, soybeans, and barley increased with increased addition of nitrogen, however, the percent utilization of fertilizer nitrogen remained constant (Table 7). Accumulation and percent utilization of fertilizer nitrogen in the aerial portions of barley corresponded closely to the accumulation and percent utilization of fertilizer nitrogen in the soybeans and fababeans and suggested that the uptake and distribution of fertilizer nitrogen was similar in the three species and that barley was a satisfactory control for the measurement of dinitrogen fixation by fababeans and soybeans. Wagner and Zapata (1982) found that barley was an adequate control for measurement of fixation by soybeans and fababeans.

The accumulation of fertilizer nitrogen in the above ground portion of lentils receiving 600 mg N pot^{-1} was similar to that of soybeans, fababeans and barley plants which received the same nitrogen treatment (Table 7). This suggested that the uptake and distribution of nitrogen in the roots and shoots of lentils at this level of nitrogen addition was similar to that of the barley, soybean and fababean plants. The relatively small accumulation and lower percent utilization of fertilizer nitrogen in the above ground portion of lentils which received 1200 mg N pot^{-1}, compared to the other species studied (Table 7), indicated that the lentils have a smaller nitrogen sink. Most of the nitrogen demand of lentils seemed to have been satisfied by the addition of 600 mg N pot^{-1}.

4.3.3 Effect of Nitrogen Treatment on the Residual Amounts of NO_3-N in the Soil after Harvesting Soybeans, Fababeans, Lentils and Barley

Nitrogen addition significantly increased the amount of nitrate nitrogen left in the soil after the termination of the experiment (Table 8). Only 1200 and 1800 mg N pot^{-1} treatments had significant amounts of nitrate nitrogen left in the soil after harvest of soybeans, fababeans and barley. Significant amounts of nitrate nitrogen, however, were found in the 600 mg N pot^{-1} treatment of lentils; further substantiating that lentils had a lesser requirement for nitrogen than soybeans, fababeans and barley. The difference in residual nitrogen remaining after harvest of lentil and barley plants which received 600 mg N pot^{-1}, even though the percent utilization of fertilizer by the aerial plant portions was similar, indicated that less accumulation of nitrogen occurred in the roots of lentils compared to barley and that barley may not have been an adequate control for measurement of fixation by lentils. The levels of residual NO_3-N in pots containing soybeans, fababeans and barley confirmed the uptake of fertilizer nitrogen by these species was similar.

Table 7. The amount of nitrogen derived from fertilizer and the present utilization of fertilizer in the aerial plant portions of fababeans, soybeans, lentils and barley.

Amount of Nitrogen Added (mg N/pot)	Amount of Nitrogen Derived from Fertilizer (mg N/pot)				Percent Utilization of Fertilizer			
	Fababeans	Soybeans	Lentils	Barley	Fababeans	Soybeans	Lentils	Barley
600	330*Aa	314 Aa	313 Aa	316 Aa	55.1 Aa	52.5 Aa	52.1 Aa	52.9 Aa
1200	719 Ba	622 Bab	391 Bc	722 Ba	59.9 Aa	51.8 Aab	32.6 Bc	60.0 Aa
1800	1021 Ca	865 Ca	396 Bb	979 Ca	56.7 Aa	48.1 Aa	22.0 Cb	54.4 Aa

* Duncan's multiple range test; means in a columns followed by the same capital letter are not significantly different at P = 0.05. Means in rows followed by the same small letter are not significantly different at P = 0.05.

Table 8. Amount of nitrate nitrogen left in the soil after harvest of the barley, soybeans, lentils and fababeans.

Amount of Nitrogen Added (mg/pot)	Residual Amount of Nitrate Nitrogen (mg N/Pot)			
	Soybean	Fababean	Lentil	Barley
0	8.8 A	8.8 A	21 A	8.6 A
600	12.0 A	11.4 A	256 B	13.7 A
1200	181 B	133 B	470 C	137 B
1800	372 C	226 C	800 D	417 C

The significant amount of residual nitrate nitrogen at the higher rates of nitrogen addition suggested that sink size limited nitrogen uptake in all species studied.

4.3.4 Effect of Nitrogen Addition on the "A" value Calculated for Barley, Soybeans, Fababeans and Lentils

The "A" value calculated for the barley, a measure of the available soil nitrogen in terms of a fertilizer standard, was constant irrespective of the amount of nitrogen applied (Table 9). The constant "A" value indicated that high rates of nitrogen addition did not change the amount or rate of soil nitrogen mineralization.

The "A" values calculated for soybeans and fababeans, a measure of available soil and fixed nitrogen in terms of a fertilizer standard, decreased significantly with each addition of nitrogen (Table 9). The "A" value of the lentils, however, was constant regardless of the amount of nitrogen added (Table 9).

Table 9. Effect of nitrogen addition on the "A" values calculated for barley, soybeans, fababeans and lentils.

Amount of Nitrogen Added (mg N/pot)	"A" Values (mg N pot^{-1})[1]			
	Barley	Soybeans	Fababeans	Lentils
600	336[2] A	1208 A	2211 A	460 A
1200	362 A	707 B	995 B	463 A
1800	366 A	485 C	618 C	471 A

1 In terms of a fertilizer standard: urea.
2 Duncan's Multiple Range Test: Means in columns followed by the same letter are not significantly different at P = 0.05.

4.3.5 The Effect of Nitrogen Addition on Symbiotic Nitrogen Fixation in Soybeans, Fababeans and Lentils as Measured by the "A" Value and ^{15}N Difference Methods

The two ^{15}N methods of measuring symbiotic nitrogen fixation (the ^{15}N assisted difference and "A" value) gave similar results in the measurement of the amount of nitrogen fixed by soybeans, fababeans and lentils (Table 10). At each level of nitrogen addition, fababeans fixed the greatest amount of nitrogen followed in turn by soybeans and lentils (Table 10).

Nitrogen addition decreased the amount of symbiotically fixed nitrogen found in the above ground portion of lentils, soybeans, and fababeans (Table 10) which indicated that combined nitrogen was used preferentially over fixed nitrogen. Such decreases have been well documented, however, there were still substantial quantities of symbiotically fixed nitrogen in the above ground portion of fababeans even with the addition of 1800 mg N pot^{-1}. In contrast, there appeared to be very little symbiotically fixed nitrogen in the above ground portion of soybeans at the 1800 mg N pot^{-1} level of nitrogen addition. Lentils fixed the least amount of dinitrogen; very little fixed nitrogen was in the above ground portion at the 600 mg N pot^{-1} level of nitrogen addition (Table 10).

The limited amount of nitrogen fixed by lentils and the significant increase in total nitrogen in the above ground portion with the addition of 600 mg N pot^{-1} suggested that lentils could not fix enough nitrogen for their nutritional requirements. Thus, lentils appeared to require supplemental nitrogen for maximum yields and protein content.

The values calculated for symbiotic nitrogen fixation by soybeans, lentils and fababeans were only for the amount of nitrogen fixed up to mid-pod fill and did not represent the total capacity of these plants to fix nitrogen.

4.3.6 Effect of Nitrogen Addition on the Nodulation of Soybeans, Fababeans and Lentils

The nodules of soybeans, fababeans and lentils appeared viable at harvest due to the leghaemoglobin observed when the nodules were dissected.

The number of nodules on the tap roots of lentils, fababeans and soybean declined with nitrogen addition (Table 11). Similar declines have been noted by Rabie (1981), Streeter (1981) and others. The decline in nodule numbers corresponded with the decline in fixation (Tables 10 and 11). It could not be discerned, however, if the decline in nodulation with nitrogen addition was due to the effect of the applied nitrogen on nodule maintenance or nodule initiation. Concepts

Table 10. Effect of nitrogen addition on symbiotic fixation of soybeans, fababeans and lentils as measured by the "A" value and ^{15}N assisted difference method.

Amount of Nitrogen Added (mg N/pot)	Amount of Nitrogen Fixed (mg N pot^{-1})†					
	Fababeans		Soybeans		Lentils	
	"A" value	^{15}N difference	"A" value	^{15}N difference	"A" value	^{15}N difference
0	-	1360 A	-	746 A	-	144 A
600	923 A*	1033 B	452 A	458 B	65 A	62 B
1200	379 B	377 C	175 B	145 C	36 B	†0 C
1800	141 C	149 D	58 C	35 D	25 B	†0 C

* Duncan's Multiple Range Test: Means in columns followed by the same letter are not significantly different P=0.05

† Negative values taken as zero.

explaining the decrease in nodular development with nitrogen addition can be found in the literature review.

The number of nodules on the tap roots of lentils was substantially less than that found on the tap roots of fababeans and soybeans (Table 11); this, suggested that the problem of fixation in lentils may have been due to nodulation.

Table 11. Nodulation on the tap roots of lentils, fababeans and soybeans.

Amount of Nitrogen Added (mg N/pot)	Nodule Numbers		
	Fababeans	Soybeans	Lentils
0	59.3 A[1]	52.7 A	11.3 A
600	49.7 AB	43.3 B	5.3 AB
1200	24.3 BC	24.0 C	3.3 B
1800	7.7 C	9.7 D	0.6 B

[1] Duncan's Multiple Range Test: Means followed by the same letter are not significantly different at $P = 0.05$.

Conclusion:

Among the three species studied, fababeans derived the greatest amount and proportion of nitrogen (88.6% by the classical difference method) by symbiotic fixation; soybeans were found to derive (81%) and lentils only (45%) of their nitrogen nutritional requirements from fixation. Fababeans and soybeans were found to be capable of deriving enough nitrogen from soil and symbiotic fixation for maximum yield and protein content. Lentils, however, seemed to need additional nitrogen for maximum yield and protein content. Also, lentils appeared to be more susceptible to the toxic effects of high rates of urea addition than fababeans or soybeans.

Nitrogen addition was found to decrease symbiotic fixation and nodular development of fababeans, soybeans and lentils. The decline in fixation was in proportion to nitrogen addition and indicated that combined nitrogen was utilized preferentially over symbiotically fixed.

The ^{15}N assisted difference method and the "A" value method appeared equally adequate for measurement of symbiotic nitrogen fixation.

CHAPTER V

LYSIMETER EXPERIMENT: ST. CLAUDE

5.1 Introduction

The results of the first growth chamber experiment indicated that fababeans fixed the greatest amount of nitrogen followed in turn by soybeans and lentils. Fababeans and soybeans appeared capable of fixing enough nitrogen for maximum production while lentils appeared to need additional nitrogen.

A lysimeter study was initiated to: 1) determine under field conditions if the addition of fertilizer nitrogen increased the yield of soybeans, fababeans and lentils, 2) evaluate two ^{15}N methods for determining the amount of dinitrogen fixed by a crop, 3) determine the amount of dinitrogen fixed by the three annual legume crops, and the effect of fertilizer nitrogen addition on dinitrogen fixation, and 4) determine the effect of high and low percent nitrogen straw residues on the "A" value method of determining dinitrogen fixation.

5.2 Materials and Methods

In the spring of 1982, a lysimeter experiment was initiated in the Haywood-St. Claude region of the Manitoba Lowlands in South Central Manitoba (NE1/4-25-8-6W).

5.2.1 Soil

The site was located on a Gleyed Rego Black Chernozem (carbonated phase) of the St. Claude series in the Almasippi association (Michalyna, 1982). The site was designated St. Claude due to its location on the St. Claude soil series.

At seeding, soil samples were taken from the four plot corners from the 0-15 cm, 15-30 cm, 30-60 cm, 60-90 cm, and 90-120 cm depths. The samples were placed in plastic bags to which 2-3 drops of toluene had been added to inhibit microbial mineralization of nitrogen. The soil was air dried at 30C for 48 hours, ground to pass through a 2 mm sieve and analyzed.

At the termination of the experiment, soil samples were obtained from each lysimeter from the 0-15 cm, 15-30 cm, 30-60 cm, 60-90 cm, and 90-120 cm depths. These samples were handled in a similar manner to samples obtained in the spring except that some samples had to be frozen until drying space was available. Prior to grinding, unground subsamples were taken from the soil obtained from one lysimeter for textural analysis. Bulk density samples were obtained in the fall from each depth for conversion of ppm NO_3-N to kg NO_3-N ha^{-1}.

5.2.2 Experimental Design and Procedure

The lysimeters, round open-ended steel cylinders with a cross sectional area of 0.1 m^2 and length of 0.305 m, were arranged into four subplots and pushed into the ground with a front end loader. Separation of the three test crops (soybeans, lentils and fababeans) and the reference crop (barley) was done to ensure equal competition for light and water (Figure 1).

The top ten centimeters of soil was removed from each lysimeter and placed on a plastic sheet. Basal applications of 100 kg P ha^{-1} as KH_2PO_4, 200 kg K ha^{-1} as KCl and KH_2PO_4, 20 kg Zn ha^{-1} as $ZnSO_4$-$7H_2O$, 15 kg Cu ha^{-1} as $CuSO_4$-$5H_2O$ and 7.4 kg S ha^{-1} as $ZnSO_4$-$7H_2O$ and

$CuSO_4 \cdot 5H_2O$, were spread and mixed. Appropriate nitrogen treatments were also mixed with the soil at this time and the soil replaced.

A 3 x 3 completely randomized factorial design containing three rates of nitrogen (0, 30 and 100 kg N ha^{-1} as 5.65 atom % ^{15}N excess urea) and three straw amendments (no straw, 5000 kg ha^{-1} of 0.5% N barley straw cut to 2.5 to 5.0 cm lengths and 5000 kg ha^{-1} of 3.0% N alfalfa straw cut to 2.5 to 5.0 cm lengths) was used for the barley and soybeans. Treatments of nitrogen alone were replicated six times while those of straw alone or straw in combination with nitrogen were replicated four times.

A completely randomized design consisting of three rates of nitrogen (0, 30, and 100 kg N ha^{-1} as 5.65 atom % ^{15}N excess urea) replicated eight times was employed for fababeans and lentils.

Imbibition of soybean, lentil and fababean seeds occurred for 24 hours prior to seeding. Wet paper towels were placed in the bottom of three large flat pans; an approximately equal volume of seed and rhizobium of the appropriate species were added, mixed and covered with three layers of wet paper towels.

The rhizobium used were Nitragin Corporation[1] S, Q and C cultures for soybeans, fababeans and lentils, respectively.

The lysimeters were seeded on June 1st. The seeds were placed into holes (made with a wooden dowel and approximately 4 cm deep) and covered with soil. Fababean, soybean, lentil and barley lysimeters received 6, 10, 15 and 25 seeds per lysimeter, respectively.

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[1] Nitragin Corporation, 3101 W. Custer Avenue, Milwaukee, WI 53209

The area outside each subplot was seeded with the appropriate species to ensure normal competition for light and moisture.

After emergence, the lysimeters were thinned to 4, 8, 13 and 25 plants per lysimeter corresponding to planting rates of 170 kg ha^{-1} for fababeans, 110 kg ha^{-1} for soybeans, 65 kg ha^{-1} for lentils and 100 kg ha^{-1} for barley, respectively.

Four replicates of lentils were harvested on August 5th while the remaining four were harvested on August 23rd. Benilate, at a rate of 15 ml 4 L^{-1} lysimeter, was applied to the lentils immediately after the first harvest to combat fusarium root rot. Benilate was also applied on August 12th and 19th. The soybean and barley lysimeters were harvested on August 27th while the fababeans were harvested on September 14th.

The soybean, fababean, and barley samples were air dried for 48 hours at 30C after which they were threshed. The seed and straw portions were placed into separate bags, oven dried at 65C and weighed. After being weighed, the seed and straw were mixed together (to give a more accurate ^{15}N analysis) and ground in a Wiley mill to pass through a 2 mm sieve. The lentil samples were air dried at 30C for 48 hours, oven dried at 65C for 48 hours, weighed and ground in a Wiley mill to pass through a 2 mm sieve. Total nitrogen (Kjeldahl N) and percent ^{15}N analysis was performed on all samples.

Due to the infestation of fusarium root rot in the lentils, which biased nitrogen uptake, dry matter accumulation and nitrogen fixation, the lentil data will not be presented.

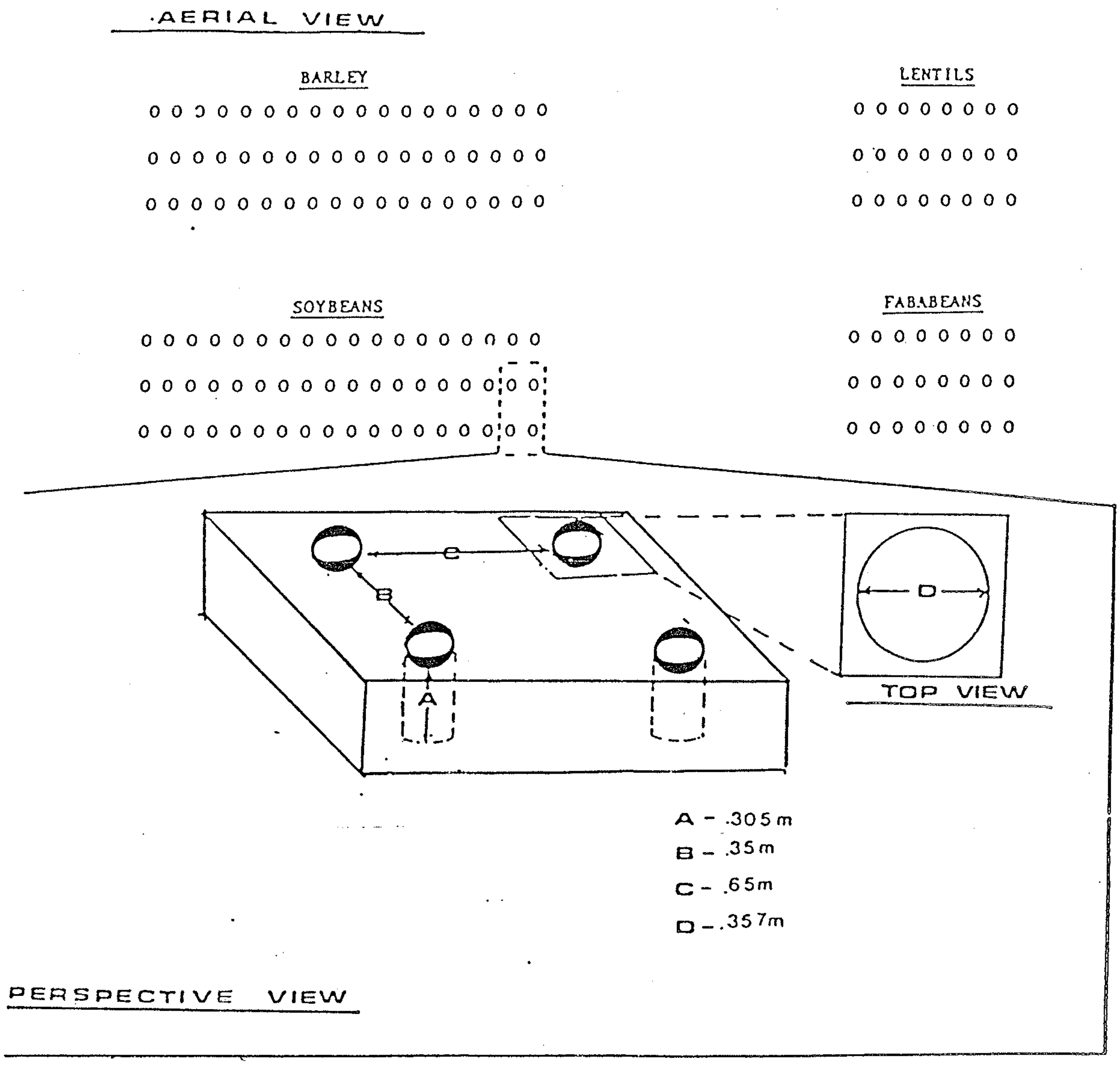


Fig. 1 Plot Diagram of Barley, Soybeans, Fababeans and Lentils in the 1982 Lysimeter Study.

5.3 Results and Discussion

5.3.1 Soil

The experiment was conducted on a loamy fine sand, the physical and chemical characteristics of which are outlined in Table 12.

Table 12. Soil Characteristics: lysimeter experiment

Soil Association = Almasippi	
Soil Series = St. Claude	
Soil type = Gleyed Rego Black Chernozem (carbonated)	
Texture = Loamy Fine Sand	
NO_3-N(kg N ha^{-1})$_{0-60cm}$	38
NO_3-N(kg N ha^{-1})$_{60-120cm}$	3
Available P(kg P ha^{-1})$_{0-15cm}$	8
Available K (kg K ha^{-1})$_{0-15cm}$	42
Extractable SO_4-S(kg S ha^{-1})$_{0-60cm}$	140
Percent Organic Matter $_{0-15\ cm}$	9
% Ca CO_3 Equivalent	30
pH	7.9
Percent Field Capacity (by weight)	21

5.3.2 Effect of Nitrogen and Straw Addition on the Dry Matter Yield, Seed Yield and Total Amount of Nitrogen Accumulated in the Above Ground Portion of Barley

The barley seed and dry matter yields were very high (Tables 13 and 14) due to excellent growing conditions.

Fertilizer nitrogen, without organic matter amendments, increased the seed yield, dry matter yield and total amount of nitrogen accumulated in the aerial portion of barley; however, the increased were not significant (Tables 13 and 14).

The amount of soil nitrogen available to the plants was greater than expected. Using the equation developed by Soper et al. (1971) based on the NO_3-N content of the soil, it was predicted that in the spring approximately 76 kg N ha^{-1} would be available to the plants. However, the above ground portion of barley plants which had not received any fertilizer nitrogen or organic matter amendments contained 109 kg N ha^{-1}; hence, mineralization was high. High mineralization of organic nitrogen was probably due to:

1) the site being recently broken; and
2) the high organic matter content in relation to soil texture (Table 12); and
3) the abundant water supply (the water table was within 1 m throughout the growing season).

Campbell and Souster (1982) found that the potentially mineralizable N in virgin soil was on the average 2.5 times greater than in cultivated soils. They also noted that losses of organic matter, potentially mineralizable N and active N fraction were greater in the coarse textured soils due to better aeration for decomposition. Myers et al. (1982) found that decomposition and mineralization of organic nitrogen was linearly related to moisture content in the available range.

The addition of barley and alfalfa residues as organic matter amendments did not significantly affect the seed yield, dry matter yield, or total nitrogen content within any rate of nitrogen fertilization (Tables 13, 14 and 15).

Table 13. Seed yield of Bonanza Barley[1] (kg ha^{-1})

Nitrogen Amendment (kg N ha^{-1})	Organic Matter Amendments		
	No Residue	Barley Residue	Alfalfa Residue
0	3975 B[2]	5294 AB	4337 B
30	5295 AB	5652 AB	4572 B
100	5514 AB	5598 AB	7189 A

1 Results expressed on an oven dried basis.
2 Duncan's Multiple Range Test: Means followed by the same letter are not significantly different at P = 0.05.

Table 14. Dry matter yield of Bonanza Barley[1] (kg ha^{-1})

Nitrogen Amendment (kg N ha^{-1})	Organic Matter Amendments		
	No Residue	Barley Residue	Alfalfa Residue
0	8113 B[2]	10624 AB	8753 B
30	10595 AB	11625 AB	9371 B
100	11611 AB	11773 AB	14327 A

1 Results expressed on an oven dried basis.
2 Duncan's Multiple Range Test: Means followed by the same letter are not significantly different at P = 0.05.

Table 15. Total amount of nitrogen accumulated in the aerial portions of Bonanza Barley[1] (kg N ha^{-1}).

Nitrogen Amendment (kg N ha^{-1})	Organic Matter Amendments		
	No Residue	Barley Residue	Alfalfa Residue
0	109 B[2]	137 AB	129 AB
30	136 AB	150 AB	116 B
100	159 AB	158 AB	202 A

[1] Results expressed on an oven dried basis.
[2] Duncan's Multiple Range Test: Means followed by the same letter are not significantly different at P = 0.05.

Thus, there was no evidence of net immobilization of any available nitrogen by microbes in decomposition of the barley residue. This lack of evidence was surprising since Tomar and Soper (1981) working on a Hochfield loamy sand in Manitoba found net immobilization of 18 and 30 kg N ha^{-1} when 5000 kg ha^{-1} of ground oat straw (0.45 % N) was mixed in the top 10 cm of soil which had received 0 and 100 kg N ha^{-1}, respectively; seed yields were decreased by 569 and 1017 kg ha^{-1}, respectively. Similar reductions in seed yields were expected in this experiment. Decomposition of the alfalfa straw was expected to give net mineralization of approximately 80 kg N ha^{-1} (based on a value of 1.4% N for the microflora (Alexander, 1977)) and result in a seed yield increase of approximately 1600 kg N ha^{-1} (Tomar and Soper, 1981). Such an increase in seed yield, dry matter yield and nitrogen content was observed only when alfalfa straw was applied in conjunction with the 100 kg N ha^{-1} fertilizer addition. At the other two levels of nitrogen addition, the seed yield, dry matter and nitrogen content of barley plants in lysimeters amended with alfalfa straw were lower than those found for plants in lysimeters amended with barley straw. The lack of evidence of net immobilization or mineralization of nitrogen with decomposition of barley and alfalfa straw suggested that either soil variability masked the net result of these processes and/or biological interchange between the pools of organic soil N and inorganic N occurred without any net effect. Bartholomew and Hilbold (1952) in greenhouse pot experiments found interchange in untreated soils, the magnitude of which was increased by the addition of alfalfa and corn residues. Decomposition of the alfalfa residue did not result in increased yield or nitrogen content when compared to the controls. Similar results were found in this experiment. Walunjkar et al. (1959) working with two North Carolina soils, indicated that N interchange was positively correlated with the organic matter content. The high organic matter content of the soil used in this study, approximately double that of Tomar & Soper (1981), may have favoured interchange to a greater extent than net immobilization or mineralization. However, since the yield response to addition of up to 100 kg N ha^{-1} (without organic matter amendments) was not significant at $P = 0.05$, it was concluded that soil variability was high.

5.3.3 The Effect of Nitrogen and Straw Addition on the Seed Yields, Dry Matter Yield, and Total Nitrogen Content of Maple Amber Soybeans

The high dry matter yield and total amount of nitrogen accumulated in the aerial portion of Maple Amber soybeans was due to the excellent growing conditions (Tables 16 and 17). The seed yield, however, was low due to an August 26th frost which terminated pod formation and filling (Table 18).

There was no significant effect of fertilizer nitrogen and/or organic matter addition on the seed yield, dry matter yield, or total amount of nitrogen accumulated in the above ground portion of the soybeans (Tables 16, 17, and 18). The lack of significant response of Maple Amber soybeans to any of the fertilizer nitrogen treatments and/or organic matter amendments indicated that soil and fixed nitrogen were sufficient for maximum production under the environmental conditions of this experiment.

Table 16. Seed yield of Maple Amber Soybeans[1] (kg ha $^{-1}$).

Nitrogen Amendment (kg N ha^{-1})	Organic Matter Amendments		
	No Residue	Barley Residue	Alfalfa Residue
0	1171 A[2]	974 A	1308 A
30	1035 A	1026 A	1098 A
100	1074 A	949 A	1094 A

1 Results expressed on an oven dried basis.
2 Duncan's Multiple Range Test: Means followed by the same letter are not significantly different at P = 0.05.

Table 17. Dry matter yield of Maple Amber Soybeans[1] (kg ha^{-1}).

Nitrogen Amendment (kg N ha^{-1})	Organic Matter Amendments		
	No Residue	Barley Residue	Alfalfa Residue
0	7236 A[2]	6963 A	8345 A
30	6725 A	7231 A	7468 A
100	6845 A	7321 A	7022 A

1 Results expressed on an oven dried basis.
2 Duncan's Multiple Range Test: Means followed by the same letter are not significantly different at $P = 0.05$.

Table 18. Total amount of nitrogen accumulated in the above ground portion of Maple Amber Soybeans (kg ha^{-1}).

Nitrogen Amendment (kg N ha^{-1})	Organic Matter Amendments		
	No Residue	Barley Residue	Alfalfa Residue
0	188 A[2]	168 A	183 A
30	179 A	182 A	203 A
100	182 A	175 A	209 A

1 Results expressed on an oven dried basis.
2 Duncan's Multiple Range Test: Means followed by the same letter are not significantly different at $P = 0.05$.

5.3.4 The Effect of Nitrogen Addition on the Dry Matter Yield, Seed Yield and Total Amount of Nitrogen Accumulated in the Above Ground Portion of Aladin Fababeans

The seed yields, dry matter yield and nitrogen accumulation of Aladin fababeans were exceptional at this site (the seed yield was approximately double the provincial average) (Table 19). The exceptional growing conditions for fababeans in 1982 probably accounted for most of the yield. The fababeans though killed by a September 13th frost were not damaged by the August 26th frost; this indicated that fababeans were more frost tolerant than soybeans.

There was no significant effect of nitrogen addition on the seed yield, dry matter yield or total nitrogen content of the aerial plant portions of Aladin fababeans (Table 19). Thus, soil and symbiotically fixed nitrogen were sufficient for maximum production under the conditions of this experiment.

Table 19. Seed yield, dry matter yield and total nitrogen accumulation in the above ground portion of Aladin Fababeans.

Nitrogen Amendment (kg N ha^{-1})	Seed Yield (kg ha^{-1})	Dry Matter Yield[1] (kg ha^{-1})	Total Nitrogen Accumulated (kg N ha^{-1})
0	4756 A[2]	13876 A	358 A
30	4616 A	14726 A	352 A
100	5712 A	15859 A	388 A

[1] Results expressed on an oven dried basis.
[2] Duncan's Multiple Range Test: Means followed by the same letter are not significantly different at P = 0.05.

5.3.5 Percent Utilization of Fertilizer Nitrogen by Barley, Soybeans and Fababeans

The percent utilization of fertilizer nitrogen by barley was not affected by rate of nitrogen addition except when amended with alfalfa straw (Table 20). Utilization of fertilizer nitrogen by soybeans was not affected by rate of fertilizer nitrogen addition, however, fertilizer utilization by fababeans increased with increased nitrogen addition (Tables 21 and 22). Regitnig (1983) in field experiments with Maple Presto soybeans found a constant percent utilization of fertilizer nitrogen regardless of rate of fertilizer nitrogen addition. Diebert et al. (1979) and Rennie et al. (1978) reported that the percent utilization of fertilizer nitrogen by nodulating and non-nodulating soybean isolines increased or remained constant with increased nitrogen addition. Diebert et al. (1976) postulated that increased utilization of fertilizer nitrogen was the result of the soybeans higher demand for nitrogen later in the growing season than that of cereals (of which the percent utilization of fertilizer nitrogen tended to decrease with increased N addition). This may also have been the case for fababeans.

Table 20. Effect of nitrogen and straw addition on the percent utilization of fertilizer by barley.

Nitrogen Amendment (kg N ha^{-1})	Organic Matter Amendments		
	No Residue	Barley Residue	Alfalfa Residue
30	39 A[1]	27.7 AB	20.3 B
100	37 A	28.1 AB	35.7 A

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

Table 21. Effect of nitrogen and straw addition on the percent utilization of fertilizer by soybeans.

Nitrogen Amendment ($kg\ N\ ha^{-1}$)	Organic Matter Amendments		
	No Residue	Barley Residue	Alfalfa Residue
30	34 A[1]B	22.1 B	33.1 AB
100	43 A	33.4 AB	33.8 AB

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

Table 22. Effect of rate of fertilizer addition on the percent utilization of fertilizer in the above ground portion of fababeans.

Nitrogen Amendment ($kg\ N\ ha^{-1}$)	Percent Utilization of fertilizer in the above ground portion
30	14.3 A[1]
100	30.1 B

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

The addition of barley straw lowered the percent utilization of fertilizer nitrogen by the aerial portion of barley and soybean plants, however, the effect was not significant (Tables 20 and 21). Since there was no indication of net immobilization of nitrogen by the decomposition of the barley straw (Tables 13, 14, and 15), the apparent decrease in the percent utilization of fertilizer nitrogen was thought to have been due to interchange of fertilizer nitrogen with straw soil nitrogen. Bartholomew and Hilbolt (1952) found decreased percent utilization of fertilizer ^{15}N when appreciable biological interchange of nitrogen occurred.

The decomposition of the alfalfa straw did not significantly affect the percent utilization of fertilizer by barley plants which received 100 kg N ha^{-1}. However, percent fertilizer utilization was significantly lowered by the decomposition of alfalfa residue at the 30 kg N ha^{-1} level of fertilizer nitrogen addition (Table 20).

The effect of the alfalfa straw on the utilization of fertilizer by the above ground portion of barley appeared to be related to the total amount of nitrogen accumulated in the plants. Barley plants which had substantial, but not necessarily significantly higher, total nitrogen accumulation with alfalfa straw addition, had percent utilizations of fertilizer similar to that of the control plants (Tables 15 and 20). However, when the total amount of nitrogen in the above ground portion was similar between plants grown on soils amended and not amended with the alfalfa straw, the percent utilization of fertilizer was lower for plants on soils amended with the alfalfa residue (Tables 15 and 20). Thus, mineralization of nitrogen and biological interchange of nitrogen (without net mineralization) may have been responsible for the similar and decreased utilization of fertilizer nitrogen, respectively, with addition of alfalfa residue to the soil.

The effect of alfalfa straw on fertilizer utilization by the above ground portion of soybeans was variable (Table 21). At 30 kg N ha^{-1} the alfalfa organic residue did not lower the percent utilization of fertilizer in the aerial portion of soybeans, however, at 100 kg N ha^{-1}

addition of alfalfa residues lowered fertilizer utilization but not significantly.

Generally, there appeared to be no significant difference in fertilizer utilization by the above ground portion of barley and soybeans; the exception occurred with 30 kg N ha^{-1} alfalfa straw treatment (Table 23). The pattern of fertilizer utilization by barley and soybeans suggested that both crops had similar fertilizer distributions and the volume of soil explored by both crops was either similar or if the soil volume explored by the two species was different, the amount of soil nitrogen available to the plant in the additional volume was insignificant. Whichever was the case, barley appeared to be an adequate control for the measurement of fixation by soybeans which confirmed the results of the first growth chamber experiment, and the results of Regitnig (1983), and Wagner and Zapata (1982).

Barley and fababean plants which had received 100 kg N ha^{-1} had a similar utilization of fertilizer nitrogen in the above-ground portion (Table 23). However, fababean plants receiving 30 kg N ha^{-1} had a significantly lower accumulation of fertilizer nitrogen in the aerial portion than barley (Table 23) which indicated that, at this level of fertilizer addition, different patterns of fertilizer utilization may have occurred.

Table 23. Comparison of the percent utilization of fertilizer by barley with that of soybeans and fababeans.

Nitrogen Amendment (kg N ha^{-1})	Organic Matter Amendment	Percent Utilization of fertilizer by Barley	Soybeans	Fababeans
30 kg N	Barley	27.7 A[1]	22.1 A	--
30 kg N	--	39.0 A	34.2 A	14.3 B
30 kg N	Alfalfa	20.3 A	33.1 B	--
100 kg N	Barley	28.1 A	33.4 A	--
100 kg N	--	37.9 AB	43.7 A	30.1 B
100 kg N	Alfalfa	35.7 A	33.8 A	--

[1] Duncan's Multiple Range Test: Means in rows followed by the same letter are not significantly different at $p = 0.05$.

5.3.6 <u>The Effect of Nitrogen and Straw Addition on the "A" Value Calculated for Barley</u>

The "A" values, a measure of the available soil N using barley plants grown on soils which had not received organic matter amendments, were constant at the two rates of nitrogen addition (Table 24). This appeared to substantiate the premise of Fried and Dean (1952); that the "A" value was rate independent.

The addition of organic matter residues tended to increase the "A" value of the soil (as measured by barley), however, the effect was significant only at the 30 kg N ha $^{-1}$, rate of fertilizer addition (Table 24). The lack of evidence that net mineralization or immobilization occurred with addition of organic matter residues to the soil, at the 30 Kg N ha^{-1} rate of nitrogen addition suggested that biological interchange of N was responsible for the rise in the "A" value. Also, if only immobilization of nitrogen occurred, there probably would have been no rise in the "A" value. Immobilization of fertilizer and soil nitrogen would have been in proportion to their respective availabilities and, hence, the "A" value would have remained constant.

Table 24. The effect of nitrogen and organic matter addition on the "A" value[1] calculated for barley.

Nitrogen Amendment (kg N ha^{-1})	Organic Matter Amendment		
	No Residue	Barley Residue	Alfalfa Residue
30	324 B[2]	523 A	558 A
100	311 B	469 AB	467 AB

[1] Kilograms soil nitrogen per hectare in terms of a fertilizer standard: urea.
[2] Duncan's Multiple Range Test: Means followed by the same letter are not significantly different at P = 0.05.

Mineralization or interchange may account for the nonsignificant rise in the "A" value of plants which had received the 100 kg N ha^{-1} - alfalfa straw treatment. The large, though non-significant, rise in the total amount of nitrogen accumulated in the above ground portion of barley suggested that net mineralization of nitrogen from the decomposition of the alfalfa organic matter amendment occurred (Table 15); however, biological interchange of N appeared to have been the dominant process with every other treatment.

5.3.7 Effect of Nitrogen and Straw Residue Addition on the "A" Value Calculated for Soybeans

The "A" value calculated for soybeans decreased with increased fertilizer N addition, however, the effect was only significant with the barley residue amendment (Table 25). Organic matter addition, whether barley straw or alfalfa straw, increased the "A" value calculated for soybeans; however, the effects were significant with only the 30 kg N ha^{-1} barley straw and 100 kg N ha^{-1} alfalfa straw treatments, respectively (Table 25).

Table 25. Effect of nitrogen and straw addition on the "A" value[1] calculated for soybeans.

Nitrogen Amendment (kg N ha^{-1})	Organic Matter Amendment		
	No Residue	Barley Straw	Alfalfa Straw
30	465 BC[2]	849 A	580 B
100	305 C	434 BC	511 B

[1] Kilograms of soil and fixed nitrogen per hectare in terms of a fertilizer standard: urea.
[2] Duncan's Multiple Range Test: Means followed by the same letter are not significantly different at P = 0.05.

5.3.8 Effect of Nitrogen Addition on the "A" Value Calculated for Fababeans

The addition of 100 kg N ha^{-1} significantly reduced the "A" value calculated for fababeans when compared to the 30 kg N treatment (Table 26).

Table 26. Effect of Nitrogen Addition on the "A" value[1] calculated for fababeans.

Nitrogen Amendment ($kg\ N\ ha^{-1}$)	"A" value
30	2165 A[2]
100	1476 B

[1] Kilograms soil and fixed nitrogen per hectare in terms of a fertilizer standard: urea

[2] Duncan's Multiple Range Test: Means followed by the same letter are not significantly different at P = 0.05.

5.3.9 Nitrogen Fixation in Soybeans and Fababeans

Only comparable treatments were used in determination of the amount of nitrogen fixed by soybeans (i.e., any barley grown in lysimeters amended with 30 kg N ha^{-1} plus barley straw were used as controls for measurement of fixation by soybeans grown in lysimeters amended with 30 kg N ha^{-1} plus barley straw) since the "A" value changed with straw amendments.

Increased fertilizer nitrogen addition decreased the amount of nitrogen fixed by soybeans as determined by the "A" value method (Table 27). At the 30 kg N ha^{-1} level of nitrogen addition, the addition of barley straw as an organic matter residue appeared to increase the amount of nitrogen fixed while alfalfa straw appeared to decrease fixation (Table 27). However, there was no other significant evidence of net mineralization or immobilization of nitrogen with the alfalfa and barley residues, respectively (Table 13) due to the high variability associated with this site. This suggested that the "A" value technique may have been more sensitive than total dry matter production or total nitrogen in determination of whether immobilization or mineralization had occurred.

The alfalfa and barley residues did not appear to affect fixation of soybeans receiving 100 kg N ha^{-1} (Table 27). At this rate of nitrogen addition there did not appear to be appreciable fixation.

Table 27. Effect of nitrogen and straw addition on the amount of dinitrogen fixed by soybeans as measured by the "A" value method (kg N fixed ha^{-1}).

Nitrogen Amendment (kg N ha^{-1})	Organic Matter Amendment		
	No Residue	Barley Straw	Alfalfa Straw
30	39 AB[1]	72 A	8 BC*
100	3 C*	11 B*C	18 B*C

[1] Duncan's Multiple Range Test: Means followed by the same letter are not significantly different at P = 0.05.
* Minus values were included in the average.

The ^{15}N assisted difference method also showed a decrease in fixation with addition of 100 kg N ha^{-1} (Table 28). However, the addition of the alfalfa and barley residues did not appear to affect fixation (Table 28).

Table 28. Effect of nitrogen and straw addition on the amount of dinitrogen fixed, by soybeans as measured by the ^{15}N assisted difference method (kg N fixed ha^{-1}).

Nitrogen Amendment (kg N ha^{-1})	Organic Matter Amendment		
	No Residue	Barley Straw	Alfalfa Straw
0	79 A[1]	32 AB	54 AB
30	33 AB	35 AB	49 AB
100	15 B	12 B*	10 B*

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

The only mathematical difference between the "A" value and the ^{15}N assisted difference method was the utilization of yield dependent criteria (total nitrogen content) by the non-fixing crop in the latter method. Plot variability in the total amount of nitrogen accumulated by the non-fixing control affected the determination of the amount of nitrogen fixed by the legumes measured by the ^{15}N assisted difference method. This was not a problem with the "A" value technique since the only yield dependent criteria utilized was the percent utilization of fertilizer by the legume. The "A" value, an isotopic dilution technique, based on the concept that a plot confronted with two or more sources of a nutrient would take up from each source in proportion to their respective availabilities. The "A" value was thought to be superior to the ^{15}N assisted difference method for measuring fixation if biological interchange of N occurred. Theoretically, interchange between soil and fertilizer nitrogen pools would result in uniform ^{15}N labelling of the active soil organic and inorganic nitrogen pools;

hence, soil derived ^{15}N would be diluted only by atmospheric derived dinitrogen. Talbott et al. (1982) stated that this was desirable since soil and symbiotically fixed nitrogen cannot be distinguished on an isotope basis. Based on the results of this experiment the "A" value method appeared to be better than the ^{15}N assisted difference method because of problems associated with the yield dependent criteria of the control crop.

The fababeans fixed substantially more nitrogen than the soybeans (Tables 28 and 29). There was no significant effect of nitrogen addition on the amount of nitrogen fixed in the aerial portion of fababeans, as measured by the ^{15}N assisted difference method and "A" value method (Table 29). Other researchers (Richards and Soper, 1979; Hill-Cottingham and Lloyd-Jones; 1980, Dean and Clark 1975) and the results of the first growth chamber experiment have shown decreased fixation with nitrogen addition. The lack of effect of fertilizer nitrogen addition on the amount of dinitrogen fixed in the aerial portion of fababeans may have been due to accumulation of the fertilizer nitrogen in the root system; indicated by the lower percent utilization of fertilizer by fababeans receiving 30 kg N ha^{-1} than soybeans or barley. However, if the reduction in fixed nitrogen were 1:1 with the amount of fertilizer taken up (Allos and Bartholomew, 1955) coupled with 50% recovery of fertilizer nitrogen, then fixation would have been reduced by 35 kg N ha^{-1}. Fixation (as measured by the "A" value technique) was reduced by 37 kg N ha^{-1} but the reduction was not significant (Table 30), due probably to the relatively small addition of fertilizer nitrogen compared to the total accumulation of nitrogen.

Table 29. Effect of nitrogen addition on the amount of dinitrogen fixed in the aerial portion of fababeans as measured by the ^{15}N assisted difference and "A" value methods.

Nitrogen Amendment (kg N ha^{-1})	Amount of Nitrogen Fixed (kg N ha^{-1})	
	^{15}N assisted difference	"A" Value
0	250 A[1]	--
30	224 A	302 A
100	239 A	265 A

[1] Duncan's Multiple Range Test: Means in columns followed by the same letter are not significantly different at $P = 0.05$.

5.3.10 Conclusions

Soil and fixed nitrogen were sufficient for maximum dry matter yield, seed yield, and nitrogen content of soybeans, and fababean plants under the conditions of this experiment. Fababeans fixed substantially more dinitrogen than soybeans. Approximately three times the amount of fixed dinitrogen was found in the aerial portion of fababeans compared to soybeans (as measured by the classical difference method). Nitrogen addition of 100 kg N ha^{-1} decreased nitrogen fixation in soybeans but had no significant effect on fababeans (as measured by the "A" value and ^{15}N assisted difference methods). The relatively small addition of fertilizer nitrogen compared to the total nitrogen accumulation may have accounted for the lack of effect of nitrogen addition on the amount of fixed nitrogen in the aerial portion of fababeans. The "A" value method appeared to be more sensitive than the ^{15}N assisted difference method particularly when organic residues had been added.

Using the "A" value method, the addition of low nitrogen residue (barley straw) appeared to increase fixation while the addition of high N residue (alfalfa straw) appeared to decrease fixation at the 30 kg N ha^{-1} level of N addition.

CHAPTER VI

SECOND GROWTH CHAMBER EXPERIMENT

6.1 Introduction

Results of the first growth chamber experiment indicated that lentils may not fix enough nitrogen for maximum yield and protein content. Lentils also seemed to be more susceptible to the toxic effects of high rates of nitrogen (as urea) than soybeans or fababeans.

Maple Presto an indeterminate early maturing cultivar of soybeans was found to need additional nitrogen for maximum yield and protein content (Regitnig, 1983). Maple Amber soybeans also indeterminate in growth appeared (from the results of the first growth chamber experiment) capable of obtaining enough nitrogen for maximum production through the fixation of atmospheric dinitrogen even on soils low in available nitrogen.

Results of the first growth chamber and lysimeter experiments as well as those of other researchers (Richards and Soper (1979), Dean and Clark (1980)) have shown that additional fertilizer nitrogen does not increase yield or protein content of fababeans.

The purpose of the second growth chamber experiment was to: 1) further substantiate that Maple Amber soybeans can fix enough nitrogen for their nutritional requirements, 2) further substantiate that lentils need additional fertilizer inputs for maximum yields, 3) determine the partitioning of fertilizer nitrogen between the roots and shoots of fababeans, soybeans, lentils, barley and non-nodulating soybeans, and 4) determine the effect of inclusion of the roots in the measurement of fixation by soybeans, fababeans and lentils.

6.2 MATERIALS AND METHOD

6.2.1 Soil

A Rego Black Chernozem of the Almasippi series in the Almasippi association (Michalyna, 1982) located near Haywood, Manitoba was selected for use because of its low NO_3- N content. The soil was obtained in the fall of 1982 from the 0-15 cm depth and stored in an unheated shed. A week prior to the initiation of the experiment, the soil was air dried at 30C, mixed, passed through a 2 mm sieve, and subsampled for analysis.

6.2.2 Experimental Design and Procedure

A completely randomized experiment, containing 19 treatments and 3 replicates was undertaken. The treatments used in this experiment are listed in Table 30.

The amounts of fertilizer applied and the method of fertilizer application were identical to that of the first growth chamber experiment except that 1.896 atom % ^{15}N excess urea was used. Inoculation and imbibition of the seeds were as described for the first growth chamber experiment. However, inoculum was not added to the non-nodulating Clay isoline soybeans.

The method of planting and the number of seeds planted were as described for the first growth chamber experiment. However, in this experiment, planting occurred 24 hours after fertilizer addition.

The procedure used in the maintenance of the pots was as described in the first growth chamber experiment.

The aerial portions of the plants were harvested at early podfill, i.e., 65 days after seeding. The samples were air dried for 48 hours at 30C, and then stored in a cold shed. After two weeks, the plant samples were taken out of the cold shed, air dried for 24 hours at 30C oven dried for 48 hours at 60C weighed and ground in a Wiley Mill to pass through a 2 mm sieve. Nitrogen and ^{14}N-^{15}N ratio analyses were performed. Subsequent to the harvest of the above ground portions the plastic inner liners containing soil and roots were taken out of the pots and placed in the cold shed. After two weeks, the soil and roots were removed from the plastic liner, placed on a 2 mm sieve and the soil washed off the roots with tap water. After washing, the root material was dried, weighed, ground to pass through a 2 mm sieve and analyzed in a manner similar to that for the aerial portions. All results are reported on an oven-dried basis.

Growth chamber conditions during the experiment were: temperature 20C day/17C night, daylength 14 hours 8 a.m. to 10 p.m., Humidity 75%, Light intensity 650 microeinsteins per m^2 with 15 percent incandescent light.

Table 30. Treatments used in growth chamber experiment 2: February 1983.

Nitrogen Addition (mg N pot $^{-1}$)	Type of Plant
0	Soybean (nodulating)
600	Soybean (nodulating)
1200	Soybean (nodulating)
1800	Soybean (nodulating)
0	Fababean
600	Fababean
1200	Fababean
1800	Fababean
0	Lentil
600	Lentil
1200	Lentil
1800	Lentil
0	Barley
600	Barley
1200	Barley
1800	Barley
0	Soybean (non-nodulating)
600	Soybean (non-nodulating)
1800	Soybean (non-nodulating)

6.3 Results and Discussion

The physical and chemical characteristics of the soil used in this growth chamber experiment are presented in Table 31.

Table 31. Soil characteristics growth chamber experiment 2: February 1983.

Soil Series	- Almasippi
Soil Type	- Gleyed Rego Black Chernozem
Texture	- Loamy Fine sand
NO_3-N (ppm)	- 13.2
Available P (ppm)	- 3.0
Available K (ppm)	- 73
Extractable SO_4-S (ppm)	- 8.0
Percent Organic Matter	- 4.3
pH	- 7.4
Conductivity (dS m^{-1})	- 0.2
Field Capacity (Determined on a weight basis)	- 21%

6.3.1 The effect of Nitrogen Addition on the Dry Matter Yield Percent Nitrogen, and Nitrogen Accumulation in Barley, Non-nodulating Soybeans, Soybeans, Fababeans and Lentils

The addition of 1800 mg N pot^{-1} as urea was toxic to the germination of soybean, non-nodulating soybean, fababean, lentil and barley seeds. However, there was no indication that addition of 1200 mg N pot^{-1} adversely affected germination and growth of barley, fababeans and soybeans (Tables 32, 33 and 34). Germination of lentil seeds did not occur in pots which had received 1200 mg N pot^{-1}; this

Table 32. The dry matter yield, percent nitrogen and the total amount of nitrogen accumulated in the root, shoot and total plant portions of barley and non-nodulating soybeans as affected by nitrogen addition.

Nitrogen Treatment (mgN/pot)	Plant Type	Yield (g/pot) Shoot	Yield (g/pot) Root	Yield (g/pot) Total Plant (Shoot + Root)	Percent Nitrogen Shoot	Percent Nitrogen Root	Percent Nitrogen Total Plant (Shoot + Root)	Total Amount of Nitrogen (mg N/pot) Shoot	Total Amount of Nitrogen (mg N/pot) Root	Total Amount of Nitrogen (mg N/pot) Total Plant (Shoot + Root)
0	Barley	8.9 [1] A	4.4 A	13.3 A	.95 A	1.09 A	1.00 A	85 A	48 A	133 A
600	Barley	29.6 B	14.9 B	44.4 B	1.14 AB	2.12 B	1.47 B	337 B	317 B	654 B
1200	Barley	45.3 C	19.1 C	64.4 C	1.42 AB	2.73 C	1.80 C	638 C	521 C	1159 C
0	Non-nod Soybean	9.8 A	4.6 A	14.4 A	1.05 A	.90 A	.98 A	101 A	30 A	141 A
600	Non-nod Soybean	27.3 B	15.2 B	42.5 B	1.54 B	1.69 B	1.60 B	416 B	261 B	679 B

1 Duncan's Multiple Range Test: Means in columns followed by the same letter are not significantly different at P=0.05.

Table 33. The dry matter yield, percent nitrogen and the total amount of nitrogen accumulated in the root, shoot and total plant portions of Aladin fababeans as affected by nitrogen addition.

Nitrogen Treatment (mgN/pot)	Yield (g/pot) Shoot	Yield (g/pot) Root	Yield (g/pot) Total Plant (shoot + root)	Percent Nitrogen Shoot	Percent Nitrogen Root	Percent Nitrogen Total Plant (shoot + root)	Total Amount of Nitrogen (mg N/pot) Shoot	Total Amount of Nitrogen (mg N/pot) Root	Total Amount of Nitrogen (mg N/pot) Total Plant (shoot + root)
0	30.9 [1] A	21.8 A	52.3 A	4.03 A	2.50 A	3.40 A	1233 A	544 A	1777 A
600	31.3 A	22.5 A	54.5 A	3.82 A	2.39 A	3.23 A	1197 A	537 A	1734 A
1200	30.2 A	22.0 A	52.3 A	3.75 A	2.49 A	3.22 A	1140 A	544 A	1685 A

1 Duncan's Multiple Range Test: Means in columns followed by the same letter are not significantly different at P=0.05.

TABLE 34 The dry matter yield, percent nitrogen and total amount of nitrogen accumulated in the root, shoot and total plant portions of Maple Amber soybeans as affected by nitrogen addition.

Nitrogen Treatment (mgN/pot)	Yield (g/pot)			Percent Nitrogen			Total Amount of Nitrogen (mg N/pot)		
	Shoot	Root	Total Plant (shoot + root)	Shoot	Root	Total Plant (shoot + root)	Shoot	Root	Total Plant (shoot + root)
0	31.7 [1]A	20.4 A	52.1 A	2.79 A	2.27 A	2.59 A	884 A	463 A	1347 A
600	30.9 A	19.9 A	50.8 A	2.96 A	2.29 A	2.70 A	915 A	454 A	1369 A
1200	30.9 A	19.6 A	50.5 A	3.03 A	2.36 A	2:75 A	9331 A	460 A	1393 A

1 Duncan's Multiple Range Test: Means in columns followed by the same letter are not significantly different at P=0.05.

TABLE 35 The dry matter yield, percent nitrogen and total amount of nitrogen accumulated in the root, shoot and total plant portions of lentils as affected by nitrogen addition.

Nitrogen Treatment (mgN/pot)	Yield (g/pot)			Percent Nitrogen			Total Amount of Nitrogen (mg N/pot)		
	Shoot	Root	Total Plant (shoot + root)	Shoot	Root	Total Plant (shoot + root)	Shoot	Root	Total Plant (shoot + root)
0	10. 6[1]A	4.5 A	15.0 A	2.71 A	1.74 A	2.43 A	286 A	79 A	365 A
600	23. 6B	15.0 B	38.6 B	2.04 B	2.27 A	2.11 B	481 B	338 B	820 B

1 Duncan's Multiple Range Test: Means in columns followed by the same letter are not significantly different at P=0.05.

substantiated that lentils were more susceptible to the toxic effect of high rates of nitrogen as urea than soybeans, fababeans and barley (Table 35).

Nitrogen addition increased the dry matter yield, and nitrogen accumulation in the root, shoot and total plant portions of barley and non-nodulating soybeans (Table 32). The concentration of nitrogen in the various plant portions also increased with nitrogen addition; however, in some instances the increase was not significant (Table 32). There was no significant difference in the dry matter yield, percent nitrogen, total nitrogen accumulation in the shoot, root or total plant portions of barley and non-nodulating soybeans which received the same treatment (Table 32). The similar uptake, accumulation and distribution of nitrogen by the barley and the non-nodulating soybeans suggested that both crops were of equal value as controls for the measurement of dinitrogen fixation. Wagner and Zapata (1982) and Regitnig (1983) found non-nodulating soybeans and barley adequate control crops for use in measuring dinitrogen fixation by soybeans.

Nitrogen addition did not increase the dry matter yield of, nitrogen concentration in and nitrogen accumulation in the root, shoot and total plant portions of fababeans and soybeans (Tables 33 and 34). The lack of response of Aladin fababeans and Maple Amber soybeans to nitrogen addition indicated the nitrogen nutritional demands of these crops were met from soil and symbiotically fixed nitrogen. The lack of increase in dry matter yield, nitrogen concentration and nitrogen accumulation of Maple Amber soybeans, with nitrogen addition, was concomitant with that of the first growth experiment and further substantiates that Maple Amber soybeans, unlike Maple Presto, were more capable of supplying their nitrogen nutritional requirement through the fixation of dinitrogen even on soils low in nitrogen.

The increased dry matter yield, nitrogen concentration and nitrogen accumulation of lentils with nitrogen addition indicated that lentils could not fix enough nitrogen for their nutritional requirements (Table 35). This substantiated the results of the first growth chamber experiment. Although this was growth chamber data and care must be taken in interpretation, the data supported the opinions of Summerfield and Mauchbauer (1982) and Slinkard and Drew (1981), and the work of Mahajan et al. (1972) that lentils grown on soils low in nitrogen need additional nitrogen for maximum yield and protein content.

6.3.2 Effect of Rate of Fertilizer Nitrogen Addition on the Amount, Percent of Nitrogen Derived from Fertilizer (% Ndff) and % Utilization of Fertilizer Nitrogen in Shoot, Root and Total Plant Portions of Barley, Non-nodulating Soybeans, Lentils, Fababeans and Soybeans

The amount of fertilizer nitrogen and the fraction of nitrogen derived from fertilizer (%Ndff) in the shoot, root and total plant portions of barley, soybeans, and fababeans increased with increased fertilizer nitrogen addition (Tables 36, 37 and 38). The amount and fraction of nitrogen derived from fertilizer in the shoot, root and total plant portions of barley and non-nodulating soybeans were not significantly different at similar rates of nitrogen addition (Table 36). This substantiated that barley and non-nodulating soybeans were of equal value as controls for measurement of fixation under the conditions of this experiment.

The amount of fertilizer nitrogen accumulated in the shoot portion of barley and non-nodulating soybeans was not significantly different than the amount accumulated in the roots; however, the %Ndff in the roots was significantly greater than in the shoots (Table 36). The similar accumulation of fertilizer nitrogen between the root and shoot portions may have been due to parallel production of the roots and shoots in early vegetative development. At the change from vegetative to reproductive development, the shoot weight continues to increase while root weight remained constant or decreased (Brouwer, 1965). This, coupled with fertilizer nitrogen being less available with time through the process of biological immobilization and interchange of nitrogen while non-labelled soil nitrogen was continually mineralized, may have diluted the ^{15}N in the shoot more than in the root and resulted in the lower %Ndff in the shoots than in the roots. Hence, the ^{14}N and ^{15}N were not uniformly mixed in the plant.

Fababeans, nodulating soybeans, lentils which had received 600 mg N pot^{-1} and fababeans and nodulating soybeans which had received 1200 mg N pot^{-1} had a lower %Ndff in the shoot than roots (Tables 37, 38 and 39). The lower %Ndff in the shoots than in the roots may have been due in part to 1) reasons previously discussed for the barley, and 2) timing of dinitrogen fixation, and 3) greater dilution of fertilizer nitrogen by fixed nitrogen in the shoots than in the roots due to the larger sink size of the shoots than the roots. Weber et al. (1971) found that nodule development and nitrogen fixation did not occur until three to four weeks after planting; hence, if shoot mass continued to increase while root mass remained constant, the %Ndff of the shoots would be less than that of the roots due to dilution from fixed nitrogen.

Fababeans, nodulating soybeans and lentils which had received 600 mg N pot^{-1} had a greater accumulation of fertilizer nitrogen in the roots than shoots; however, the converse was true for fababeans and soybeans which had received 1200 mg N pot^{-1} (Tables 37, 38 and 39). The greater accumulation of fertilizer nitrogen in the shoot portion of fababeans and soybeans which had received 1200 mg N pot^{-1} while the %Ndff of the roots was greater than the shoots was related to the relative sink size of the roots and shoots. The greater sink size of the shoot allowed for greater accumulation of fertilizer nitrogen and a lesser fraction of nitrogen derived from fertilizer (%Ndff) in the shoot than in the root portion (from the dilution of fertilizer N by symbiotically fixed and soil nitrogen).

Table 36. Effect of rate of nitrogen addition on the accumulation and concentration of fertilizer nitrogen in the root, shoot and total plant portions of barley and non-nodulating soybean plants.

Nitrogen Treat-ment (mg/pot)	Plant Type	Accumulation of Fertilizer Nitrogen (mg N/pot)			Percent Nitrogen Derived from Fertilizer (%Ndff)		
		Shoot	Root	Total Plant (shoot+root)	Shoot	Root	Total Plant (shoot+root)
600	Barley	221[1]Aa	271 Aa	493 A	6.6 Aa	8.6 Aa	7.6 A
1200	Barley	509 Ba	488 Ba	997 B	8.0 Ba	9.4 Bb	8.5 B
600	Non-Nod Soybean	271 Ab	237 Aa	508 A	6.5 Aa	9.1 ABb	7.5 A

[1] Duncan's Multiple Range Test: Means in column followed by the same capital letter are not significantly different at P = 0.05. Means in rows followed by the same small letter are not significantly different at P = 0.05.

Table 37. Effect of rate of nitrogen addition on the accumulation and concentration of fertilizer nitrogen in the root, shoot and total plant portion of fababeans.

Nitrogen Treat-ment (mg/pot)	Accumulation of Fertilizer Nitrogen (mg N/pot)			Percent Nitrogen Derived from Fertilizer (%Ndff)		
	Shoot	Root	Total Plant (shoot+root)	Shoot	Root	Total Plant (shoot+root)
600	211[1]Aa	290 Ab	501 A	1.2 Aa	5.4 Ab	3.7 A
1200	540 Ba	426 Bb	970 B	4.7 Ba	7.8 Bb	7.0 B

[1] Duncan's Multiple Range Test: Means in column followed by the same capital letter are not significantly different at P = 0.05. Means in rows followed by the same small letter are not significantly different at P = 0.05.

Table 38. Effect of rate of nitrogen addition on the accumulation and concentration of fertilizer nitrogen in the root, shoot and total plant portion of soybeans.

Nitrogen Treatment (mg/pot)	Accumulation of Fertilizer Nitrogen (mg N/pot)			Percent Nitrogen Derived from Fertilizer (%Ndff)		
	Shoot	Root	Total Plant (shoot+root)	Shoot	Root	Total Plant (shoot+root)
600	214[1]Aa	286 Ab	499 A	2.4 Aa	6.3 Ab	3.7 A
1200	608 Ba	365 Bb	968 B	6.5 Ba	7.9 Bb	7.0 B

1 Duncan's Multiple Range Test: Means in column followed by the same capital letter are not significantly different at P = 0.05. Means in rows followed by the same small letter are not significantly different at P = 0.05.

Table 39. Amount of fertilizer nitrogen accumulated in the shoot, root and total plant portion of lentils.

Nitrogen Treatment (mg N/pot)	Accumulation of Fertilizer Nitrogen (mg N/pot)			Percent Nitrogen Derived from Fertilizer (%Ndff)		
	Shoot	Root	Total Plant (shoot+root)	Shoot	Root	Total Plant (shoot+root)
600	220[1]A	264 A	484	4.6 A	7.8 B	5.9

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

The percent utilization of fertilizer nitrogen by the total plant portions of the barley, non-nodulating soybeans, Maple Amber soybeans, Aladin fababeans, and lentils were not significantly different at the two rates of nitrogen addition (Table 40). This indicated that the roots of these species exploited similar volumes of soil and that the plants had not reached the limit of their ability to utilize fertilizer nitrogen. The shoot to root ratio of percent utilization of fertilizer increased with increased addition of fertilizer nitrogen which indicated that fixed nitrogen was partitioned to the shoots.

Table 40. Utilization of fertilizer by the shoot, root and total plant portion of barley, non-nodulating soybeans, fababeans, lentils and soybeans.

Nitrogen Treatment (mg/pot)	Crop	% Utilization of Fertilizer Shoot	Root	Total Plant
600	Barley	37.00 BCDa	45.35 Aa	82.30 A
1200	Barley	41.70 BCDa	41.36 ABa	82.33 A
600	Non-nod Soybeans	45.2 ABa	39.5 ABa	84.69 A
600	Soybeans	37.9 CDa	47.63 Ab	83.50 A
1200	Soybeans	50.3 ABa	30.36 Cb	80.67 A
600	Fababeans	35.3 Da	48.28 Ab	83.51 A
1200	Fababeans	44.4 ABCa	35.48 BCb	80.98 A
600	Lentils	36.6 BCDa	44.06 Aa	80.70 A

[1] Duncan's Multiple Range Test: Means in columns followed by the same capital letter are not significantly different at P = 0.05; Means in rows followed by the same small letter are not significantly different at P = 0.05.

6.3.3 Effect of Nitrogen Addition on the "A" Value calculated for Barley, Non-nodulating Soybeans, Soybeans, Fababean and Lentils

There was no significant difference between the "A" values calculated for the root, shoot, and total plant portions of barley plants which had received 600 mg N pot^{-1} and 1200 mg N pot^{-1}. The constant "A" value indicated that the two rates of nitrogen addition did not change the amount of available soil nitrogen. The "A" values of the non-nodulating soybeans shoot, root, and total plant portions were not significantly different than that of barley (Table 41); indicating that the crops were equal as controls for the measurement of dinitrogen fixation.

Table 41. Effect of nitrogen addition on the "A" values calculated barley and non-nodulating soybeans.

Treatment (mg N/pot)	Crop	"A" Value[1] (mg N/pot)		
		Shoot	Root	Total
600	Barley	313.3 A[2]	99.6 A	194.3 A
1200	Barley	303.7 A	76.2 A	206.7 A
600	Non-nodulating Soybeans	326 A	60.6 A	195 A

1 A measure of available soil nitrogen in terms of a fertilizer standard: urea.
2 Duncan's Multiple Range Test: Means followed by the same letter are not significantly different at P = 0.05.

The "A" value calculated for the shoot and total plant portions of fababeans and soybeans decreased with increased nitrogen addition (Tables 42 and 43). The "A" value calculated for the root portion of fababeans decreased with increased nitrogen addition while that of soybean roots did not (Tables 42 and 43). The decrease in "A" values of the total plant with nitrogen addition suggested that fertilizer nitrogen was utilized preferentially over fixed. Fababeans had the

Table 42. Effect of nitrogen addition on the "A" values calculated for fababeans.

Treatment (mg N/pot)	"A" Value[1] (mg N/pot)		
	Shoot	Root	Total Plant
600	2813 A[2]	513 A	1477 A
1200	1375 B	336 B	883 B

[1] A measure of available soil and fixed nitrogen in terms of a fertilizer standard: Urea.
[2] Duncan's Multiple Range Test: Means followed by the same letter are not significantly different at P = 0.05.

Table 43. Effect of nitrogen addition on the "A" values calculated for soybeans.

Treatment (mg N/pot)	"A" Value[1] (mg N/pot)		
	Shoot	Root	Total Plant
600	1989 A[2]	353.6 A	1040 A
1200	658 B	315 AB	525 B

[1] A measure of available soil and fixed nitrogen in terms of a fertilizer standard: Urea.
[2] Duncan's Multiple Range Test: Means followed by the same letter are not significantly different at P = 0.05.

Table 44. "A" value calculated for the shoot, root and total plant portion of lentils.

Treatment (mg N/pot)	"A" Value[1] (mg N/pot)		
	Shoot	Root	Total Plant
600	721	169	416

[1] A measure of available soil and fixed nitrogen in terms of a fertilizer standard:Urea

highest "A" values while lentils had the lowest of the fixing crops studied (Tables 42, 43 and 44).

6.3.4 The Effect of Nitrogen Addition on Symbiotic Nitrogen Fixation by Fababeans, Soybeans, and Lentils as Measured by the "A" Value and ^{15}N Assisted Difference Techniques

The two ^{15}N methods for measuring nitrogen fixation (the "A' value and ^{15}N assisted difference) and the two non-fixing control crops (barley and non-nodulating soybeans) gave similar measurement of the amount of nitrogen fixed by fababeans, soybeans and lentils (Tables 45, 46 and 47).

Barley which had received 600 and 1200 mg N pot^{-1} were utilized as respective non-fixing controls for fababeans and soybeans which had received 600 and 1200 mg N pot^{-1}. While this may not have been the intended use of the "A" value technique, the lack of significant difference in available soil nitrogen at the two rates of nitrogen addition (as measured by the "A" value technique (Table 41)) and the similar results obtained in measurement of fixation using the non-nodulating soybeans which had 600 mg N pot^{-1} and barley which received 600 and 1200 mg N pot^{-1} as controls indicated that both procedures were adequate in the determination of the amount of dinitrogen fixed. The similar results in the measurement of nitrogen fixation with the two control crops indicated that the crops were equal as controls in the measurement of nitrogen fixation. Zapata and Wagner (1982) also found that barley and non-nodulating soybeans were equal controls for the measurement of fixation by soybeans.

The order of fixation for the crops studied (from greatest to least) was fababeans, soybeans and lentils (Tables 45, 46 and 47).

This was concomitant with the results of the first growth chamber experiment.

Inclusion of root mass in the determination of the amount of nitrogen fixed by fababeans and soybean plants which had received 0 and 600 and 0 mg N pot^{-1}, respectively resulted in significantly higher values for fixed nitrogen (Tables 45 and 46). Fababeans and soybeans which had not received any fertilizer nitrogen had 30% and 34%, respectively of their fixed nitrogen in the root system which was greater than expected. Pate _et_ _al._ (1979) found that white puline retained only 15% of total fixed nitrogen in the root system; this was similar to the amount of fixed nitrogen retained in root system of lentils (15%). Hence, use of just the shoot portion underestimated the amount of nitrogen fixed. However, the inclusion of roots in the determination of fixation by soybeans and fababeans at higher rates of nitrogen addition did not significantly affect the values for fixed nitrogen (Tables 45 and 46). Very little fixed nitrogen was allocated to the root portion of plants at higher rates of nitrogen addition.

Increased nitrogen addition decreased fixation by soybeans and fababeans through preferential utilization of fertilizer nitrogen (Tables 45 and 46).

Inclusion of roots nor the addition of nitrogen significantly affected the total amount of nitrogen fixed by lentils (Table 47). The lack of decline in the amount of dinitrogen fixed by lentils with nitrogen addition was probably due to partitioning of the nitrogen towards increased growth. Allos and Bartholomew (1965) indicated that increased fixation with nitrogen addition was due to increased biomass production.

Table 45. Amount of nitrogen fixed by fababeans as measured by the ^{15}N assisted difference and "A" value techniques.

Amount of Nitrogen Added (mg N/pot)	Amount of Nitrogen Fixed (mg N/pot)					
	"A" Value			^{15}N Assisted Difference		
	Shoot	Total Plant	Root	Shoot	Total Plant	Root
0	--	--	--	1148[1]Ab	1645 Ac	497 Aa
600	876 Ab	1071 Ac	195 Ac	870 Bb	1074 Bc	204 Ba
1200	473 Ba	546 Ba	73 Ab	480 Cb	543 Cb	63 Ba
0*	--	--	--	1132 Ab	1637 Ac	505 Aa
600**	871 Ab	1070 Ac	199 Aa	838 Bb	1068 Bc	230 Ba
1200**	462 Bb	555 Bb	93 Aa	460 Cb	548 Cb	88 Ba

1 Duncan's Multiple Range Test: Means in columns followed by the same capital letter are not significantly different at P = 0.05; Means in rows followed by the same small letter are not significantly different at P = 0.05.

* Fixation was determined by using non-nodulating soybeans to which no nitrogen had been added as the controls.

** The non-nodulating soybeans which had received 600 mg N were used as a control crop in the determination of the amount of dinitrogen fixed.

Table 46. Amount of nitrogen fixed by soybeans as measured by the ^{15}N assisted difference and "A" value techniques.

Amount of Nitrogen Added (mg N/pot)	Amount of Nitrogen Fixed (mg N/pot)					
	"A" Value			^{15}N Assisted Difference		
	Shoot	Total Plant	Root	Shoot	Total Plant	Root
0	--	--	--	799[1]Ab	1216 Ac	417 Aa
600	595 Ab	705 Ac	110 Aa	590 Bb	707 Bb	117 Ba
1200	177 Bb	269 Bb	92 Aa	203 Cb	255 ab	52 Ba
0*	--	--	--	783 Ab	1207 Ac	424 Aa
600**	590 Ab	704 Aab	114 Aa	559 Bb	702 Bb	143 Ba
1200**	166 Ba	268 Ba	102 Aa	182 Ca	261 Ca	79 Ba

1 Duncan's Multiple Range Test: Means in columns followed by the same capital letter are not significantly different at P = 0.05; Means in rows followed by the same small letter are not significantly different at P = 0.05.

* Fixation was determined by using non-nodulating soybeans to which no nitrogen had been added as the controls.

**The non-nodulating soybeans which had received 600 mg N were used as a control crop in the determination of the amount of dinitrogen fixed.

Table 47. The effect of nitrogen addition on symbiotic nitrogen fixation by lentils.

Amount of Nitrogen Added (mg N/pot)	Amount of Nitrogen Fixed (mg N/pot)					
	"A" Value			^{15}N Assisted Difference		
	Shoot	Total Plant	Root	Shoot	Total Plant	Root
0	--	--	--	201[1]Ab	233 Ab	32 Aa
600	147 Ab	179 Ab	32 Aa	145 Ab	179 Ab	34 Aa
0*	--	--	--	185 Ab	224 Ab	39 Aa
600**	142 Ab	178 Ab	36 Aa	131 Ab	170 Ab	39 Aa

1 Duncan's Multiple Range Test: Means in columns followed by the same capital letter are not significantly different at P = 0.05; Means in rows followed by the same small letter are not significantly different at P = 0.05.

* Fixation was determined by using non-nodulating soybeans to which no nitrogen had been added as controls.

**The non-nodulating soybeans which had received 600 mg N were used as a control crop in the determination of the amount of dinitrogen fixed.

6.3.5 Conclusions

The following conclusions were derived from this experiment:

1) Even when available soil nitrogen was low, Maple Amber soybeans and Aladin fababeans fixed enough nitrogen for maximum production if environmental conditions were favourable. Given that this experiment was conducted under conditions similar to that used by Regitnig (1983), it was further concluded that the Maple Amber cultivar unlike Maple Presto could fix enough nitrogen for its nutritional requirements.

2) Lentils grown on soils low in nitrogen, even when environmental conditions were favourable, needed additional nitrogen for maximum production; nitrogen fixation did not adequately meet their nutritional requirements. Also, lentils appeared to be more susceptible to the toxic effects of high rates of nitrogen addition (as urea) than fababeans or soybeans.

3) There was no significant difference in the use of barley and non-nodulating soybeans as controls for the measurement of fixation, nor was there any significant differences between the ^{15}N assisted difference and "A" value methods in measurement of dinitrogen fixation.

4) Soil, fertilizer and fixed nitrogen was not uniformly distributed between the root and shoot portions of fababeans, soybeans and lentils.

5) Preferential utilization of combined nitrogen by soybeans and fababeans caused a decreased fixation with increased nitrogen addition. The lack of decrease in fixation of lentils with nitrogen addition was probably due to partitioning of the extra nitrogen towards increased growth.

6) The use of just the shoot portion in measuring the amount of dinitrogen fixed by fababeans, soybeans and lentils resulted in underestimation of fixation by 30, 34 and 15%, respectively for plants which had received 0 mg N pot^{-1}. However, at high rates of nitrogen addition, inclusion of the roots did not significantly improve the estimate of the amount of dinitrogen fixed.

CHAPTER VII

FIELD EXPERIMENT 1983

7.1 Introduction

Knowledge of the physiological stage of development at which dinitrogen fixation occurs in soybeans is important with regard to timing of fertilizer application if the cultivar cannot fix enough for its own nutritional requirement due to its natural physiology or poor nodulation.

Israel (1981) in field experiments found that Ransom and Davis cultivars inoculated with USDA 110 rhizobium fixed nitrogen at two distinctly different physiological stages. The Davis cultivar was found to fix most of its nitrogen during vegetative development while the Ransom fixed most of its nitrogen during reproductive development. Regitnig (1983) in field experiments found that Maple Presto fixed most of its nitrogen at mid-podfill. Regitnig also found that Maple Presto soybeans did not fix enough nitrogen for its own nutritional requirements. However, results of two growth chamber experiments and the lysimeter experiment indicated that Maple Amber soybeans did supply their nutritional requirements through the fixation of atmospheric dinitrogen.

A field experiment was undertaken to:

1. determine the physiological stage of growth at which maximum fixation of dinitrogen occurred in Maple Amber soybeans,
2. determine whether additional nitrogen increased the seed yield of Maple Amber soybeans,

3. determine the effect of nitrogen addition at flowering on the yield and nitrogen content of Maple Amber soybeans, and
4. evaluate ^{15}N and acetylene reduction methods for measuring dinitrogen fixation.

7.2 Materials and Methods

7.2.1 Soil

There were two experimental sites. One site was located in the Morden-Winkler region of the Manitoba lowlands in Southern Manitoba (NE 1/4 12-4-5w) while the other site was located in the St. Claude region of South Central Manitoba (SW corner of SW 1/4 33-8-7W). The experimental site in the Morden region was located on a Gleyed Rego Black Chernozem (carbonated phase) of the Greysville series. The experimental site in the St. Claude region was located on a Gleyed Regosol of the Long Plain series in the Almasippi Association (Michalyna, 1984). The sites were designated Greysville and Long Plain.

Soil samples were obtained from the 0-15 cm, 15-30 cm, 30-60 cm, 60-90 cm and 90-120 cm depths from the four plot corners at each site, at seeding. The samples were air dried at 30C for 48 hours. Chemical and physical analyses were performed on the samples by methods described in Chapter 3.

7.2.2 Experimental Design

7.2.2.1 Experimental Design Greysville

Maple Amber and non-nodulating soybeans (an isoline of Clay) were separated into adjacent plots to facilitate seeding and sharing of the non-nodulating control crop by two researchers. The non-nodulating isoline of Clay was used since it was the earliest maturing non-nodulating isoline available. The use of an uninoculated Maple Amber as a control for measurement of fixation was considered dubious since the sites had been cropped (in the last 5 years) to soybeans; contamination by native rhizobium would have rendered the uninoculated soybeans ineffective as a control crop.

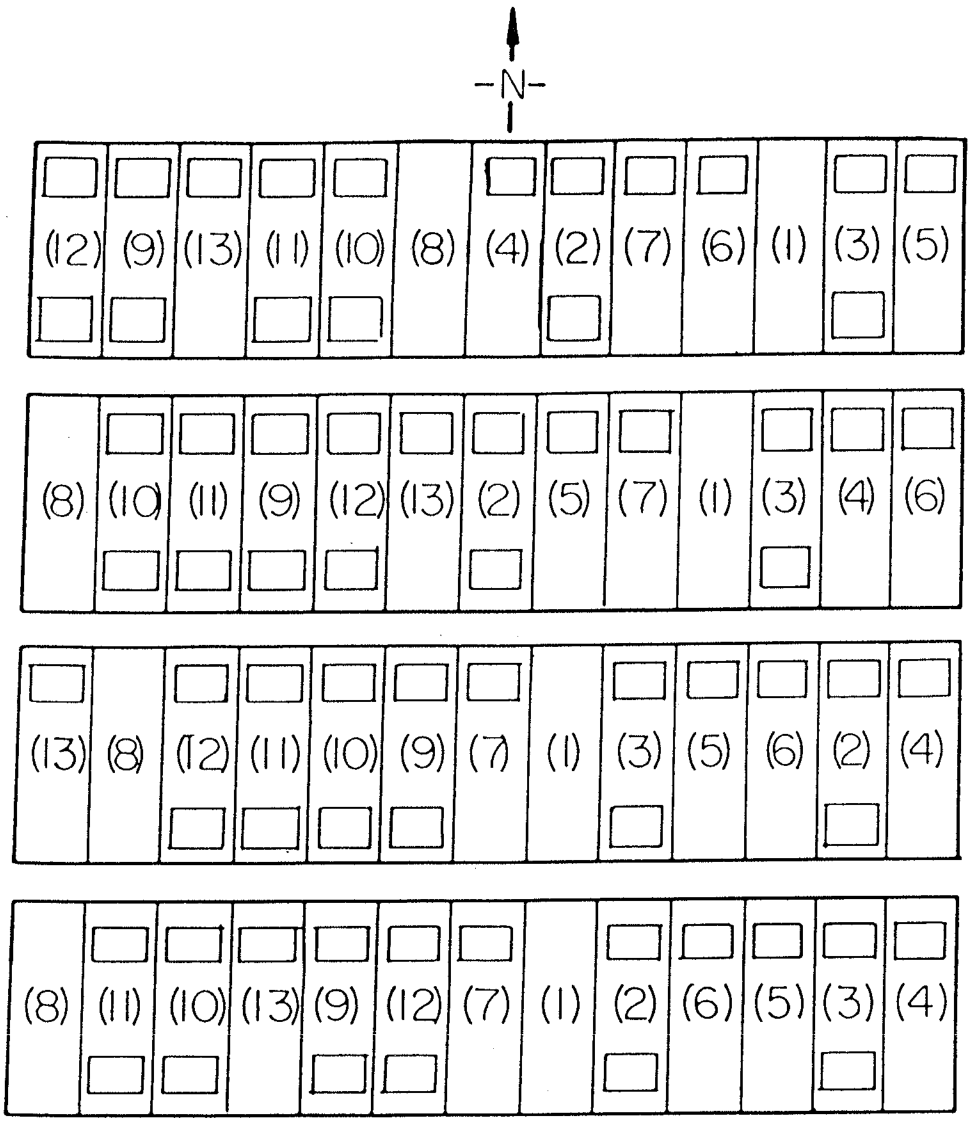
Two 1.0 meter square areas were selected and staked in each of two subplots which received 30 kg N ha^{-1} and seeded to Maple Amber soybeans while only one 1.0 meter square area was selected and staked in the third. The 1.0 meter square areas were sprayed with a solution containing 3.176 atom % ^{15}N atom excess urea at the appropriate rate; unlabelled urea was broadcast over the remainder of the plot. Two 1.0 meter square areas were selected and staked in each of two subplots which received 30 kg N ha^{-1} and seeded with non-nodulating soybeans; unlabelled urea was broadcast over the remainder of the plot. This arrangement allowed for five and eight harvests, respectively of Maple Amber and non-nodulating soybeans throughout the growing season. Five harvests through the growing season allowed for determination of the stages in physiological development at which fixation occurred in Maple Amber soybeans. The difference in the number of harvests of Maple Amber and non-nodulating soybeans was due to sharing of the non-nodulating soybeans for the study of seasonal fixation by Maple Amber soybeans and Easton lentils (Table 48, Fig. 2).

One 1.0 meter square area was selected and staked in subplots which received 100 and 200 kg N ha^{-1} at seeding and 100 kg N ha^{-1} at flowering, and 100 kg N ha^{-1} at seeding and seeded to Maple Amber and non-nod soybeans, respectively. A solution containing 1.616 atom % ^{15}N atom excess urea was sprayed on each staked area at the appropriate rate; unlabelled urea was broadcast over the remaining subplot area (Table 48, Fig. 2).

All subplots were rototilled after nitrogen application. Prior to seeding, 17 kg P ha^{-1} as triple super phosphate was banded to approximately 15 cm with an Allis Chambers Drill. The plots were

Table 48. Soybean Treatments: Greysville

Plot Number	Nitrogen Amendment (kg/ha)	Time of N Application	Type of Soybean
1	0	--	Nodulating
2	30	Preplant	Nodulating
3	30	Preplant	Nodulating
4	30	Preplant	Nodulating
5	100	Preplant	Nodulating
6	200	Preplant	Nodulating
7	100	Flowering	Nodulating
8	0	--	Non-nodulating
9	30	Preplant	Non-nodulating
10	30	Preplant	Non-nodulating
11	30	Preplant	Non-nodulating
12	30	Preplant	Non-nodulating
13	100	Preplant	Non-nodulating



( )-Treatment Number □-Applied ^{15}N

FIG. 2 Plot Diagram of the Greysville Site seeded on May 27th with a 9 row Allis Chambers Drill (17.8 cm between rows) at 104 and 130 kg ha^{-1} for Maple Amber soybeans and non-nodulating soybeans, respectively. Different seeding rates were used to achieve approximately equal plant populations of 90 plants/m^2.

Maple Amber soybeans were inoculated with Nitragin[1] Corporation S culture in both the slurry and granular form. Twice the recommended rate of 44 grams slurry inoculum per 100 grams of seed was mixed with the seed immediately prior to seeding. The granular form was placed with the seed at a rate of 69 kg inoculum ha^{-1}. The two forms of inoculum and the high rates of application ensured that nodulation was not limited by the number of rhizobia present.

Though this site had adequate moisture when selected in early May, drying of the seed bed during the eight hours between rototilling and seeding resulted in poor germination. Thus, water was applied to all ^{15}N areas and to 1.0 meter square non-^{15}N areas in each subplot on June 2nd and 9th at a rate of 10 L per meter square. Emergence was not improved and the entire plots of both cultivars were reseeded on June 9th over existing seed rows with a Planet Junior. The Maple Amber soybeans used in reseeding were inoculated with Nitragin Corporation S culture - slurry form at twice the recommended rate of 44 grams inoculum per 100 grams of seed. After emergence, both cultivars were thinned to approximately 90 plants per meter square.

Weed control was facilitated by hand weeding throughout the season and application of Hoe grass at a rate of 3.75 L ha^{-1} on June 16th.

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[1] Nitragin Corporation, 3101 W. Custer Ave., Milwaukee, WI, 53209.

Malathion was applied at a rate of 16 mL per 15 L of water on August 9th, 16th and 23rd to control grasshoppers.

Three one meter rows were harvested from the non-^{15}N portions of the 0, 30, 100 and 200 kg N ha^{-1} treatments of Maple Amber soybeans at five physiological stages of development (Table 49) for determination of dry matter production and acetylene reduction assay.

The ^{15}N portions of the 30 kg N ha^{-1} treatments of Maple Amber and non-nodulating soybeans were harvested at five physiological developmental stages by cutting 3 rows, 0.9 meters in length, from the centre of the sprayed areas. At maturity, 3 rows, 0.9 meters in length, were cut from the ^{15}N portion of the 100 kg N ha^{-1}, 100 kg N ha^{-1} at flowering and 200 kg N ha^{-1} treatments of Maple Amber soybeans. Two 0.9 meter rows were harvested from the 0 kg N ha^{-1} and the ^{15}N portion of the 100 kg N ha^{-1} treatments of non-nodulating soybeans at the fourth and fifth harvests (Table 49).

Table 49. Stages of development of soybeans at various harvests: Greysville.

Harvest Number	Stage of Development	Date
1	3-4 Trifoliate Leaf	June 29th
2	Early Flowering	July 16th
3	Early Pod Formation	Aug. 2nd
4	Mid-podfill	Aug. 22nd
5	Maturity	Sept. 25th

Plant samples were divided, where possible, into leaflets, stems and petioles, pods and seeds. The samples were then air dried, oven dried, weighed, and ground to pass through a 2 mm sieve. Analysis was performed by methods outlined in Chapter 2.

At each harvest, acetylene reduction assay was performed. Four plants were selected at random from the non-^{15}N portion of each Maple Amber soybeans treatment plot. A volume of soil with a surface radius of approximately 8 cm around the stem and a depth of 15 cm was removed with a spade. The soil was shaken from the roots and each root sample placed in a 900 mL Mason jar with a serum stopper in the lid. The lid was placed on the jar and 20 mL of acetylene was added with a graduated 30 mL syringe. The samples were incubated for one hour to allow for conversion of acetylene to ethylene. A 20 mL gas sample was taken from each container. After dispelling a few millilitres, 10 mL of gas was placed into a 10 mL vacu-tainer. Nodule numbers were determined after the acetylene reduction sample had been obtained. Subsequent methodology used in the acetylene reduction analysis is outlined in Chapter 2.

7.2.2.2 Experimental Design Long Plain

The plot design at the Long Plain site was similar to that used at the Greysville site, except that ^{15}N labelled urea was not used and only one treatment plot per replication in the Maple Amber and non-nodulating soybeans received 30 kg N ha^{-1} (Table 50, Figure 3).

Seeding and inoculation were as described in Section 7.2.2.1 except that application of water and reseeding did not occur. Seeding occurred on May 27. Weeds were controlled throughout the growing season by hand weeding.

At five physiological developmental stages (Table 50), six 1.0 meter rows from each treatment plot of Maple Amber soybeans were harvested for determination of dry matter production and acetylene reduction assay. At maturity, six 1.0 meter rows were harvested from each treatment plot of non-nodulating soybeans for use as controls in determination of the amount of dinitrogen fixed by Maple Amber soybeans received similar rates of nitrogen addition. The non-nodulating soybeans which had received 100 kg N ha^{-1} were also used as the controls for measurement of the amount of nitrogen fixed by Maple Amber soybeans which had received 200 kg N ha^{-1}.

Plant samples were divided, where possible, into leaflets, stem, petioles, pods and seeds. These samples were air dried, oven dried at 60^{C}, weighed and ground to pass through a 2 mm sieve. The methods used for subsequent analysis are outlined in Chapter 2.

Acetylene reduction assay and nodule counts were completed by methods outlined in Section 7.2.2.1 and Chapter 2.

Table 50. Soybean treatments: Long Plain

Treatment Number	Nitrogen Treatment (kg N ha^{-1})	Type of Soybean
1	0	Nodulating
2	30	Nodulating
3	100	Nodulating
4	200	Nodulating
5	0	Non-nodulating
6	30	Non-nodulating
7	100	Non-nodulating

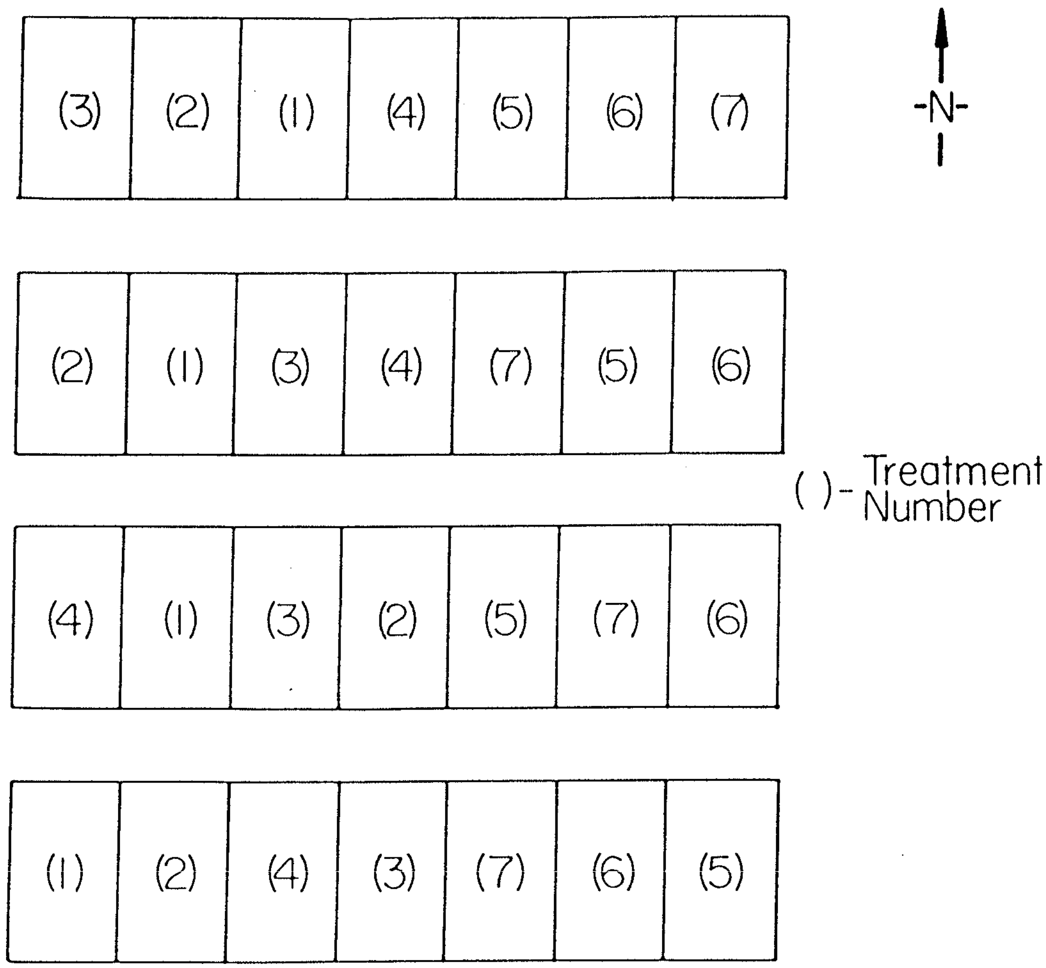


FIG. 3 Plot Diagram of the Long Plain Site

Table 51. Stages of development of soybeans at various harvests: Long Plain.

Harvest Number	Stage of Development	Date
1	3-4 Trifoliate Leaf	July 4th
2	Early Flowering	July 19th
3	Early Pod Formation	Aug. 7th
4	Mid-podfill	Aug. 30th
5	Maturity	Oct. 13th

7.3 Results and Discussion

7.3.1 Soil

The physical and chemical characteristics of the soils used in the field experiment are presented in Table 52. Though both sites had similar amounts of precipitation during the growing season, differences in texture and depth to the water table resulted in the Long Plain site being droughty while at the Greysville site there was no evidence of water stress after reseeding.

Table 52. Soil Test Results Greysville and Long Plain.

	Site	
	Greysville	Long Plain
Soil Series	Greysville	Almasippi
Soil Type	Gleyed Rego Black Chernozem	Gleyed Regosol
Texture	Clay loam	Fine sand
NO_3-N (kg ha^{-1}) 0-60 cm	29	24
60-120 cm	32	12
Available K (kg ha^{-1}) 0-15 cm	423	113
Available P (kg ha^{-1}) 0-15 cm	81	49
Extractable SO_4-S (kg ha^{-1}) 0-60 cm	High	30
Percent Organic Matter	5.0	3.0
pH	7.5	7.9
Conductivity -d Sm^{-1}	0.3	0.2
Carbonate Content	Low	Low

7.3.2 Dry Matter and Nitrogen Accumulation Pattern of Maple Amber Soybeans

The dry matter and nitrogen accumulation pattern of Maple Amber soybeans for each rate of nitrogen addition, at each site, were similar. Thus, only the accumulation pattern for soybeans which received 0 kg N ha^{-1} was presented in the body of this thesis. The dry matter and nitrogen accumulation pattern of Maple Amber soybeans which received 30, 100, and 200 kg N ha^{-1} are contained in Appendix A.

The dry matter accumulation pattern of Maple Amber soybeans at the Greysville site followed a sigmoid curve; maximum rate of dry matter accumulation occurred between early flowering and mid-podfill. Decreased plant mass from mid-podfill to maturity was due to leaflet and petiole abscission (Table 53, Figure 4).

Leaflet mass increased until early pod formation remained constant from early pod formation to mid-podfill and decreased to maturity. Stem and petiole mass increased until early flowering and remained constant from early flowering to maturity even though petiole abscission occurred (Table 53, Figure 4). Similar results were found by Beaver and Cooper (1982).

Pod mass increased from early pod formation to mid-podfill, after which it remained constant while seed mass increased from mid-podfill to maturity (Table 53, Figure 4). Similar results were obtained by Hanway and Weber (1971a).

Dry matter accumulation of Maple Amber soybeans at the Long Plain site followed a similar pattern to that of soybeans at the Greysville site; however, drought conditions at this site limited dry matter accumulation and yield (Table 54, Figure 5). Only 50 percent of the yield at Greysville was achieved at Long Plain. Carlson et al. (1982) found moisture stress decreased in soybean yields by up to 50 percent. The magnitude of the yield reduction was a function of the cultivar. The earlier decline in dry matter, leaflet mass and pod mass of the soybeans at the Long Plain site, compared with soybeans at the Greysville site, was probably also due to moisture stress (Tables 53 and 54, Figures 4 and 5 respectively).

TABLE 53 Seasonal dry matter and nitrogen accumulation of Maple Amber soybean- 0 kg N ha^{-1} treatment: Greysville.

Harvest No	Mass of (kg ha^{-1})					Nitrogen Accumulation in (kg N ha^{-1})				
	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant
1	156 A[1]	48 B	0.0 A	0.0 A	203 A	7.4 CD	1.2 D	0.0 A	0.0 A	8.6 A
2	590 B	324 B	0.0 A	0.0 A	923 A	22.6 BC	4.9 CD	0.0 A	0.0 A	27.5 A
3	1393 C	1467 A	70.d A	0.0 A	2930 B	63.9 B	23.5 A	2.6 B	0.0 A	90.0 B
4	1316 C	1702 A	806 A	1012 A	4368 C	36.5 A	17.7 B	13.7 D	65 B	135 C
5	0.0 A	1302 A	865 B	1825 C	3991 D	0.0 D	7.4 C	7.3 C	122 C	136 C

1 Duncan's Multiple Range Test: Means in columns followed by the same letter are not significantly different at P=0.05.

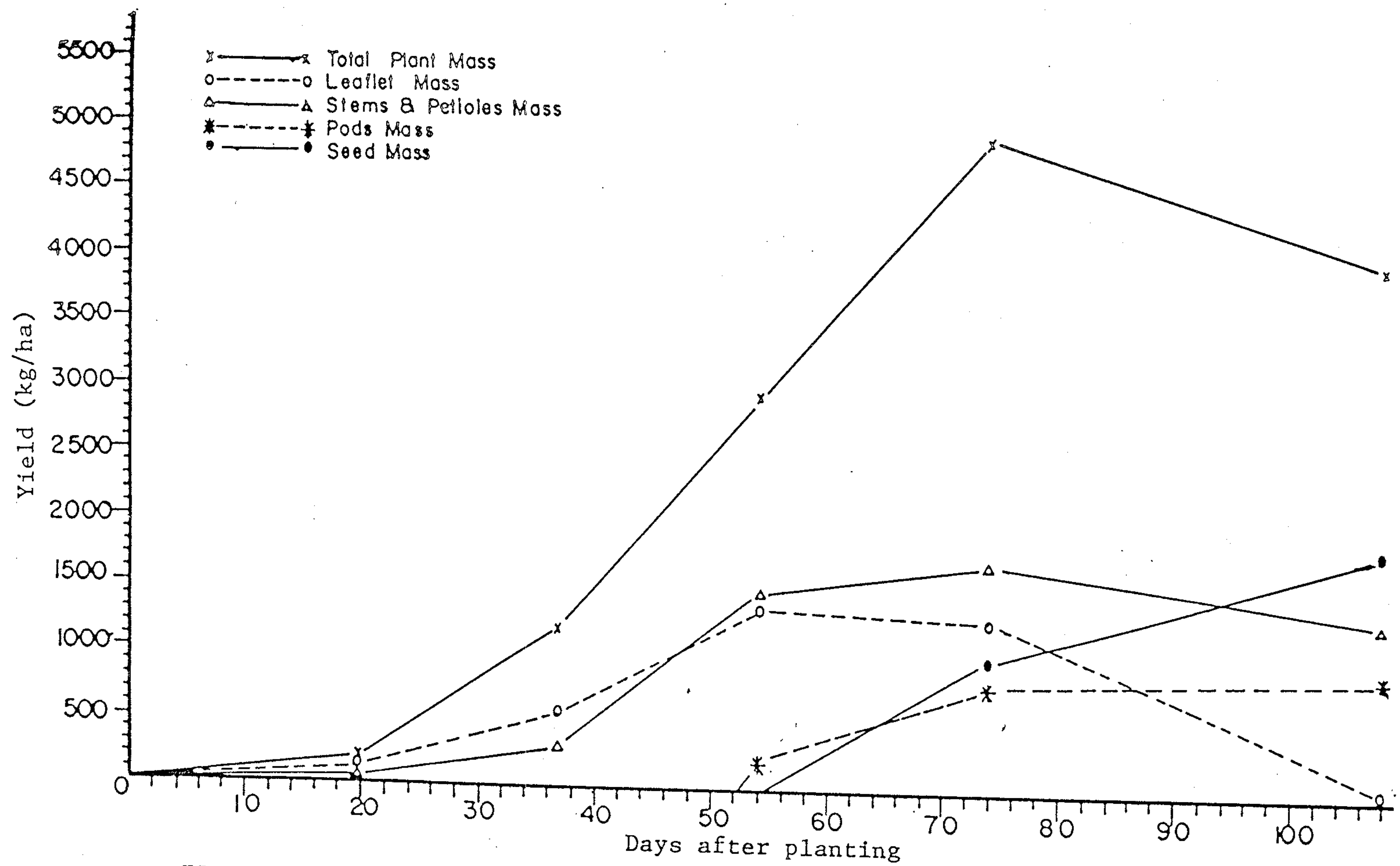


FIG. 4. SEASONAL DRY MATTER ACCUMULATION OF MAPLE AMBER SOYBEANS 0 KG N/HA GREYSVILLE

TABLE 54. Seasonal dry matter and nitrogen accumulation of Maple Amber soybean 0 kg N ha^{-1} treatment: Long Plain.

Harvest No.	Mass of (kg ha^{-1}) Leaflets	Stems and Petioles	Pods	Seeds	Total Plant	Nitrogen Accumulation in (kg N ha^{-1}) Leaflets	Stems and Petioles	Pods	Seeds	Total Plant
1	162 C[1]	65.3 C	0 C	0 B	227 B	7.13 C	1.66 B	0 B	0 B	8.79 B
2	441 B	304 B	0 C	0 B	745 B	18.09 BC	4.82 B	0 B	0 B	22.9 B
3	754 B	686 A	501.4 A	0 B	1939 A	30.25 A	12.23 A	16.97 A	0 B	59.5 A
4	144 C	288 B	339.5 B	625 A	1398 A	2.58 C	1.67 B	1.98 B	40.7 A	46.9 A
5	0.0 C	266 B	468 AB	703 A	1437 A	0.0 C	2.54 B	3.56 B	48.5 A	54.6 A

1 Duncan's Multiple Range Test: means in columns followed by the same letter are not significantly different at P=0.05.

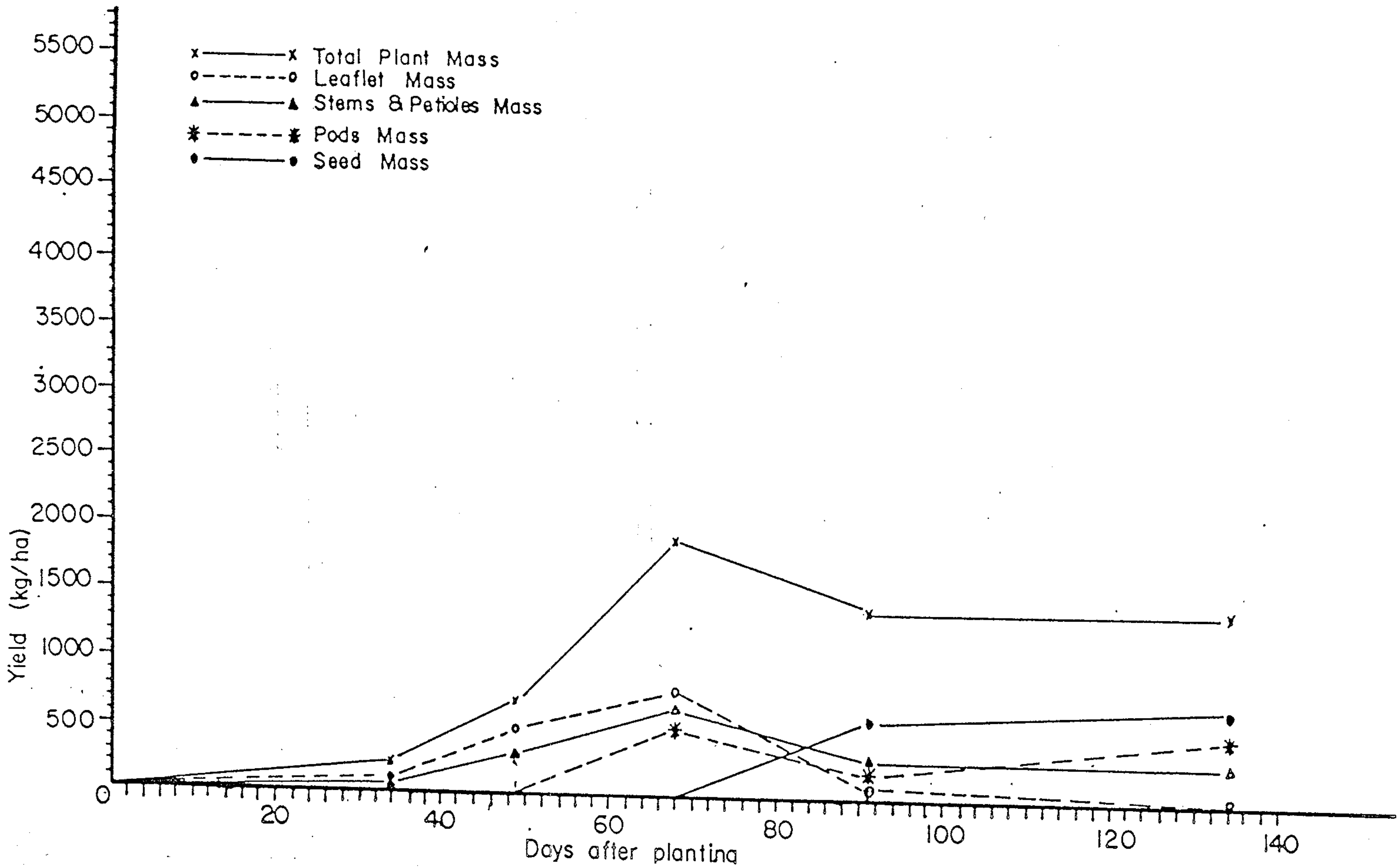


FIG. 5. SEASONAL DRY MATTER ACCUMULATION OF MAPLE AMBER SOYBEANS 0 KG N/HA LONG PLAIN

Nitrogen accumulation followed a sigmoid curve similar to that of dry matter accumulation; the maximum rate of nitrogen accumulation occurred at the same stage of physiological development as dry matter accumulation (Table 53, Figs. 4 and 6, and Table 54, Figs. 5 and 7) respectively. The total amount of nitrogen in the plant, however, was constant from mid-podfill to maturity (Tables 53 and 54, Figs. 6 and 7 respectively). Maximum accumulation of nitrogen in the leaflet portion occurred at early podfill after which, the amount of nitrogen in the leaflets declined (Tables 53 and 54, Figs. 6 and 7 respectively). Since no significant decrease in dry matter accumulation occurred from early pod formation to mid-podfill, the decline in leaflet nitrogen was due to a redistribution of the nitrogen toward other plant parts, probably pods and seeds.

Nitrogen accumulation in the stem and petioles increased until early pod formation after which it declined (Tables 53 and 54, Figures 6 and 7). Redistribution of stem and petiole nitrogen towards the seeds and pods probably accounted for the decline. Zeiher et al. (1982) noted that the proportion of seed N from N redistribution was related to the amount of available N in the vegetative tissue and pod walls. Also noted was that later maturing cultivars received more of their seed N from redistribution than early maturing cultivars. At maturity, most of the nitrogen was in the seed (Tables 53 and 54, Figures 6 and 7). The constant amount of nitrogen in the plant from mid-podfill (nitrogen harvest) to maturity suggested that very little nitrogen was in the abscised leaves and petioles due to translocation of the nitrogen to the seeds. Hanway and Weber (1971b), however, found

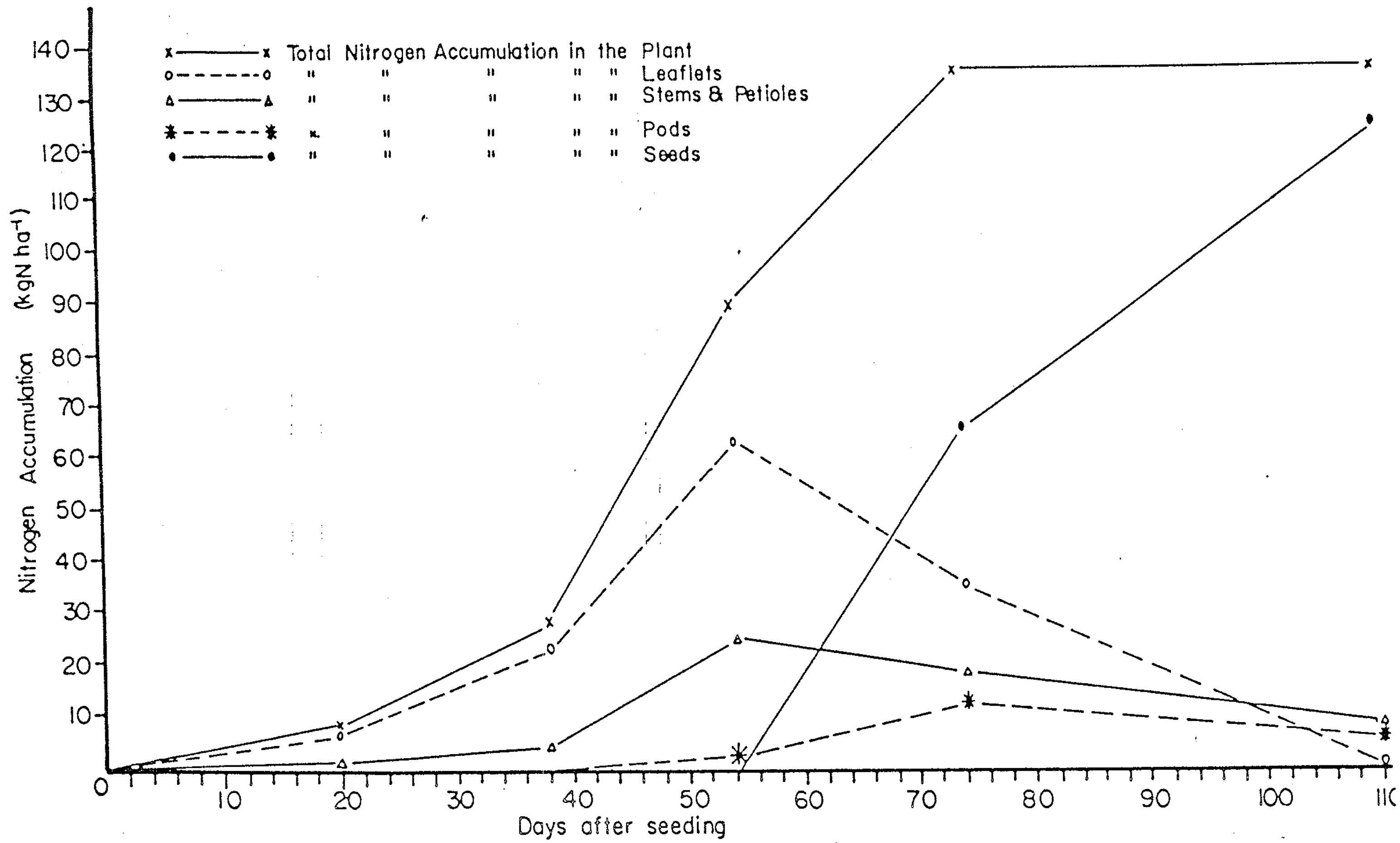


FIG. 6. SEASONAL NITROGEN ACCUMULATION OF MAPLE AMBER SOYBEANS 0 KG N HA^{-1}
GREYSVILLE

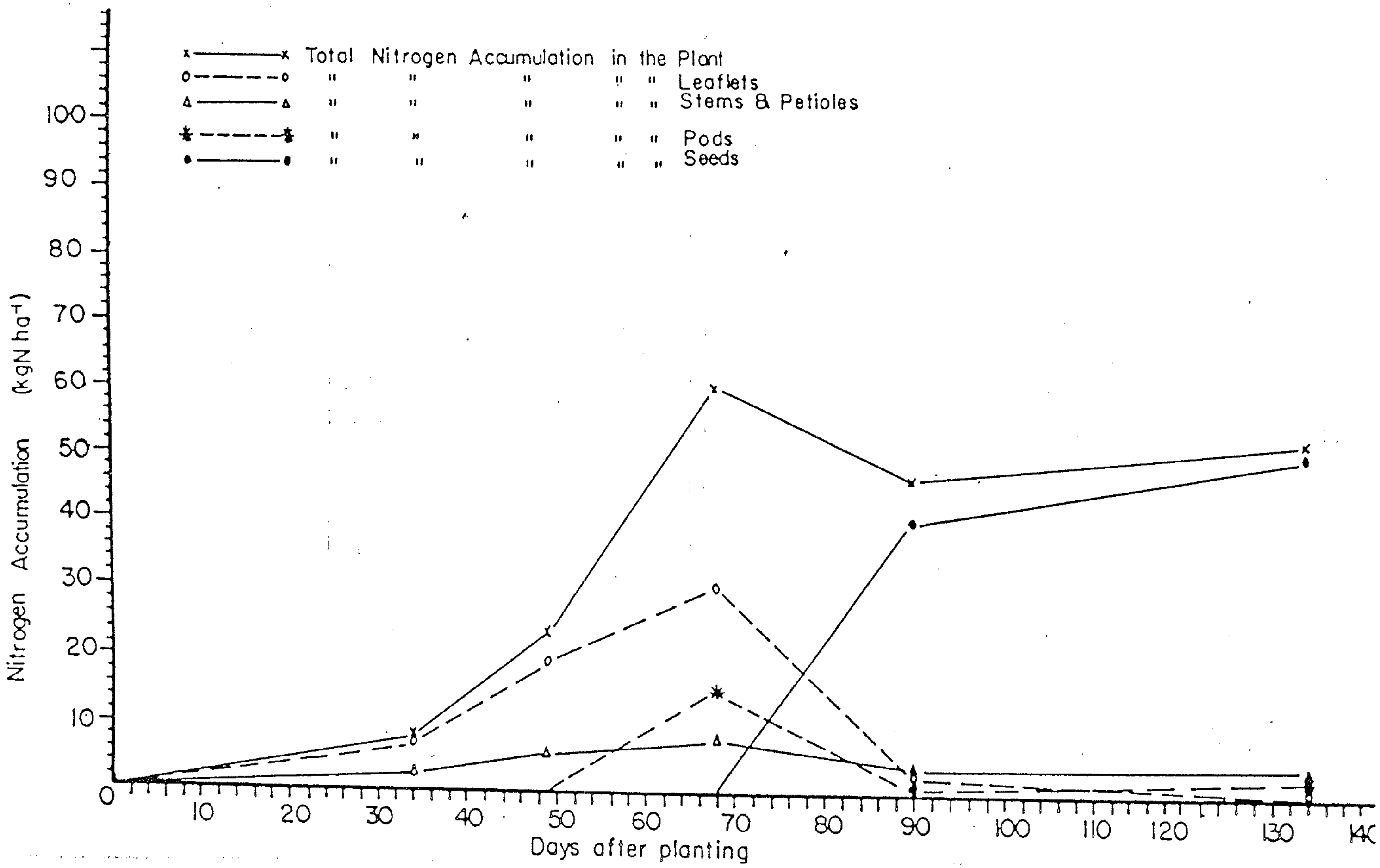


FIG. 7. SEASONAL NITROGEN ACCUMULATION OF MAPLE AMBER SOYBEANS 0 KG N HA$^{4-1}$
LONG PLAIN

(by direct measurement) that approximately 20% of the nitrogen in Hawkeye soybeans was in fallen leaflets and petioles.

At each physiological stage of development, the proportion of dry matter production and nitrogen accumulation in the various plant portions was relatively constant regardless of plant size (Appendix B). Hanway and Weber (1971a) found in a two year study, that the mass of various plant parts relative to total plant mass were similar at the same physiological stage of development. Beaver and Cooper (1982) found little difference between Corsoy (maturity group II) and Williams (maturity group III) soybeans in the percentage of maximum total vegetative dry matter in leaflets, petioles, stems and branches.

At the first harvest, 77% of the dry matter and 87% of the nitrogen was accumulated in the leaflets of Maple Amber. With time, a decline in the proportion of dry matter and nitrogen in the leaflets occurred (Tables 55 and 56). Changes in the allocation of photosynthate appeared to precede changes in nitrogen allocation (Tables 55 and 56). At maturity, the seeds contained 45-50% of total plant dry matter and 89% of the total nitrogen (Tables 55 and 56).

7.3.3 Effect of Nitrogen Addition on Dry Matter Accumulation, Seed Yield and Nitrogen Accumulation

Dry matter and nitrogen accumulation of Maple Amber soybeans at the five harvests and two sites as affected by nitrogen addition are presented in Tables 57 to 67.

At the first harvest, there was no significant effect of nitrogen addition up to 200 kg N ha^{-1} on any of the parameters measured for soybeans at the Greysville site, except percent nitrogen in the stems

TABLE 55 Seasonal changes in the fractionation of dry matter and nitrogen in leaflets, stems and petioles, pods, and seeds, 0 kg N ha^{-1} treatment: Greysville.

Harvest No	Percent of Total Plant: Mass in Leaflets	Mass in Stems and Petioles	Mass in Pods	Mass in Seeds	Nitrogen in Leaflets	Nitrogen in Stems and Petioles	Nitrogen in Pods	Nitrogen in Seeds
1	77 A[1]	23 A	0 A	0 A	87 A	13 BC	0 A	0 A
2	65 B	36 B	0 A	0 A	82 A	18 B	0 A	0 A
3	47 C	51 C	2.5 A	0 A	69 B	28 A	2.9 B	0.0 A
4	27 D	35 B	16.7 C	21.5 B	27 C	13 BC	10.3 C	50 B
5	0 E	29 A	21.8 D	45.8 C	0 D	6 C	5 D	89 C

1 Duncan's Multiple Range Test: Means in columns followed by the same letter are not significantly different at P=0.05.

TABLE 56 Seasonal changes in the fractionation of dry matter and nitrogen in leaflets, stems and petioles, pods, and seeds, 0 kg N ha^{-1} treatment: Long Plain.

Harvest No	Percent of Total Plant							
	Mass In				Nitrogen in			
	Leaflets	Stems and Petioles	Pods	Seeds	Leaflets	Stems and Petioles	Pods	Seeds
1	71.3 A[1]	28.8 A	0 C	0 C	81.0 A	19.0 A	0.0 C	0 B
2	59.8 B	41.5 B	0 C	0 C	79.5 A	23.0 A	0.0 C	0 B
3	38.8 C	35.4 A	25.8 B	0 C	50.6 B	20.8 A	28.7 A	0 B
4	11.0 D	18.7 C	24.4 B	43.9 B	5.5 C	3.4 B	4.1 B	87.9 A
5	0.0 E	13.9 C	32.3 A	49.6 A	0.0 D	4.6 B	6.4 B	89.0 A

1 Duncan's Multiple Range Test: Means in columns followed by the same letter are not significantly different at P=0.05.

and petioles (Table 57). There was, however, a trend towards lower mass and higher percent nitrogen in the various plant portions with nitrogen addition. These effects negated each other and resulted in no significant effect of N addition on the total amount of nitrogen in the plants or plant portions. At the Long Plain site, nitrogen addition of 100 and 200 kg N ha^{-1} significantly increased the percent nitrogen in the leaflets, stems and petioles and total plant (Table 58). The total amount of nitrogen in the stems and petioles and the total plant mass declined significantly with nitrogen additions of 100 and 200 kg N ha^{-1}, respectively (Table 58). The mass of leaflets and stems and petioles were not significantly affected by any of the nitrogen treatment imposed though a trend of decreased mass with increased nitrogen addition was evident (Table 58).

The trend of lower mass and higher percent nitrogen in the various plant portions with increased nitrogen addition appeared indicative of the additional nitrogen delaying either emergence or physiological development. Regitnig (1983) found that increased nitrogen addition delayed physiological development of Maple Presto soybeans. If any delay in physiological development with nitrogen addition occurred it was not visually apparent at either site, hence, it must have been small.

Table 57 Effect of nitrogen addition on the yield and nitrogen accumulation of Maple Amber soybeans: Greysville - First Harvest

Nitrogen Addition	Mass of (kg/ha)					Percent Nitrogen in					Total Amount of Nitrogen in (kg N/ha)				
(kg N/ha)	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant
0	155.6[1]A	48.3 A	0.0 A	0.0 A	204 A	4.99 A	2.54 A	0.0 A	0.0 A	4.43 A	7.4 A	1.2 A	0.0 A	0.0 A	8.6 A
30	138.8 A	43.2 A	0.0 A	0.0 A	182 A	5.20 A	3.53 AB	0.0 A	0.0 A	4.80 A	7.2 A	1.5 A	0.0 A	0.0 A	8.8 A
100	125.0 A	39.5 A	0.0 A	0.0 A	164 A	5.67 A	3.71 AB	0.0 A	0.0 A	5.41 A	7.0 A	1.4 A	0.0 A	0.0 A	8.9 A
200	125.0 A	36.2 A	0.0 A	0.0 A	161 A	5.78 A	4.20 B	0.0 A	0.0 A	5.48 A	6.9 A	1.6 A	0.0 A	0.0 A	8.6 A

1 Duncan's Multiple Range Test: Means in Columns Followed by the Same Letter are not Significantly Different at P=0.05

Table 58 Effect of nitrogen addition on the yield and nitrogen accumulation of Maple Amber soybeans: Long Plain - First Harvest

Nitrogen Addition	Mass of (kg/ha)					Percent Nitrogen in					Total Amount of Nitrogen in (kg N/ha)				
(kg N/ha)	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant
0	162[1]A	65 A	0.0 A	0.0 A	227 A	4.4 A	2.5 A	0.0 A	0.0 A	3.9 A	7.1 A	1.7 A	0.0 A	0.0 A	8.8 A
30	142 A	58 A	0.0 A	0.0 A	200 AB	4.9 AB	2.7 A	0.0 A	0.0 A	4.3 AB	7.0 A	1.6 AB	0.0 A	0.0 A	8.6 A
100	119 A	49 A	0.0 A	0.0 A	168 B	5.1 B	3.0 B	0.0 A	0.0 A	4.5 B	5.9 A	1.5 AB	0.0 A	0.0 A	7.6 A
200	117 A	46 A	0.0 A	0.0 A	163 B	5.2 B	3.1 B	0.0 A	0.0 A	4.5 B	6.1 A	1.4 B	0.0 A	0.0 A	7.3 A

1 Duncan's Multiple Range Test: Means in Columns Followed by the Same Letter are not Significantly Different at P=0.05

Nitrogen addition significantly increased the percent and total amount of nitrogen in the plant and various plant portions of Maple Amber soybeans at the second harvest at Greysville (Table 59). There was no significant effect of N addition on the mass of the total plant or plant portions (Table 59). At this stage of development if nitrogen fixation was not yet active, and there was little available soil nitrogen such a response to additional nitrogen was probable.

At the second harvest at the Long Plain site, nitrogen addition did not significantly affect leaflet mass, or the total amount of nitrogen in the leaflets, stem and petioles, or total plant (Table 60). Nitrogen addition of 100 kg N ha^{-1} resulted in a significantly higher percent nitrogen in the leaflets, stems and petioles and total plant (Table 60). Stem and petiole and total plant mass were significantly decreased by N additions of 100 and 200 kg N ha^{-1} (Table 60).

The lack of response in total plant nitrogen (above ground portion) or amount of nitrogen in various plant portions was due to higher percent nitrogen in plants and plant portions which had lower mass. The lower total plant mass with nitrogen addition of 100 and 200 kg N ha^{-1} was due entirely to the decreased stem and petiole mass; the reason for the decrease was not known. However, it was evident at this harvest (by visual assessment) that the plants were under moisture stress. The dry matter yield of soybeans at this site was only one-half to three-quarters that of soybeans at the Greysville site.

At the third harvest of soybeans at the Greysville site, there was no significant effect of nitrogen addition on any of the parameters measured except percent nitrogen in the leaflets, total amount of

Table 59 Effect of nitrogen addition on the yield and nitrogen accumulation of Maple Amber soybeans: Greysville - Second Harvest

Nitrogen Addition (kg N/ha)	Mass of (kg/ha)					Percent Nitrogen in					Total Amount of Nitrogen in (kg N/ha)				
	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant
0	592[1] A	324 A	0.0 A	0.0 A	923 A	3.8 A	1.5 A	0.0 A	0.0 A	3.0 A	22.6 A	4.9 A	0.0 A	0.0 A	23.8 A
30	810 A	441 A	0.0 A	0.0 A	1251 A	4.8 B	2.5 B	0.0 A	0.0 A	3.8 B	39.0 B	10.8 B	0.0 A	0.0 A	47.6 B
100	765 A	375 A	0.0 A	0.0 A	1140 A	4.7 B	2.6 B	0.0 A	0.0 A	4.0 BC	36.1 B	9.8 B	0.0 A	0.0 A	46.0 B
200	752 A	375 A	0.0 A	0.0 A	1127 A	5.1 B	2.7 B	0.0 A	0.0 A	4.3 C	37.8 B	10.4 B	0.0 A	0.0 A	48.1 B

1 Duncan's Multiple Range Test: Means in Columns Followed by the Same Letter are not Significantly Different at P=0.05

Table 60 Effect of nitrogen addition on the yield and nitrogen accumulation of Maple Amber soybeans: Long Plain - Second Harvest

Nitrogen Addition (kg N/ha)	Mass of (kg/ha)					Percent Nitrogen in					Total Amount of Nitrogen in (kg N/ha)				
	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant
0	441[1] A	304 A	0.0 A	0.0 A	745 A	4.1 A	1.6 A	0.0 A	0.0 A	3.1 A	18.1 A	4.8 A	0.0 A	0.0 A	22.9A
30	382 A	263 A	0.0 A	0.0 A	645 AB	4.1 A	1.5 A	0.0 A	0.0 A	3.0 A	15.8 A	4.0 A	0.0 A	0.0 A	19.9A
100	332 A	203 B	0.0 A	0.0 A	536 B	4.5 B	2.2 B	0.0 A	0.0 A	3.7 B	15.1 A	4.5 A	0.0 A	0.0 A	19.6A
200	421 A	212 B	0.0 A	0.0 A	533 B	4.4 AB	1.9 AB	0.0 A	0.0 A	3.4 AB	14.5 A	3.9 A	0.0 A	0.0 A	18.1A

1 Duncan's Multiple Range Test: Means in Columns Followed by the Same Letter are not Significantly Different at P=0.05

nitrogen in the stems and petioles, pod mass and percent nitrogen in the pods (Table 61). The trend, however, was towards greater mass, percent nitrogen and total nitrogen accumulation in the various plant portions (the pods being the exception) with increased nitrogen addition (Table 61). The lower mass and total nitrogen accumulation in the pods of soybeans which had received 100 and 200 kg N ha^{-1} was due to a delay in physiological development by approximately three days (visual inspection).

There was no consistent trends in any of the parameters measured for the third harvest of soybeans at the Long Plain site (Table 62). In some parameters, various nitrogen treatments were found to be significantly different from others; however, this was probably due more to variability within the plot related to moisture stress than any specific treatment effect. That moisture stress affected yields was visually ascertained at sampling time and could also be seen by the difference in yields of the soybeans at the two sites (Table 61 and 62).

At the fourth harvest at the Greysville site, soybeans which had received 100 and 200 kg N ha^{-1} had a greater mass of leaflets, percent nitrogen in leaflets, stems and petioles and seeds, and accumulation of nitrogen in the leaflets, stems and petioles and total plant than soybeans which had received 0 and 30 kg N ha^{-1} (Table 63). The other parameters measured were not significantly affected by nitrogen addition (Table 63). Delayed maturity and translocation of nitrogen from vegetative to reproductive organs may have been responsible for the difference between soybeans which had received 100 and 200 kg N

Table 61 Effect of nitrogen addition on the yield and nitrogen accumulation of Maple Amber soybeans: Greysville - Third Harvest

Nitrogen Addition	Mass of (kg/ha)					Percent Nitrogen in					Total Amount of Nitrogen in (kg N/ha)				
(kg N/ha)	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant
0	1393[1] A	1467 A	70 AB	0.0 A	2391 A	4.6 A	1.7 A	3.6 A	0.0 A	3.3 A	64 A	24 A	2.6 A	0.0 A	90 A
30	1522 A	1585 A	83 A	0.0 A	3299 A	4.4 A	1.5 A	3.3 B	0.0 A	3.3 A	68 A	24 A	2.7 A	0.0 A	112 A
100	1960 A	1899 A	31 B	0.0 A	4070 A	5.0 B	2.0 A	3.6 A	0.0 A	3.6 A	100 A	37 AB	1.1 A	0.0 A	145 A
200	2141 A	2071 A	53 AB	0.0 A	4083 A	5.1 B	2.2 A	3.6 A	0.0 A	3.6 A	108 A	45 B	2.0 A	0.0 A	146 A

1 Duncan's Multiple Range Test: Means in Columns Followed by the Same Letter are not Significantly Different at P=0.05

Table 62 Effect of nitrogen addition on the yield and nitrogen accumulation of Maple Amber soybeans: Long Plain - Third harvest

Nitrogen Addition	Mass of (kg/ha)					Percent Nitrogen in					Total Amount of Nitrogen in (kg N/ha)				
(kg N/ha)	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant
0	754[1] A	686 A	501 A	0.0 A	1939 AB	4.0 A	1.8 A	3.4 A	0.0 A	3.1 A	30 A	12 A	17 A	0.0 A	60 A
30	869 A	786 A	625 A	0.0 A	2280 B	4.2 B	1.7 A	3.4 A	0.0 A	3.1 A	37 A	13 A	21 A	0.0 A	71 A
100	896 A	783 A	476 AB	0.0 A	2155 AB	3.4 A	2.0 AB	3.5 A	0.0 A	3.3 A	39 A	16 A	16.5 AB	0.0 A	71 A
200	661 A	570 A	342 B	0.0 A	1573 A	4.2 B	2.2 B	3.4 A	0.0 A	3.3 A	38 A	13 A	12 B	0.0 A	52 A

1 Duncan's Multiple Range Test: Means in Columns Followed by the Same Letter are not Significantly Different at P=0.05

ha^{-1} and those which had received 0 and 30 kg N ha^{-1} in some of the parameters measured. For example, delayed maturity would delay leaflet and petiole abscission and result in a greater mass of leaflets and petioles with increased rates of nitrogen addition.

At the fourth harvest of soybeans at the Long Plain site, there were no consistent trends related to nitrogen addition in any of the parameters measured, except percent nitrogen, in the various plant portions which increased with increased addition of nitrogen (Table 64). As stated, inconsistent results with regards to nitrogen addition were probably due to the large plot variability associated with this site due to moisture stress.

At the fifth harvest (maturity), the parameters of stem and petiole mass, pod mass, seed yield, percent nitrogen in the pods, seeds and total plant of Maple Amber soybeans at the Greysville site were not significantly affected by nitrogen additions of up to 200 kg N ha^{-1} at seeding or of 100 kg N ha^{-1} at flowering (Table 65). The percent and total amount of nitrogen in the stems and petioles and percent nitrogen in the seeds were greatest with addition of 200 kg N ha^{-1} (Table 65). There was, however, a significant response in seed yield of the non-nodulating Clay isoline to addition of 100 kg N ha^{-1} (Table 66). This indicated that soil and symbiotically fixed N were sufficient for maximum production of Maple Amber soybeans under the condition of this experiment and that available soil nitrogen alone was not enough for maximum production. These results were concurrent with those found in the first and second growth chamber experiments.

Table 63 Effect of nitrogen addition on the yield and nitrogen accumulation of Maple Amber soybeans: Greysville - Fourth Harvest

Nitrogen Addition (kg N/ha)	Mass of (kg/ha)					Percent Nitrogen in					Total Amount of Nitrogen in (kg N/ha)				
	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant
0	1316[1] A	1702 A	806 A	1012 A	4869 A	2.8 A	1.0 A	1.7 A	6.4 A	2.8 A	37 A	18 A	14 A	65 A	136 A
30	1344 A	1813 A	887 A	833 A	4895 A	2.9 A	1.0 A	2.0 A	6.6 AB	2.7 A	39 A	18 A	17 A	59 A	132 A
100	1616 B	2086 A	666 A	856 A	5224 A	3.2 B	1.4 B	1.8 A	6.8 B	2.9 AB	52 B	29 A	12 A	59 A	151 AB
200	1733 B	2424 A	685 A	834 A	5224 A	3.2 B	1.9 C	2.2 A	7.0 B	2.9 AB	56 B	45 B	15 A	58 A	173 B

1 Duncan's Multiple Range Test: Means in Columns Followed by the Same Letter are not Significantly Different at P=0.05

Table 64 Effect of nitrogen addition on the yield and nitrogen accumulation of Maple Amber soybeans: Long Plain - Fourth Harvest

Nitrogen Addition (kg N/ha)	Mass of (kg/ha)					Percent Nitrogen in					Total Amount of Nitrogen in (kg N/ha)				
	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant	Leaflets	Stems and Petioles	Pods	Seeds	Total Plant
0	144[1] B	288 A	340 A	625 A	1398 A	1.8 A	0.6 C	0.6 B	6.5 B	3.4 A	2.6 B	1.7 B	2.0 A	41 A	47 A
30	163 B	295 A	381 A	621 A	1510 A	1.9 A	0.6 C	0.7 B	6.4 B	3.3 A	3.0 B	1.8 B	2.4 A	43 A	50 A
100	313 A	311 A	458 A	848 A	2078 A	1.9 A	0.8 B	0.7 B	6.7 AB	4.1 A	5.8 A	3.4 A	3.2 A	56 A	69 A
200	228 AB	459 A	302 A	560 A	1401 A	2.0 A	1.0 A	1.0 A	7.0 A	3.5 A	4.5 AB	2.9 A	2.9 A	39 A	40 A

1 Duncan's Multiple Range Test: Means in Columns Followed by the Same Letter are not Significantly Different at P=0.05

Table 65 Effect of nitrogen addition on the yield and nitrogen accumulation of Maple Amber soybeans: Greysville - FIFTH HARVEST

Nitrogen Addition (kg N/ha)	Mass of (kg/ha)				Percent Nitrogen in				Total Amount of Nitrogen in (kg N/ha)			
	Stems and Petioles	Pods	Seeds	Total Plant	Stems and Petioles	Pods	Seeds	Total Plant	Stems and Petioles	Pods	Seeds	Total Plant
0	1302[1] AB	865 A	1824 A	3991 A	0.57 BC	0.8 A	6.7 AB	3.4 B	7.4 AB	7.3 A	121.5 A	136 A
30	1249 AB	1011 A	2075 A	4335 A	0.47 C	0.7 A	6.5 B	3.4 B	5.9 B	7.5 A	134.0 A	148 A
100	1218 AB	963 A	2062 A	4242 A	0.52 BC	0.8 A	6.6 AB	3.5 B	6.5 B	7.7 AB	134.0 A	148 A
200	1424 A	836 A	1995 A	4215 A	0.70 A	0.8 A	6.8 A	3.5 B	9.9 A	6.5 A	134.0 A	149 A
100[2]	1032 B	979 A	2006 A	4017 A	0.63 AB	0.8 A	6.7 AB	3.7 A	6.6 B	7.8 A	135.0 A	150 A

1 Duncan's Multiple Range Test: Means in Columns Followed by the Same Letter are not Significantly Different at P=0.05

2 100 kg N/ha added at flowering

Table 66. Effect of nitrogen addition on seed yield of non-nod soybeans: Greysville.

Nitrogen Addition ($kg\ ha^{-1}$)	Yield ($kg\ ha^{-1}$)
0	1311 A[1]
30	1189 A
100	1841 B

1 Duncan's Multiple Range Test: Means followed by the same letter are not significantly different at P = 0.05.

At the fifth harvest of Maple Amber soybeans at Long Plain site nitrogen addition did not significantly affect any of the parameters measured except percent and total amount of nitrogen in the seed and percent nitrogen in the plant; these were significantly greater for soybeans which had received 200 kg N ha^{-1}, and 100 and 200 kg N ha^{-1}, respectively (Table 67). However, it should be noted that an increase in all parameters occurred with increased N addition. The great variability at this site, due primarily to moisture stress (as previously stated), made it undiscernible whether the trends of increased mass, percent nitrogen and total nitrogen content with increased N addition were real or not. It should be noted, however, that Zablotowicz et al. (1981) stated that moisture stress decreased fixation before it had any effect on potential yield, hence, under conditions of moisture stress, it was possible to have yield responses to the addition of nitrogen.

Table 67 Effect of nitrogen addition on the yield and nitrogen accumulation of Maple Amber soybeans: Long Plain - FIFTH HARVEST

Nitrogen Addition (kg N/ha)	Mass of (kg/ha)				Percent Nitrogen in				Total Amount of Nitrogen in (kg N/ha)			
	Stems and Petioles	Pods	Seeds	Total Plant	Stems and Petioles	Pods	Seeds	Total Plant	Stems and Petioles	Pods	Seeds	Total Plant
0	266[1] A	468 A	703 A	1437 A	1.0 A	0.8 A	6.9 A	3.8 B	2.5 A	3.6 A	48.5 A	54.6 A
30	295 A	581 A	894 A	1770 A	1.0 A	0.7 A	6.7 A	3.7 B	2.9 A	4.4 A	59.5 A	66.9 A
100	354 A	586 A	1052 A	1997 A	1.0 A	0.7 A	7.1 B	4.1 A	3.5 A	4.0 A	74.8 A	82.3 A
200	353 A	606 A	1069 A	2025 A	1.1 A	0.9 A	7.2 B	4.2 A	3.5 A	5.1 A	76.7 B	85.4 A

1 Duncan's Multiple Range Test: Means in Columns Followed by the Same Letter are not Significantly Different at P=0.05

7.3.4 The Use of a Non-nodulating Isoline of Clay as a Control for Seasonal Fixation by Maple Amber Soybeans at the Greysville Site.

The influence of stage of physiological development on nitrogen uptake, nitrogen distribution in the plants and nitrogen fixation has been well established and hence, when fixation of nitrogen was determined at various stages of growth it was important that the control and fixing crops were at the same stage of development. If not, the possibility of error in determination of the amount of nitrogen fixed increased. The fraction of mass and nitrogen in the various plant portions was found to be independent of plant size and dependant on stage of growth (Appendices B and C).

The similar fractionation of mass and nitrogen in the various plant portions of the non-nod and Maple Amber soybeans indicated that both cultivars were at approximately the same stage of physiological development at each harvest (Appendix C).

There was no significant difference in the percent utilization of fertilizer nitrogen by non-nodulating and Maple Amber soybeans at the Greysville site at each harvest, except the first (Table 68). With both cultivars, no significant increase in fertilizer utilization occurred after early flowering. Brouwer (1965) noted that at the change from vegetative to reproductive development, the shoot mass of oats increased while the root mass remained constant or decreased. Thus, the reproductive tissue within the shoots of soybeans was probably the dominant nitrogen sink from flowering to maturity and any uptake of fertilizer nitrogen would be directed towards these tissues.

Maple Amber soybeans utilized 9.8 percent of the fertilizer nitrogen applied at flowering, hence, it was evident that the roots were capable of nutrient uptake during early reproductive development. Loss of approximately 1344 kg ha^{-1} and 500 kg ha^{-1} of mass through leaflet and petiole abscission respectively from mid-podfill to maturity did not result in any decline in fertilizer nitrogen utilization (Table 68). Translocation of the fertilizer nitrogen towards the reproductive tissue was partly responsible for the lack of decline in fertilizer N utilization.

Table 68. The % utilization of fertilizer nitrogen by Maple Amber and non-nodulating Soybeans at five physiological stages of development.

Harvest Number	Physiological Stage	% Utilization of Fertilizer Nitrogen Maple Amber Soybeans	 Non-nodulating Soybeans
1	3-4 Trifoliate Leaf	3.2[1]Aa	6.3 Ab
2	Early Flowering	9.4 Ba	14.1 Ba
3	Early Pod Formation	13.6 Ba	13.2 Ba
4	Mid-podfill	9.8 Ba	11.7 Ba
5	Maturity	13.1 Ba	13.1 Ba

[1] Duncan's Multiple Range Test: Means in columns followed by the same letter are not significantly different at P = 0.05. Means in rows followed by the same small letter are not significantly different at P = 0.05.

7.3.5 Seasonal Fixation Profile of Maple Amber Soybeans: Greysville.

The "A" values calculated for Maple Amber and non-nodulating soybeans increased until mid-podfill; after which there was no significant change (Table 69). This indicated that these stages (mid-podfill and maturity) were equally adequate for determination of the total amount of dinitrogen fixed by Maple Amber. Since the "A" value measured the amount of available soil and fixed nitrogen and soil nitrogen for Maple Amber and non-nodulating soybeans respectively, the lack of significant difference in "A" values at mid-podfill and maturity suggested that there was no change in soil and fixed nitrogen availability or both changed concomitantly if no change in percent utilization occurred.

Table 69. Seasonal changes in the "A" value calculated for Maple Amber and non-nod soybeans: Greysville.

		"A" Value (kg N ha^{-1} urea)	
Harvest Number	Physiological Stage	Maple Amber Soybeans	Non-nodulating Soybeans
1	3-4 Trifoliate Leaf	115[1]A	136 A
2	Early Flowering	240 A	172 A
3	Early Pod Formation	404 B	244 B
4	Mid-podfill	1261 C	644 C
5	Maturity	1149 C	652 C

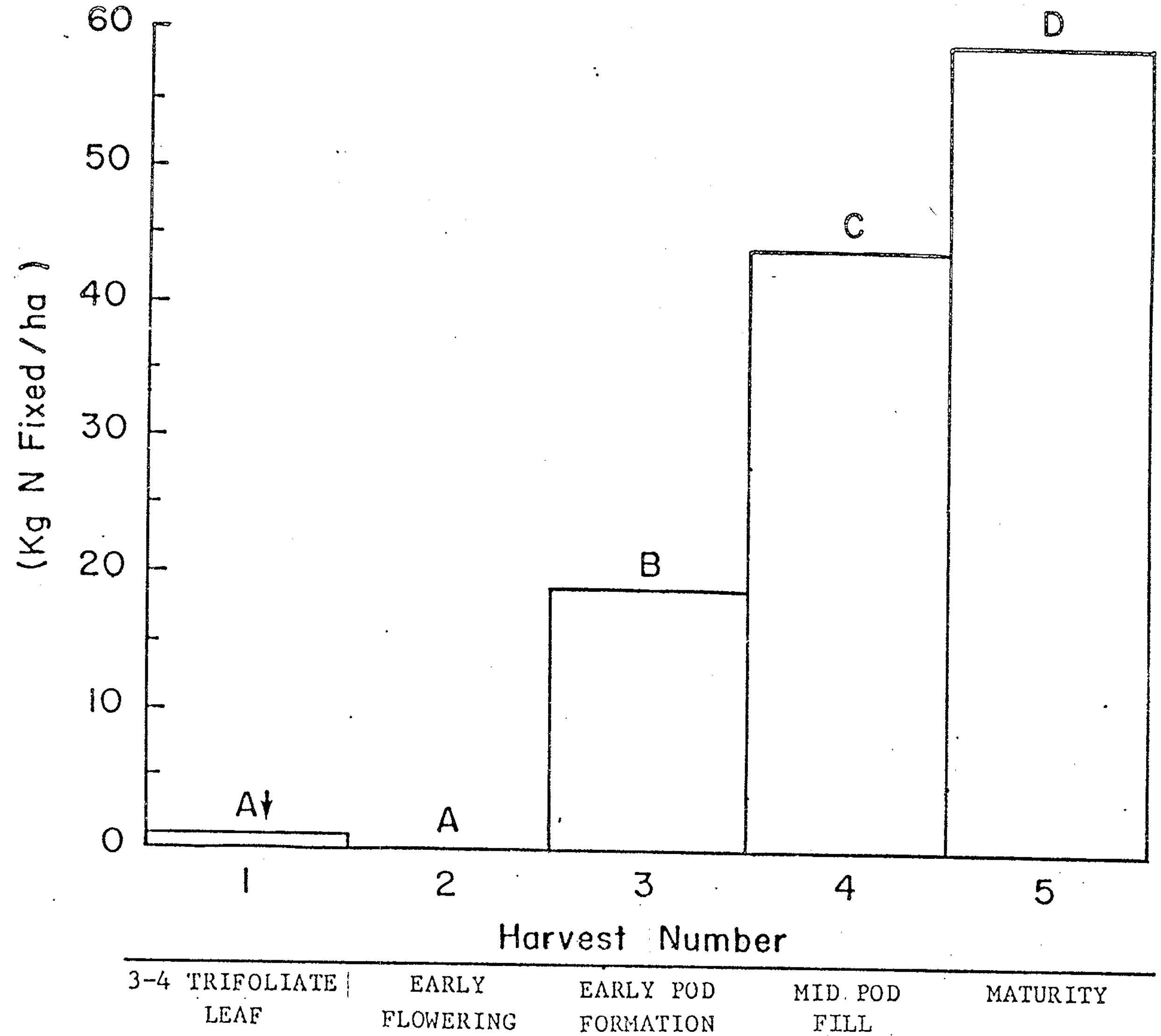
[1] Duncan's Multiple Range Test: Means in columns followed by the same letter are not significantly different at P = 0.05.

The ^{15}N assisted difference method and "A" value method gave similar profiles of seasonal dinitrogen fixation except at the fourth harvest (Figures 8 and 9). The difference between the two methods in the measurement of the amount of nitrogen fixed at mid-podfill was due to a difference in fertilizer utilization by the two cultivars; the effect of fertilizer utilization on the measurement of dinitrogen fixation by these two methods was discussed in the literature review. The ^{15}N assisted difference method indicated that appreciable nitrogen was fixed from mid-podfill to maturity, while the "A" value method indicated no appreciable fixation occurred. The "A" value method was probably more valid since acetylene reduction assay indicated only minimal nitrogenase activity from mid-podfill to maturity (Figure 10). Quantitative determination of dinitrogen fixation by acetylene reduction assay was not attempted for reasons outlined in literature review.

Israel (1981) showed that the physiological stage of development at which fixation occurs was dependent on the cultivar. Ransom and Davis cultivars when inoculated with USDA -31 and 110 Rhizobium fixed the majority of their nitrogen during vegetative and reproductive development respectively. The "A" value method, ^{15}N assisted difference method and acetylene reduction assay, in this experiment, all indicated that very little fixation and nitrogenase activity occurred during vegetative development (Figs. 8, 9, and 10). Both ^{15}N assisted methods of measuring fixation showed maximum rate of fixation was during reproductive development (Figs. 8 and 9). Nitrogenase activity, as measured by acetylene reduction assay, however, increased until mid-podfill after which it declined rapidly (Fig. 10). Therefore, it was apparent that nitrogen fixation occurred during reproductive development in Maple Amber soybeans; the stage of maximum rate of dry matter and nitrogen accumulation and hence the period of greatest demand for carbohydrate/photosynthate and nitrogen. Regitnig (1983) also found with Maple Presto soybeans that the maximum rate of fixation occurred during reproductive development.

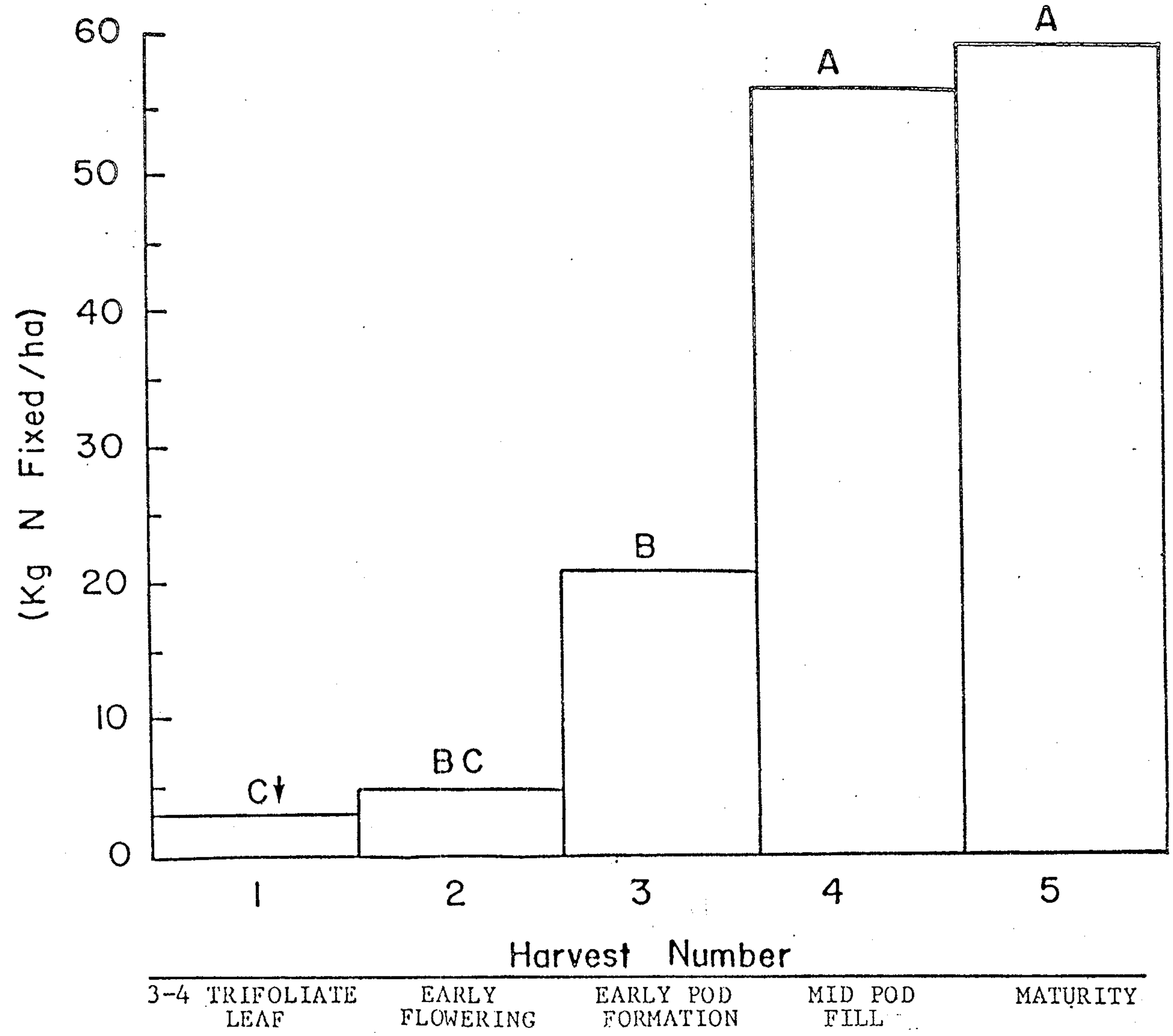
7.3.6 The Effect of Nitrogen Addition on the Percent Utilization of Fertilizer by, "A" Value of and Nitrogen Fixation in Maple Amber Soybeans: Greysville.

Increased nitrogen addition increased the percent utilization of fertilizer nitrogen by the aerial portion of Maple Amber and non-nodulating soybeans (Table 70). Deibert et al. (1979) also found that the percent utilization of fertilizer increased with increased nitrogen addition. They postulated that this was due to soybeans' utilization of N at later growth stages than other crops (cereal grains). They noted that non legume crops normally showed decreased fertilizer nitrogen utilization with increased rate of addition. The increase in fertilizer utilization with nitrogen addition could have also been due to accumulation of fertilizer nitrogen in the root system at lower rates of N addition. Results from the second growth chamber experiment showed that at the lower rate of N addition (600 mg N pot^{-1}) that 37.9 and 47.6 percent of the fertilizer nitrogen applied was utilized by shoot and root portions of Maple Amber soybeans, respectively; in contrast, 50.3 and 30.4 percent of applied nitrogen was utilized by the shoots and roots respectively of plants which had received 1200 mg N pot^{-1}. The reasons for such a change are discussed



↓DUNCAN'S MULTIPLE RANGE TEST: COLUMNS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT P=0.05

FIG. 8. THE AMOUNT OF NITROGEN FIXED AT VARIOUS PHYSIOLOGICAL STAGES OF DEVELOPMENT CALCULATED BY THE ^{15}N ASSISTED DIFFERENCE METHOD



↓ DUNCAN'S MULTIPLE RANGE TEST: COLUMNS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT, P=0.05

FIG. 9. THE AMOUNT OF NITROGEN FIXED AT VARIOUS PHYSIOLOGICAL STAGES OF DEVELOPMENT CALCULATED BY THE "A" VALUE METHOD

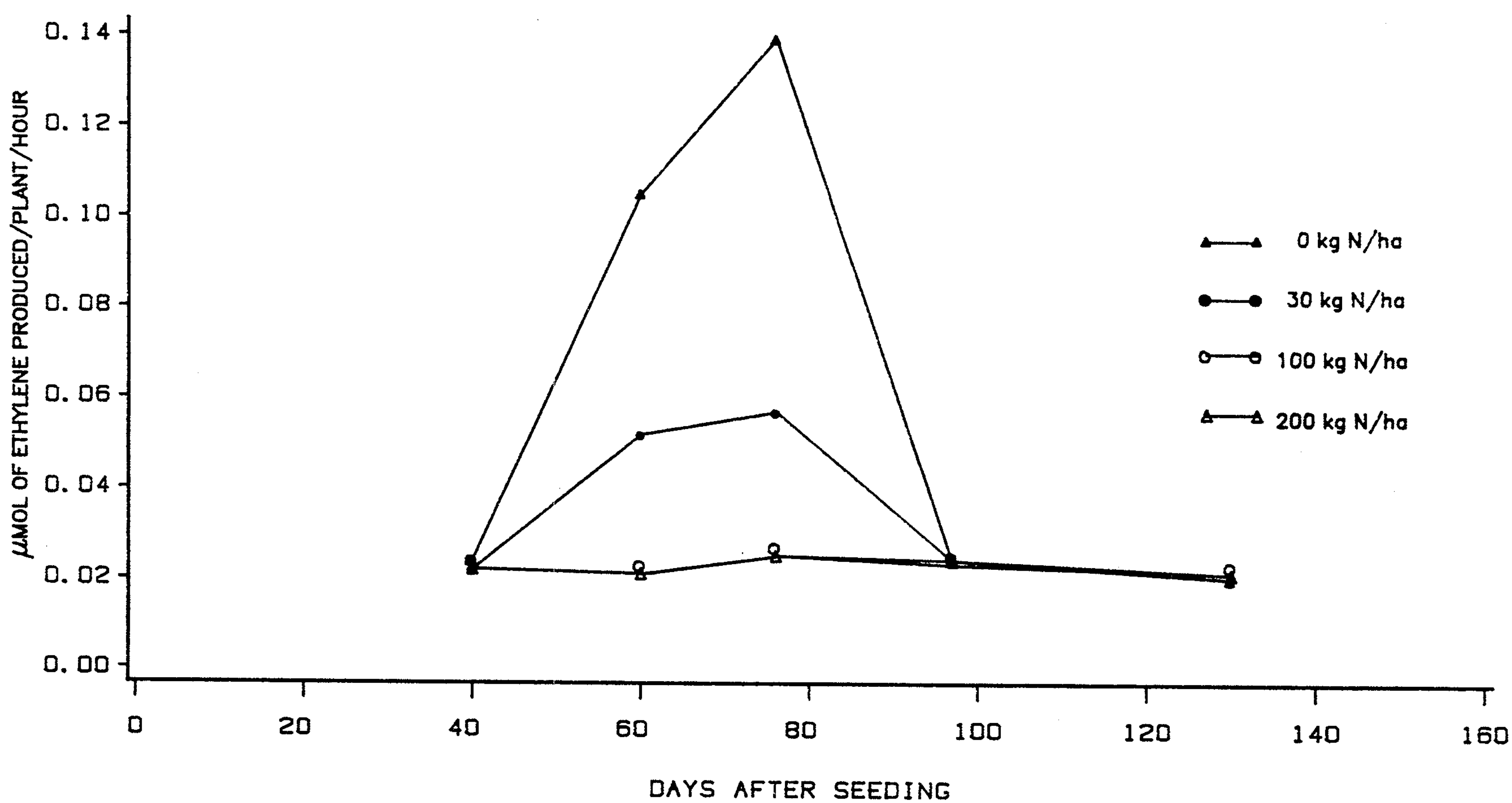


FIG. 10. SEASONAL ACETYLENE REDUCTION PROFILE OF MAPLE AMBER SOYBEANS GREYSVILLE

in Section 6.3.2. It should be noted, however, that the plants in the growth chamber study had not reached maturity at harvest, hence direct comparison may not be entirely valid.

Table 70. The effect of nitrogen addition on the utilization of fertilizer at maturity.

Nitrogen Addition (kg N ha^{-1})	% Utilization of Fertilizer Nitrogen	
	Maple Amber Soybeans	Non-nodulating Soybeans
30	13.1[1]Aa	13.1 Aa
100	21.9 Ba	17 Ba
200	19 B	---
100*	9.8 A	---

* Nitrogen added at Flowering.
[1] Duncan's Multiple Range Test: Means in columns followed by the same capital letter are not significantly different at P = 0.05; Means in rows followed by the same small letter are not significantly different at P = 0.05.

The utilization of fertilizer nitrogen by Maple Amber soybeans which had received 100 kg N ha^{-1} at flowering was lower than that of plants which had received the nitrogen at seeding (Table 71). In contrast, Deibert et al. (1979) found no difference in fertilizer utilization between soybean plants which had received nitrogen at seeding or flowering; however, these plots were sprinkler irrigated. Therefore, the low utilization of fertilizer by Maple Amber soybeans at flowering was probably due to inadequate rainfall and/or movement of surface applied fertilizer into the root zone and/or timing of the rainfall. If rainfall events occurred late in the growing season during a period of decline in root activity, fertilizer utilization would have been low.

There was no significant difference between Maple Amber and non-nodulating soybeans in the utilization of fertilizer at the 30 and 100 kg N ha^{-1} rates of fertilizer addition hence it appeared that the fertilizer nitrogen was equally available to both cultivars.

Nitrogen addition of 100 and 200 kg N ha^{-1} decreased the "A" value calculated for Maple Amber soybeans (Table 71); in contrast, the "A" value calculated for the non-nodulating soybeans remained constant (Table 71). The "A" value calculated for the non-nod soybeans, a measure of available soil nitrogen, indicated that the "A" value technique was independent of the rate of fertilizer addition (the original premise of Fried and Dean (1952)) and that the amount or rate of soil nitrogen mineralization was not changed by nitrogen addition. The "A" value calculated for Maple Amber soybeans measured available soil and fixed nitrogen. Since no decline in available soil nitrogen occurred with nitrogen addition the decline in the "A" value with addition of 100 and 200 kg N ha^{-1} was probably due to a decline in available fixed nitrogen.

The ^{15}N assisted difference method and the "A" value method gave similar estimates of the amount of dinitrogen fixed by Maple Amber soybeans at each rate of nitrogen addition (Table 72). Hence, it appeared, at final harvest, that both techniques were equally suitable for measuring dinitrogen fixation. Nitrogen fixation did not occur in Maple Amber soybeans which had received 100 and 200 kg N ha^{-1}. These results were concomitant with those of the lysimeter experiment.

Table 71. The effect of nitrogen addition on the "A" value calculated for Maple Amber and non-nodulating soybeans at maturity: Greysville.

Nitrogen Addition (kg N ha^{-1})	"A" Value (kg N ha^{-1} urea)	
	Maple Amber Soybeans	Non-nodulating Soybeans
30	1149[1]A	652 A
100	624 B	681 A
200	601 B	--

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

Appreciable fixation did not occur in Maple Amber soybeans which had received 100 kg N ha^{-1}. Also, in both instances the estimate of the amount of nitrogen fixed (as measured by the classical difference method) was between 70 and 80 kg N ha^{-1}. In contrast, the greatest amount of fixation reported by Regitnig (1983) for Maple Presto soybeans was 47 kg N ha^{-1}, this appeared to substantiate premises from the growth chamber experiments that Maple Amber soybeans appeared to be better capable of supplying their own nitrogen nutritional requirements than Maple Presto.

Acetylene reduction assay was also used in this study. At the first, fourth and fifth harvests there was very little nitrogenase activity regardless of treatment (Table 73). This was in agreement with the results obtained for seasonal fixation with the "A" value technique. At the second and third harvests nitrogen addition significantly decreased the nitrogenase activity of the nodules (Table 73) and subsequently dinitrogen fixation (Table 72).

Table 72. The effect of nitrogen addition on the amount of nitrogen fixed by Maple Amber soybeans at the fifth harvest: Greysville.

Nitrogen Added (kg N ha^{-1})	Amount of Nitrogen Fixed (kg N ha^{-1})	
	"A" Value Method	^{15}N Assisted Difference Method
0	--	71 A
30	59[1]A	59 A
100	0*B	0*B
200	0*B	0*B

[1] Duncan's Multiple Range Test: Means in columns followed by the same letter are not significantly different at P = 0.05.
* Minus values taken as an indication of zero fixation.

Table 73. Ethylene production rate (umol ethylene $plant^{-1}$ hr^{-1}) at various harvests: Greysville.

Nitrogen Added (kg N ha^{-1})	Harvests				
	1	2	3	4	5
0	0.024 A[1]	0.105 A	0.139 A	0.024 A	0.020 A
30	0.022 A	0.052 B	0.057 AB	0.024 A	0.020 A
100	0.022 A	0.021 B	0.025 B	0.023 A	0.021 A
200	0.022 A	0.021 B	0.025 B	0.024 A	0.021 A

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

The acetylene reduction assay like the two ^{15}N methods of measuring fixation indicated that little nitrogenase activity and hence fixation occurred in nodules of Maple Amber soybeans which had received 100 kg N ha^{-1}; thus, it appeared that the acetylene reduction assay, though a point estimate of fixation, provided good qualitative information on the effects of nitrogen addition on nitrogen fixation when samples were obtained at the proper stages of development. Other researchers such as Regitnig (1983), and Semu and Hume (1979) have used the assay to provide qualitative information in studies of fixation. Use of this technique for quantitatively measuring fixation was considered dubious for reasons outlined in the literature review and reviews by LaRue and Patterson (1981), and Hardy et al. (1973) and thus was not attempted.

Nitrogen addition also decreased and delayed nodule development on Maple Amber soybeans (Table 74). Maximum numbers of nodules per plant were attained by early flowering. The decrease and delay in nodulation with nitrogen addition has been well documented in the literature (Rabie, 1981; Streeter, 1981; Ham et al., 1975; Aba-Shakra et al., 1972; Johnson and Hume, 1972). Theories with regard to the cause of suppression of nodule development with N addition can be found in the literature review. The number of nodules per plant on roots of Maple Amber soybeans were of similar order of magnitude as that found by Regitnig (1983) on the roots of Maple Presto and Nangu (1980) on the roots of Malayan, Orba, and TGm 686 soybeans. The number of nodules found on the roots of Maple Amber soybeans in the growth chamber experiment were approximately double that found in the field experiment. Regitnig (1983) found that the number of nodules on the roots of field grown Maple Presto soybeans were at least half that found on the roots of Maple Presto soybeans grown in the growth chamber. Therefore, the difference in nodule numbers on roots of field and growth chamber grown soybeans was probably due to differences in root environment and method of sampling.

Table 74. Nodules per plant at various harvests: Greysville

Nitrogen Added (kg N ha^{-1})	Harvests 1	2	3	4	5
0	2.3 Ab[1]	12.5 Aa	8.3 aA	10.2 Aa	9.5 Aa
30	2.0 Ab	8.5 ABa	8.5 aA	7.0 Ba	6.2 Ba
100	0.6 Bb	4.0 BCa	5.4 aB	5.3 Ba	2.9 Ca
200	0.0 Bb	1.0 Ca	2.7 aC	0.7 Ca	1.5 Ca

[1] Duncan's Multiple Range Test: Numbers followed by the same capital letters in columns are not significantly different at P = 0.05. Numbers followed by the same small letters in rows are not significantly different at P = 0.05.

7.3.7 Nitrogen Fixation by Maple Amber Soybeans: Long Plain

The classical difference and acetylene reduction methods of measuring fixation indicated that no appreciable fixation of dinitrogen occurred by Maple Amber soybeans at the Long Plain site (Table 75). Moisture stress, which resulted in decreased yield, appeared to inhibit fixation. A number of workers (Tu and Hiezkamp, 1977; Sprent, 1977; Sprent, 1972; and Kuo and Boersma, 1971) have found decreased yield and fixation with moisture stress. Although there was no direct measurement of the magnitude of the moisture stress at this site, visual observation indicated that the stress was severe; the leaves of corn on adjacent plots were rolled even in early morning.

There was no difference between Maple Amber and non-nodulating soybeans which had received similar rates of N in any of the yield parameters measured except percent nitrogen in the seed at the 0 kg N ha^{-1} rate of N addition. Maple Amber soybeans had a significantly greater concentration of nitrogen in the seed than non-nodulating soybeans; however, the nitrogen content in the seed and total plant portion of Maple Amber was less than in the non-nod soybeans (Table 75). Hence, the greater concentration of nitrogen in Maple Amber at 0 kg N ha^{-1} rate of nitrogen addition was probably due to less dilution of nitrogen.

At this site, there was an apparent trend towards increased dry matter and seed yield and nitrogen content in the seed and total plant with nitrogen addition. Zablotowicz _et_ _al_. (1981) noted that moisture stress decreased fixation before it had any effect on yield; hence, yield response to N addition may have occurred under the limiting moisture conditions of this experiment. However, the lack of positive response of the non-nodulating soybeans to N addition (in actuality the response appeared negative) indicated that the apparent response of Maple Amber to N addition was due to plot variability.

The acetylene reduction profile of the Maple Amber soybeans at the Long Plain site was similar to that of Maple Amber soybeans at the Greysville site; however, the amount of ethylene produced was considerably reduced (Fig. 11), due to the moisture stress at this

Table 75. Seed Yield, percent nitrogen in the seed, total amount of nitrogen in the seed, plant yield and total amount of nitrogen in the plant of Maple Amber and non-nodulating soybeans: Long Plain.

Amount of Nitrogen Added (kg N ha^{-1})	Property	Soybean Culture	
		Maple Amber	Non-nodulating
0	Seed Yield (kg/ha)	703[1]A	1123 A
0	% Nitrogen in Seed	6.91 A	5.84 B
0	Total Nitrogen in Seed (kg N/ha)	48.5 A	64.5 A
0	Total Plant Yield (kg/ha)	1137 A	2283 A
0	Total Nitrogen in Plant (kg N/ha)	55 A	74 A
30	Seed Yield (kg/ha)	894 A	948 A
30	% Nitrogen in Seed	6.68 A	6.21 A
30	Total Nitrogen in Seed (kg N/ha)	60 A	58 A
30	Total Plant Yield (kg/ha)	1770 A	1862 A
30	Total Nitrogen in Plant (kg N/ha)	67 A	66 A
100	Seed Yield (kg/ha)	1052 A	923 A
100	% Nitrogen in Seed	7.14 A	6.36 A
100	Total Nitrogen in Seed (kg N/ha)	75 A	57 A
100	Total Plant Yield (kg/ha)	1997 A	1734 A
100	Total Nitrogen in Plant (kg N/ha)	82 A	64 A

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

site. Although there was an apparent decrease in nitrogenase activity with N addition, the decrease was not significant (Table 76, Fig. 11).

Nodule numbers decreased at the Long Plain site with N addition (Table 77). Similar results were found at the Greysville site. Although the number of nodules on soybeans at both sites were similar for plants which had received the same N treatment, visual inspection

Table 76. Ethylene production (umol ethylene plant^{-1} hr^{-1}) at various harvests: Long Plain.

Nitrogen Added (kg N ha^{-1})	Harvest 1	2	3	4	5
0	0.025 A[1]	0.021 A	0.043 A	0.031 A	0.025 A
30	0.026 A	0.019 A	0.041 A	0.028 A	0.025 A
100	0.023 A	0.020 A	0.036 A	0.026 A	0.023 A
200	0.022 A	0.021 A	0.027 A	0.024 A	0.024 A

[1] Duncan's Multiple Range Test: Means in columns followed by the same letter are not significantly different at $P = 0.05$.

indicated that the nodules on soybeans from the Long Plain site were smaller. Hanus, et al. (1981) indicated that hydrogenase activity of the nitrogenase enzyme may protect nodule integrity during periods of moisture stress. Thus, the apparent decline in ethylene production with N addition is probably due to hydrogenase activity for maintenance of nodule integrity under conditions of moisture stress and the decline in nodule numbers with N addition.

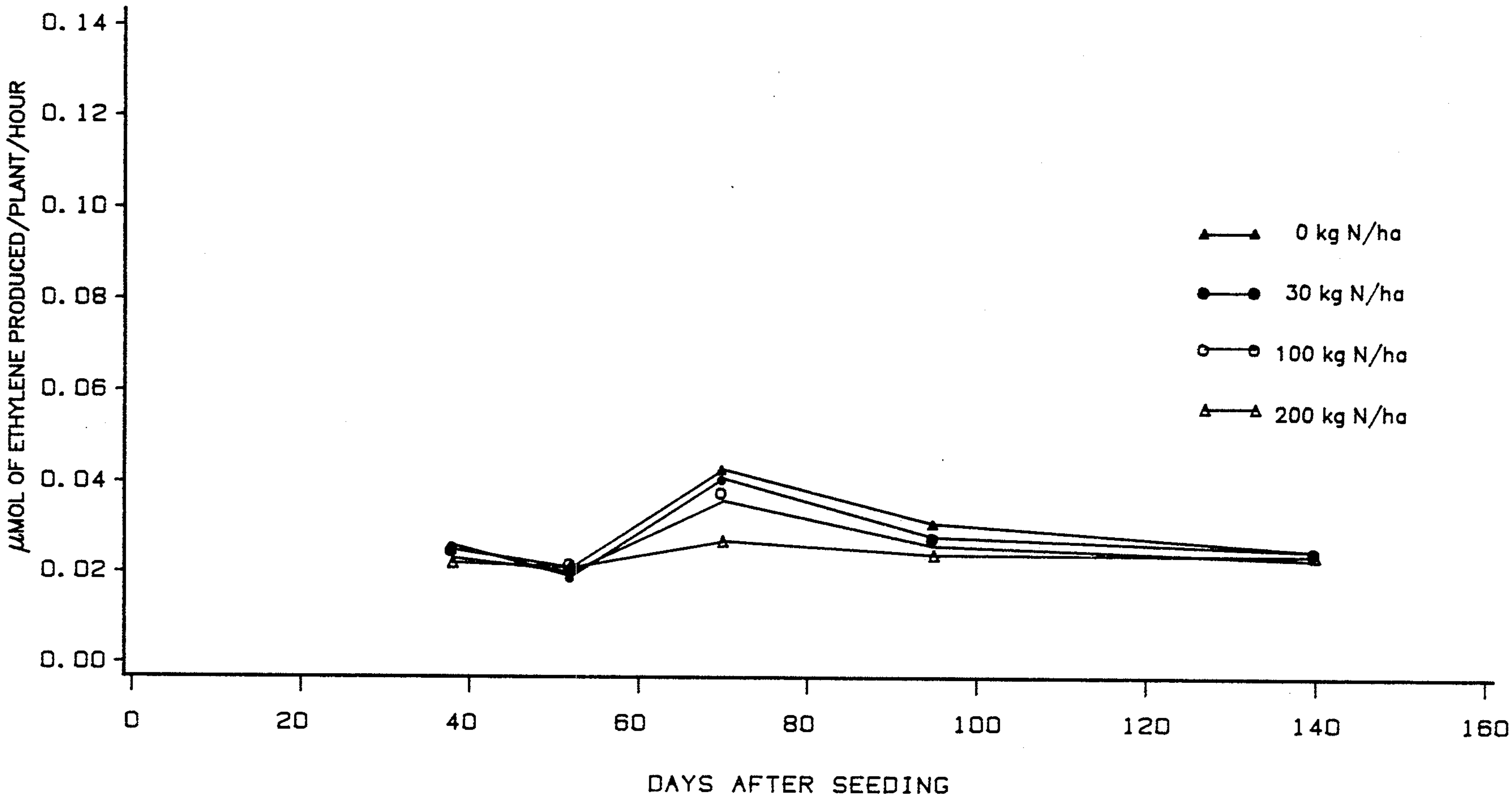


FIG. 11. SEASONAL ACETYLENE REDUCTION PROFILE OF MAPLE AMBER SOYBEANS LONG PLAIN

Table 77. Nodules per plant at various harvests: Long Plain.

Nitrogen Added (kg N ha^{-1})	Harvests 1	2	3	4	5
0	2.5 Ab[1]	10.4 Aa	10.5 Aa	7.8 Aa	2.8 ABb
30	1.2 Bc	6.0 Bb	8.7 Aa	5.2 Bb	3.7 Ab
100	0.9 Bc	3.8 BCbc	5.0 Ba	3.5 BCbc	4.2 Ab
200	0.6 Ba	1.2 Ca	3.0 Ba	1.8 Ca	1.03 Ba

[1] Duncan's Multiple Range Test: Numbers followed by the same capital letters in columns are not significantly different at P = 0.05. Numbers followed by the same small letters in rows are not significantly different at P = 0.05.

7.3.8 Conclusions

The following can be concluded:

1) Maximum rate of dry matter and nitrogen accumulation of Maple Amber soybeans occurred during reproductive development (early flowering to mid-podfill).

2) Moisture stress lowered the yield potential of Maple Amber soybeans at the Long Plain site by 50% when compared to soybeans at the Greysville site.

3) There was no significant effect of nitrogen addition on dry matter yield, seed yield or nitrogen distribution in Maple Amber soybeans at either site. However, at some harvests nitrogen addition appeared to delay physiological development.

4) At each stage of physiological development the proportion of dry matter and nitrogen in the various plant portions was found to be

constant regardless of plant size. This was used to determine that Maple Amber and non-nod soybeans were at similar stages of physiological development at each harvest.

5) It appeared that non-nod Clay soybeans were an adequate control for determination of amount of fixation by Maple Amber soybeans throughout the growing season.

6) Changes in allocation of photosynthate appeared to precede changes in allocation of nitrogen.

7) There was, for the most part, no significant difference in fertilizer utilization of non-nod and Maple Amber soybeans at the Greysville site. Fertilizer nitrogen applied at seeding did not appear to be utilized after flowering. However, nitrogen applied at flowering was utilized, but the percent utilization was less than that of spring applied fertilizer.

8) There was no decline in fertilizer nitrogen utilization and very little reduction in total nitrogen from mid-podfill to maturity though a loss in mass of 1344 kg ha^{-1} and 500 kg ha^{-1} occurred due to leaflet and petiole abscission, respectively. Hence, it appeared that the majority of nitrogen in the aerial portion of the plant was translocated from vegetative to reproductive tissue.

9) The ^{15}N assisted difference and "A" value methods gave similar estimates of seasonal fixation for Maple Amber soybeans. However, in the one case of discrepancy between the two methods (measurement of the amount of nitrogen fixed from mid-podfill to maturity) the "A" value and acetylene reduction assay both indicated no fixation occurred while the ^{15}N assisted difference method indicated

appreciable fixation. Hence, in this case, the "A" value method appeared more valid than the ^{15}N assisted difference method.

10) Nitrogen addition significantly decreased fixation of Maple Amber soybeans at the Greysville site. At rates of 100 and 200 kg N ha^{-1} no appreciable fixation occurred. Fixation of atmospheric dinitrogen by Maple Amber soybeans at the Long Plain site was not apparent due to moisture stress.

11) Acetylene reduction assay was found to be useful for point determinations of nitrogenase activity.

12) Nitrogen addition decreased nitrogenase activity of the nodules during reproductive development on the soybeans at the Greysville site. Nitrogenase activity of nodules on soybeans at the Long Plain site was considerably less than that found at the Greysville site. There was no significant effect of N addition on nitrogenase activity.

13) Nitrogen addition at both sites delayed and decreased nodulation.

GENERAL CONCLUSIONS

Field, lysimeter and growth chamber experiments showed that nitrogen additions of 200 kg N ha^{-1}, 100 kg N ha^{-1} and 1200 mg N pot^{-1} respectively did not increase the yield or protein content of Maple Amber soybeans. Thus, Maple Amber soybeans, unlike Maple Presto, appeared capable of supplying their nitrogen nutritional requirements through the fixation of atmospheric dinitrogen. The amount of dinitrogen fixed (by the classical difference method) was 79 kg N ha^{-1}, 71 kg N ha^{-1} and 1640 mg N pot^{-1} for lysimeter, field and growth chamber experiments, respectively. The maximum fixation of dinitrogen occurred from early flowering to mid-pod formation for Maple Amber and corresponded to the period of maximum dry matter and nitrogen accumulation. Nitrogen addition was found in all cases to suppress fixation by Maple Amber; in field and lysimeter experiments 100 kg N ha^{-1} was found to completely inhibit fixation. Nitrogen addition was also found to delay nodulation and decrease nodule numbers. Moisture stress lowered the yield potential of Maple Amber soybeans at the Long Plain site by 50% when compared to soybeans at the Greysville site.

The nitrogen nutritional requirements of Aladin fababeans and lentils were evaluated in growth chamber and lysimeter experiments, and growth chamber experiments respectively. The results showed that fababeans could fix enough nitrogen for their nutritional requirements: 250 kg N ha^{-1} and 1645 mg N pot^{-1} (by the classical difference method) in lysimeter and growth chamber experiments respectively. Lentils however, only fixed 200 mg N pot^{-1} in growth chamber experiments and needed additional nitrogen for maximum yield. Lentils were also found to be more susceptible to the toxic effects of high rates of nitrogen addition as urea than soybeans and fababeans. In both cases, nitrogen addition decreased fixation and nodule development. The suppression of fixation appeared to be directly proportional to fertilizer nitrogen accumulation.

Comparison of the amount of nitrogen fixed by soybeans, fababeans and lentils showed that fababeans were by far the best fixers of dinitrogen while lentils fixed the least.

Various methods of assessing dinitrogen fixation were used. ^{15}N assisted difference method, classical difference method, "A" value method, acetylene reduction assay and nodule counts. The ^{15}N assisted difference method and the "A" value method in most cases gave similar estimates of the amount of dinitrogen fixed by the legumes. However, discrepancies between the two methods occurred when the control and the legume had different fertilizer nitrogen utilization. In such cases, the "A" value method was thought to give a better estimate of fixation. Acetylene reduction assay and nodule counts were suitable in qualitative estimates of fixation. Utilization of only the aerial plant portion for measurement of symbiotic nitrogen fixation by legumes under estimated fixation at lower rates of nitrogen addition. Results also showed that the fertilizer nitrogen was not uniformly distributed in the plant parts but tended to accumulate preferentially in the roots.

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Appendix A

SEASONAL DRY MATTER AND NITROGEN ACCUMULATION OF MAPLE AMBER SOYBEANS RECEIVING 30, 100 AND 200 KG N HA^{-1} AT THE GREYSVILLE AND LONG PLAIN SITES.

30 kg N ha^{-1} treatment: Greysville.

Harvest No.	Mass of (kg ha^{-1}) Leaflets	Stems and Petioles	Pods	Seeds	Total Plant	Amount of Nitrogen in (kg N ha^{-1}) Leaflets	Stems and Petioles	Pods	Seeds	Total Plant
1	138.8 C[1]	43 C	0.00 A	0 A	182 A	7.2 C	1.5 C	0 C	0 A	8.8 C
2	809 B	441 C	0.00 A	0 A	1250 C	39 B	10.8 B	0 A	0 A	48 C
3	1337 A	1343 AB	80.8 A	0 A	2761 B	61 A	20.5 A	2.6 C	0 A	98 B
4	1344 A	1813 A	857 B	662 B	4896 A	39 B	17.9 A	17.2 A	59 B	131 AB
5	0.0 C	1249 B	1011 C	2075 C	4335 A	0.0 C	5.9 BC	7.5 B	134.4 C	148 A

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

100 kg N ha^{-1} treatment: Greysville.

Harvest No.	Mass of (kg ha^{-1}) Leaflets	Stems and Petioles	Pods	Seeds	Total Plant	Nitrogen Accumulation in (kg N ha^{-1}) Leaflets	Stems and Petioles	Pods	Seeds	Total Plant
1	124 C[1]	39 A	0.00 A	0.0 B	164 A	1.00 C	1.4 A	0.00 A	0.0 A	8.9 A
2	765 C	375 A	0.00 A	0.0 A	164 A	1.00 C	1.4 A	0.00 A	0.0 A	46.0 B
3	2141 A	1899 B	31.0 A	0.0 A	4070 B	108 A	36.5 B	2.63 B	0.0 A	145 C
4	1616 B	2086 B	666 B	8.56 B	5225 C	52 B	28.6 B	12.1 C	59 B	151 C
5	0.0 D	1218 C	962 C	2062 C	4242 BC	0.00 C	6.5 A	6.9 D	134 C	148 C

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

APPENDIX A - cont'd

100 kg N ha^{-1} treatment: Long Plain.

Harvest No.	Mass of (kg ha^{-1}) Leaflets	Stems and Petioles	Pods	Seeds	Total Plant	Amount of Nitrogen in (kg N ha^{-1}) Leaflets	Stems and Petioles	Pods	Seeds	Total Plant
1	117 C[1]	45.7 D	0.0 B	0.0 B	163 B	5.9 C	1.38 C	0.0 B	0.00 B	7.3 B
2	332 B	203 C	0.0 B	0.0 B	535 B	15.1 B	4.5 B	0.0 B	0.0 B	19.6 B
3	896 A	783 A	476 A	0.0 B	2155 A	39.0 A	15.5 A	14.0 A	0.0 B	70.9 A
4	313 B	459 B	458 A	847 A	2077 A	5.8 C	3.5 B	3.2 B	56.3 A	66.7 A
5	0.0 D	354 B	586 A	1052 A	1997 A	0.0 D	3.5 B	4.0 B	74.8 A	82.3 A

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

200 kg N ha^{-1} treatment: Long Plain.

Harvest No.	Mass of (kg ha^{-1}) Leaflets	Stems and Petioles	Pods	Seeds	Total Plant	Nitrogen Accumulation in (kg N ha^{-1}) Leaflets	Stems and Petioles	Pods	Seeds	Total Plant
1	118.8 CD[1]	49.3 D	0.0 C	0 C	168 A	6.11 C	1.5 B	0.0 C	0 C	7.6 C
2	421.5 B	212 C	0.0 C	0 C	533 C	14.25 B	3.9 B	0.0 C	0 C	18.1 C
3	661 A	570 A	342 B	0 C	1573 AB	27.60 A	12.3 A	11.7 A	0 C	52.0 B
4	228 C	311 B	302 B	560 B	1401 B	4.5 C	2.9 B	2.9 BC	38.9 B	49.1 B
5	0.0 D	353 B	606 A	1069 A	2025 A	0.0 D	3.5 B	5.1 B	76.7 A	85.4 A

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

Appendix B

RAW DATA VERIFYING THE CONSISTENCY OF THE FRACTIONATION METHOD O KG N TREATMENT, SECOND HARVEST: GREYSVILLE SITE.

Mass of (kg ha^{-1})		Fraction of Plant Mass in		Nitrogen Accumulation in (kg N ha^{-1})		Fraction of N in	
Leaflets	Stems and Petioles	Leaflets	Stems and Petioles	Leaflets	Stems and Petioles	Leaflets	Stems and Petioles
368	232	61	39	14.5	18.5	79	21
442	254	64	36	17.6	21.7	81	19
700	369	66	34	26.3	31.1	84	16
886	441	67	33	32.0	38.6	83	17

Fractionation of plant mass and nitrogen in the various plant portions at the first harvest: Greysville site.

Soybean Cultivar	Percent Plant Mass in Leaflets	Percent Plant Mass in Stems and Petioles	Percent Plant Nitrogen in Leaflets	Percent Plant Nitrogen in Stems and Petioles
Maple Amber	77 A[1]	23 A	83 A	17 A
Non-nodulating	78 A	23 A	84 A	16 A

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

Fractionation of plant mass and nitrogen in the various plant portions at the second harvest: Greysville site.

Soybean Cultivar	Percent Plant Mass in Leaflets	Percent Plant Mass in Stems and Petioles	Percent Plant Nitrogen in Leaflets	Percent Plant Nitrogen in Stems and Petioles
Maple Amber	65 A[1]	35 A	78 A	22 A
Non-nodulating	60 A	40 A	79 A	21 A

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

APPENDIX C - cont'd

Fractionation of plant mass and nitrogen in the various plant portions at the third harvest: Greysville site.

Soybean Cultivar	Percent Plant Mass in Leaflets	Percent Plant Mass in Stems and Petioles	Percent Plant Nitrogen in Leaflets	Percent Plant Nitrogen in Stems and Petioles
Maple Amber	53 A[1]	47 A	78 A	22 A
Non-nodulating	49 B	51 B	79 A	21 A

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

Fractionation of plant mass and nitrogen in the various plant portions at the fourth harvest: Greysville site.

Soybean Cultivar	Percent Plant Mass in				Percent Plant Nitrogen in			
	Leaflets	Stems and Petioles	Pods	Seeds	Leaflets	Stems and Petioles	Pods	Seeds
Maple Amber	28 A[1]	35 A	18 A	19 A	30 A	13 A	13 A	45 A
Non-nodulating	29 A	36 A	16 A	19 A	32 A	13 A	11 A	43 A

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

Fractionation of plant mass and nitrogen in the various plant portions at the fifth harvest: Greysville site.

Soybean Cultivar	Percent Plant Mass in				Percent Plant Nitrogen in			
	Leaflets	Stems and Petioles	Pods	Seeds	Leaflets	Stems and Petioles	Pods	Seeds
Maple Amber	--	28 A[1]	24 A	48 A	--	4 A	6 A	90 A
Non-nodulating	--	37 B	19 A	44 A	--	8 A	7 A	85 A

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